

## Section 9. Laboratory Considerations

---

|   |             |
|---|-------------|
| <b>9.1. Overview and General Guidance .....</b>   | <b>9--1</b> |
| <b>9.2. Specimen Labeling .....</b>   | <b>9-4</b>  |
| <b>9.3. Procedures for Specimens that cannot be evaluated .....</b>   | <b>9-4</b>  |
| <b>9.4. Use of LDMS.....</b>  | <b>9-5</b>  |
| 9.4.1. LDMS Codes for Specimen Log In.....  | 9-6         |
| 9.4.2. Logging in Time for PK Samples:.....   | 9-7         |
| 9.4.3. Entering weight measurements of vaginal swabs for PK in LDMS: .....  | 9-7         |
| <b>9.5. Urine Testing for Pregnancy and Urinary Tract Infection .....</b>   | <b>9-9</b>  |
| 9.5.1. Specimen Collection.....   | 9-9         |
| 9.5.2. Pregnancy Testing .....  | 9-9         |
| 9.5.3. Urinary Tract Infection .....  | 9-9         |
| <b>9.6. Blood Specimens for Chemistry, Hematology, HIV testing, Syphilis, Plasma Archive, MK-2048 and Vicriviroc Blood Levels .....</b>                     | <b>9-10</b> |
| 9.6.1. Specimen Collection and Initial Processing .....   | 9-10        |
| 9.6.2. Chemistry (Alanine transaminase, Aspartate aminotransferase, and Creatinine), Hematology (CBC with Diff and Platelets), and Coagulation .....        | 9-10        |
| 9.6.3. HIV Testing.....   | 9-10        |
| 9.6.4. Syphilis Testing .....   | 9-12        |
| 9.6.5. Plasma Archive.....  | 9-13        |
| 9.6.6. Blood for PK (MK-2048, VCV).....   | 9-13        |
| <b>9.7. Vaginal Specimens for Gram Stain, Vaginal Fluid pH, Vaginal Wet Mount, GC/CT NAAT, Trichomonas, Vaginal Secretions for PK, and IVR for PK .....</b> | <b>9-14</b> |
| 9.7.1. Gram Stains of Vaginal Fluid.....  | 9-14        |
| 9.7.2. Vaginal pH and Wet Preps, if indicated for BV and/or Yeast .....   | 9-15        |
| 9.7.3. Testing for GC/CT (Neisseria gonorrhoea and Chlamydia trachomatis) by NAAT .....   | 9-17        |
| 9.7.4. Test for Trichomonas vaginalis .....   | 9-17        |
| 9.7.5. Vaginal Swabs for PK (Collection Time Targets for PK Blood and Vag) .....  | 9-17        |
| 9.7.6. Testing of Intravaginal Ring (IVR) for Remnant Content Analysis .....  | 9-20        |
| <b>9.8. Cervical Specimens: Pap Test and Biopsy for PK.....</b>   | <b>9-21</b> |
| 9.8.1. Cervical Biopsy.....   | 9-21        |
| 9.8.2. Papanicolaou (Pap) Test (*only if indicated) .....   | 9-21        |
| <b>Appendix 9-1: HIV ANTIBODY TESTING ALGORITHM .....</b>   | <b>9-22</b> |

---

### 9.1. Overview and General Guidance

As transmission of HIV and other infectious agents can occur through contact with contaminated needles, blood, and blood products, all study staff must take appropriate precautions when collecting and handling biological specimens. Sites must have appropriate written safety procedures in place before study initiation. Guidance on universal precautions available from the US Centers for Disease Control can be found at the following website: <http://www.cdc.gov/hai/>.

The tests to be performed at each visit during the MTN-028 study are listed in Table 9-1. Sites are responsible to ensure that specimen volumes do not exceed what is described in the informed consent process. The total blood volumes calculated in Table 9-1 include additional blood that may be collected for any clinically indicated testing. The MTN LC may request details of collection containers and volumes for this purpose, as shown in Table 9-2.

**Table 9-1: Overview of Laboratory Tests by visit for MTN-028**

| Sample Type             | Test or Procedure                                  | VISIT Day        |                    |          |       |       |        |        |                    |                    |                     |                   |    |
|-------------------------|--|------------------|--------------------|----------|-------|-------|--------|--------|--------------------|--------------------|---------------------|-------------------|----|
|                         |  | SCR              | ENR (Day 0)        | Days 1,2 | Day 3 | Day 7 | Day 14 | Day 21 | Day 28             | Days 29,30,31      | Day 35 Final Clinic | Early Termination |    |
| Urine                   | hCG  | X                | X                  | *        | *     | *     | X      | *      | X                  | *                  | *                   | *                 |    |
|                         | Dipstick UA  | X                | *                  | *        | *     | *     | *      | *      | X                  | *                  |                     |                   |    |
|                         | Urine culture                                      | *                | *                  | *        | *     | *     | *      | *      | *                  | *                  |                     |                   |    |
| Blood                   | CBC with differential and platelets                | X                | X                  |          |       |       |        |        | X                  |                    | *                   | *                 |    |
|                         | Chemistries (Creatinine, AST, ALT)                 | X                | X                  |          |       |       |        |        | X                  |                    | X                   | X                 |    |
|                         | HIV-1 serology                                     | X                | X                  | *        | *     | *     | *      | *      | *                  | *                  | X                   | X                 |    |
|                         | HBsAg  | X                |                    |          |       |       |        |        |                    |                    |                     |                   |    |
|                         | INR  | X                |                    |          |       |       |        |        |                    |                    |                     |                   |    |
|                         | Anti-HCV   | X                |                    |          |       |       |        |        |                    |                    |                     |                   |    |
|                         | Syphilis serology                                  | X                | *                  | *        | *     | *     | *      | *      | *                  | *                  | *                   | *                 |    |
|                         | Plasma for Archive                                 |                  | X                  | *+       | *+    | *+    | *+     | *+     | *+                 | *+                 | *+                  | *+                |    |
|                         | Plasma for Blood PK                                |                  | (hr 1, 2, 4, 6)    | X        | X     | X     | X      | X      | X                  | (hr 0, 1, 2, 4, 6) | X                   | X                 | X* |
|                         | Vaginal  | Vaginal fluid pH | *                  | *        | *     | *     | *      | *      | *                  | *                  | *                   | *                 | *  |
|                         | KOH wet mount for candidiasis                      | *                | *                  | *        | *     | *     | *      | *      | *                  | *                  | *                   | *                 |    |
|                         | Saline wet mount for BV                            | *                | *                  | *        | *     | *     | *      | *      | *                  | *                  | *                   | *                 |    |
|                         | Vaginal NAAT GC/CT                                 | X                | *                  | *        | *     | *     | *      | *      | *                  | *                  | *                   | *                 |    |
|                         | Rapid Trichomonas                                  | X                | *                  | *        | *     | *     | *      | *      | *                  | *                  | *                   | *                 |    |
|                         | Vaginal Swab for PK                                |                  | (hr 0, 1, 2, 4, 6) | X        | X     | X     | X      | X      | (hr 0, 1, 2, 4, 6) | X                  | X                   | X*                |    |
|                         | Gram stain   |                  | X                  |          | X     |       |        |        | X                  |                    | X                   | X                 |    |
| Cervical                | Collect Pap test                                   | *                |                    |          |       |       |        |        |                    |                    |                     |                   |    |
|                         | Cervical Tissue for PK                             |                  |                    |          |       |       |        |        | X                  |                    |                     |                   |    |
| IVR                     | Collect study product                              |                  |                    |          |       |       |        |        | X                  |                    |                     | X                 |    |
| Blood Volume Total (mL) | Approximate, check local laboratory requirements ♪ | 28               | 66                 | 46       | 46    | 46    | 46     | 46     | 94                 | 46                 | 54                  | 54                |    |

X = required; \* = perform, if clinically indicated; ¥ = do not collect if vaginal ring was out for 3 or more days  
 ♪ Maximum volume needed for study requirement, if all specimens are collected including "if clinically indicated".  
 + Plasma for confirmation of viral load and HIV drug resistance testing.

Table 9-2 also shows where laboratory procedures may be performed: study site clinics or laboratories, approved commercial laboratories, and laboratories within the MTN Laboratory Center (MTN LC), including the MTN Pharmacology Core at the University of Colorado, Colorado Antiviral Pharmacology Laboratory (CAVP). Regardless of whether tests are performed in clinic or laboratory settings, study staff that performs the tests must be trained in properly associated QC procedures prior to performing the tests for study purposes (i.e. training documentation should be available for inspection at any time).

