Module 3 – Ensuring Specimen Integrity for Viral Load Testing

Learning objectives:

By the end of the module, participants will be able to:

• List the specimens rejection criteria for viral load testing
• Describe the correct specimen collection procedures for viral load testing
• Perform correct procedures for separation of plasma from whole blood
• Properly prepare dried blood spots (DBS) for viral load testing
• Properly store and package specimens for transport
• Track specimen referral status to ensure timely return of all test results

Target audiences: all cadres involved in pre-testing sample collection, processing, storage, and packaging (Clinicians, Phlebotomists, Counselors, and Laboratorians)

Pre-requisites: Module 1

Participant handouts: 3-1, 3-2, 3-3, 3-4, 3-5, 3-6, 3-7, 3-8, 3-9, 3-10, 3-11, 3-12

Special preparations before facilitating: None

<table>
<thead>
<tr>
<th>Icon</th>
<th>Meaning</th>
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<tbody>
<tr>
<td>🗒️</td>
<td>Refer to Handout</td>
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<td>🔨</td>
<td>Customize the slide for local context</td>
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### Module-at-a-glance

<table>
<thead>
<tr>
<th>Segment</th>
<th>What you do</th>
<th>Time</th>
<th>Handout</th>
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<tbody>
<tr>
<td><strong>Module opening</strong></td>
<td>State the module objective.</td>
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</tr>
<tr>
<td><strong>1. Specimen Type used for Viral Load Testing</strong></td>
<td>Explain the slides according to the content notes.</td>
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<tr>
<td></td>
<td>Gauge participants’ knowledge with the Knowledge Check question.</td>
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<tr>
<td><strong>2. Specimen Collection and Processing</strong></td>
<td>Explain the slides according to the content notes.</td>
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<td>Gauge participants’ knowledge with the Knowledge Check questions.</td>
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<td></td>
<td>Explain the slides according to the content notes.</td>
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<td>3-1, 3-2, 3-3</td>
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<tr>
<td></td>
<td>Conduct activity 3A: Is this DBS Valid according to content notes.</td>
<td></td>
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<td></td>
<td>Explain the slide according to the content notes.</td>
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<td>3-4</td>
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<tr>
<td><strong>3. Specimen Packaging and Transport</strong></td>
<td>Explain the slides according to the content notes.</td>
<td>1:20</td>
<td>3-5, 3-6, 3-7, 3-8</td>
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<tr>
<td></td>
<td>Conduct activity 3B: Packaging Specimens for Shipment to Referral Sites according to content notes.</td>
<td></td>
<td>3-6, 3-7</td>
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<tr>
<td></td>
<td>Explain the slides according to the content notes.</td>
<td></td>
<td>3-9, 3-10</td>
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<td></td>
<td>Gauge participants’ knowledge with the Knowledge Check questions.</td>
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<tr>
<td><strong>4. Specimen Tracking</strong></td>
<td>Explain the slides according to the content notes.</td>
<td>0:15</td>
<td>3-11</td>
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<tr>
<td><strong>5. Specimen Rejection Criteria</strong></td>
<td>Conduct activity 3C: Tracking Referral Specimens according to content notes.</td>
<td>0:40</td>
<td>3-12</td>
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<td></td>
<td>Session B (next day) - DBS packaging</td>
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<td>3-7</td>
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<tr>
<td><strong>Module closing</strong></td>
<td>Invite participants to supply words to complete each key message</td>
<td>0:10</td>
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</tbody>
</table>

**TOTAL MODULE DURATION:** 6:00
1. **Specimen Type used for Viral Load Testing**

3. **Heading - The Quality of Specimens Affects Viral Load Test Results**
   Specimen integrity is the cornerstone of a quality viral load test result. To protect specimen integrity, they must be properly collected in the correct type of tube, stored at the correct temperature, properly processed and within the proper timeframe, transported in the right temperature and packaging. Coordination between the clinic, specimen transport method and the lab is important to ensure timely pickup and proper delivery of specimens.

   The testing laboratory will reject any specimens that arrive with an incorrect temperature or improper packaging, or without the appropriate paperwork. You will learn the specimen rejection criteria later in this module so you know how to prevent your specimens from being rejected.

4. **Heading - Keep Your Turn Around Time Short**
   Turnaround time (TAT) is always a challenge. There are many steps along the way that can impact TAT and each step should be tracked to determine where and if there is a breakdown; improvement projects should be initiated to eliminate the breakdowns to reduce the TAT.

5. **Heading - Methods for Blood Collection**
   There are several methods of blood collection – venipuncture, finger prick, and heel stick (for babies).
   - The preferred method for viral load testing is *venous blood in EDTA tube*.
   - *Finger prick* has been utilized for viral load testing. However, this method has limited data supporting its validity and currently is not recommended by manufacturers.
   - Countries who want to use this specimen collection method for viral load testing should evaluate this form of specimen collection to ensure DBS created from finger prick is as effective as venipuncture.
   - *Heel stick* for creation of DBS is not currently widely used for viral load testing but is the cornerstone for EID testing.

6. **Heading - Specimens Used for Viral Load: Plasma and Dried Blood Spots (DBS)**
   Before testing, whole blood collected must be processed into either plasma or dried blood spot (DBS) specimens for viral load testing. The *gold standard* specimen for viral load testing has been plasma. DBS created from venous blood was validated only recently.

