

Module 4

Pre-test Information and DBS Collection for Infant Virological Testing



Module 4 Table of Contents

Module 4, Part 1: Overview for the Trainer	2
Session 4.1: Infant HIV Testing: Pre-test Information.....	4
• Exercise 1.....	6
Session 4.2: Dried Blood Spot Collection, Drying, Packing and Shipment.....	8
• Exercise 2.....	14
• Exercise 3.....	16
Module 4, Part 2: Course Content	20
Session 4.1 Course Content: Infant HIV Testing: Pre-test Information	20
Session 4.2 Course Content: Dried Blood Spot Collection, Drying, Packing and Shipment.....	23
Appendix 4A: Listening and Learning Skills Checklist	32
Appendix 4B: Infant PCR Laboratory Requisition Form	33
Appendix 4C: Specimen Delivery Checklist	34
Appendix 4D: Relevant Pages of Child Health Card	35
Appendix 4E: Collection of DBS from Infants for PCR Testing	36
Appendix 4F: Drying and Packaging DBS Samples for Transport	37
References	40

Module 4, Part 1: Overview for the Trainer



Total Module Time: 250 minutes (4 hours, 10 minutes)

Session 4.1: Infant HIV Testing: Pre-test Information

Activity/Method	Time
Interactive trainer presentation and large group discussion	20 minutes
Exercise 1: Infant HIV testing pre-test information: Role play followed by small group practice	40 minutes
Questions and answers	5 minutes
Total Session Time	65 minutes

Session 4.2: Dried Blood Spot Collection, Drying, Packing and Shipment

Activity/Method	Time
Interactive trainer presentation and large group discussion	100 minutes
Exercise 2: DBS specimen collection practice: Pair work	50 minutes
Exercise 3: Packing samples and receiving results: Demonstration in large group	20 minutes
Questions and answers	5 minutes
Review of key points	10 minutes
Total Session Time	185 minutes

Materials Needed



- Slide set for Module 4
- Flip chart and markers
- Tape or Bostik (adhesive putty)
- Ensure participants have:
 - Copies of the Participant Manual

For Exercises 2 and 3:

- Baby doll or model to show positioning of infant during heel or toe prick
- DBS cards, 1 for each participant
- 2mm lancets, 1 for each participant
- Gloves, preferably powder-free, 1 pair for each participant
- Pens
- Disinfectant for skin, such as 70% spirits
- Gauze or cotton wool
- Glassine paper, 1 for each participant
- Sealable plastic bags, 1 for each pair of participants
- Envelopes for mailing
- Markers for labelling envelopes

	<ul style="list-style-type: none"> • Desiccant packets, about 10 for each pair of participants • Humidity cards, 1 for each pair of participants • Drying racks • Sharps containers and a hazardous waste bag to dispose of used DBS cards • Hand hygiene with soap and water or water-free hand cleaner • Mother health or ANC card • Child health card (1 for each participant, if not in the appendices) • Laboratory requisition forms (1 for each participant, if not in appendices) • Specimen delivery checklist (1 for each pair of participants, if not in appendices) • Clinic database (access via laptop computer, where possible) or copy of the register/log used for tracking DBS specimens and results, e.g., DBS Specimen Tracking Register/Log <u>or</u> Baby Testing and Follow-up Register/Log
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Special Instructions

	<ul style="list-style-type: none"> • Ensure copies of the nationally approved laboratory requisition forms, specimen delivery checklist and child health cards appear in the appendices; if not, have copies available for participants to complete during the exercises. • Ensure you also have available copies of the registers/logs or databases and other tools in which infant HIV testing services are tracked. • Review the 2nd and 3rd exercises, which require the gathering of a lot of supplies and take a number of steps to complete. You may want to bring in a trainer skilled in DBS collection to assist you with these exercises.
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Session 4.1: Infant HIV Testing: Pre-test Information



Total Session Time: 65 minutes (1 hour, 5 minutes)

Session Objective

After completing this session, participants will be able to:

- Conduct the HIV pre-test information session for HIV-exposed infant testing



Trainer Instructions

Slides 1–4

Step 1:

Session Objective

Begin by reviewing the Session 4.1 learning objective. Ask participants if they have any questions.

Step 2:

Routine HIV Testing

Provide an overview of routine HIV testing of infants whose mothers are living with HIV using the slides and Session 4.1 Course Content.



Make These Points

- Routine HIV testing requires caregiver agreement and a private opportunity to refuse testing.
- WHO recommends the routine testing of all infants whose mothers are living with HIV. Testing may be conducted by the healthcare provider or a trained lay provider.



Trainer Instructions

Slides 5–14

Step 3:

HIV Testing of HIV-exposed Infants: The Pre-test Session

Provide an overview of the steps in the pre-test session for the HIV-exposed infant attending the 4–6-week (or after) postnatal visit, using the slides and Session 4.1 Course Content.



Make These Points

- The core pre-test session for the HIV-exposed infant undergoing routine, diagnostic HIV testing at the 4–6 week visit includes the following key points: [1]
 - **Assess:** assess caregiver’s knowledge of HIV
 - **Routine testing:** explain that we routinely test all HIV-exposed babies for HIV
 - **Explain the procedure:** discuss the steps of the testing procedure
 - **Confidentiality:** explain that the discussion is private
 - **Result:** explain what a positive or negative result means
 - **Return:** discuss when to return for results and routine care
 - **Questions:** ask if the caregiver has any questions

Participants will need to memorize these points as they form the core of any infant/child pre-test session, regardless of scenario.

Step 4:

Ask participants the following:

- *What do you say during the pre-test session to the caregiver of an HIV-exposed infant who is being tested for HIV at 4–6 weeks of age?*

List these key points on flipchart paper, try to organize them based on the 7 key points. See Table 4.1 in Session 4.1 Course Content for additional detail.

Step 5:

Point out where, on the mother’s or child’s health card, the pre-test session and HIV testing or refusal of HIV testing should be documented.



Trainer Instructions

Slide 15

Step 6:

Lead participants through Exercise 1, which provides an opportunity to apply their knowledge of the infant pre-test information session to a role play.