**Table 9-2: Overview of Laboratory Testing Locations, Specimens, and Methods for MTN-028**

| Test  | Testing Location       | Specimen Type                    | Tube or Container and tube size (recommended) | Kit or Method  |
|---|------------------------|----------------------------------|---|--|
| Urine Pregnancy Test (hCG)  | In clinic              | Urine                            | Plastic screw top cup                         | Beckman Coulter ICON 25  |
| Urine Dipstick and Culture*   | Local lab              | Urine                            | Plastic screw top cup                         | Siemens Multistix® 10 SG or Uristix 4 or other MTN LC approved methodology |
| Complete Blood Count with Differential and Platelets  | Local Lab              | Consult Local Lab Requirements   |   | Local methodology  |
| Chemistries (AST, ALT, Creatinine)  | Local Lab              | Consult Local Lab Requirements   |   | Local methodology  |
| Prothrombin Time Coagulation (INR)  | Local Lab              | Consult Local Lab Requirements   |   | Local methodology  |
| Hepatitis B (HBsAg)   | Local Lab              | Consult Local Lab Requirements   |   | Local methodology  |
| HCV   | Local Lab              | Consult Local Lab Requirements   |   | Local methodology  |
| Syphilis Serology   | Local Lab              | Consult Local Lab Requirements   |   | Local methodology  |
| HIV serology  | Clinic/Local Lab       | Plasma, serum, or whole blood    | EDTA or plain, 4-mL                           | FDA approved tests   |
| Plasma for Archive or Confirmation of Viral Load and HIV Resistance Testing                             | Clinic/Local Lab       | Plasma                           | EDTA 10-mL tube                               | MTN LC procedure<br>MTN LC Virology  |
| Plasma for Blood PK (MK-2048,VCV)   | CAVP                   | Plasma                           | EDTA 10-mL tube                               | CAVP collection procedure  |
| Vaginal pH*   | In clinic              | Vaginal swab                     | N/A   | S/P pH Indicator Strips  |
| Vaginal Saline Wet Preparation (for BV and/or KOH wet mount)*   | In clinic              | Vaginal swab                     | tube with 6 drops of saline                   | MTN LC procedure   |
| Vaginal NAAT for GC/CT  | Local lab              | Urine or vaginal swab            | Kit specific Transport tube                   | BD Probetec or Gen-Probe Aptima  |
| Trichomonas Rapid Test  | Local lab or in clinic | Vaginal swab (supplied with kit) | OSOM: Sterile tube with no additives          | OSOM kit   |
| Vaginal Swab for PK   | CAVP                   | Swab                             | 2.0-mL Cryovial                               | CAVP collection procedure  |
| Vaginal Smear for Gram-stain  | MTN LC                 | Vaginal Swab                     | 2 Slides                                      | MTN LC procedure   |
| Pap Test**  | Local Lab              | Consult Local Lab Requirements   |   | Local methodology  |
| Cervical Biopsy for PK  | CAVP                   | Tissue                           | 2.0 mL cryovial                               | MTN LC collection procedure  |
| Used Intravaginal Ring for PK residual assessment   | Merck Designated lab   | Used IVR                         | Biohazard labeled 3"×5" amber Zippit pouch    | MTN LC /Merck procedure  |
| *Perform only if clinically indicated per local SOP.  |                        |                                  |   |  |
| *Perform if participant does not have a documented satisfactory Pap within 3 years prior to Enrollment. |                        |                                  |   |  |

Ideally, one method, type of test kit, and/or combination of test kits will be used for each protocol specified test throughout the duration of the study. If for any reason a new or alternative method or kit

must be used after study initiation, site laboratory staff must perform a validation study of the new method or test prior to changing methods. The MTN LC must be notified before the change and can provide further guidance on validation requirements.

Specimens that will be stored and shipped to the MTN LC or CAVP are highlighted in Table 9-3. These are the samples that will be entered into LDMS (section 9.4).

**Table 9-3: Overview of Specimens for Storage and Shipment**

| <b>Specimen</b>   | <b>Processing</b>   | <b>Ship to</b> | <b>Shipping schedule</b>  |
|---|---|----------------|---|
| <b>Plasma for Archive (at enrollment) or for Confirmation of Viral Load and HIV Resistance (at f/u)</b> | Prepare as many 1.5-mL aliquots as possible.<br>If sample is collected and held at room temp, freeze $\leq -70^{\circ}\text{C}$ within 4 hours.<br>If refrigerated after collection, freeze $\leq -70^{\circ}\text{C}$ within 24 hours. | MTN LC         | Store frozen at site until notified by MTN LC. However, if plasma for HIV confirmation, ship immediately to MTN LC Virology Core.   |
| <b>Plasma for Blood PK (MK-2048,VCV)</b>  | Centrifuge and aliquot into two or more cryovials with a minimum of 1.5-mL in each. Freeze within 8 hrs of blood collection.  | CAVP, MTNLC    | Store frozen at site until conclusion of study or notified by MTN LC.   |
| <b>Vaginal Swab for PK</b>  | Record Pre- and Post-collection weight of swab.<br>Freeze at $\leq -70^{\circ}\text{C}$ within 2 hours of collection  | CAVP           | Store frozen at site until conclusion of study or notified by MTN LC.   |
| <b>Vaginal smear for Gram-stain</b>   | Make 2 slides. Room temp. Label with LDMS label.  | MTN LC         | Store one set of slides that will be batch-shipped at conclusion of the study. Store 2nd set of slides (as backup) at site until all slides from first set are confirmed as received. |
| <b>Cervical Biopsy for PK</b>   | Perform Pre (without biopsy) and Post (with biopsy) weights. Flash-freeze. Store at $\leq -70^{\circ}\text{C}$ .  | CAVP           | Store frozen at site until conclusion of study.   |
| <b>Used Intravaginal Ring for PK Residual Assessment</b>  | Place IVR in amber pouch  | MTN LC         | 2-8°C storage at site until conclusion of study.  |

Provided in the remainder of this section is information intended to standardize laboratory procedures across sites. Adherence to the specifications of this section is essential to ensure that primary and secondary endpoint data derived from laboratory testing will be considered acceptable to all regulatory authorities across study sites.

## 9.2. Specimen Labeling

All containers into which specimens are initially collected (e.g., urine collection cups, blood collection tubes) will be labeled with SCHARP-provided Participant ID (PTID) labels. Although PTIDs are pre-printed on these labels, study staff must write the specimen collection date on each label. The visit code also may be written on the label. Use an indelible ink pen (e.g., Sharpie) if information is handwritten such as the date or collection time point.

When specimens are tested at the local lab, any additional labeling required for on-site specimen management and chain of custody will be performed in accordance with site SOPs. Refer to Table 9-4 for tests that will be entered into LDMS and labeled with LDMS-generated labels.

## 9.3. Procedures for Specimens that cannot be evaluated

Specimen collection will be repeated (whenever possible) if samples cannot be evaluated per site SOPs. Site clinic and laboratory staff will monitor specimen collection, processing and management

as part of ongoing quality assurance (QA) procedures and take action as needed to address any issues or problems.

If additional specimens need to be collected for the same test due to either laboratory error (lost, broken tube, clerical, etc.) or clinical error, a protocol deviation form may be required. The MTN LC must be notified in the following cases:

- Any time a participant must return to the clinic for specimen collection
- When PK specimens are missed
- Insufficient blood volume is collected for the plasma archive
- Any time specimens have been mishandled, possibly compromising specimen integrity
- Any situation that may indicate a protocol deviation

If site staff has any questions regarding time windows or collection processes, call MTN LC staff as soon as possible for guidance.

#### **9.4. Use of LDMS**

The Laboratory Data and Management System (LDMS) is a program that must be used by all sites for the storage and shipping of sample types listed in Table 9-3. LDMS is supported by the Frontier Science Foundation (FSTRF). Detailed instructions for use of LDMS are provided at <https://www.fstrf.org/ldms> (may require a password).

All sites will be required to maintain the current version of LDMS and monitor updates relating to use of the LDMS. It is crucial to be aware of proper label formats to ensure that specimens are correctly labeled. Sites will be responsible to back up their LDMS data locally (frequency determined by site) and to export their data to FSTRF (at least weekly).