7. **Heading - Plasma vs. DBS for Viral Load Testing: Pros and Cons**
   Tradeoffs exist with each sample type, and these should be balanced against clinical and operational considerations.

   For example, dried blood spots may eliminate the need for cold chain transport, but this may restrict the type of viral load platform to be used and the applicable clinical thresholds. On the other hand, EDTA-derived plasma requires centrifugation. Small and inexpensive battery-operated centrifuges may be a solution; however, where logistics for adequate sample transport to a laboratory do not exist, this will require dedicated staff at the clinic level trained in phlebotomy, sample processing, storage and transport as well as safety precautions associated with sample manipulation and waste management.

   Ask participants what are the pros and cons of using plasma versus DBS for viral testing in their setting. List their contributions on the flipchart before showing the answers on the slide.
<table>
<thead>
<tr>
<th>Slide Number</th>
<th>Content Notes for PowerPoint Slides</th>
</tr>
</thead>
</table>
| **8** | **Heading - Knowledge Check**  
Gauge participants’ knowledge with the Knowledge Check question. |

2. **Specimen Collection and Processing**

| 10 | **Heading - Example of Valid and Invalid Venous Blood Specimens**  
Whole blood samples received into the laboratory should be inspected to insure that the blood is a valid specimen. Invalid whole blood specimens include specimens that are hemolyzed or clotted.  
- Hemolysis occurs if the specimen is shaken too vigorously causing lysis of the red blood cells. This can happen with improper mixing or during transport if the specimens are packaged or handled inappropriately.  
- Clotting can occur if the EDTA tube is not mixed immediately or properly by inversion after blood collection. |

| 11 | **Heading - Example of Valid and Invalid Plasma Samples**  
Valid plasma samples are clear and have a slight yellow tint with defined buffy coat and red blood cell layers. Hemolyzed plasma specimens appear bright pink to red in color after centrifugation. Lipemic plasma occurs when there is an elevated amount of fat in the blood and the plasma is white and thick.  
Other specimens that are contaminated can have extra layers of unidentifiable cells or debris. Contaminated specimens could be caused by issues with the EDTA collection tube or butterfly or tubing associated with blood collection. Be sure to inspect all of these items prior to use, but do not open EDTA tubes. Contamination can be seen without opening. |

| 12-13 | **Heading - Knowledge Check**  
Gauge participants’ knowledge with the Knowledge Check questions. |
### Heading – Plasma using Venous Blood

Although it is outside the scope of this module, management must make sure phlebotomists are competent and follow the proper procedures when collecting venous blood. Refer the following three tools from the [SLMTA curriculum](https://www.slmta.org/tool-kit/english) to ensure phlebotomist competency:

- 701 - Phlebotomy Checklist
- 702 - Phlebotomy Key Competencies
- 703 - Phlebotomy Patient Survey


- Collect whole blood into commercially available anticoagulant-treated tubes e.g., EDTA-treated (lavender tops) or citrate-treated (light blue tops). Heparinized tubes cannot be used as it inhibits PCR reactions. Cells are removed from plasma by centrifugation for 10 minutes at 1,000-2,000 x g (ideally 1500) using a refrigerated centrifuge.
- The resulting supernatant is designated plasma. Following centrifugation, it is important to immediately transfer the liquid component (plasma) into a clean polypropylene tube using a Pasteur pipette. The samples should be maintained at 2-8°C while handling. If the plasma is not analyzed immediately, the plasma should be apportioned into 0.5 ml aliquots, stored, and transported at −20°C for short term storage of −70°C for longer (a month or more) storage. It is important to avoid freeze-thaw cycles and do not freeze thaw more than 3 times. Samples which are hemolyzed, icteric, or lipemic can invalidate certain tests.

### Heading - Centrifugation of Whole Blood into Plasma

Centrifugation of whole blood at the correct speed and time is critical to proper plasma separation. Centrifugation can be measured using RPM or xg (RCF). RPM (rotations per minute) vary based on rotor in use and therefore is different for each type of rotor even when utilizing the same centrifuge. Xg or RCF (relative centrifugal force) is a constant measurement and does not vary by manufacturer or rotor. Therefore RCF should be utilized to standardize the speed at which the tubes are spun. A standard speed and time for separation of plasma from blood is 1500 xg for 10 minutes. The centrifuge should be cleaned on a regular basis and must be calibrated yearly.

