

**MOSAIC HIV Drug Resistance Monitoring Studies**

Template Manual of Procedures

*Template 1.0 | 4 August 2023*

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# Introduction

This Manual of Procedures (MOP) specifies procedural information for the Maximizing Options to Advance Informed Choice for HIV Prevention (MOSAIC) HIV Drug Resistance (HIVDR) monitoring studies.

## Protocol Specifications

Each country team is responsible for maintaining archived and current versions of the study protocol, including annual renewal documents in their essential protocol documents. All PrEP facilities participating in each protocol are expected to operate under the protocol version that is currently approved by the local institutional review board/ethics committee (IRB/EC).

## Sources of Procedural Information

The MOP serves to supplement the study protocol. It does not replace or substitute the protocol or its contents. In the event this manual is inconsistent with the information and guidance provided in the protocol, the specifications in the protocol will take precedence. In the event study implementation questions are not adequately addressed by the applicable study protocol or this manual or if any inconsistencies between the two documents are identified, please notify the *[insert title of in-country manager*], who will in turn notify the [*insert title/organization of global or headquarters staff if applicable*]. The [*insert title/organization*] staff should also be contacted for any general questions on protocol implementation or study procedures.

## Selection of PrEP Facilities

The protocol is designed such that all facilities providing PrEP (including private, public, NGO or other) are eligible to participate in the study. Each HIVDR protocol team will work with the ministry of health and/or or protocol investigators to specifically determine the PrEP facilities participating in each study. These may include facilities providing oral PrEP, PrEP ring, and/or injectable PrEP, depending on local regulatory approval and PrEP availability.

## Protocol Investigator Responsibilities

Each investigator listed in the protocol must conduct their applicable study in accordance with the International Conference on Harmonisation Consolidated Guidance for Good Clinical Practice (GCP). Although clinic staff are not necessarily operating in clinical research centers, it is expected that clinic staff will:

* Document all observations, including eligibility criteria, for each participant in adequate and accurate source documents
* Ensure data on lab requisition forms and case report forms are accurate, complete, legible, and timely
* Document changes or corrections to the lab requisition forms and case report forms with initials and date, and ensure that the original entry is not obscured
* Maintain SOPs, job aids, or other materials for easy reference
* Ensure confidentiality of medical records by maintaining records in locked rooms, as per country-specific guidelines for protecting health information
* Store signed informed consent forms separately from other study documents that include the PrEP ID number or barcode

## IRB/EC Submissions

The [*insert title of project managers, as applicable*] in each country are encouraged to request that their IRB/ECs acknowledge receipt for all documents submitted to them, and to request that the IRBs/ECs note both the effective date and the expiry dates of all approvals. Documentation of all correspondence to and from all responsible IRBs/ECs (i.e., complete copies of all submissions, responses, and approvals) should be maintained in essential document files (Table 1). The following are documents for IRB/EC submission and approval requirements pertinent to the MOSAIC HIVDR studies:

* Initial protocol and subsequent protocol amendments, including informed consent forms
* Study status reports/updates (per IRB/EC policy)
* Protocol deviations (per IRB/EC requirements)
* Protocol investigator qualification documentation, including CVs and proof of ethics training such as a training completion certificates
* Other documentation required/requested by the IRB/EC
* Final study report/closure report

## Essential Document Files

[*Insert project management staff as applicable],* and PrEP clinic staff, are responsible for proper collection, management, storage, quality control, and quality assurance of study-related documentation.

Table 1 provides a list of essential documents utilized for HIVDR studies. The description of these documents is provided with a recommended location where they should be filed during the conduct of these studies.