**LDMS Help:** Questions related to use of LDMS in MTN-028 may be directed to MTN LC or LDMS Technical (User) Support. Usual business hours for LDMS User Support are 12:00 am - 6:00 pm (ET) from Monday through Friday. Contact LDMS User Support at:

Email: [ldmshelp@fstrf.org](mailto:ldmshelp@fstrf.org)  
Phone: +716-834-0900, ext 7311  
Fax: +716-834-8432

All other hours and weekends, an on-call user support specialist will be available if you are locked out of your LDMS or are experiencing errors that prevent you from completing your LDMS lab work. Use the LDMS Web Pager utility to page LDMS User Support. Alternatively, you may e-mail the paging system directly at [ldmspager1@fstrf.org](mailto:ldmspager1@fstrf.org). Please allow at least 15 minutes to get a response before sending another e-mail to the paging system.

**Discrepancy Reports:** Each site must export its LDMS data to Frontier Science (FSTRF) on a weekly basis. Exported data are used by the MTN Statistical and Data Management Center (SDMC) to generate a monthly specimen repository report and to reconcile data entered in LDMS with data entered on study case report forms (CRFs). Any discrepancies identified during the reconciliation are included in a monthly discrepancy report for each site. Sites are expected to resolve all discrepancies within two weeks of receipt of the report. The MTN LC is responsible for reminding sites to adhere to the two week timeframe and for following up with sites that do not resolve discrepancies within two weeks.

The MTN SDMC reviews the discrepancy reports for critical samples (e.g., plasma needed for confirmatory HIV testing) that appear to be missing and works with MTN LC and site staff to undertake appropriate corrective action. All corrective action should be documented in paper-based clinic and/or laboratory records as appropriate, and entered in the details section of LDMS. The MTN LC and SDMC will discuss and document any items that, although resolved, appear 'irresolvable' in LDMS.

### 9.4.1. LDMS Codes for Specimen Log In

The table 9-4 should be used as a guide when logging in MTN-028 specimens for storage or shipping. Please use the LDMS codes listed below when logging in specimens for each test listed. LDMS tracking sheets for Enrollment/Day 28 (multi PK time-point) and other visits (single PK time-point) can be found in the Study Implementation Materials section on the MTN-028 webpage.

| Table 9-4 LDMS Specimen Management Guide to Logging in MTN-028 Specimens*  |                  |                    |   |                                  |  |               |                             |       |                     |
|--|------------------|--------------------|---|----------------------------------|--|---------------|-----------------------------|-------|---------------------|
| Sample   | Primary Specimen | Primary Derivative | Aliquot Derivative                                    | Aliquot Sub additive/ derivative | Other Specimen ID (optional)                       | # of Aliquots | Aliquot Volume              | Units | Time or Weights     |
| Plasma for Archive or Confirmatory Test  | <b>BLD</b>       | <b>EDT</b>         | <b>PL1</b> (single spin);<br><b>PL2</b> (double spin) | <b>N/A</b>                       | <b>EPA</b> (enrollment);<br><b>CON</b> (follow-up) | 2-5           | 1.5 mL<br>in 2-mL cryovials | mL    | --                  |
| Plasma for PK (MK-2048, VCV)   | <b>BLD</b>       | <b>EDT</b>         | <b>PL1</b>  | <b>N/A</b>                       | <b>PK</b>  | 2-5           | 1.5 mL<br>in 2-mL cryovial  | mL    | See 9.4.2           |
| Vaginal Smear for Gram Stain   | <b>VAG</b>       | <b>NON</b>         | <b>SLD</b>  | <b>GRS</b>                       | --   | 2             | 2 smears                    | Each  | --                  |
| Vaginal PK Swab  | <b>VAG</b>       | <b>NON</b>         | <b>SWB</b>  | <b>N/A</b>                       | --   | 1             | 1 swab                      | mG    | See 9.4.2 and 9.4.3 |
| Cervical Biopsies for PK   | <b>CVB</b>       | <b>NON</b>         | <b>BPS</b>  | <b>N/A</b>                       | --   | 1             | 1 biopsy in 2- mL cryovial  | mG    | See 9.4.2 and 9.4.3 |
| Used Vaginal Ring for PK residual assessment   | <b>IVR</b>       | <b>NON</b>         | <b>IVR</b>  | <b>N/A</b>                       | --   | 1             | 1 pouch                     | Each  | --                  |
| *List of Codes and their definitions:<br><b>BLD:</b> Whole Blood <b>IVR:</b> Used Intravaginal Ring <b>PL1/2:</b> Single or double spun plasma <b>BPS:</b> Biopsy<br><b>VAG:</b> Vaginal Swab <b>EDT:</b> EDTA <b>SLD:</b> Slide <b>GRS:</b> Gram stain slide<br><b>CVB:</b> Cervical Biopsy <b>NON:</b> No Additive <b>SWB:</b> Swab <b>N/A:</b> Not Applicable |                  |                    |   |                                  |  |               |                             |       |                     |

### 9.4.2. Logging in Time for PK Samples

In this study, there will be multi PK time-point visits, and in LDMS, in addition to time of collection, the TIME and TIME UNIT field are used to note the specific time point on your aliquot labels. In figure 9-1, the single tube of blood for PK is entered in the primary area (see yellow rectangle A), and the three aliquots of 1.8-mL plasma that are derived from the sample are entered in the lower section for the derivative (see blue rectangle B).

- The collection time, using the 24-hour clock notation, is entered in the Specimen Time area (Figure 9-1, red rectangle C). For this example, it is 16:00.
- During multiple PK time-point visits, the PK time-point information is entered in Time and Time Unit area (Figure 9-1, green rectangle D). This blood was for the 4-HR time point.

Figure 9-1: LDMS Screen for Time Entry.

| Group | TYPE1 | ID1       | TYPE2    | ID2   | TYPE3 | ID3 | Visit | Unit |
|-------|-------|-----------|----------|-------|-------|-----|-------|------|
| 1 MTN | PID   | 302600026 | PROTOCOL | 013.0 | ID3   |     | 2.00  | Vol  |
| 2     |       |           |          |       |       |     |       |      |
| 3     |       |           |          |       |       |     |       |      |
| 4     |       |           |          |       |       |     |       |      |
| 5     |       |           |          |       |       |     |       |      |
| 6     |       |           |          |       |       |     |       |      |

Spec. Date: 15/Nov/2011 Exp. Date: 0

# of Tubes: 1 Primary Type: BLD Other Spec ID:

| Specimen # | Global Spec ID | Primarv     | Addive | Volume | Units   | Spec Time | Time | Time Unit |
|------------|----------------|-------------|--------|--------|---------|-----------|------|-----------|
| 1          | 414V11002715   | HC6012KK-00 | BLD    | EDT    | 8.00 ML | 16:00     | 4:00 | Hours     |

# of Aliquots: 3 Vol: 1.8 Units: ML Derivative: PL1 Sub Add/Der: N/A Other Spec ID:

| Specimen | Global Spec ID | Primarv     | Add | Der | Sub Add/Der | Volume | Units   | Cond | Other Spec Id |
|----------|----------------|-------------|-----|-----|-------------|--------|---------|------|---------------|
| 1        | 414V11002721   | HC6012KK-01 | BLD | EDT | PL1         | N/A    | 1.80 ML | SAT  |               |
| 2        | 414V11002721   | HC6012KK-02 | BLD | EDT | PL1         | N/A    | 1.80 ML | SAT  |               |
| 3        | 414V11002721   | HC6012KK-03 | BLD | EDT | PL1         | N/A    | 1.80 ML | SAT  |               |

### 9.4.3. Entering weight measurements of vaginal swab and cervical biopsy for PK in LDMS:

In the derivative area for the primary sample, The VOLUME and UNIT field is used for displaying weight measurements with proper units. Once the net-weight is attained by subtracting the pre-weight from the post-weight, the result can be entered into LDMS as shown in figure 9-2, red rectangle.

- In the primary sample area (section A), use table 9-4 to enter correct code for the sample. Make sure to place the correct collection time under Spec Time field. Click the 'add' button to the right. This will add the sample to field. Under Units, enter EA (for each) and enter '1' for Volume (See Figure 9-2).
- To enter the actual weights, make an aliquot in Section B for the primary sample by entering a '1' in the # of Aliquots field. For Volume, enter the net-weight and select 'MG' (milligrams) for UNITS. Enter the correct derivative and Sub-Add/Der codes, then click the add button (See Figure 9-2).