In the lab, utilize the available equipment to show how to toggle between RPM and RCF and how the number varies. If possible show and discuss the use of a nomagraph to determine RCF when all you have is information on RPM.
<table>
<thead>
<tr>
<th>Slide Number</th>
<th>Content Notes for PowerPoint Slides</th>
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<tbody>
<tr>
<td>16</td>
<td><strong>Heading – Spotting and Drying the DBS Card from Venipuncture EDTA Tubes</strong>&lt;br&gt;Refer participants to the job aide (3-2) and walk them through the steps. There will be a laboratory session for hands-on practice of making the DBS specimens.&lt;br&gt;&lt;br&gt;Provide additional tips:&lt;br&gt;  - The area where DBS are prepared needs to be inside, away from wind and dust, not exposed to direct sunlight and must be in a clean and non-cluttered space.&lt;br&gt;  - Always use Universal Safety Precautions. This includes:&lt;br&gt;    - Treat all blood samples as though they are infectious.&lt;br&gt;    - Wash hands.&lt;br&gt;    - Wear gloves, apron/lab coat and if no hood is available use a face shield&lt;br&gt;    - Dispose of waste appropriately.&lt;br&gt;  - If there are no pipettes available a transfer pipette or a capillary can be used. Just ensure that the spot is completely filled.&lt;br&gt;  - You must change tips between each patient and only one tube should be opened at any given time.</td>
</tr>
<tr>
<td>17</td>
<td><strong>Heading – Spotting and Drying the DBS Card from Finger Prick Using a Capillary Tube</strong>&lt;br&gt;Refer participants to the job aide (3-3) and walk them through the steps.</td>
</tr>
<tr>
<td>18</td>
<td><strong>Heading - A Valid DBS Specimen</strong>&lt;br&gt;A valid DBS specimen should be labeled with a patient ID or unique identifier, a date and the spots should be completely filled. If there is not enough blood for 5 complete spots, a minimum of 3 completely filled spots should be created. It is okay if the blood goes outside of the circle.</td>
</tr>
<tr>
<td>19-26</td>
<td><strong>Heading - Activity 3A: Is this DBS Valid?</strong>&lt;br&gt;Read the activity slide (#19) before showing slide #20. Allow participants 3 minutes to write down their responses before unveiling the right answers using Slides #21-#26.</td>
</tr>
<tr>
<td>27</td>
<td><strong>Heading - How to Prevent Unacceptable DBS Samples?</strong>&lt;br&gt;Refer participants to the job aide (3-4). Highlight the criteria for acceptable DBS and unacceptable DBS samples. Emphasize that unacceptable DBS samples will be rejected by the testing laboratory. Discuss things they can do to prevent unacceptable DBS samples. Stress the points below:&lt;br&gt;  - Ensure that all patient information (name and/or unique identifier) is present and legible&lt;br&gt;  - Ensure the date is included&lt;br&gt;  - Ensure the circles are completely filled in&lt;br&gt;  - Ensure there are at least 3 god spots per card&lt;br&gt;  - Ensure the spots are not layered or clotted or contaminated with water or alcohol&lt;br&gt;  - Ensure the spots are completely dry prior to packaging</td>
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<tr>
<td>28</td>
<td><strong>Heading - Recommended vs. Minimum Specimen Volume for Viral Load Testing</strong>&lt;br&gt;The minimum specimen volume is set because below that level testing cannot be done. When specimen collected does not meet that minimum requirement, it will be rejected. On the other hand, we would like to collect more than just minimum specimen volume in case retesting is required due to instrument failure or operator errors.</td>
</tr>
<tr>
<td>29-33</td>
<td><strong>Heading - Knowledge Check</strong>&lt;br&gt;Gauge participants' knowledge with the Knowledge Check questions.</td>
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<tr>
<td>3.</td>
<td><strong>Heading – Guidelines for Specimen Handling Conditions</strong>&lt;br&gt;After correct labeling and packaging, specimens should be placed at the correct storage temperature. Specimens should be stored in dedicated refrigerators and freezers and should not be stored with reagents. Each specimen type has very specific requirements to ensure their integrity for testing and different manufactures often have different suggested storage conditions. Always refer to the manufacturer for ideal specimen storage. In general,&lt;ul&gt;&lt;li&gt;Whole blood should be separated into plasma within 6 hours of collection. If this is not possible, whole blood can be stored up to 24hrs at 2-8C and should be transported at this same temp with frozen ice packs. Whole blood can never be frozen for later use in viral load testing.&lt;/li&gt;&lt;li&gt;Plasma can be stored at 2-8C for up to 5 days. And prior to freezing, plasma can be transported at this temperature with frozen ice packs. For storage from 5-60 days plasma can be held at -20C but ideally plasma should be placed at -70C for any long term storage. Once plasma is frozen, it must be transported frozen to avoid freeze thaw cycles.&lt;/li&gt;&lt;li&gt;DBS if packaged properly can be stored at ambient temperature for up to 2 weeks and can be transported at this same temperature. For storage after 2 weeks, DBS should be kept at -70C.&lt;/li&gt;&lt;/ul&gt;</td>
</tr>
<tr>
<td>35</td>
<td><strong>Heading – Packaging Viral Load Samples for Transportation</strong>&lt;br&gt;All specimen types should be transported as described utilizing the triple packaging system. Local and international laws should be utilized to avoid any transport issues.</td>
</tr>
<tr>
<td>36</td>
<td><strong>Heading – Example of Triple Packaging System</strong>&lt;br&gt;All triple packaging includes 3 layers.&lt;ul&gt;&lt;li&gt;The first later consists of the primary specimen container. For viral load this is a tube or filter paper.&lt;/li&gt;&lt;li&gt;The second packaging container contains absorbent material and is leak proof to prevent spillage. This can be a specially designed screw top container or something like a zip top bag. Often the second layer of packaging has a rack or similar item to keep specimens from moving around too much.&lt;/li&gt;&lt;li&gt;The outer container should have biohazardous labeling when necessary (DBS does not require this) and should contain all relevant forms and paperwork to accompany the specimens. The outer container can consist of a box or cooler. The outside of the 3rd container should remain clean so as to easily be handled without PPE.&lt;/li&gt;&lt;/ul&gt;Refer participants to and walk them through job aide 3-6.</td>
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<td>Slide Number</td>
<td>Content Notes for PowerPoint Slides</td>
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</table>
| **38**       | **Heading - Packaging Whole Blood and Plasma for Transport**  
Refer participants to and walk them through job aide 3-7. |
| **39**       | **Heading - Packaging DBS for Transport**  
Refer participants to and walk them through job aide 3-8. There will be a practical session later with spotting the DBS cards. |
### Heading - Activity 3B: Packaging Specimens for Shipment to Referral Sites