Exercise 1

Exercise 1: Infant HIV testing pre-test information: Role play	
Purpose	To review the content of the pre-test session for infants who are 4–6 weeks of age.
Duration	40 minutes
Advance Preparation	<ul style="list-style-type: none"> • Review the infant HIV pre-test session as it normally plays out in the 4–6 week visit. • Brief your co-trainer on the scenario for the large group role play. If you don't have a co-trainer, identify a participant or co-worker who has experience conducting pre-test sessions who can assist you. • Review both Table 4.1 and Appendix 4A, refer participants to both of these tools for use during this exercise.
Introduction	My co-trainer and I will role play (in front of the group) a healthcare provider and a caregiver whose infant is about to be tested for HIV. Once we have completed the role play, participants will break into groups of 2 to practise role playing the HIV pre-test session with client scenarios. You should refer to Table 4.1 for key points for pre-testing counselling and Appendix 4A for guidance on the Listening and Learning skills.
Activities	<p>Large group role play (10 minutes)</p> <ol style="list-style-type: none"> 1. The trainer and co-trainer should model the following scenario to participants (convened in the large group). The role play should take about 5 minutes (at most) and the debrief another 5 minutes. <p>Scenario:</p> <p>Aisha, who has been HIV-infected and on ART for over 2 years, has brought her 6-week old daughter, Kiki, in for her first routine care visit. Aisha is aware that Kiki will be tested for HIV today. Kiki has already been weighed, immunized and examined by the nurse and Aisha has been provided with co-trimoxazole. Aisha has been provided with support for her own health as well as support for infant feeding. HIV testing is the final step for today's visit.</p> <p>Aisha asks about Kiki's risk of being infected. She is very worried about her baby testing HIV-positive and blames herself for making her baby sick.</p> <p>The person role playing the healthcare provider should start the discussion using the key points in Table 4.1. Participants should turn to Appendix 4A and tick off the counselling skills used by the healthcare provider.</p> <ol style="list-style-type: none"> 2. Upon completion of the role play, ask participants: <ol style="list-style-type: none"> A. How do you think the role play went? B. What went well?

	<p>C. What would you have wanted to change? D. Did the healthcare provider cover all of the steps in Table 4.1? E. Did the healthcare provider have an opportunity to demonstrate any listening and learning skills (see Appendix 4A)? F. Did the role play seem realistic? G. If not, why not? H. Any other comments?</p> <p>Small group role play (20 minutes)</p> <p>3. Invite participants to break into pairs to practice providing pre-test counselling for an infant’s 4–6 week HIV test. For the first role play, one participant should role play the healthcare provider and the other the mother. For the second role play the pair should swap roles. The participant role playing the mother should make up their own patient scenario based on a patient they know, friend, or family member. The mothers should feel free to ask questions of the health care provider.</p> <p>4. Give participants 15–20 minutes to role play and debrief using the same 8 questions that appear above (lettered A–H).</p> <p>Report back and large group discussion (10 minutes)</p> <p>5. Bring the large group back together and ask the group:</p> <ul style="list-style-type: none"> • What did you think of the role play? • Was it easy? Or difficult? • If so, why? • What did you learn from the role play that you will use in your work? • Who can list the 7 key points of the pre-test session?
Debriefing	Review that the routine pre-test session follows a simple script that covers 7 points. Pre-test counselling allows the caregiver to understand the key aspects of infant testing and to make an informed decision of whether to consent for testing.



Trainer Instructions

Step 7:

Allow 5 minutes for questions and answers on this session.

Session 4.2: Dried Blood Spot Collection, Drying, Packing and Shipment



Total Session Time: 185 minutes (3 hours, 5 minutes)

Session Objectives

After completing this session, participants will be able to:

- Understand the steps in collecting a blood sample using dried blood spot (DBS) procedure
- Dry, pack and store DBS specimens to send to the laboratory
- Distinguish between valid and invalid DBS samples
- Record DBS results in the designated database or register/log and follow up on missing or delayed results



Trainer Instructions

Slides 16–18

Step 1:

Session Objectives

Read the session objectives as listed above. Ask participants if they have any questions before moving on.

Step 2:

DBS, Introduction

Provide an overview of DBS testing and its advantages.



Make These Points

- The collection of blood for DBS sampling is less traumatic than venipuncture, uses only a small volume of blood and is reliable for safe specimen collection and delivery.



Trainer Instructions

Slides 19–21

Step 3:

The Healthcare Provider's Responsibilities

Provide an overview of the healthcare provider's general responsibilities in DBS collection. This section is a summary of the skills that will be taught in this session.

Step 4: Getting Started: Documentation

Share copies of the laboratory requisition form (Appendix 4B), specimen delivery checklist (Appendix 4C) and the designated clinic register/log (whether it is electronic or paper-based) with participants. Both forms and the register/log will be needed later in this session.



Make These Points

- The accurate completion of the laboratory requisition form, specimen delivery checklist, and clinic registers is the responsibility of the clinician and important for timely and accurate test results.



Trainer Instructions

Slides 22–25

Step 5:

Necessary Supplies

Tell participants that they will now learn how to collect DBS specimens, starting with a discussion of the supplies needed for collection.

As you list the required supplies for DBS specimen collection, describe what each item is used for, holding up each one for participants to see as you describe it.

Pass items around to participants so they can examine them. Let them know that they will be practising with these supplies later in the training. Collect the items before moving on to the next section so that you can use them in the DBS specimen collection discussion.

Step 6:

Universal Precautions for Handling Body Fluids and Sharps

Participants should be familiar with universal precautions, this section is simply a reminder. Introduce this topic by asking:

- *What are some examples of safety practices that should be followed when collecting blood specimens?*



Make These Points

- It is essential that all healthcare providers follow universal precautions when drawing or taking blood from any patient for any reason.



Step 7:

Trainer Instructions

Slides 26–39

Procedure for DBS Blood Sample Collection

Summarize the entire procedure by explaining that with DBS testing, whole blood is collected onto filter paper and dried (rather than sending tubes of blood to the laboratory).

There are a total of 9 steps in the procedure for DBS sample collection – Refer to slide set and Appendix 4E. Go through the steps one-by-one, showing the slides with photographs as you go along.

Be sure to show the DBS supplies, including DBS card, used at each step as visual aids. If possible, use a doll or a model to demonstrate the positioning of the infant.

Note: Appendix 4E is also available at:

[https://www.childrenandaids.org/HEI Toolkit](https://www.childrenandaids.org/HEI_Toolkit)

Give participants an opportunity to ask questions.



Make These Points

- The 9 steps in the procedure for DBS sample collection are should be followed for every patient (a standard operating procedure). You can post the DBS collection poster (Appendix 4E) in your clinic to help all providers remember the steps in DBS collection.
- Participants are responsible for learning these steps and following them exactly. In the beginning, they may need to refer to their Participant Manuals when collecting DBS specimens to ensure that they don't miss any steps.
- Even a healthcare provider who is expert at these steps should take the time to review them every now and again to ensure he/she is not forgetting anything. Missed steps lead to incomplete paperwork or improperly collected specimens, which means delayed results.