In the example in figure 9-2: Pre-weight Swab: 3073.2 mg, Post weight Swab: 3139.7 mg, Net weight of Swab is 66.5 mg (3139.7 -3073.2 = 66.5). Enter '66.5' under VOLUME and select 'MG' for Units, 'SWB' for Derivative, and 'N/A' for Sub-Add/Der, then press add.

Figure 9-2: LDMS Screen for Weight Entry

|   | Group | TYPE1 | ID1       | TYPE2    | ID2   | TYPE3 | ID3 | Visit | Unit     |
|---|-------|-------|-----------|----------|-------|-------|-----|-------|----------|
| 1 | MTN   | PID   | 335000159 | PROTOCOL | 024.0 |       |     |       | 6.00 Vol |
| 2 |       |       |           |          |       |       |     |       |          |
| 3 |       |       |           |          |       |       |     |       |          |
| 4 |       |       |           |          |       |       |     |       |          |
| 5 |       |       |           |          |       |       |     |       |          |
| 6 |       |       |           |          |       |       |     |       |          |

Spec. Date: 26/Jun/2014 Exp. Date: 0  
 Recd. Date: 26/Jun/2014 Recd. Time: 15:30 Export ID:

Remote  Imported

Enter Specimen ID

# of Tubes: 1 Primary Type: VGL Other Spec ID:  Spec. Time: 15:09

| Specimen # | Global Spec ID | Primary     | Additive | Volume | Units | Spec Time | Time  | Time Unit | Cond |
|------------|----------------|-------------|----------|--------|-------|-----------|-------|-----------|------|
| 1          | 414V14006368   | FC60756V-00 | VGL      | NON    | 1.00  | EA        | 15:09 |           | SAT  |

**A**

# of Aliquots: 1 Vol: 66.5 Units: MG Derivative: Sw/B Sub Add/Der: N/A Other Spec ID:

| Specimen | Global Spec ID | Primary     | Add | Der | Sub Add/Der | Volume | Units | Cond | Other Spec Id |
|----------|----------------|-------------|-----|-----|-------------|--------|-------|------|---------------|
| 1        | 414V14006369   | FC60756V-01 | VGL | NON | Sw/B        | N/A    | 66.50 | MG   | SAT           |

**B**

9.4.4. LDMS Entry for Vaginal Smear for Gram Stain

For Vaginal Smear for Gram Stain, the one swab that was used to inoculate the two slides is the primary sample. After the primary sample information is entered, then added, the two slides are entered as aliquots. An example is shown in figure 9-3. Note that after the 2 aliquots are added, a pop-up message will warn the user that the total aliquot volume exceeds the primary volume. Ignore the message and continue.

Figure 9-3 LDMS Entry for Vaginal Smear for Gram Stain

|   | Group | TYPE1 | ID1       | TYPE2    | ID2   | TYPE3 | ID3 | Visit | Unit     |
|---|-------|-------|-----------|----------|-------|-------|-----|-------|----------|
| 1 | MTN   | PID   | 335000159 | PROTOCOL | 024.0 |       |     |       | 6.00 Vol |
| 2 |       |       |           |          |       |       |     |       |          |
| 3 |       |       |           |          |       |       |     |       |          |
| 4 |       |       |           |          |       |       |     |       |          |
| 5 |       |       |           |          |       |       |     |       |          |
| 6 |       |       |           |          |       |       |     |       |          |

Spec. Date: 26/Jun/2014 Exp. Date: 0  
 Recd. Date: 26/Jun/2014 Recd. Time: 15:30 Export ID:

Remote  Imported

Enter Specimen ID

# of Tubes: 1 Primary Type: VGL Other Spec ID:  Spec. Time:

| Specimen # | Global Spec ID | Primary     | Additive | Volume | Units | Spec Time | Time | Time Unit | Cond |
|------------|----------------|-------------|----------|--------|-------|-----------|------|-----------|------|
| 1          | 414V14006370   | DC60756X-00 | VAG      | NON    | 1.00  | EA        |      |           | SAT  |

**A**

# of Aliquots: 2 Vol: 1 Units: EA Derivative: SLD Sub Add/Der: GRS Other Spec ID:

| Specimen | Global Spec ID | Primary     | Add | Der | Sub Add/Der | Volume | Units | Cond | Other Spec Id |
|----------|----------------|-------------|-----|-----|-------------|--------|-------|------|---------------|
| 1        | 414V14006371   | DC60756X-01 | VAG | NON | SLD         | GRS    | 1.00  | EA   | SAT           |
| 2        | 414V14006371   | DC60756X-02 | VAG | NON | SLD         | GRS    | 1.00  | EA   | SAT           |

**B**

## 9.5. Urine Testing for Pregnancy, Urinary Tract Infection, and Urinalysis

### 9.5.1. Specimen Collection

- The participant should not have urinated within one hour prior to urine collection.
- Provide the participant with a sterile, plastic, preservative-free screw-top urine collection cup labeled with a SCHARP-provided PTID label.
- Instruct the participant to collect the portion of the urine flow that is required by the test
- If the urine is to be used for culture, instruct the participant to clean the labia prior to specimen collection and to collect a midstream urine sample.
- Instruct the participant to screw the lid tightly onto the cup after collection.

### 9.5.2. Pregnancy Testing

Pregnancy status is a critical participant safety consideration in MTN-028. The Beckman Coulter ICON 25, Quidel QuickVue One-Step hCG urine, Quidel QuickVue Combo hCG urine/serum pregnancy, or Fisher HealthCare Sure-Vue Urine hCG test must be used at all sites. All sites must maintain an adequate inventory of the pregnancy test kits at all times. Inventory should be monitored closely and re-supply orders placed at least 8-12 weeks in advance of actual need (or longer if needed per site procurement policies and procedures). The date and time of pregnancy testing must be documented.

The pregnancy test is performed according to site SOPs and the package insert (i.e. a negative result is based on the recommended total time for test to be considered complete.) Do not perform any other urine pregnancy tests for confirmatory purposes. If the urine pregnancy test cannot adequately be interpreted because of interfering factors (e.g. excess blood or extreme cloudiness due to amorphous material), the sample can be spun down and the urine supernatant can be used. If the test continues to have interferences such as gross hemolysis making the test difficult to read, then another urine sample will need to be collected.

In the rare event in which a participant becomes pregnant, study product use will be permanently discontinued. The participant will be terminated from the study.

### 9.5.3. Urinary Tract Infection

Urine Dipstick and/or Culture: Perform the tests according to the package insert for the dipstick and your local SOP for culture.

For initial diagnosis and treatment of a UTI use your local standard of care (if you use a dipstick for leukocytes and nitrites record the results on the CRF "Safety Laboratory Results"). However, for confirmation of a UTI and reporting as an AE you must perform a urine culture if this is not part of your local standard of care. The culture results are not recorded on the CRF.

**See also section 8.9 Clinical Considerations of the SSP for additional information.**

### 9.5.4 Urinalysis for Renal Function

Tests for glucose and protein are required at screening and day 28 visits. Perform the test according to the package insert and your local procedure. Record the results on the CRF "Safety Laboratory Results".

## 9.6. Blood Specimens for Chemistry, Hematology, HIV testing, Syphilis, Plasma Archive, MK-2048 and Vicriviroc (VCV) Blood Levels

The blood tests performed at each study visit vary depending on the time point of the visit and potentially the clinical presentation of the participant. Perform all tests according to site SOPs and package inserts.

### 9.6.1. Specimen Collection and Initial Processing

Label all required tubes with a SCHARP-provided PTID label at the time of collection. After collection:

- Allow serum tubes (no additive or serum separator tubes) to clot, then centrifuge per site SOPs.
- Lavender top tubes (additive = EDTA) should be gently inverted at least eight times after specimen collection to prevent clotting. If whole blood for hematology testing and plasma is to be taken from the same tube, hematological tests must be completed before the tube is centrifuged and aliquoted. If whole blood is to be used for multiple tests, ensure that the tube is well mixed before removing any specimen. EDTA tubes will be used for plasma MK-2048 and Vicriviroc PK levels, plasma archive at enrollment, and if applicable, plasma for confirmation of viral load and HIV resistance testing.
- Light blue top tubes (additive = Na Citrate) are used for coagulation determinations. These tubes should be gently inverted at least 4 times after specimen collection to prevent clotting.