***Table 1. Essential Files and Location***

| Document | Description | Suggested Location |
| --- | --- | --- |
| Case Report Form (CRF) | Paper form completed by site healthcare worker (HCW) for each participant, to capture required protocol information | Forms sent with sample shipment. Copy is immediately sent to [*country project managers*], CRF data uploaded into central study database by [*country project manager*]. Original forms sent to [*country project managers*] at periodic intervals. |
| Laboratory Requisition Form (LRF) | Paper form completed by site healthcare worker (HCW) and receiving laboratory for each participant |  |
| Communications | Relevant correspondence, including meeting notes, memos, and notes to file | [*Country project managers and headquarters staff, as applicable*] |
| Informed Consent Form (ICF) | ICF provides description of study to potential participants | Participant chart\* |
| Investigator qualification, such as CVs and GCP/HSP certification | Documents for investigators listed in protocols, to demonstrate qualifications to conduct the study | [*Country project managers*] |
| IRB/EC approvals and correspondence, including protocol deviation tracking | Copies of all materials submitted to and approved by the IRB/EC | [*Country project managers*] |
| Laboratory documents, including certifications and accreditations | Documentation that demonstrated ability of laboratories to perform HIVDR and drug level tests | Laboratory file |
| Participant ID code listing | A secure, confidential listing that allows site HCW to verify participant identity | Participant chart\* |
| Manual of Procedures | Procedural manual that is used across all MOSAIC HIVDR studies; final version 1.0 and any subsequent updates to be filed | [*Country project managers and headquarters staff, as applicable*] |
| Site assessment reports | Documentation of site assessment report and findings, conducted by [*country project managers*] | [*Country project managers*] |
| Shipping and storage records for samples | Documents that record shipping dates and details of specimens shipped to laboratories | Laboratory file |
| Training records | Documentation of training completed by site staff and PrEP implementing partners | [*Country project managers and headquarters staff, as applicable*] |

\* The participant chart may also be the patient’s PrEP file. If electronic files are utilized, then the facility should also file participant-related paper documentation (such as the signed informed consent form) in a separate folder or binder that is stored in a locked cabinet or locked room.

## Communications

Applicable clinic and laboratory staff will communicate directly with the [*country project managers*] or through a designated person throughout the process of specimen collection, receipt, testing and storage. [*Country project managers*] have routine conference calls with the [*headquarters staff as applicable*] to review ongoing study implementation updates.

# 2. Accrual and Informed Consent Introduction

This section provides information on requirements and procedures for recruiting participants into MOSAIC HIVDR-sponsored protocols. This section also presents information related to informed consent requirements for study participants.

# 2.1 Participant Accrual

PrEP facilities will recruit all individuals with recent PrEP use, who seroconvert, into MOSAIC-sponsored studies. Definitions of “recent PrEP use” is specified in each protocol. PrEP users who seroconvert, and provide consent, will be recruited until the sample size is reached, or the study closes. Potential participants will be referred to the study as soon as possible after identification of HIV seroconversion, i.e., the first positive rapid HIV test.

Each protocol specifies the approximate accrual plan for all participating sites. The [*country* *project manager*] is responsible for ensuring that the total accrual does not exceed the number specified in the protocol. Accrual will remain open, and participants may enroll throughout the timeline in the study protocol. As each protocol approaches the completion date, the [*country project manager*] will ensure all study partners, PrEP facilities and key investigators are aware of accrual closure.

Each [*country project manager*] will document the number of participants enrolled in the study through study-specific tracking spreadsheets, databases or other documentation method.

# 2.2 Participant Eligibility

MOSAIC HIVDR-sponsored protocols specify minimal screening and eligibility criteria including participant informed consent, review of records by clinic staff to confirm HIV seroconversion and confirmation of recent PrEP use. Each study team should refer to their applicable protocol for exact eligibility criteria, however, informed consent must be obtained before conducting any other procedures. See section 2.3 for more information on informed consent.

Should clinic staff or the [*country project manager*] identify that an ineligible participant has inadvertently been enrolled into the study, the [*country project manager*] should complete a protocol deviation form, and inform the protocol chair of the study, applicable IRB/EC and the [*headquarters team*] for guidance on subsequent action to be taken.

# 2.3 Informed Consent

Informed consent is a process by which an individual voluntarily expresses their willingness to participate in research, after having been informed of all aspects of the research that are relevant to their decision. It is not merely a form or a signature, but a process, involving information exchange, comprehension, voluntariness, and documentation.

Each study protocol will specify use of a written or oral consent process, as approved by the IRB/EC. Each protocol will include either an IRB-approved informed consent form, or oral consent information sheet, that is reviewed with the participant and completed at the time of enrollment into the study, after the individual has been informed about the reason for the study, their role in it, any potential benefits or risks to them, and who to contact if they have further questions.