Trainer Instructions

Slides 40–46

Step 9:

Drying and Packaging DBS for Transport to the Laboratory

Explain the steps for drying, packing, labelling, and storing DBS specimens one-by-one. Use the slides with photos and Appendix 4F to list the supplies needed and illustrate your explanation. Again, use your supplies and forms to demonstrate the process while you present the information.

Appendix 4F is also available at:

https://www.childrenandaids.org/HEI_Toolkit

Step 10:

Show participants how to complete the specimen delivery checklist (if not in the appendices, be sure to bring copies).



Make These Points

- Like the procedure for DBS Blood Sample Collection, drying, packing and sending the samples to the laboratory is also a standard procedure that needs to be followed in a set order. Participants will become familiar with the procedure after repetition.



Trainer Instructions

Slides 47–69

Step 13:

Valid and Invalid DBS Specimens

In this section participants will see examples of valid and invalid specimens. Trainers should use the slide set to show examples of valid and invalid DBS specimens.

Step 14:

For the example of invalid specimens, ask participants:

- *Why do you think the specimen is not acceptable?*
- *What do you think could have happened to invalidate the specimen?*
- *What procedures should have been followed to prevent this problem?*

Summarize using *Table 4.2. Common DBS errors and how to prevent them.*



Make These Points

- It is important that specimens for DBS are correctly collected, dried and packaged. Invalid specimens can result in laboratory errors or delays.



Step 15:

Trainer Instructions

Slide 65

Responsibilities of the Laboratory

Briefly provide an overview of responsibilities of the local laboratory and where to send specimens.



Make These Points

- It is important that healthcare providers understand the responsibilities of the laboratory staff as well as their own responsibilities.



Step 16:

Trainer Instructions

Slides 66–75

Ways to Return Results from the Lab to the Health Facility

Provide a summary of electronic methods used to return results using the slide set and Session 4.2 Course Content. Start the discussion by asking:

- *How are HIV test results returned to your clinic?*

Step 17:

Steps When Receiving Results

Remind participants that early infant HIV diagnosis using DBS specimens does NOT end when the specimen is sent to the lab. Encourage discussion about these next steps by asking:

- *What do you do in your clinic when DBS test results are returned?*
- *What do you do if you do not receive results?*

Refer to the slide set and Session 4.2 Course Content.

Step 18:

Tracking No Shows

Ask participants:

- *Do you think your clinic's procedure is effective, i.e., are you able to get results to caregivers quickly?*
- *What improvements need to be made to ensure caregivers get their infants' results efficiently?*



Make These Points

- There are a number of electronic methods that can be used to return test results to the clinic as soon as they are available at the laboratory.
- Once the results come back from the lab, they need to be recorded in the patient record and the clinic's designated register so that the result is available when the mother and infant return to the clinic. Once the post-test counselling session has been conducted, the child health card should be updated; in addition, the patient record and designated register should be updated once again.
- There should be a system in place for tracking caregivers of infants with positive NAT to ensure they come to clinic immediately for post-test counselling and ART initiation. This same system should be used to identify clients who have dropped out of care so that they can be brought back into care.



Step 19:

Trainer Instructions

Slides 76–78

Missing and Delayed Results

Discuss missing and delayed results. Engage participants by asking:

- *What is turn-around time (TAT)?*
- *What is reasonable TAT? When will you say result is "lost"? [Answer: it depends on the country and TAT, but generally TAT should be 28 days or less.]*
- *What do you do if results are deemed "lost"?*



Make These Points

- Turnaround time (TAT) is the time from sample collection to sharing of results with the client.

- It is important that every clinic that orders NAT tracks when specimens are sent and when results are received. Timely, life-saving treatment is dependent on timely results.
- The designated (electronic or paper-based) register/log is an important tool to track results and identify delayed results.



Trainer Instructions

Slides 79–80

Step 20:

Conduct Exercises 2 and 3. Exercise 2 is an opportunity to go through the process of collecting a DBS sample, whereas Exercise 3 reviews the process of shipping the DBS samples and receiving the results.

Be sure to remind participants that they will be practising universal precautions during this exercise, as they would in the clinical setting.

Exercise 2

Exercise 2: DBS specimen collection practice: Pair work

Purpose	To give participants opportunity to practise the DBS collection method and complete the required forms.
Duration	50 minutes
Advance Preparation	<p>Gather together the following materials:</p> <ul style="list-style-type: none"> • Baby doll or model to show positioning of infant during heel or toe prick • Filter paper blood collection cards (DBS cards), 1 for each participant • 2mm lancets or glucolet, 1 for each participant • Gloves, preferably powder-free, 1 pair for each participant • Pens • Disinfectant for skin, such as 70% spirits • Gauze or cotton wool • Weighing paper • Sharps containers and a hazardous waste bag to dispose of used DBS cards • Hand hygiene with soap and water or water-free hand cleaner • Laboratory requisition forms (1 per participant if not in appendices) • 1 Basket or box ready for the forms, centrally placed • 1 DBS card drying rack, placed centrally

	<ul style="list-style-type: none"> • Designated register used to track specimens and results (if the register is electronic, then project it onto a screen using a computer and LCD projector) • Mother health or ANC card • Child health card (1 per participant if not in appendices)
<p>Introduction</p>	<p><i>In this exercise we are each going to practise taking DBS specimens using a finger stick. This blood collection exercise is just for practice and the DBS specimens will neither be tested nor given to anyone.</i></p> <p><i>It is extremely important that each of you observes Universal Precautions during this exercise as you would in any clinic setting.</i></p> <p>At this point the trainer should distribute the supplies necessary for DBS collection to each group.</p>
<p>Activities</p>	<p>Small group work (30 minutes)</p> <p>Ask participants to break into pairs. Ask:</p> <ul style="list-style-type: none"> • <i>If you were in a clinical setting and you were about to collect a DBS specimen, what documentation would you need to complete first? [Answer: The laboratory requisition form and DBS card should be completed before you take the specimen.]</i> <p>Project the laboratory requisition form on the screen and take about 1 minute to explain how it is completed. Participants should complete this form for their “patient” (i.e., the individual with whom they were paired to practise DBS collection). Then ask:</p> <ul style="list-style-type: none"> • <i>What else would you have to do before you collect a DBS specimen? [Answer: Complete the name, DOB, and other fields on the DBS card.]</i> <p>Project a correctly completed DBS card on the screen. Walk around the room to make sure everyone has completed both the laboratory requisition form and DBS card correctly.</p> <p>Get participants started on their DBS collections. Inform participants that if anyone does not want their finger pricked, they should not feel required to do so; they can also use a doll as a model.</p> <p>Have “Appendix 4E: Collection of DBS from Infants for PCR Testing” available as a reference.</p> <ul style="list-style-type: none"> • One participant in each pair should perform all steps of DBS collection on the other person by finger stick (or use a doll/model). • Ensure used lancets are properly disposed of immediately after use. • Once the specimen has been collected, the healthcare provider should place the filter DBS card in the drying rack and the laboratory requisition form in the basket near the drying rack. • Once this process is completed, participants should switch roles and repeat the process using the second, clean, set of equipment.