Referral testing requires proper packaging and shipping of patient specimens to preserve their integrity and suitability and to protect all persons involved in their transportation. In this activity, participants learn the importance of safe and effective specimen packing and practice appropriately packing samples with available materials of varying levels of sophistication.

#### Preparations before the activity:
- Set up three work stations (tables), each equipped with one set of materials below and necessary PPE.
- Do not use actual specimens, but obtain the specimen collection containers or materials (i.e., glass slide) to simulate the specimens to be packaged.
- Label all specimens and their accompanying test requisitions with patient ID information, date/time of collection, collector’s initials, and ID number. To simplify the patient name selection you may use “Patient A, Patient B, etc.”

<table>
<thead>
<tr>
<th>Set 1 (Standard – commercially available)</th>
<th>Set 2 (Rudimentary Packaging)</th>
<th>Set 3 (ad hoc packaging)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test requisition forms</td>
<td>Test requisition forms</td>
<td>Test requisition forms</td>
</tr>
<tr>
<td>6 Specimens</td>
<td>6 Specimens</td>
<td>6 Specimens</td>
</tr>
<tr>
<td>• 3 blood specimens</td>
<td>• 3 blood specimens</td>
<td>• 3 blood specimens</td>
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<tr>
<td>• 3 DBS cards</td>
<td>• 3 DBS cards</td>
<td>• 3 DBS cards</td>
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<tr>
<td>Specimen tracking form(s)</td>
<td>Specimen tracking form(s)</td>
<td>Specimen tracking form(s)</td>
</tr>
<tr>
<td>Absorbent sheaths and/or sheeting</td>
<td>Several paper towels</td>
<td>Several paper towels</td>
</tr>
<tr>
<td>Clear plastic bags</td>
<td>Secondary container(s)</td>
<td>Secondary container(s)</td>
</tr>
<tr>
<td>Pathoshield Carton</td>
<td>1 zip top bag large</td>
<td>Clear plastic wrap</td>
</tr>
<tr>
<td>Cello tape / Shipping Tape</td>
<td>1 specimen rack</td>
<td>1 tea box or small box supplies came in (like blood collection supplies box)</td>
</tr>
<tr>
<td>Scissors</td>
<td>Several sheets of newspaper</td>
<td>Rubber bands, tape or rope/string for closure</td>
</tr>
<tr>
<td>Biohazard shield emblems</td>
<td>Lab tape or whatever is available</td>
<td>Some sort of ice packs</td>
</tr>
<tr>
<td>Cooler box w/ ice packs</td>
<td>Scissors</td>
<td></td>
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<tr>
<td></td>
<td>Cooler box w/ ice packs</td>
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</table>

#### Conduct the activity:
- Divide participants evenly into 3 groups and assign each group to one work station.
- Use Slide #40 to provide instructions for the activity.
- After 15 minutes, debrief by unpacking each box and provide your feedback making sure all participants can see what you are doing.
- Stress that, regardless of the sophistication of the packaging materials, it should still be possible to properly package and ship specimens for referral. Fancy materials are not necessary.
- Total activity takes about 30 minutes (introducing, conducting, and debriefing).

For more detail, refer to Module 7, Packaging Specimens for Shipment activity in the SLMTA curriculum ([https://www.slmta.org/tool-kit/english](https://www.slmta.org/tool-kit/english)).
<table>
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<tr>
<th>Slide Number</th>
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</table>
| 41           | **Heading – Guidelines for Sample Transport**  
Sample transport begins at the clinic where packaging takes place and ends at the testing laboratory where specimens are received. The specimens must be properly packaged according to all safety guidelines and ice packs used must be frozen. Transport documents must be filled out, checked and signed prior to the hand off to the transport driver. The transport driver must then acknowledge receipt of the specimens. The drivers must be trained in safe practices for bloodborne pathogens and have contact information if a spill occurs or be trained and have the necessary materials to deal with a spill. Upon arrival at the testing facility, specimens should be checked for any issues prior to the driver leaving the facility.  
Refer participants to job aide 3-9. Walk them through the handout and answer any questions they may have. |
| 42           | **Heading - Chain of Custody for Sample Transport**  
Transport documents must be filled to maintain and prove chain of custody. Each step in the process must provide a signature to acknowledge and accept responsibility for this chain of custody. Date and time should be recorded at every hand off. The package should inspected at every exchange to ensure there are no issues with leakage or spills.  
Refer participants to job aide 3-9. Walk them through the handout and answer any questions they may have. |
| 43-45        | **Heading - Knowledge Check**  
Gauge participants’ knowledge with the Knowledge Check questions. |
| 47           | **Heading - Keeping Track of All Referral Samples at Clinics**  
There should be a designated individual at each clinic responsible for checking to determine if samples were received at the laboratory in a timely fashion and in good condition. This individual or another should also be the point of contact for the lab for issues with specimens and return of results. A single point of contact makes it much easier for labs to know who to communicate with when needed. For the same reason, a single point of contact at the laboratories for the clinic should also be established.  
Refer participants to job aide 3-11. Walk them through the handout and answer any questions they may have. |
| 48           | **Heading - Clinics Should Routinely Track These Indicators**  
Tracking these indicators will help ensure that all specimens collected are accounted for and results will be returned for patient management. |
| 5            | **Specimen Rejection Criteria** |
### Slide Number | Content Notes for PowerPoint Slides
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50 | **Heading - Activity 3C: Sample Rejection Criteria**
Assuring that specimens are acceptable is critical to the quality of viral load testing. In this activity participants will be divided into 3 groups, each assigned to develop a list of sample rejection criteria for whole blood, plasma, or DBS. After 20 minutes, debrief by conducting a large group discussion of each group’s list. Provide your feedback and pull out all items pertaining to all three specimen types and write them on a separate flipchart paper under “General.”