*Note: If locally available tube top colors do not correspond with the tube additives specified above, use appropriate tubes based on the additives, not the listed tube top colors.*

### 9.6.2. Chemistry (Alanine transaminase, Aspartate aminotransferase, and Creatinine), Hematology (CBC with Diff and Platelets), and Coagulation

Testing will be performed per local standard of care.

- Tests performed for Chemistry
  - Liver Function:
    - Alanine transaminase (ALT),
    - Aspartate aminotransferase (AST).
  - Renal Function:
    - Creatinine
    - Creatinine Clearance Calculator, using participant's weight in conjunction with the Cockcroft-Gault formula. See EXCEL worksheet in the Study Implementation Materials section of the MTN-028 protocol on the MTN website.
- Hematology tests (Complete blood counts (CBC) with five-part differentials)
  - Hemoglobin,
  - Hematocrit,
  - Platelets,
  - White Blood Cell Count and differential
  - Red Blood Cell Count
- Coagulation Test: Prothrombin Time to include an INR.

### 9.6.3. HIV Testing

EDTA plasma, whole blood (fingerstick or venipuncture) and serum can be used to test for HIV using tests that have been validated at the study site. All HIV testing in laboratories must be done under Clinical Laboratory Improvement Amendment (CLIA) certification. All tests and associated QC procedures must be documented on local laboratory log sheets or other laboratory source documents.

HIV infection status will be assessed using an FDA-approved HIV immunoassay per the HIV testing algorithm (see appendix 9-1 in this section or appendix II of the MTN-028 protocol). Rapid tests, such as Oraquick, are considered immunoassays and can be used with whole blood (fingerstick or venipuncture). The first specimen drawn for immunoassay and confirmatory testing is considered Sample 1. If Sample 1 is HIV positive by the confirmatory test a second specimen (Sample 2) is drawn to confirm the first results.

**Notify the MTN LC immediately if any kit inventory or quality control problems are identified, so that appropriate action can be taken.**

#### 9.6.3.1 HIV Rapid Test Result Interpretation

- If SAMPLE 1 immunoassay result is negative, the participant will be considered HIV-seronegative.
- If the SAMPLE 1 immunoassay result is positive or indeterminate, an FDA-approved confirmatory test should be performed on SAMPLE 1.
  - Go to 9.6.3.2 if SAMPLE 1 is Screening or Enrollment sample
  - Go to 9.6.3.3 if SAMPLE 1 is Follow-up Visit sample
- If there is insufficient sample to perform the confirmatory test, then additional blood must be drawn. This re-draw will still be regarded as Sample 1 per the algorithm.

#### 9.6.3.2 HIV Confirmatory Test for Screening or Enrollment Visit

- Until enrolled, treat enrollment testing same as screening participants.
- If the confirmatory test for SAMPLE 1 is negative, indeterminate or invalid, contact the MTN Virology Core: [mtnvirology@mtnstopshiv.org](mailto:mtnvirology@mtnstopshiv.org) for guidance.
  - It is not recommended for participants with discrepant HIV testing results to continue enrollment.
- If the confirmatory test is positive for the screening visit, the participant is considered seropositive and is not eligible for enrollment.

#### 9.6.3.3 HIV Confirmatory Test for Follow-Up Visits

- If at a follow-up visit, the confirmatory test on SAMPLE 1 is negative, indeterminate or invalid, contact the MTN Virology Core for guidance:
  - 412-383-8138
  - [mtnvirology@mtnstopshiv.org](mailto:mtnvirology@mtnstopshiv.org).
- If the confirmatory test is positive at a follow-up visit, a second sample of blood (SAMPLE 2) will be drawn for additional confirmatory testing, HIV RNA resistance testing and plasma storage at the MTN Virology Core.
  - Draw enough whole blood to store a total of 5 mL of plasma to send to the virology core. The virology core can work with less but 5 mL is the desired amount to complete all testing.
  - ***NOTE: Draw extra blood with Sample 2, if required for local standard of care or at discretion of clinician. This blood is sent directly to a local lab following their procedures.***
- Processing of SAMPLE 2 is similar to Plasma for Archive:
  - Log into LDMS, but with special ID = CON.
  - Centrifuge at 1500xg and aliquot 1.5 mL plasma into 2-mL cryovials and freeze at <-70°C.
- Alert the MTN Virology Core, 412-383-8138, about shipment.
- Package and ship 3 aliquots immediately on dry ice to:

Dr. Urvi Parikh  
University of Pittsburgh  
3550 Terrace St.  
Scaife Hall S804  
Pittsburgh, PA 15261

- MTN Virology Core will provide test results to the site.
  - If positive, the participant is HIV positive.
  - If negative, indeterminate or invalid, the MTN Virology Core will supply guidance.

#### 9.6.4. Syphilis Testing

RPR tests may be performed on either serum or plasma. Serum is the specimen of choice for VDRL and syphilis confirmatory tests. However, other sample types may be allowed according to the particular test's package insert. All testing and QC procedures must be performed and documented in accordance with study site SOPs.

Syphilis testing can be performed using FDA approved tests in one of two ways:

1. Perform a non-treponemal screening test, such as Rapid Plasma Reagin (RPR) or Venereal Disease Research Laboratory (VDRL) test, followed by a confirmatory test for *Treponema pallidum*.
  - Any FDA approved *Treponema pallidum* confirmatory test can be used such as the Enzyme Immunoassay (EIA), microhemagglutinin assay for *Treponema pallidum* (MHA-TP), *Treponema pallidum* hemagglutination assay (TPHA), *Treponema pallidum* particle agglutination (TPPA), or fluorescent treponemal antibody (FTA-ABS).
  - All positive RPR or VDRL results must have a titer reported.
    - For reactive RPR or VDRL tests observed during screening, a confirmatory test is performed and appropriate clinical management action must be taken prior to enrollment in the study.
    - Enrolled participants considered positive should include repeat non-treponemal assay tests at quarterly intervals following syphilis diagnosis to evaluate treatment effectiveness.
    - If the RPR or VDRL titer does not decrease four-fold or revert to seronegative within three months after treatment, further investigation and/or treatment may be warranted.
  
2. Perform syphilis assessment using a specific FDA approved treponemal test (such as EIA, MHA-TP, TPHA, TPPA, or FTA-ABS) and confirming positive test results with a non-treponemal assay (RPR or VDRL).
  - If the confirmatory non-treponemal assay is reactive at screening visit, appropriate clinical management action must be taken prior to enrollment in the study.
    - If the RPR or VDRL is negative, this may indicate prior treatment, late latent disease, or a false positive test.
      - MTN LC recommends additional testing using an alternative treponemal test other than the original treponemal test used for the original assessment so the participant can be correctly evaluated. (Of note, the FTA-ABS should not be used as the alternative confirmatory test due to performance issues).
        - If the second confirmatory test is negative, the participant is not considered infected with syphilis.
        - If the second confirmatory test is positive, the participant has had prior exposure to syphilis and depending on clinical scenario may or may not require treatment.

Please consult the MTN LC with any questions related to Syphilis testing to confirm treatment effectiveness and/or interpretation of unusual test results.

Questions related to result interpretation concerning eligibility and enrollment in the study should be directed to the MTN-028 Protocol Safety Physicians ([mtn023safetymd@mtnstopshiv.org](mailto:mtn023safetymd@mtnstopshiv.org)).

#### 9.6.5. Hepatitis B Surface Antigen and Hepatitis C Antibody

This testing will be done on serum or EDTA plasma per local SOPs

#### 9.6.6. Plasma Archive

For plasma archive, use collection tubes with EDTA anticoagulant. Aliquot plasma into 2-mL cryovials, store at  $\leq -70^{\circ}\text{C}$ , and batch onsite until the MTN LC study team requests shipping and/or testing.

- LDMS will be used to label and track the specimens.
- If sample is collected and held at room temp, freeze within 4 hours. If refrigerated or placed on ice after collection, freeze within 24 hours.
- Spin blood at room temperature in a centrifuge according to one of these techniques:
  - Single spun: Spin blood at 1500xg for 10 minutes, remove plasma.
  - Double spun: Spin blood at 800xg for 10 minutes, place plasma in a tube to spin again at 800xg for 10 minutes, remove plasma.
- Prepare as many 1.5-mL aliquots as possible, at least 3-mL total volume.
- If total volume is less than 0.5 mL, redraw as soon as possible.
- If less than 1 mL of plasma is available, store that plasma and inform the MTN LC for instruction.
- If samples are hemolyzed, store the aliquots as per normal and enter comments in LDMS.
- The MTN LC will send instructions to the site when shipping and/or testing is required.