	<ul style="list-style-type: none"> • Participants will have approximately 20 minutes to practise (10 minutes/person). • Walk around the room and provide support as needed. • Once all participants have had a chance to practise DBS collection, have them put their DBS cards in the drying rack and dispose of used gloves, lancets, cotton wool, etc. Reconvene the group to discuss the activity. <p>Large group debrief (5 minutes) Ask participants:</p> <ul style="list-style-type: none"> • How was the process of pricking your client’s finger, collecting the blood specimen and filling the circles on the filter paper? • What did you learn? • What mistakes were made? (If not offered by participants, the trainer should share mistakes he/she observed, in a manner that is supportive to the learning process rather than judgmental.) • How is the specimen collection process different on an infant (than on a fellow trainee)? • Reassure participants that their specimen collection techniques will improve and become more efficient as they gain experience. <p>Large group discussion on next steps (15 minutes) Ask participants:</p> <ul style="list-style-type: none"> • What other documentation needs to be completed? [Answer: the designated clinic register. Have the volunteers complete the register—or enter information into the database—with information about their patient. They can make up a hypothetical patient. Where possible, complete the register using the computer and projected on the screen.] <p>Also discuss with participants any other documentation that needs to be completed as per country or clinic protocol, e.g., individual patient chart, child health card, etc. Where possible, have available copies of these forms and point out the page and field where this information is recorded.</p>
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Exercise 3

Exercise 3: Packing samples and receiving results: Demonstration in large group	
Purpose	To review the process of packing samples for shipment to the laboratory and what to do when results are returned.
Duration	20 minutes
Advance Preparation	For this exercise you will need: <ul style="list-style-type: none"> • A table at the front of the room, to represent the area where blood samples are dried and stored in a clinic setting.

	<ul style="list-style-type: none"> • Blood samples, you may use the samples from Exercise 2 (if they are dry). Or, have ready 3 “pretend” sample cards, perhaps colored with a red marker pen in each of the 5 spots to represent drops of blood. Always use universal precautions, even if using “pretend” sample cards, so that you model good practice. • Gloves • Glassine paper • Sealable plastic bags, 1 for each pair of participants • Envelopes for mailing • Markers for labelling envelopes • Desiccant packets, 10 for each pair of participants • Humidity cards, 1 for each participant • Hand hygiene with soap and water or water-free hand cleaner • Specimen delivery checklist (bring copies if not in the appendices) • Drying rack for drying specimens • Basket or box ready for the forms <p>Ask for 3 volunteers to play the role of the healthcare provider. Have them come to the front of the room.</p>
Introduction	These 3 volunteers will review the process of packing DBS cards for shipment to the laboratory and receiving results.
Activities	<p>Volunteer demonstration, packing samples for shipment (15 minutes) Once they have completed the designated register, ask:</p> <ul style="list-style-type: none"> • <i>You have seen your patient, collected blood samples, completed the laboratory requisition forms, paper or electronic register, and patient cards/charts. The samples are now dried. What do you do next? [Answer: Package each sample. Volunteers should go through the process of packaging each individual sample step-by-step, as explained “Appendix 4F: Drying and Packaging DBS Samples for Transport”.]</i> <p>Then ask:</p> <ul style="list-style-type: none"> • <i>Now you have each sample properly packaged. The samples are ready to go to the lab. What do you do next? [Answer: Complete the specimen delivery checklist. Where possible, have your volunteers demonstrate how this should be completed on the computer or by actually filling in the form for several DBS cards. As you record each specimen on the check list, you should place the specimen and its corresponding requisition form in a large envelope for transport to the lab. After all specimens have been recorded, the specimen check list should go in the envelope to be sent to the lab.]</i> <p>Volunteer demonstration, receiving results (5 minutes) Thank the volunteers, and ask for 3 more volunteers to play the role of healthcare providers. Have a sheet with the lab results available. Ask:</p>

	<ul style="list-style-type: none"> • <i>It has now been 4 weeks since you sent your DBS cards to the laboratory. Your patients' lab results are now available. Here are the results from the lab. (Hand these to the volunteers). What do you do next? [Answer: Record the results in the designated register as well as the individual patient chart. File results reports either in patient file or a secure place]</i> <p>Have a volunteer record at least one patient result in the designated register. Remind participants that when this patient returns, the healthcare provider will go to the clinic register or patient chart to obtain the patient test result. Hold the register up (or, if electronic, project it onto the screen) for everyone to see, pointing out which columns are completed and how they are completed.</p> <p>End the exercise by thanking everyone and asking if there are any questions.</p>
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Step 21:

Trainer Instructions

Slides 81–83

Module key points

Ask participants what they think the key points of the module are. What information will they take away from this module? Summarize the key points of the module, using participant feedback and the content below.



Module 4: Key Points

- HIV testing of HIV-exposed infants is routinely conducted at the 4–6 weeks, 9 months and 18 months of age (or 3 months after stopping breastfeeding, whichever is later). The pre-test counselling should cover 7 points:
 1. **Assess:** assess caregiver's knowledge of HIV
 2. **Routine testing:** explain that we routinely test all HIV-exposed babies for HIV
 3. **Explain the procedure:** discuss the steps of the testing procedure
 4. **Confidentiality:** explain that the discussion is private
 5. **Result:** explain what a positive or negative result means
 6. **Return:** discuss when to return for results and routine care
 7. **Questions:** ask if the caregiver has any questions
- Dried blood spot (DBS) refers to small drops of whole blood that are collected on strips of special filter paper that are then dried. If properly dried and stored, specimens remain stable for an extended

period of time and can be transported with minimal special handling to a central laboratory.

- When results are received, record them in the designated register/database as well as the patient chart.
- If a client does not come for the scheduled post-test counselling session, ensure s/he is tracked by phone, text or e-mail if available. If she does not respond to electronic messaging, or does not have a phone, reach her in person via outreach staff.
- If a positive NAT result is received, trace the caregiver immediately for post-test counselling and ART initiation.
- Actively follow up delayed results. If the patient shows for the post-test session and the results are not available, contact the laboratory. If the sample was lost or rejected by the laboratory, use the visit as an opportunity to take a new specimen for testing.