51 | **Heading – Sample Rejection Criteria**
Review handout 3-12 to conclude activity 3C.

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### Laboratory Practical Session

52 | **Heading - Making and Packaging DBS from EDTA Tubes - A Practical Session**

<table>
<thead>
<tr>
<th>Supplies needed for making DBS</th>
<th>Supplies needed for packaging</th>
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<tbody>
<tr>
<td>▪ Remnant whole blood samples</td>
<td>▪ Zip top air tight bags</td>
</tr>
<tr>
<td>▪ 200 uL pipettes and tips, or transfer pipettes*</td>
<td>▪ Silica packets-1 per dbs</td>
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<tr>
<td>▪ DBS cards (two for each person)</td>
<td>▪ Humidity cards</td>
</tr>
<tr>
<td>▪ Pens for labeling</td>
<td>▪ Glassine paper</td>
</tr>
<tr>
<td>▪ Serological pipettes or something to utilize to keep DBS off the benchtop when spotting</td>
<td>▪ PPE</td>
</tr>
<tr>
<td>▪ Drying racks</td>
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<tr>
<td>▪ All associated PPE</td>
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<tr>
<td>▪ Preferably hood to work in</td>
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</table>

*If there are no pipettes available, a transfer pipette or a capillary can be used. Just ensure that the spot is completely filled.

Emphasize that tips must be changed between each patient and only one tube should be opened at any given time.

1. Walk the participants through Job Aide 3-2 and then have each participant create their own blood spot with proper PPE and technique. Split into pairs or groups of 3 and have them critique each other.
2. Come back together as a group and discuss the issues/difficulties if any. This practical session should take approximately 30 minutes to complete.
3. After allowing DBS to dry overnight, break up into the same groups from the previous day and following Job Aide 3-8 to package the DBS cards. Allow time for participants to critique each other. Come back together as a group and discuss the issues/difficulties if any. This practical session should take approximately 30 minutes to complete.

53-54 | **Heading – Module 6: Key messages**
Invite participants to supply words to complete each key message.
Whole Blood/Plasma Collection for HIV Viral Load Testing

- Use non-powdered gloves
- Use EDTA vacutainer tube (purple top) only.
- Collect about 5 ml of blood from adult patients
- Separate plasma from whole blood within 6 hours of collection by centrifugation at 1500xg for 10 minutes at room temperature.
- Transfer plasma to a sterile polypropylene (Cryo) tube. Be careful not to disrupt the buffy coat
- Transfer a minimum sample volume of 1.2 mL or 1200 uL aliquots into each of two properly labeled polypropylene tubes.
- Be sure that the polypropylene tubes are properly screw-capped.

Do not use Heparin or ACD as an anticoagulant.
Spotting and Drying the DBS Card

1. Position DBS card to avoid contamination using a rack, serological pipette, or the packaging from the vacutainer.
2. Mix blood by inverting EDTA tube 10 times.
3. Open EDTA tube.
4. Utilize a pipette set to 70 ul. If not available, utilize disposable transfer pipette being sure to squeeze the end of the pipette before putting it into the tube.
5. Plate in tube and release end of pipette.
6. Ensure the internal circle is fully spotted with blood. Do not layer successive drops of blood or apply blood more than once in the same collection circle.
7. Repeat the same procedure for the remaining 4 circles. Apply blood to only one side of the filter paper.

- Allow the specimen to fully air dry horizontally (minimum 4 hours but preferably overnight) at ambient temperature on a drying rack.
- Keep away from direct sunlight, dust, and bugs.
- Do not dry with heat, stack, or allow DBS collection cards to touch anything during the drying process.
- Use separate and well labeled racks for HIV and viral load DBS cards.
Spotting and Drying the DBS Card from Finger Prick Using a Capillary Tube

1. Collect supplies
   - Gloves (powder free)
   - Blood Collection Card
   - Lancet
   - Capillary tube
   - Alcohol swabs
   - Gauze
   - Pen
   - Appropriate disposal Containers

2. Complete all paperwork and label card. Include:
   - Name
   - Date
   - Sample ID
   - Collector ID
   - Any other necessary information

3. Choose puncture site and prepare the site
   - Wear gloves and proper protective equipment
   - Warm the area
   - Clean the puncture site with an alcohol swab and let dry for 30 sec

4. Use lancet to puncture the edge of the chosen finger. Discard the first drop using gauze.

5. As blood appears, hold capillary flat and touch tip to blood sample. Capillary action draws blood to the fill ¾ of the tube. To avoid clotting, immediately load 2 drops of blood into one pre-printed circle on the filter paper. Repeat until at least 3 pre-printed circles are completely

6. Place DBS on drying rack without DBS touching anything then let dry for at least 4 hours or overnight. Avoid DBS contact with sunlight.