#### 9.6.7. Blood for PK of Vicriviroc (MK-4176) and MK-2048

On single time-point days (visit days 1, 2, 3, 7, 14, 21, 29, 30, 31, & 35), The participant will self-collect the vaginal PK swab approximately within 1 hour after the blood is drawn for PK. On multiple time-point days (visit days ENR and 28), optimally the participant will self-collect the vaginal PK swab within 5 minutes after the blood PK sample is drawn. See section 9.7.5 for details.

NOTE: If it is an Early Termination visit and the vaginal ring has been out for 3 or more days, do not collect samples for vaginal fluid PK and plasma PK.

Collect blood into a labeled 10-mL EDTA Vacutainer tube using either an indwelling venous catheter or direct venipuncture.

1. Mix blood sample with the anticoagulant using gentle inversions (8 to 10 times).
2. Centrifuge the sample at approximately 1500xg for 10 minutes. The centrifugation must be completed and sample placed in the freezer within 8 hours of blood collection.
3. Use a pipette to aliquot approximately 1.5 mL of the resulting plasma into 2-mL cryovials. One of these will serve as the primary sample; the others will serve as a back-up in case the primary samples are accidentally destroyed during shipment.
4. Prepare two storage boxes and label one as "primary samples" and the other as "back-up samples". Transfer the tubes from each participant in chronological order into the storage boxes. All samples will be tracked in LDMS.
5. Store the boxes with samples at  $\leq -70^{\circ}\text{C}$  until shipped.

#### SHIPPING:

- MTN LC will coordinate sample shipments throughout course of study if necessary and at its conclusion.
- All shipments will be on dry ice that will be sufficient for a 24-hour period and can be initiated Monday through Wednesday to insure that samples arrive in the lab during the work week.
- The back-up samples will be retained at the site until advised by the MTN LC or MTN-028 leadership team. One purpose of the extra aliquots is to be available in case the shipment is not received in the proper condition (e.g. thawing of samples).

### 9.7. Vaginal Specimens for Gram Stain, Vaginal Fluid pH, Vaginal Wet Mount, GC/CT NAAT, Trichomonas, Vaginal Fluid for PK, and IVR for Remnant Drug Content Analysis

Refer to Pelvic Exam checklist of this SSP manual for further information on the required sequence of specimen collection and diagnostic procedures to be performed during study pelvic exams.

#### 9.7.1. Gram Stains of Vaginal Fluid

Dried vaginal fluid smears will be prepared for Gram staining and assessment for bacterial vaginosis at the MTN LC. Two slides (one designated as primary and the other as secondary) will be prepared at each required time point and both will be entered into LDMS. The primary slide will be shipped to the MTN LC and the secondary will be archived on site until written notification is received from the Statistical Center for HIV/AIDS Research & Prevention (SCHARP) that the slide may be discarded.

Instructions for slide preparation and shipping are provided below:

1. Use a pencil to write the PTID and specimen collection date on the frosted end of the slide. This is the side of the slide that the specimen is to be applied.



2. Immediately following specimen collection from the lateral vaginal wall via swab (Dacron or cotton), roll the swab across each of the slide. (Be sure to collect the specimen from opposite the vaginal wall used for the wet mount specimen collection.) Do not place the swab in saline, transport medium, or any transport container prior to slide preparation.
3. A SCHARP-provided PTID label is to be placed on the underside of the slides (on the frosted end, under the pencil markings); write the specimen collection date in indelible ink (e.g. Sharpie pen) on each label.



4. Allow the specimens to air-dry on the slides. Do not heat-fix.
5. Vaginal smears for gram stain are to be logged into LDMS (specimen type = VAG)

and label the slides with LDMS labels. Place the LDMS label on the frosted end of the slide on top of the pencil markings (same side as sample).



6. The primary slides will be positioned in a plastic slide holder and sent to the MTN LC. If there is no culture on the visit for which a gram stain is collected, then hold the gram stain slides until other samples are to be sent to the Magee-Womens Research Institute. (See shipping instructions below).
7. Store the secondary slide in the slide box location assigned in LDMS at room temperature. (This is a backup slide in case the first is lost, broken, or unreadable).

#### 9.7.2. Vaginal pH and Wet Preps, if indicated for Bacterial Vaginosis (BV) and/or Yeast

BV will be diagnosed based on the presence of any three of the four Amsel's criteria:

- Homogenous vaginal discharge
- Vaginal pH greater than 4.5
- Positive whiff test
- At least 20% clue cells.

Wet prep assessments used to diagnose BV and candidiasis are discussed in section 9.7.2.2 and summarized in Table 9-5.

CLIA regulations require semi-annual **wet mount proficiency testing**. The MTN LC administers a web-based proficiency test approximately every six months. Wet mount slides on the MTN web pages are posted for this purpose every 6 months.

- Contact Lorna Rabe of the MTN LC ([lrabe@mwri.magee.edu](mailto:lrabe@mwri.magee.edu)) to register names of clinicians who need to take the test.
- The registrants take the test and enter their answers directly on the website.
- The MTN LC sends a report of the results, including any necessary corrective action, to the Laboratory Manager.

Contact the MTN LC for additional information and guidance on performing and documenting the proficiency testing. Also contact the MTN LC when new laboratory staff is hired, so that appropriate training can take place prior to such staff performing wet mounts for study purposes.

##### 9.7.2.1 Vaginal Fluid pH, if indicated for BV

Vaginal fluid pH will be assessed if clinically indicated for bacterial vaginosis. pH Indicator Strips (pH range 3.6 to 6.1) with brand names S/P Cardinal Health, Baker-pHIX, Whatman, or Machery-Nagel must be used at all sites.

Vaginal fluid pH swab (Dacron or cotton) may be collected in any of 2 ways depending on if a speculum is used at that particular visit:

- Obtained by the clinician during the pelvic examination
  - Collected by the clinician in a non-speculum exam
- Note: a speculum is not required for pH sample collection.

##### Vaginal Fluid pH Procedure:

1. Swab onto the pH strip (Do not insert the pH strip into the vagina).

2. Match the resulting color of the indicator strip to the color scale provided with the strips to determine the pH value.
3. Record the pH value directly onto the appropriate case report form (CRF). It is not necessary to record pH values onto laboratory log sheets or other source documents prior to recording values onto CRFs.

| <b>Table 9-5 Summary of Wet Prep Assessments and Diagnostic Criteria</b> |  |   |
|--|--|---|
| <b>Assessment</b>  | <b>Saline Prep</b>   | <b>KOH Prep</b>   |
| Whiff Test   | Not applicable   | Positive if fishy amine odor detected                   |
| Yeast  | Positive if pseudohyphae and/or budding yeast are observed. Pseudohyphae and budding yeast may be obscured by epithelial cells. These cells will be lysed by KOH, thus pseudohyphae and budding yeast not observed in saline prep may be observed in KOH prep. | Positive if pseudohyphae or budding yeast are observed. |
| Clue Cells   | Individual cells rather than clusters of cells should be examined. Positive if at least 20% clue cells observed. Cells must be completely covered with bacteria ( <i>Gardnerella vaginalis</i> and/or anaerobic GNR) to be counted as clue cells.              | Not applicable (clue cells are lysed by KOH)            |

#### **9.7.2.2 Vaginal Fluid Wet Mount Testing, if indicated for BV and Yeast (KOH)**

Wet mount procedures for this study are only performed if indicated, and consists of two different preparations: Potassium Hydroxide (KOH) and Saline. These procedures are for diagnosis of BV and candidiasis as summarized in Table 9-5.