Ideally, the informed consent process takes place on the same day as the participants’ HIV positive test, in the language of their choice. The [*country project manager*] is responsible for ensuring the current IRB-approved consent form or oral consent information sheet, in English and any other approved language, is available to PrEP clinics for use prior to sample collection. If an updated version of the consent form/information sheet is approved by the applicable IRB/EC, the [*project manager*] will ensure that updated copies are available in collection kits and outdated blank forms are removed from PrEP clinics.

Country teams may be able to enroll participants under the age of 18 (age of consent) if they have received prior approval from their IRB/EC. Documentation of IRB/EC considerations for enrolling participants under 18 years of age must be filed with other study essential documents. PrEP clinics must be informed of any considerations for enrolling minors in the study.

For participants who do not provide consent, no procedures should be performed and no data that can be linked to the participant’s name or other personal identifier(s) should be recorded.

Irrespective if there is a written or oral consent process, it is the responsibility of the clinic staff involved in the informed consent process to:

* Deliver all required information in a private setting, and in a manner that is understandable to the potential study participant
* Assure that informed consent is obtained in a setting free of coercion and undue influence
* Confirm that the participant comprehends the information
* Document the process

An illiterate participant can be consented and enrolled, provided they are otherwise willing and eligible. Specific procedures for consenting illiterate clients are outlined by the IRB/EC of record.

Participants should be offered a signed copy of the consent or information sheet, if applicable, though they may opt to not receive a copy.

# Study Procedures Introduction

This section provides information regarding study procedures for MOSAIC HIVDR-sponsored studies, including enrollment, sample collection and results provision. Information on laboratory procedures is provided in Section 4.

# 3.1 Visit Location

Given the nature of the study procedures, all visit procedures are expected to be completed at the study clinic.

# 3.2 Overview of Study Visit Procedures

Ideally, all study procedures are expected to occur on one day, at the time of the first reactive HIV test result (see Figure 1). If for some reason procedures are not completed on the day of identified seroconversion, clients will be asked to return to the clinic to complete the procedure as soon as possible as within 5 days; assuming they are still eligible per protocol requirements. Template job aids, standard operating procedures and other support materials will be provided to country project managers. These materials may be adapted for country-specific procedures.

Upon identification of a potential participant, the study staff person will open a “MOSAIC HIVDR Kit” and conduct informed consent and eligibility determination per Section 2 of this manual.

Typically, MOSAIC HIVDR DBS Kits will include the following:

* Alcohol or spirit swabs
* EDTA vacutainer blood collection tube
* Blood collection safety needle and cap
* Vacutainer holder
* Lancet device
* Gauze
* Fabric bandage
* Two unused DBS cards
* Transfer pipette (plastic dropper)
* Two sealable plastic bags
* Desiccant packs
* Humidity indicator cards
* Barcode labelled stickers
* Laboratory Requisition form
* Case Report form
* Informed Consent form
* Chain of Custody form
* Pre-addressed shipping envelope

For sites enrolling PrEP ring users, kits will also be provided that have PrEP ring collection materials, including a storage bag, container for rinsing the ring, and paper towels/gauze.

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Clinic staff are requested to inform the applicable [*country project manager*] about the potential participants and the [*country project manager*] can then provide any needed support, instructions and guidance to ensure the samples are collected and shipped according to protocol.

***Figure 1. Study Procedure Flowchart***

**Client DOES NOT consent**

**Client consents**

**No further evaluation needed.**

**If HIV NEGATIVE**

**If HIV POSITIVE**

**3.3 Data Collection**

After informed consent is conducted, the clinic staff person will add the participant ID number, or affix project-specific barcode-labeled stickers, to:

* Each of the two Dried Blood Spot (DBS) cards
* Blood collection tube
* Laboratory Requisition form (LRF)
* Case Report Form (CRF)
* Ring collection bag (if indicated)
* Client’s medical file

Each country-specific protocol should reference their approved CRF to confirm all data fields are completed per protocol requirements. The clinic staff person will complete the CRF and initial section of the LRF by reviewing the client’s medical file as well as interviewing the client as indicated.

The participant ID number only (which may be the National PrEP number) will be included on the form, to ensure confidentiality of the study participants. Importantly, the clinic staff member should do an initial Quality Control (QC) check to confirm the information on the CRF and LRF is written correctly, and that no personal identifying information is included. Both the CRF and LRF will be sent to the laboratory, along with the collected samples.