- Dispose of used lancet and gauze using appropriate biohazard disposal container.
DBS Samples for Early Infant Diagnosis and Viral Load

Acceptable DBS

Each sample tested must meet the following criteria:

- **Identifying information**
  - on the DBS card must be clear and match accompanying paperwork.
  - Name: [Example Name]
  - Date: [Example Date]

- At least 3 good spots must be obtained. Samples should fill the circles and be as centered as possible especially in the case of perforated cards.

- After drying, DBS should be dark and a uniform color, indicating proper collection and drying technique.

Unacceptable DBS - The Lab Will Reject These Samples

- **Can't Read Identification**
  - PROBLEMS: The ID information cannot be read, so this sample should not be tested.
  - SOLUTIONS:
    - Label the DBS card neatly before taking a sample.
    - If the name can't be read, another sample is needed.

- **Not Enough Blood**
  - PROBLEMS: These spots do not have enough blood to be tested. Remember, at least 3 GOOD SPOTS are needed for testing.
  - SOLUTIONS:
    - If finger or heel prick, making sure the area is warm and properly positioned will help blood flow well for sample collection.
    - Don't squeeze right on the puncture site, squeeze the whole foot or hand.
    - If blood has stopped flowing from where you pricked, a second prick at another site may be necessary.
    - A sick or dehydrated person may need venipuncture.
    - Ensure pipette is set to 70 µL.

- **Layered or Clotted Blood**
  - PROBLEMS:
    - Note the spots are darker in the center. This happens if you put wet blood on top of dry blood.
    - This may also have been made by using clotting blood.
  - SOLUTIONS:
    - Touch the card to a blood drop when it looks heavy and ready to fall. This is the right amount of blood.
    - If a drop is too small, and is still wet, another drop can be placed on top. Otherwise, move to another spot.
    - Never use clotting or clotted blood to make a DBS.

- **Serum Rings/Alcohol Contamination**
  - PROBLEMS:
    - The yellow ring around the blood spots means the blood is contaminated or separated
    - The alcohol may not have dried
    - This can happen from squeezing the puncture site - plasma may leak out instead of blood.
    - Also could be due to lack of mixing of EDTA tube prior to spotting.
  - SOLUTIONS:
    - After cleaning the area, allow the alcohol or spirits to dry for 30 seconds before blood draw or prick.
    - Use gentle squeezing of the whole hand or foot to encourage blood flow. Never directly “milk” or squeeze the wound site.
    - If making DBS from EDTA tube, be sure to invert tube to mix blood several times prior to pipetting.

- **Poor Collection Technique**
  - PROBLEMS:
    - Blood may have been clotting when dropped on the cart.
    - More than one drop was applied to each circle.
    - None of the circles are filled well.
  - SOLUTIONS:
    - Wait for a large drop to collect before touching blood to the paper.
    - A drop of blood that falls on its own is the perfect size.
    - Never touch the skin, a needle, syringe, or pipette tip to the paper when applying blood.
Guidelines for Specimen Handling Conditions

- After specimens are appropriately labeled, place them at the correct temperature until packing and transport.
- Use a designated refrigerator or freezer with temperature tracking.
- Do not remove specimens from the designated storage refrigerator or freezer until packing occurs.

- Keep at 15-30°C for up to 6 hours or at 2-8°C for up to 24 hours prior to centrifugation for plasma
- Transport at 2-8°C on frozen ice packs

- Store at 2-8°C for up to 5 days
- After 5 days, store plasma at -20°C for up to 60 days. For >60 days, store at -70°C
- If transported after freezing, specimens must be kept frozen.
- Do not freeze-thaw-freeze more than 3 times.

- Keep at ambient temperature up to 2 weeks for storage and transport
- After 2 weeks, freeze at -70°C for long term storage

Whole blood (EDTA tubes)  
Plasma  
Dried blood spots (DBS)

Based on Appendix F from Guidance for Developing a Specimen Transport and Referral System for Viral Load and Infant Virologic HIV Diagnosis Testing Networks
Triple Packaging System

Primary Receptacles
- Contains the specimen
- Must be watertight and leak proof
- Must be appropriately labeled as to content.
- Wrapped in enough absorbent material to absorb all fluid in case of breakage or leakage.

Secondary Packaging
- Encloses and protects the primary receptacle
- Must be watertight and leak proof
- Several wrapped primary receptacles may be placed in a single secondary packaging.
- Contains ice packs if needed

Outer Packaging
- Protects secondary packaging from physical damage while in transit
- Contains specimen data forms, letters, and other types of information that identify or describe the specimen and identify the shipper and receiver, and any other documentation required.
- Must be a sturdy container with a latch or able to be taped shut

Specimen Packaging Diagram

DO NOT put any patient information on outer container or secondary containers or lids.