#### **Preparation and Examination of Wet Prep Slides**

Materials:

Pencil

2 SCHARP labels, 3 if using optional tube

2 frosted end slides

Glass or plastic tube, optional

Sterile physiologic saline

10% KOH

Dacron Swab

2 cover slips

Microscope, 10x and 40X magnification

1. Use a pencil to write the PTID and specimen collection date on one side of the frosted end of two microscope slides. Affix a SCHARP-provided PTID label to the other side of the slides (on the frosted end, under the pencil markings)
2. Immediately following collection from the lateral vaginal wall via swab (Dacron or cotton), smear vaginal fluid specimens onto each slide. Alternatively, the swab may be placed in a glass or plastic tube with approximately six drops (100 µL) sterile physiologic saline to allow for non-immediate slide preparation. In this case, vaginal fluid specimens should be smeared onto the two slides upon receipt from the collecting clinician.
3. Apply one drop of 10% KOH to one slide and immediately perform whiff test for a “fishy” amine odor. Then apply cover slip.
4. Apply one drop of sterile physiologic saline to the second slide, emulsify with

the vaginal fluid specimen, and then apply cover-slip. Examine immediately at 10X magnification for epithelial cells, budding yeast, and pseudohyphae. Examine at 40X magnification to determine whether observed epithelial cells are clue cells and quantitate the cells. Clue cells are irregularly bordered squamous epithelial cells that are completely covered with bacteria (*Gardnerella vaginalis*). Clue cells must comprise at least 20 percent of the observed epithelial cells in order for the saline prep to be considered positive for clue cells.

5. Examine the KOH slide at both 10X and 40X magnification for yeast and pseudohyphae.

#### **RESULTS:**

- If wet prep slides are read in-clinic by clinical staff, results may be recorded directly on to appropriate case report forms (CRFs).
- If slides are read by lab staff (either in the local laboratory or a designated in-clinic lab area), results must be recorded on laboratory log sheets or other laboratory source documents and then transcribed onto appropriate CRF.

#### **9.7.3. Testing for GC/CT (*Neisseria gonorrhoea* and *Chlamydia trachomatis*) by NAAT**

Testing for chlamydia and gonorrhoea is performed at screening and when clinically indicated. Sites can choose to use the BD Probetec or Gen-Probe Aptima. If the site does not have access to these tests, they can send the samples to the MTN LC for testing. Contact the MTN LC prior to sending specimens for GC/CT testing.

- Use the Gen-Probe vaginal collection swab and transport tube
- Affix a SCHARP-provided PTID label onto the transport tube.
- Swab the lateral wall of the vagina.
- Immediately place the swab in the transport tube, break off the shaft of the swab, and cap the tube.
- Transport the specimen at ambient temperature to the local laboratory

#### **9.7.4. Test for *Trichomonas vaginalis* using OSOM Rapid Test**

Testing for *Trichomonas* is done using the OSOM Rapid *Trichomonas* test (manufactured by Sekisui Diagnostics)

- Use the rayon swab provided with the kit for collection
- Affix a SCHARP-provided PTID label to a clean glass or plastic tube with a cap.
- Collect specimen using kit-provided swab from the lateral vaginal wall (fluids also may be collected from the posterior fornix; avoid collecting specimens from the cervix).
- Immediately place the swab in the labeled tube, break off the shaft of the swab, and cap the tube.
- Testing is expected to be performed during the participant visit. However, specimens may be stored at room temperature for 24 hours or refrigerated for 36 hours before testing.

#### **9.7.5. Vaginal Swabs for PK**

PK collection times need to be recorded on the LDMS sample tracking sheet. In addition to sample collection, this section discusses acceptable 'windows' on collection time points and action to be taken if collection is outside of this.

#### **Collection Timing and Target Times for Vaginal Swabs and Blood for PK**

Enrollment or Day 28, visits with multiple PK collection time-points.

- When to start the timer

- At Enrollment, start timer upon ring insertion.
  - There is no blood draw at time 0, use this additional time to instruct participant on self-collection.
- On Day 28, start timer upon ring removal.
  - There is blood draw for PK at time 0.
- All serial collections are based on the starting time-point.
- When each time-point is due:
  - Blood will be drawn first,
  - Ideally, the participant will self-collect the PK swab within 5 minutes of the blood draw.
- Make sure that specimen times are accurate in case there are delays in sample collection. Correct recording will allow the interval of time to be correctly gauged.
- Missed or delayed blood draw time-point:
  - There will be no bearing on the next time point.
    - Example: Although the '1 hour' time-point draw was 15 minutes late (drawn at 75 minutes), the 2 hour PK blood would still be drawn at the 2 hour (120 minute) mark.
  - If a collection is missed entirely, notify the MTN-028 management team.

#### Follow-up Visits with single PK collection time point

The blood is drawn, and then the participant should collect the self-collected swab within 1 hour. The protocol states approximately within 1 hour.

#### In the case that the ring is removed prior to a visit:

- Vaginal swab for PK should still occur, preferably after at least 8 hours have passed from the time of re-insertion. However, if less than 8 hours, still collect the samples. In any case, comment on the LDMS tracking sheet and Ring Adherence CRF, item 4.
- This ring removal / re-insertion should be noted on LDMS tracking sheet and the Ring Adherence CRF.
- If it is an Early Termination visit and the vaginal ring has been out for 3 or more days, do not collect samples for vaginal fluid PK and plasma PK.

#### **Procedure for Vaginal Fluid Sampling for PK assessment and weighing swab**

1. Each day of collection of vaginal swab for PK, perform QC that would be required for the analytical scale to accurately weigh samples to a weight of at least 0.1 milligrams. Do not turn off balance until weighing for the day is completed.
2. Materials for each time point:
  - 2 SCHARP labels with PTID, visit number, visit date, time point.
  - 2-mL Nalgene cryovials
  - Polyester-Tipped (Dacron) Swab
  - Ziplock biohazard sample bags
  - Urine cup (without lid) or similar lightweight container, placed on middle of scale, to contain items to be weighed. (Some balances have an optional basket.)
  - A rack that will hold the cryovial
  - For clinical staff, scissors to cut swab shaft
  - Calculator
3. Handle items to be weighed with gloves.
4. Place identically-labeled SCHARP label on each the cryovial and a biohazard sample ziplock bag.
5. Perform pre-weight.

- a. Zero the urine cup or similar container
  - b. Place the labeled 2-mL cryovial in the urine cup.
  - c. Place the packaged sterile Dacron swab upright in the urine cup. (Make sure it is not leaning on a part of the scale.)
  - d. Record this pre-weight on the LDMS Tracking Sheet.
  - e. Place the cryovial and the packaged Dacron swab in a biohazard sample ziplock bag with the matching label to the tube.
  - f. If multiple time points or multiple participants on that day, pre-weights for all time points may be obtained with careful observation of time-point labels.
6. Make sure you have the correct participant time-point and instruct the participant to wash their hands before the exam.
- a. In the exam room instruct the participant that none of the items in the bag should be thrown into the garbage – only into the ziplock bag.
  - b. Prep for the participant:
    - i. Have the rack ready.
    - ii. Unscrew the lid of the 2-mL cryovial and place the tube in the rack, the lid in the ziplock bag.
    - iii. Start the peel of the packaging of the swab. (Sometimes not a sufficient separation)
  - c. The participant will peel the packaging and remove the Dacron swab to collect vaginal fluid (slow count to 10).
    - i. The participant will place the swab in the tube and the swab packaging into the ziplock bag.
  - d. Cutting or bending to break the swab shaft. (!!!Potential to lose swab shaft!!!)
    - i. If clinical staff will cut the shaft, a suggestion, for leverage, is to not use tip of blades to cut, but make sure shaft of swab is at the pivot point of the scissors, then cut.
    - ii. If clinical staff or participant will perform a repeated bend to break the shaft with dominant hand, while doing so, it may be easiest to hold the top of the tube with the forefinger and thumb of the other hand.
  - e. Place the cut shaft in the ziplock bag.
  - f. Screw the lid back on the cryovial and place sample in the bag with the swab packaging and the swab shaft.
7. Perform Post Weight:
- a. Zero the urine cup or similar lightweight container.
  - b. Weigh the capped cryovial containing the absorbed swab tip, the swab packaging and the remainder of the swab shaft (Suggestion: Place the swab shaft into the packaging and have it upright during weighing.)
  - c. Make sure that the post-weight is larger than the pre-weight.
  - d. Record post-weight on the LDMS Tracking sheet.
8. Within 2 hours, place the sample tubes in the freezer at  $\leq -70^{\circ}\text{C}$ .

#### Shipping of PK swab samples

- LC will coordinate sample shipments throughout course of study if necessary and at its conclusion.
- The back-up samples will be retained at the site until advised by the LC or MTN-028 leadership team.
- All shipments will be on dry ice that will be sufficient for a 24 hour period and can be initiated Monday through Wednesday to insure that samples arrive in the lab during the work week.