Step 22: Ask if there are any questions or clarifications.

Module 4, Part 2: Course Content

Session 4.1 Course Content: Infant HIV Testing: Pre-test Information

Routine HIV Testing

WHO recommends ***routine*** HIV testing for infants whose mothers are living with HIV. Like other diagnostic tests, routine HIV testing in paediatric settings requires the following:

- **Consent:** HIV testing must be voluntary; in paediatric testing, **consent** for infant testing is obtained by providing brief pre-test information to the parent or guardian and getting verbal agreement for testing. Written consent is not required.
- **Opportunity to refuse testing:** The caregiver should be informed of the process for HIV testing and the right to decline testing.

Testing of infants may be conducted by healthcare providers—nurses, midwives, doctors, counsellors, social workers, and laboratorians—or lay providers who are trained and supervised. In their 2015 and 2016 guidelines, WHO recognized the importance of task sharing with lay providers to support implementation of infant HIV testing. [2] [3] A lay provider refers to any person who performs functions related to healthcare delivery who has been trained to deliver specific services but has not received a formal certificate or degree. Lay providers play an important role in task sharing, i.e., the redistribution of tasks between cadres of healthcare providers with the aim of relieving upper level healthcare providers of certain day-to-day activities so that they can focus on other duties.

HIV Testing of HIV-exposed Infants: The Pre-test Session

Although PMTCT interventions are very effective, they do not eliminate the risk of HIV transmission to the infant, so routine HIV testing of HIV-exposed infants is necessary. The benefits of testing infants in the first 6 weeks of life include:

- Provide reassurance to families, particularly families whose infants test HIV-negative
 - Counselling after a negative result at 6 weeks should emphasize that there can be continued transmission during the breastfeeding period, so it is important to remain in care, keep taking ART every day, and re-test the infant according to national guidelines until after the end of breastfeeding
- Identify infected infants early so that they can initiate ART early, thereby increasing their chance of survival.

This session focuses on how to discuss the topic of routine, diagnostic early infant HIV testing with the caregiver of an HIV-exposed infant during the 4–6-week clinic visit. The pre-test information may be provided in a group setting and then continued with a one-to-one discussion between the caregiver and health care provider. In any counselling session, the healthcare provider is expected to use “listening and learning skills”. The listening and learning skills are summarized in Appendix 4A: Listening and Learning Skills Checklist.

The key points and model scripts for pre-test counselling are listed in Table 4.1. Note that scripts should be adapted to meet the client’s situation, as suggested by her understanding of her own HIV infection, level of anxiety, and her questions. The script will also be adapted based on test used, infant age, and whether the test is an initial HIV test or follow-up test.

Table 4.1: The pre-test session, HIV-exposed infant nucleic acid testing (NAT) [1]

Key point	<i>Script/Key points NAT (0–18 months of age)</i>
1. Assess the parent/caregiver’s and child’s knowledge of HIV and the diagnostic procedure.	Use a question and answer format to gauge level of understanding: <ul style="list-style-type: none"> • <i>What is HIV?</i> • <i>How is HIV passed from mother to baby?</i> • <i>How can mother-to-child HIV transmission be prevented?</i> Emphasize the importance of the breastfeeding mother taking and adhering to her ART regimen.
2. HIV testing is routine for all HIV-exposed infants/benefit of testing	<ul style="list-style-type: none"> • <i>Even if you and the baby received medicine for PMTCT, there is still a small chance that he/she is HIV-infected. Because of this small chance, we routinely test babies for HIV at this age. If your baby is HIV-infected, the sooner we know the sooner we can start him/her on ART; if we can start ART before s/he gets sick s/he’ll stay healthier longer. We will do a test today, and your baby should be re-tested at regular intervals [follow national guidelines] until a final test after the end of breastfeeding.</i>
3. How the test will be conducted	<ul style="list-style-type: none"> • <i>We will take a few drops of blood by pricking the [heel, toe or finger based on age]. The blood will be collected on a card that will be sent for testing. [Script should be adapted to process and test kit to be used.]</i> • <i>Although we strongly recommend testing, you have the right to decline.</i> • <i>If the test result is not available same day: We will schedule you for an appointment to return for your result.</i>
4. Confidentiality	<ul style="list-style-type: none"> • <i>The test result and anything we discuss today is confidential/private and will not be shared with anyone else unless you give permission.</i>
5. Explanation of result. 5a. What a positive result means	<ul style="list-style-type: none"> • <i>If the baby’s test result indicates that he/she is HIV-positive, he will be started on ART right away. ART will help him to stay healthy.</i>
5b. What a negative result means	<ul style="list-style-type: none"> • <i>If the baby tests HIV-negative, then this is the first test in a series of tests to confirm that your baby does not have HIV.</i>

	<p>Adapt based on infant age: <i>Your baby will also be tested at 9 months of age and 3 months after breastfeeding ends (or at 18 months if you stop breastfeeding early).</i></p>
6. Return	<ul style="list-style-type: none"> • <i>Point of care (POC) testing: Your result will be ready in an hour.</i> • <i>You will need to return to the clinic in ___ weeks for the baby's result. If the result comes back early, we may also contact you so that you can get the test result as soon as it is available. If you agree that we can contact you, we'd like to make sure that we have your correct phone number and address. [Confirm contact information]</i>
7. Client questions	<ul style="list-style-type: none"> • <i>What questions do you have about HIV testing?</i>

Session 4.2 Course Content: Dried Blood Spot Collection, Drying, Packing and Shipment

DBS, Introduction

HIV infection in children under 18 months of age can be diagnosed with virological testing using nucleic acid testing (NAT) technologies. NAT can be conducted on either of the following:

- **High throughput instruments:** NAT is most commonly conducted using high throughput instruments that must be operated by trained laboratorians and are based in regional laboratories. These instruments can use DBS specimens, which facilitate storage and shipping to the laboratory. OR
- **PoC/near PoC instruments.** The Alere™ q HIV-1/2 Detect uses drops of freshly collected blood samples, whereas the Cepheid AB Xpert® HIV-1 Qual Assay can use either drops of freshly collected blood or DBS.

DBS technology

DBS technology has been used widely in the United States and Europe since the early 1970s to screen newborns for genetic disorders. More recently, DBS technology has been used to test samples for HIV using NAT. DBS is also used for viral load testing.

In DBS collection, small drops of whole blood are collected on strips of special filter paper, and the paper is then dried. The procedure for taking a DBS specimen involves obtaining blood from a heel, toe, or finger with a lancet and applying it directly onto filter paper.