If any mistakes are discovered, a single line should be drawn through the error, with the staff person initialing and dating the correction. Prior to sending a CRF or LRF with personal identifying information, a certified copy should be made for internal document filing; however, the copy that is sent to the laboratory should have the information completely blacked out, with initials and date.

Once the [*country project manager*] receives the paper-based CRF from the laboratory, they will upload data into the [*insert as applicable*] central database, utilizing quality control procedures to ensure the entries into the database match the information on the CRF. The [*headquarters office*] will conduct periodic quality assurance measures, comparing the database with the collected CRFs. Any discrepancies will be provided to the [*country project manager*] who will confirm and correct these data with the applicable clinic site.

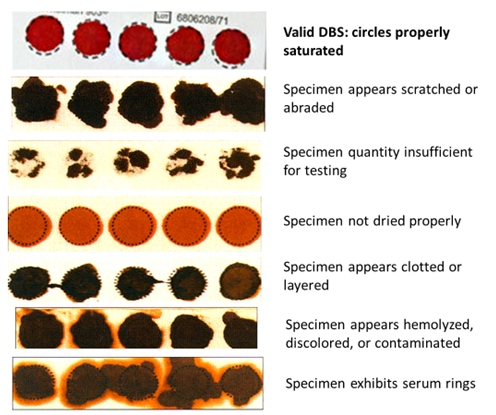
All study-related information will be stored securely at the applicable PrEP clinic, in the [*country project manager*] office, and in the [*headquarters office]*; using locked cabinets and password protected files.

**3.4 Blood Collection**

A healthcare worker trained in phlebotomy will collect venous blood from consented participants (upper limit of blood volume specified in the respective protocols and consent forms) for preparation of a dried blood spot (DBS) sample and/or to process a plasma sample for HIV drug resistance testing.

The health care worker preparing the DBS should be instructed to perform the following procedures:

* Wash hands and put on gloves once hands are dry before starting procedure.
* Lay out both DBS cards onto a clean surface.
* Label the DBS card with the unique barcode sticker provided in the sample collection kit or the participant PrEP ID# and the date of sample collection (the same day as the preparation of the DBS sample).
* Gently invert the blood tube at least 5 times to ensure complete mixing, then draw up at least 0.5 mL of blood using the transfer pipette.
* Starting from left to right on the first DBS card, fill each circle on the first DBS card by slowly depressing the bulb.  Attention should be made to not touch the cards directly and to ensure blood drops fall in the center of each circle
  + When absorbed, the blood drops should fill the entire outline of each circle (see Figure 2).
* Once both cards have been successfully spotted with 5 spots per card, bend the flap behind the card (if available) and place the cards on a surface in a clean, dry space or on a DBS drying rack.
* The cards should be stored with blood spots facing up and dried overnight or for at least 3 hours at ambient temperature.
* Protect the cards from rodents, insects, and direct sunlight.
* Do not stack the cards on top of each other or allow them to touch other surfaces during the drying process.
* Prepare remaining blood in tube to include in shipment if applicable per standard procedures (i.e., for backup plasma testing).
* Dispose of all consumable materials and unused blood (if not being shipped to laboratory) in accordance with local protocols and clean working areas with a 10% sodium hypochlorite or chlorhexidine solution when finished.
* Once the blood spots have dried, close the flap to cover the blood spots (if applicable). Make sure the DBS is card labeled with Participant PrEP ID# or barcode sticker, and date of sample collection.

***Figure 2: Examples of Valid and Invalid Dried Blood Spots***

Source: GE Healthcare Life Sciences. 2010. Simple spot check. GE Document 28984392

The dried, labeled and dated DBS cards will then be individually packaged in a glassine envelope. The glassine envelope will be placed in a single gas-impermeable, sealable plastic bag containing desiccant packs to remove residual moisture and containing the humidity indicator card. The sealable plastic bag, containing the DBS cards (considered non-infectious when shipping), will then be placed in the envelope provided in the collection kit with the completed CRF/LRF to ship to the centralized laboratory for drug resistance testing and storage. Country specific guidance may require that leftover blood in the blood tube (considered infectious when shipping) be shipped to the testing laboratory as well.