Biohazard Label should be on Secondary Container. 
DO NOT put Biohazard Label on Outer Container.
Specimen Packaging and Transport

- Utilize PPE when packaging specimens.
- Ensure specimens are in the appropriate transport media (primary containers) for the specimen collected and the test requested (primary containers). Ensure that primary containers will not leak.
- Determine the requirements temperature (ambient temperature vs refrigerated) and the referral timeframe (i.e., 6 hours) for the specimen collect and the test requested.
- Consult the driver/courier schedule to ensure that the sample will reach the testing center within the necessary referral timeframe.
- Place cold packs on the bottom (ideally) or sides of a secure leak-proof secondary container to properly preserve the specimens during shipping (specimens shipped at ambient temperature may not require cool packs, although it is often still advisable in warm climates.)
- Place the primary container(s) in the secondary container with sufficient absorbent material—paper towels, cotton balls, commercial products—to absorb the entire contents of the primary containers.
  - Ambient temperature specimens can be transported in the same secondary packaging as refrigerated specimens, but should be packed as far away from the cool pack as possible and be insulated by at least one layer of absorbent material.
- Ensure secondary container(s) is labeled properly with a biohazard sticker or stamp.
- Place secondary container(s) in an outer shipping container that can be secured with a screw top, latch mechanism or sealed with tape.
- Place test requisition forms in a plastic sheath (if possible) or zip top bag inside the outer shipping container with specimen tracking form.
- Confirm that the contact information for the laboratory is clearly marked on the outer shipping packaging and/or in paperwork inside the outer packaging.
- Note the date and time of pick-up on the specimen tracking form/transport log and/or the driver/courier logbook.
- Ensure that the drivers/couriers have received safety training in the transportation of specimens.
- Disinfect the bench where the specimens were packaged.
Cross Section of Refrigerated Specimen Packaging

- Ambient or room temperature specimens
- Refrigerated specimens
- Paper towels / absorbent material
- Cool Pack
- Secondary Container
- Outer Container
Job Aid Specimen Packaging-Whole Blood and Plasma

**Step 1:** Place cooler in box for transport

**Step 2:** Add **frozen** ice packs to cooler. Temp for whole blood should be 2-8°C.

**Step 3:** Cover frozen ice packs with absorbent material (paper towels, kimwipes, etc.)

**Step 4:** Add specimen racks, place in zip-top bag, close and add to cooler.

**Step 5:** Add racks and more frozen ice packs to minimize movement.

**Step 6:** Close cooler with lid and keep closed unless more specimens are added.
Packaging DBS Samples for Transport

1. Dry completely before packaging. Package within 24 hours of creation.
2. Wrap each DBS card with a glassine paper so that DBS cards will not have direct contact with each other.
3. Insert up to 10 wrapped cards into a special sealable plastic bag.
4. Add 10 desiccant packets to each bag. Ensure desiccants are at the bottom of the bag.
5. Add at least one humidity card per bag. Gently press the bag to remove most of the air before sealing.
6. Organize DBS according to specimen IDs.
7. Place the bag(s) of DBS, Requisition form, and chain of custody form into a large rip-resistant envelope.
8. Label the envelope with name of collection site (clinic), and date samples were sent.
9. Avoid leaving under sun and heat. Ship within 2 weeks. After 2 weeks, DBS should be frozen at -70°C.
Guidelines for Sample Transport

Ensure all collected specimens and accompanying paper work are picked up timely in a cooler box.

Before pickup/delivery, the driver:
- Makes sure each cooler box has:
  ✓ frozen ice packs
  ✓ a thermometer/data logger
  ✓ a specimen transport log
- Inspects the spill kit in vehicle weekly and replace any missing items.

After receiving the cooler box, the driver:
- Completes and sign the transport log.
- Only makes approved stops along the route.
- Upon arrival, delivers the cooler box and paper work to the designated receiver; ensures that person signs and fills in the time and date of receipt.
- Picks up any cleaned (decontaminated) cooler boxes and racks for return to the clinics/health centers.
- In case of a spill of biohazardous waste in the transport vehicle, reports the incidence and disposes the waste in bins designated for biohazardous waste.

Based on Appendix H from Guidance for Developing a Specimen Transport and Referral System for Viral Load and Infant Virologic HIV Diagnosis Testing Networks
# SPECIMEN TRANSPORT LOG