Advantages of DBS testing include the following:

- A lower volume of blood is required for testing, so specimen collection is easier and requires less training.
- DBS specimens have a longer lifespan than whole blood or plasma, are more stable and therefore easier to transport and store.
- DBS specimens can be shipped to distant laboratories, because they can be transported relatively easily (in comparison to fluid samples) without compromising the specimen.
- Because specimens are dried, they pose little biohazard risk. Therefore, they are safer to handle than whole blood specimens.[4]

The Healthcare Provider's Responsibilities

The healthcare provider's responsibilities are to ensure the proper collection of valid DBS samples for NAT and complete and accurate labelling and documentation. The healthcare provider must:

- Collect valid samples.
- Label the samples correctly.
- Dry, package and store samples appropriately until they are transported to the laboratory
- Correctly complete all related documentation.

The following procedure is for the collection of DBS specimens that are sent to a laboratory for testing. Point-of-care testing follows different procedures depending on the platform used; providers using point-of-care testing should receive additional training on these methods.

Getting Started: Documentation

Although every country will have different paperwork for documentation, most countries will have the following:

1. **The laboratory requisition form**, which is sent with the samples to the laboratory, includes information that the lab will need to conduct the test on the blood sample.

In general, this form requests information such as:

- child's name
- date of birth
- mother's name
- child/patient identification number
- name of project site
- name of provider requesting the test
- information about where to send the results
- date and time of specimen collection

This form needs to be completed for each specimen sent to the lab. Note: The child/patient identification number is very important, since it is used to identify the child. Often for confidentiality reasons, the lab will not provide results by name but by number. Making sure this number is correctly recorded on the form is critical. The laboratory requisition form appears in Appendix 4B.

2. **Specimen delivery/transport checklist** tracks quantity and quality of specimens along the chain of custody. The specimen delivery/transport checklist appears in Appendix 4C.
3. **Electronic (database) or paper-based register/log** used for recording and tracking NAT specimens and results, e.g., EID/NAT/DBS Specimen Tracking Register/Log or Baby Testing and Follow-up Register/Log. As different countries have different methods for recording and tracking specimens and results, hereafter it will be referred to as the Infant NAT Register.

Necessary Supplies

The supplies needed to collect a DBS specimen from an infant or child include the following:

- DBS cards
- 2mm lancets
- Gloves, preferably powder-free
- Pen
- Laboratory requisition forms
- Disinfectant for skin, such as 70% spirits
- Gauze or cotton wool
- Sharps container

The supplies required for DBS drying and packaging include:

- Gloves, preferably powder-free

- Drying racks
- Glassine paper
- Sealable plastic bags
- Desiccant packets
- Humidity cards
- Envelopes for mailing
- Marker/pen
- Specimen delivery checklist

Universal Precautions for Handling Body Fluids and Sharps

Safety practices that should be followed when collecting blood specimens include the following:

- Wear gloves when in contact with blood, body fluids, secretions, excretions, mucous membranes and contaminated items.
- You must also wear gloves when handling the DBS card (even before it has blood on it).
- Wash hands before putting on gloves and immediately after removing them. If using gloves with powder, wash the powder off the gloves after putting them on.
- All sharps should be handled with extreme care and disposed of in sharps containers to prevent injury to others.
- Clean up spills of infectious material/fluids promptly.
- Ensure that patient care equipment, supplies and linen contaminated with infectious material/fluids is either discarded or disinfected or sterilized adequately.
- In the event of a sharps injury, inform supervisor immediately and follow national protocol for post-exposure prophylaxis (PEP).

Procedure for DBS Blood Sample Collection

The following is the procedure for DBS sample collection:

1. **Gather all necessary supplies** listed above.
2. **Complete all necessary documentation** including the clinic register(s), laboratory requisition form, and label the DBS card with the baby's name, birth date, site code, date of collection, time of collection, and any other requested information. Write clearly and be sure to complete all fields.

NAME BABY'S NAME DOB: 11/3/2006

DATE 3/10/06 15:00

CE no var Lot-No. S85 608PW-01 Expiry Date: 2007-08

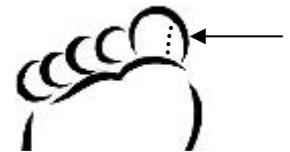
Do not touch the circles with anything other than the infant's blood. Your hands, glove powder, ink, or dirt will affect the result. It is important not to contaminate cards by contact with blood from other sources such as touching another DBS card.

3. **Decide where you will prick the infant** according to baby's size and age:

- **Small infants** up to about the age of 4 months and up to 5 kg: prick the heel. The best area is the lateral section of the heel. Do not prick the back of the heel where the bone is.

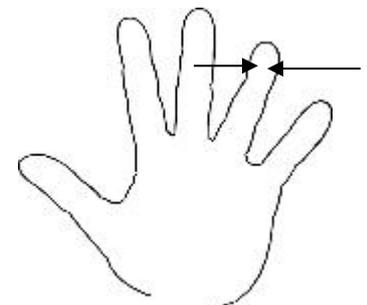


- **Larger infants** between 4 and 10 months old, or 5–10 kg: prick the big toe. Never stick the other toes in children; it is too easy to hit and damage the bone.



- The lateral side or outside part of the big toe works best. Do not prick the very end of the toe where the bone is close to the skin.

- **Older infants** over 10 months or more than 10 kg—prick the finger. The best finger is the ring finger on the left hand as this finger will be the least used by the baby. Select the lateral side of the fingertip. Do not stick the very end of the finger where the bone is close to the skin. The thumb is not recommended because it will be the most painful



4. **Wash hands and then put on powder-free gloves.** If powdered gloves are used, rinse gloved hands to get the powder off.

5. **Position the baby and clean the area to be pricked:**

Have the mother sit on the examination bed with baby on her lap. Show her how to properly hold the baby depending on the site to be pricked. The baby's foot or hand should be below the level of his heart so blood will flow more easily. Warm the area you will prick, especially if the infant is cold. The mother can hold the baby's foot or hand in her hand. Rubbing it gently may help. A cloth or nappy soaked in warm water can be used—keep on for about 3 minutes.

Clean the area you will prick with 70% spirit or alcohol swabs, and let it dry for 30 seconds. It is important to let the disinfectant dry so that the spirit does not mix with the blood when you prick the site.

6. Gently squeeze and release the area to be pricked until it is ready to be bled. Prick the infant's heel, toe or finger with a 2mm lancet. Do not use a needle or scalpel or longer lancet. The lancets are the correct length to puncture safely without damaging baby's bone.
7. Wipe away the first drop of blood with dry cotton wool, and allow a large drop to form on the puncture site.
8. Touch the DBS card gently against the large drop and allow it to completely fill the circle on the paper. The first drop should fill the circle. Do not press the paper against the heel, toe or finger; just allow the droplet to touch the paper.