When sending whole blood, ensure tubes are capped tightly and they are placed in a secondary, leak-proof container (sealable bag) containing absorbent material. That bag should then be placed in a tertiary, rigid shipping container. Proper labeling should be included on the package per national regulations, including potential requirements for labeling the package as infectious, even if the package only include DBS cards. If both DBS and whole blood are being shipped at the same time to the same place, it is acceptable to include the DBS package in the whole blood shipping container.

The DBS sample should be shipped as soon as possible and should not exceed storage at ambient temperature for more than 5 days total including the time needed for shipping. If DBS cannot be shipped to the centralized laboratory within 3 days of collection, DBS must be stored at -80ºC, or temporarily at -20ºC until long-term storage at -80ºC is available. Whole blood for plasma processing should be shipped as soon as possible, ideally within 24 hours. Whole blood may be refrigerated at 4ºC or at room temperature (15-30ºC), until shipment; do not freeze whole blood. If the temperature exceeds 30ºC, then the [*country project manager*] should try to arrange for ice packs or refrigerate where possible.

If the facility or local lab spins the blood to create a plasma sample, this will need to be shipped on dry ice. In this situation, the facility/lab should contact the [*country project manager*] for additional guidance.

**3.5 Used Ring Collection**

If the participant is using the PrEP ring, the used ring that is returned to the clinic by the participant should be collected for residual drug testing at the time of their first positive HIV test and enrollment into the study. The used ring may contain vaginal secretions and is therefore treated as a biohazard.

The participant will be instructed to place the used ring in a clean container, or plastic bag, in the clinic. The health care worker will then perform the following procedures:

* Collect the container (if used) and add water (tap or bottled water is acceptable)
* Move the ring around in the water or swirl the container to remove vaginal material (note: the health care worker may also hold ring under water if a container is not used)
* Take the ring out of the water and blot dry with paper towels or gauze
* The ring should be dry before storing in the study provided sealable plastic bag
* Dispose of blotting materials and contaminated water according to the clinic’s biohazard policy. The container used to rinse the ring can be discarded if disposable, or cleaned and re-used if a glass container.

Note: the ring can be rinsed in the clinic or the laboratory, but ideally clinics and laboratories will be instructed to keep this process consistent with whatever method they are trained on. If the rings will be rinsed in the laboratory, clinic staff will be instructed to collect the ring and immediately place it in the provided sealable plastic bag.

The ring sealable plastic bag will then be placed in the envelope provided in the collection kit with the completed CRF/LRF, and DBS samples, to ship to the centralized laboratory for storage and shipment to monitor drug levels. The ring may remain stored at ambient temperature until shipment.

**3.6 Sample Shipment and Communication**

Table 2 provides an overview of shipment and communication plans for each study. Further details are included in [*insert if applicable: project-specific flow charts* *or other support materials*]. It is best practice for sites to alert the [*country project manager*] before shipping a sample in case modified procedures are required.

***Table 2. Project Shipment Overview***

|  |  |  |  |
| --- | --- | --- | --- |
| Project/Study | Sample Type | Shipment | Communication |
| *[insert project name]* | *[Whole Blood, DBS, and/or Plasma]* | *[Briefly describe shipment process to applicable lab, including courier and any LRFs or specimen tracking forms]* | *[Note any key communication plan for laboratory to notify project of test results]* |

**3.7 HIV Drug Resistance Results**

Once available, test results will be provided to the PrEP facility that collected the sample as well as the [*country project manager*]. Copies of the result should be initialed and dated by the site clinicians and filed in the participant’s chart. Disclosure of available test results should be provided to the participant, as well as to their ART provider, as per protocol requirements. The [*country project manager*] should refer any questions regarding interpreting drug results to the appropriate resource available in country or with the testing laboratory.

**3.8 Study Monitoring**

[*Project staff*] may review study records at clinics and laboratories. The purpose of these visits is to

* Assess the quality of study implementation and documentation
* Troubleshoot and provide technical assistance and/or retraining related to implementation issues and problems
* Share information on successful implementation strategies identified at other sites or laboratories
* Identify action items as needed to address study implementation issues and problems

[*Project staff*] will document the visit and associated findings in an assessment report. The report will summarize observations and include action items. A copy of the report is stored at the [*country project manager office and in the headquarters office*] records.