## COMMUNITY CLINIC SPECIMEN DELIVERY TRANSPORT LOG

**Date**: __/__/___

<table>
<thead>
<tr>
<th>Participant ID: Name and ID # only</th>
<th>Specimen Type</th>
<th># of Specimen Collected</th>
<th>Test Requested</th>
<th>Specimen Collection Verification</th>
<th>Receiving Lab: Specimen Rejected</th>
<th>Date Results Received at Clinic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Name: ____________</td>
<td>□ Whole Blood</td>
<td>____________</td>
<td>□ HIV RNA (VL)</td>
<td>Specimen(s) collected by: ____________</td>
<td>Yes □ Reason for rejection:</td>
<td><em><strong>/</strong></em>/___</td>
</tr>
<tr>
<td>ID #: ____________</td>
<td>□ DBS</td>
<td>____________</td>
<td>□ EID</td>
<td>Date: ____________</td>
<td>□ Clotted</td>
<td></td>
</tr>
<tr>
<td>Name: ____________</td>
<td>□ Whole Blood</td>
<td>____________</td>
<td>□ HIV RNA (VL)</td>
<td>Specimen(s) collected by: ____________</td>
<td>Yes □ Reason for rejection:</td>
<td><em><strong>/</strong></em>/___</td>
</tr>
<tr>
<td>ID #: ____________</td>
<td>□ DBS</td>
<td>____________</td>
<td>□ EID</td>
<td>Date: ____________</td>
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<tr>
<td>Name: ____________</td>
<td>□ Whole Blood</td>
<td>____________</td>
<td>□ HIV RNA (VL)</td>
<td>Specimen(s) collected by: ____________</td>
<td>Yes □ Reason for rejection:</td>
<td><em><strong>/</strong></em>/___</td>
</tr>
<tr>
<td>ID #: ____________</td>
<td>□ DBS</td>
<td>____________</td>
<td>□ EID</td>
<td>Date: ____________</td>
<td>□ Insufficient volume</td>
<td></td>
</tr>
</tbody>
</table>

**To be completed by designated clinic packing staff**: Nurse □ Lab Assist □ Phlebotomist Date: ___/___/___
- Requisition form complete? Yes □ No □ If No, please explain: ____________
- Total number of tubes confirmed? Yes □ No □ If No, please explain: ____________
- Specimen packed according to standard operating procedure? Yes □ No □ If No, please explain: ____________
- Ice packs frozen? Yes □ No □ If No, please explain: ____________
- Data logger properly placed in the cooler box? Yes □ No □ If No, please explain: ____________
- Clinic packaging Staff Signature: ____________

**Verification to be completed by Clinical Manager**: Date: ___/___/___
- Requisition form complete? Yes □ No □ Verified by Manager initials: ____________
- Total number of tubes confirmed? Yes □ No □ Verified by Manager initials: ____________
- Specimen packed according to standard operating procedure? Yes □ No □ Verified by Manager initials: ____________
- Ice pack frozen? Yes □ No □ Verified by Manager initials: ____________
- Data logger properly placed in the cooler box? Yes □ No □ Verified by Manager initials: ____________
- Clinic packaging Staff Signature: ____________

**To be completed by designated driver for transport**: Date: ___/___/___
- Pickup Date: ___/___/___
- Pickup Time: ___/___/___
- Confirm lab request form is available? Yes □ No □ If No, please explain: ____________
- Confirm packaging condition? Yes □ No □ If No, please explain: ____________
- Driver Signature: ____________

**To be completed by the receiving Lab** (LAB NEEDS TO RETAIN A COPY):
- Receiving Lab: ____________
- Lab Receipt Date: ___/___/___
- Lab Receipt Time: ___/___/___
- Received lab request form with shipment? Yes □ No □ If No, please explain: ____________
- Received transport log with shipment? Yes □ No □ If No, please explain: ____________
- Driver Signature: ____________

**To be completed by the receiving Lab** (LAB NEEDS TO RETAIN A COPY):
- Receiving Lab: ____________
- Lab Receipt Date: ___/___/___
- Lab Receipt Time: ___/___/___
- Received lab request form with shipment? Yes □ No □ If No, please explain: ____________
- Received transport log with shipment? Yes □ No □ If No, please explain: ____________
- Driver Signature: ____________

**To be completed by the receiving Lab** (LAB NEEDS TO RETAIN A COPY):
- Receiving Lab: ____________
- Lab Receipt Date: ___/___/___
- Lab Receipt Time: ___/___/___
- Received lab request form with shipment? Yes □ No □ If No, please explain: ____________
- Received transport log with shipment? Yes □ No □ If No, please explain: ____________
- Driver Signature: ____________
# Health Facility Laboratory Viral load Log Sheet

<table>
<thead>
<tr>
<th>S.No</th>
<th>Unique Identification</th>
<th>Name</th>
<th>Age</th>
<th>Sex</th>
<th>VL Sample Type collected (Whole blood, Plasma, DBS)</th>
<th>Date &amp; time of Collection</th>
<th>Date &amp; time of sample Referral</th>
<th>Dispatched by</th>
<th>Report received by, Date &amp; time Received by (Name)</th>
<th>VL result (copies/ml &amp; log, or ND)</th>
<th>Date VL result provided to VL focal person</th>
<th>Reason for VL test</th>
<th>Remark</th>
</tr>
</thead>
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</tbody>
</table>
## Sample Rejection Criteria

### General
- Poorly labelled specimen
- Mismatched sample and form labeling
- Missing labels on container or tracking form
- Samples without request forms/tracking forms
- Request form without samples
- Name and signature of requester missing
- Mismatched information on request form and specimen
- Inappropriate specimen packing
- Inappropriate specimen for the test requested

### Whole Blood
- Wrong container/anticoagulant used
- EDTA tubes that arrive with less than 3.5 mLs of whole blood
- EDTA tube specimens that arrive hemolyzed.
- EDTA tube that arrives more than 24 hours after specimen collection.

### DBS
- DBS cards with insufficient blood spots (need to have at least 2 good spot).
- DBS cards with clotting present in spots.
- DBS cards that have serum rings indicating contamination around the spots.

### Plasma
- Plasma that arrives at a temperature above 8°C.
- Plasma tubes that contain less than 1.5 mL.