Fill all circles if possible. Three complete circles are needed by the laboratory. If a circle is poorly done, move to the next one.



A DBS card with 5 completely filled spots. [Note: Name is changed to protect patient identity.]

Do not “milk” or squeeze the area that has been punctured as this will cause tissue fluid to mix with blood and contaminate the sample. If there is not enough blood, you can gently pump and release or apply gentle pressure to the whole lower leg, foot or hand (above the puncture site, depending on where the prick is made.) If this is not successful it may be necessary to prick the infant in another location to complete the collection.

Note: it is important not to touch the circles with anything other than the infant's blood. Fingerprints, glove powder, ink or dirt will all affect the result.

9. When sufficient circles have been filled, clean the puncture site and press cotton wool against it until it stops bleeding. Do not use a bandage. Ensure the wound is clean and bleeding has stopped for at least 5 minutes. Complete documentation and recheck the wound before the baby leaves your care.

These steps are summarized in “Appendix 4E: Collection of DBS from Infants for PCR Testing.” Appendix 4E is a one-page job aid on DBS collection that you can use in your clinic.

Drying and Packaging DBS for Transport to the Laboratory

See “Appendix 4F: Drying and Packaging DBS Samples for Transport” for instructions on drying and packaging DBS specimens in preparation for sending them to the laboratory.

Valid and Invalid DBS Specimens

When specimens are not collected correctly, the specimen may be rejected by the laboratory or the result may be inaccurate.

Try to fill all 5 spots on the card completely. If that is not possible, at least 3 completely filled spots are needed. Remember: 3 well-filled spots are better than 5 partially filled spots.

Some common errors and how to prevent them are shown in Table 4.2.

Table 4.2. Common DBS errors and how to prevent them

Error/Problem	Solution
Labelling and documentation errors	<ul style="list-style-type: none"> Complete DBS card, lab requisition forms and all other documentation completely, accurately and with legible handwriting
Yellow rings around the spots - could be due to disinfectant or tissue fluid contamination	<ul style="list-style-type: none"> Allow disinfectant to dry completely before pricking the patient Do not squeeze the skin directly around the spot where the patient was pricked
Scratches or smudges on the filter paper	<ul style="list-style-type: none"> Do not scratch or dab blood on the filter paper surface
Drops are too small	<ul style="list-style-type: none"> Warm the site Apply pressure (but not right at the site) Consider choosing another site For fingerpick: ensure hand is below the level of the heart
Caking or layering blood on the filter paper	<ul style="list-style-type: none"> Do not put a new drop of blood on top of a drop that is already dry
Drop is not centred on the card	<ul style="list-style-type: none"> Ensure site is centred directly over the centre of the spot Show caregiver how to hold the infant/child firmly so that he/she is not moving during the procedure
Specimens are not dried or packaged properly	<ul style="list-style-type: none"> Follow instructions in Appendix F for drying and packaging DBS specimens. Do not start packaging until DBS specimens are completely dry (4 hours or longer).

Responsibilities of the Laboratory

Note: This section should be adapted based on local/national standard operating procedures.

1. The laboratory should acknowledge receipt of all specimens by completing and returning the specimen delivery checklist.
2. Any specimen rejected at the laboratory should be identified on the specimen delivery checklist. The laboratory should immediately inform the sending health facility that the sample was rejected and reason for sample rejection, so that the facility can trace the infant and organize the drawing of a new sample as soon as possible.
3. All specimens should be entered in the designated register as per Standard Operating Procedures. The laboratory will keep the second copy of the checklist for reference purposes.
4. There should be a system so that the laboratory can notify a health facility of a positive infant virologic test result on the same day of testing, since it is important that the infant is started on ART right away.

Ways to Return Results from the Lab to the Health Facility

Even when results are returned as hard copies via courier or postal service, WHO recommends use of technology to expedite return of results to the clinic and, in turn, to the patient. Examples of methods for returning results electronically include the following.

- **Short message service (SMS) printers:** The SMS printer is a small battery-operated printer that can take a SIM card and has the capability of receiving messages without the need for a handset (see Figure 4.1). SMS printers can receive and print test results without computer or internet access. SMS printers will function where there is cellular network coverage.
- **SMS text messages:** Automated text messages may be used to notify health providers of test results and to notify caregivers that results are available at the health facility. Text messages to caregivers should encourage them to come to the clinic to get test results and should not disclose any confidential information.
- **Secure webpage or electronic medical record system.** Another option is for the laboratory to develop password protected clinic-specific webpages. Results would then be posted on the webpage by laboratory staff to be accessed by clinic staff who visit the web-based portal and input the correct password. This system is a viable option where clinics and laboratories have reliable access to the internet and where web-based results can be printed so that documentation of test result can be added to the patient medical record. Some electronic medical record systems can also be accessed by the lab to record test results and flag positive results to the health care provider.
- **Telephone:** Telephone calls may be used to notify caregivers of test results, especially for urgent follow-up of positive test results. Given the need for documentation, phone communication of results should be followed-up with a paper printout (sent by courier or post) from the laboratory for the patient medical record.

In many settings, test results will be returned using a combination of methods, e.g., urban centres with access to the internet might access results using a web-based portal while remote sites rely on SMS printers.

Point-of-care platforms for infant virological testing have the potential to allow for same-day return of results and avoid the challenges of specimen transport and delayed turn-around time (TAT).

Figure 4.1 Example of an SMS printer

Steps When Receiving Results

The steps to take when receiving infant HIV test results at the health facility are the same regardless of method of return (electronic or paper):

- Document receiving the results and date receipt/review of results (electronically or with date stamp if paper copy).
- Write the results in the designated register and/or clinical database; and the individual patient chart. If a paper copy of result is received, file it in the patient chart.
- If HIV-positive: re-enter baby's name— together with the necessary details for follow-up and linkages—in the designated register/patient chart to enable prompt follow-up of infant and ensure appropriate person is notified to urgently trace the infant's caregiver to come to the clinic for test result, counselling and prompt ART initiation.



It is important that the designated register is completed accurately. Information from the designated register is used for monitoring and reporting the number of specimens sent for testing, tracking return of results, calculating positivity rates, tracking time it takes for results to be returned, tracking infants in need of post-test counselling and ART. This information is vital for programme monitoring and evaluation.