### 9.7.6. Testing of Intravaginal Ring (IVR) for Remnant Content Analysis

Used rings will be analyzed for residual levels of MK-2048 and Vicriviroc, and will be collected at day 28 or early termination visit. The used rings may contain vaginal secretions and therefore treated as a biohazard. The rings will remain in the amber pouch and stored at room temperature until further notice from the MTN LC. Rings that are defective or inserted briefly and removed for various reasons may be destroyed at the site via biohazard procedures.

#### Important notes:

- Hour 0 Blood and Vaginal swab for PK should be collected immediately before ring removal.
- If the ring is removed by the participant prior to the clinic visit and will not be reinserted, instruct the participant to rinse and dry the ring and place it in a container that is stored at room temperature. At the clinic, the used ring is still prepared for residual drug analysis. After the used ring is taken out of the container that the participant used to return it, follow directions starting with step 1 of "Removal of ring by clinician".

#### Materials:

- A disposable container or a reusable container that was cleaned using 10% bleach solution for 20 minutes or sterilized.
- Tap water
- PPE: lab coat, gloves, face guard
- Paper towel or gauze
- 3"x5" amber Zippit pouch with affixed biohazard label
- SCHARP label for amber pouch

#### Removal of ring by clinician:

1. Wear lab coat, gloves, and protective face guards when performing this step.
2. The clinician will remove the used ring and place in a clean container with tap water.
3. Move the ring around in the water or swirl the container to remove vaginal material.
4. Take the ring out of the water and blot dry with paper towels or gauze.
5. The ring should be dry before storing in pouch.
6. Dispose of blotting materials and contaminated water according to your institution biohazard policy.

#### Preparation of used ring for storage on-site:

1. Site staff will place the ring into a new 3"x5" amber Zippit pouch (see figure 9-4) that was provided by LC to store the rings.
2. Label the pouch with the participant ID number and visit number.
3. Add a biohazard sticker if one is not already attached to the pouch, making sure not to cover the identifier information.
4. Store the used ring within the biohazard labeled amber pouch at 2-8°C.
5. The use of LDMS is required to log in all used rings.
6. At the end of the study, LC will contact site to coordinate shipment.

**Figure 9-4: 3"x5" amber Zippit pouch**



## **9.8. Cervical Specimens: Pap Test and Biopsy for PK**

Pap smears are only required if clinically indicated or if a participant has not had a documented normal test within 12 months prior to Enrollment.

A cervical biopsy will be collected as described in the site SOP, and will be using standard cervical biopsy instruments (Kevorkian, Tischler, etc) with a bite size measuring 3 x 5 mm. Topical anesthetic will not be used, however, oral nonsteroidal anti-inflammatory drugs (NSAIDs) for pain management are allowed. Bleeding may be controlled through a combination of applied pressure, silver nitrate and/or monsel's solution.

### **9.8.1. Papanicolaou (Pap) Test (\*only if indicated)**

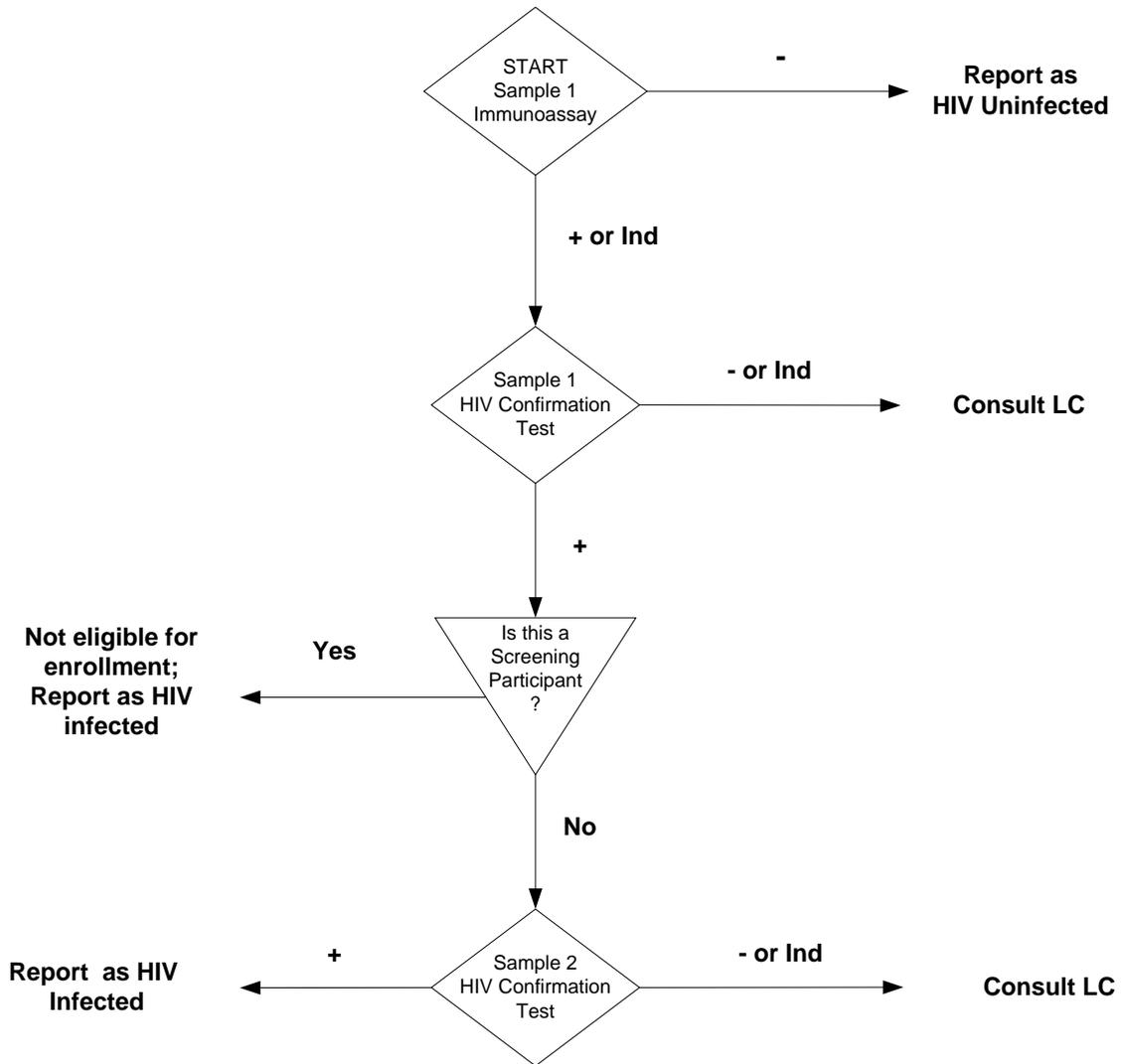
If a Pap is required, ecto- and endocervical cells will be collected after all tissues have been visually inspected, and all other required specimens have been collected. The testing will be done at the site's local laboratory. Specimen collection, testing and QC procedures must be performed and documented in accordance with study site SOPs.

### **9.8.2. Cervical Biopsy for PK**

One biopsy will be collected for a tissue PK level at Visit day 28.

1. Label one 2 ml cryovial (Nunc or Nalgene) with a SCHARP label and the appropriate sample and study identification information.
2. Weigh the labeled cryovial using an analytical scale with a sensitivity rating of 0.1 milligrams or better. Document this pre-weight on the LDMS tracking sheet.
3. Directly transfer the biopsy to the designated pre-weighed cryovial.
4. Obtain the post-weight, which should be greater than the pre-weight, for the cryovial containing the biopsy using an analytical scale and document on the LDMS tracking sheet.
5. Calculate the net weight, which should be greater than zero.
6. Immediately freeze the cryovial containing the PK biopsy in dry ice ethanol bath (dry ice with enough ethanol to make a slushy consistency) or liquid nitrogen.
7. Document the time when the cryovial containing the biopsy is frozen on the LDMS tracking sheet.
8. Store the labeled cryovials containing the frozen biopsies at  $\leq -70^{\circ}\text{C}$ .
9. All pre and post weights are also to be logged by the processing lab onto an excel weight worksheet supplied by LC. The net-weights will be calculated by the formula in the worksheet and entered into the LDMS system.
10. LC will coordinate shipments throughout if necessary and at the end of the study. All shipments will be on dry ice and can be initiated Monday through Wednesday to insure that samples arrive in the lab during the work week.

**Appendix 9-1: HIV ANTIBODY TESTING ALGORITHM**



Ind: Indeterminate test results  
LC: Laboratory Center