# Laboratory Procedures Introduction

This section provides information and instructions for laboratory staff related to the processing, storing, shipping, and testing of laboratory specimens for the MOSAIC HIV drug resistance (HIVDR)-sponsored studies. The laboratory will communicate directly with the [*country project managers*] throughout the process of specimen receipt, testing, and storage.

## Current Protocol Specifications

All specimens collected will be obtained from a clinic or healthcare facility that collected the blood sample, and/or PrEP ring, from a PrEP client who seroconverted (had an HIV positive rapid test) and consented to having their sample tested for HIVDR and drug levels. Additionally, samples may undergo viral load testing to address any challenges with HIVDR testing. Unless otherwise specified, the samples will be shipped via courier service from the clinic site at ambient temperature to the testing laboratory.

The specimens received must be processed in the laboratory by qualified personnel and all specimens will be handled according to Good Clinical Laboratory Practices (GCLP) and Universal Precautions.

Performance of this procedure will expose personnel to biohazardous material. All specimens must be handled as infectious material as outlined in your Laboratory’s Safety Manual. The technologist must take all precautions and adhere to all prescribed policies when collecting and handling biological specimens.

This procedure may expose you to:

* Bloodborne pathogens

To perform this procedure, laboratory personnel must use:

* Gloves
* Disinfectant: Diluted bleach (1:10 v/v solution)

This section of the MOP gives basic guidance to laboratory staff but is not an exhaustive procedure manual. This section must be supplemented with laboratory Standard Operating Procedures (SOP) for specimen management, processing, and testing.

## Equipment and Materials Required for Dried Blood Spot (DBS) Processing and Storage

**Equipment:**

* -70°C freezer for storage

**Materials:**

* Gloves
* Freezer boxes or storage containers large enough to contain DBS cards in their sealed bags
* Extra desiccant packs and sealable bags if needed

## Procedures for Sample Processing and Storage

## Receipt of Specimen Shipment

The laboratory will be notified that a shipment is arriving by the [*country program manager*] or by the PrEP site. Upon arrival of a shipment, the following is done:

1. Document shipment’s received date and initials onto the Laboratory Requisition Form (LRF) and the laboratory’s specimen logging notebook or electronic system.
2. Check that the LRF is filled in with all details required, scan a copy of the LRF and the CRF and send via email to the [*country project manager*].
3. If the LRF or CRF is not completely filled, or there are any errors or deviations noted, the project manager will follow up with the PrEP site to complete/correct the form. A completed and corrected LRF will be re-sent to the laboratory for filing, as needed.
4. Check contents of received envelope according to Table 3.

***Table 3. Checklist for Sample Receipt***

|  |
| --- |
| **Each envelope should include:**   * EDTA tube with whole blood, plasma, and/or two DBS cards with 5 spots of blood on each card in a sealed plastic bag with a desiccant and humidity indicator card. * A completed corresponding LRF, CRF, and Chain of Custody form (if applicable) * Used ring in a sealed bag (if applicable) |
| **LRF:**   * The barcodes and/or participant ID on each DBS cards, blood tube, and/or ring storage pouch, must match the client ID on the LRF * The LRF must be completely filled out; any missing information is to be reported to the [*country project manager*] |

## Notification of Specimen Shipment Receipt

Notify the [*country project manager*] (via phone and/or email) and add a note on the corresponding LRF of any deviations identified during the shipment review as described in Table 3, including but not limited to:

* Missing LRF or CRF
* Missing information on the LRF (e.g., date of sample collection)
* Bag does not contain desiccant and/or humidity indicator card
* Desiccant changes color due to excess humidity
* Humidity is indicated on humidity indicator card
* Bag’s seal is faulty
* Spots are left blank on the DBS
* Spots are not incorrectly made on the DBS (e.g., insufficient volume)
* Damage to the card
* Issues with blood in EDTA tube (e.g., clotting or other issue) (if included)

## Specimen Processing and Arrangement for Storage

Once the shipment is reviewed and the [*country project manager*] is notified, as described above, the following steps occur to prepare for sample processing and storage:

1. If EDTA blood tube received:
   1. Process the blood tube for plasma separation and collection according to the laboratory’s established SOPs. Aliquot the plasma into appropriately labeled cryo-tubes.
   2. Spot one or two extra DBS card as back up and for PK evaluation
2. Repackage the DBS cards into a new sealable plastic bag and replace the desiccant pack, with a new desiccant.
3. For the DBS cards: Label each freezer box (or a large bag for study samples) according to your laboratory’s standard freezer organizational practices. Ensure there is a humidity indicator card in each [box or bag]. One DBS sample will be used for viral load and/or HIVDR testing. The 2nd DBS card will be shipped to laboratory in [*insert country name as applicable*] for estimation of drug levels.
4. For the plasma aliquots in cryo-tubes: store the labeled plasma aliquots in a labeled cryobox at -80°C for HIVDR testing.
5. For the PrEP rings: store the ring at room temperature, it will be shipped to a laboratory in [*insert country name*].
6. Store the DBS cards (and/or plasma aliquots) in a sealed bag or freezer box and store at -80ºC until needed for HIVDR testing or for shipment to [*insert country name as applicable*] for drug resistance or drug level testing. Indicate on the laboratory specimen log the details of the sample and the location of the storage in the -80°C freezer.
7. Store the LRF and CRF in a secured, locked, cabinet until requested.

## Specimen HIV-1 Drug Resistance (HIVDR) Testing

The laboratory will conduct the following procedures for HIVDR Testing:

1. Testing of DBS (and/or plasma) samples for HIVDR testing will be performed as per the applicable laboratory HIVDR testing SOP. Procedures will include:
   1. Extraction of nucleic acids: performed using the bioMérieux NucliSENS® System, the ViroSeq HIV-1 Genotyping System, Qiagen, or a validated commercial or laboratory-developed system.
   2. PCR Amplification of extracted nucleic acids and Sanger sequencing of HIV-1 *polymerase* (*protease*, *reverse trascriptase* & i*ntegrase* region) using either the WHO accredited ABI Thermo-Fisher HIV-1 DR test kit, the ViroSeq HIV-1 Genotyping System or a validated commercial or laboratory-developed procedure.
2. Figure 3 describes the general systematic flow of HIV RNA and HIVDR testing that will be performed across laboratories; however, each laboratory will test samples according to their standard operating procedures.
3. Once the results are ready, results are emailed to the [*country project manager*] and applicable site as required.
4. Copies of the results are filed in secure MOSAIC files at the laboratory, or within a secure electronic cloud-based electronic storage system.

***Figure 3: Laboratory Testing Flow***

\*Skip step for DBS samples, if RNA PCR assay using DBS is not available or if there is limited sample volume (<2 ml plasma or <5 DBS spots).

\*\*Copies/mL thresholds for successfully testing HIVDR may differ at individual labs; specific testing algorithms will be described in lab-specific SOPs or other guidance documents.

\*\*\*Sample may be sent to [insert laboratory] for troubleshooting or specialized testing.

HIV RNA PCR\*

Report Results to MOSAIC and National Program

Sanger Sequencing

(*pro-RT-int)*

≥ 1000 copies/mL\*\*

< 1000 copies/mL\*\*

Successful sequence

Report RNA Results to MOSAIC\*\*\*

Inform country project manager\*\*

Failed testing

**Plasma or DBS**

* + 1. Returned Vaginal Rings for Residual Drug Analysis

Vaginal Rings should arrive at the laboratory rinsed and ready for storage. If a ring arrives un-rinsed, refer to section 3 of this manual for guidance on rinsing the ring.

Vaginal rings will be stored at room temperature in the laboratory until the MOSAIC team requests shipment.

## Procedures for Shipment of Samples to Back-up Laboratory

The following procedures will be followed once the laboratory is notified to prepare a shipment of MOSAIC samples to a back-up laboratory or laboratory outside the country per study requirements:

1. The study PI will seek permission from local IRB as needed prior to sample shipment.
2. Once IRB approved, the [*country project manager*] will notify the laboratory to initiate shipment.
3. The stored DBS (at ≤-70**°**C), will be batched and shipped to [*insert country PK laboratory*] on dry ice. The used PrEP rings should be batched and shipped to [*insert country laboratory*] in ambient room temperature.
4. Once the permission is granted for shipment, the laboratory will ensure that all the correct documents (including the import/export permits for the samples) for the shipment are available and the shipping manifest prepared by the laboratory.
5. The international shipping courier will provide the dry ice and boxes for shipment and the IATA certified Lab Tech will pack the DBS (and/or plasma) samples for shipment.