- When the caregiver receives the infant's test result, enter the post-test counselling date in the patient chart and designated register.
- When counselling the caregiver (see Module 5), ensure she understands that the child will be retested:
 - If the 6-week NAT result is negative, then retest at 9 months **and** at either 18 months or 3 months after completion of breastfeeding (whichever is later) [Adapt as needed based on national guidelines].
 - If the NAT result is positive, the healthcare provider should collect a second DBS specimen to be retested for confirmation of the first result. However, the child should be initiated on ART immediately; do not wait for the confirmatory test result to be returned.

Tracking No Shows

Every clinic should have a mechanism—whether paper-based or electronic—for identifying patients that did not attend for testing, scheduled clinic visits or for their post-test result. The Infant DBS Register can be used to identify patients who have not returned to receive test results. Clinics with computer-based records and appointment systems have the option of developing a database to assist with identifying patients who have missed appointments or are lost to follow-up.

Health care providers should ask patients at the time of enrolment about permission for tracing by phone or home visit as needed for reporting of critical test results, if missed appointments or other urgent medical issues. If the patient consents, locator information (address/location and phone number) should be documented at the health facility.

Once a patient has been identified as missing an appointment or lost to follow-up, that individual must then be traced. Where the technology is available, invite patients back into care using SMS messages, phone calls, or e-mail. Where that technology is not available, then consider making a home visit. Patients who have not yet collected a positive HIV test result, should be reached in person via an outreach worker or other peer navigator if they do not respond within 1–2 days of an electronic message.¹

Missing and Delayed Results

Healthcare providers have an important role to play in ensuring that all requested test results are returned in a timely manner. In their 2016 guidelines, WHO states that “it is strongly recommended that test results from NAT in infants be returned to the clinic and child/mother/caregiver as soon as possible, but at the very latest within 4 weeks of specimen collection.”[3]

HIV-positive infants should be identified and followed up as soon as possible to enable early initiation in care and treatment with full cooperation of their caregivers and HIV healthcare providers.

Responsibilities for ensuring timely results are:

- Health facility: should use the designated register to identify results not received within 28 days, and immediately notify the laboratory.
- Laboratory: should check if samples were received, when was the test done and date when the results were dispatched.

Re-collecting specimens from infants where no results were found

If results have not been received by the health facility within 28 days and tracing the result with the laboratory is unsuccessful, use the post-test appointment to take another DBS specimen and re-send for testing. If the infant did not have an appointment or does not show for the appointment, phone or visit the infant’s caregiver as soon as it is known that the results have been lost to ask them to come in for retesting.

Appendix 4A: Listening and Learning Skills Checklist

Skill	Specific strategies, statements, behaviours	(✓)
Skill 1: Use helpful non-verbal communication	• Shows a relaxed and natural attitude	
	• Adopts an open posture	
	• Leans forward when talking	
	• Makes eye contact	
	• Sits squarely facing client	
	• Other (Specify)	
Skill 2: Ask open-ended questions	• Uses open-ended questions to get more in-depth information from the client	
	• Asks questions that reflect interest, care and concern rather than interrogation or judgement	
	• Other (Specify)	
Skill 3: Use responses and gestures that show interest	• Nods, smiles reassuringly; uses encouraging responses (such as “yes,” “okay,” “Mmm,” or “aha”)	
	• Clarifies statements effectively	
	• Takes time to summarise information the client shares	
	• Comments on client’s challenges while also indicating client’s strengths	
	• Other (Specify)	
Skill 4: Reflect back and re-formulate what your client says	• Reflects emotional responses back (re-formulates) to the client using different words	
	• Other (Specify)	
Skill 5: Empathize—show that you understand how she feels	• Demonstrates empathy: shows an understanding of how the client feels	
	• Avoids sympathy. Sympathy is when the healthcare provider moves the focus to herself (“I know how you feel, my sister has HIV.”) whereas empathy focuses on the client (“You’re really worried about what’s going to happen now that you’ve tested HIV-positive.”)	
	• Other (Specify)	
Skill 6: Avoid words that sound judgemental	• Avoids judging words such as good, bad, correct, proper, right, wrong, adequate, inadequate, satisfied, sufficient, fail, failure, succeed, success, etc.	
	• Uses words that build confidence and give support (for example, recognises and praises what a client is doing right)	
	• Other (Specify):	

Adapted from: WHO, CDC (2008). PMTCT Generic Training Package. Module 5 HIV Testing and Counselling for PMTCT. [5]

Appendix 4B: Infant PCR Laboratory Requisition Form

Countries should include their Infant PCR Laboratory Requisition Form in this appendix.

Appendix 4C: Specimen Delivery Checklist

Countries should include their Specimen Delivery Checklist in this appendix.

Appendix 4D: Relevant Pages of Child Health Card

Countries should include the relevant pages of their Child Health Card in this appendix.

Appendix 4E: Collection of DBS from Infants for PCR Testing

Collection of Dried Blood Spots (DBS) from Infants for PCR Testing

<p>1. Gather necessary supplies</p> <ul style="list-style-type: none"> • Gloves • DBS Card • Lancet (2mm) • Skin Disinfectant • Gauze or Cotton Wool • Pen • Sharps Container 		<p>2. Complete all necessary paperwork</p> <ul style="list-style-type: none"> • Clinic Register(s) • Laboratory Request/Report Form • DBS Card
<p>3. Choose the area to be pricked and ask the mother to warm this area by gently rubbing it with her hands.</p> <ul style="list-style-type: none"> • Infants 6wks-4mo: heel • Infants 4mo-10mo: big toe • Infants >10mo or >10kg: finger 		
<p>4. Wash and glove hands. If gloves have powder, wash off powder.</p>		<p>7. Wipe away the first spot of blood, allow a large drop of blood to collect.</p> 
<p>5. Position the baby with the foot or hand down, clean the spot to be pricked with skin disinfectant, allow to dry for 30 seconds.</p>		<p>8. Touch the filter paper gently against the large drop and allow it to completely fill the circle. Collect at least 3 full circles.</p> 
<p>6. Gently squeeze and release the area to be pricked until it is ready to be bled, prick the infant in the selected spot with the 2mm lancet.</p>		<p>9. Clean area with gauze and apply gentle pressure to stop bleeding. Ensure the wound is clean and bleeding has stopped for at least five minutes. Complete documentation and recheck the wound before the baby leaves your care.</p> 
		<p>Valid DBS Specimen</p> 



Appendix 4F: Drying and Packaging DBS Samples for Transport

Drying and Packaging Dried Blood Spot (DBS) Samples

Supplies



DBS Card



Drying Rack



Glassine Paper



Desiccant Packs



Humidity Indicator



Individual Bag (Left)/
Batch Bag (Right)

Drying

1. Leave DBS on a drying rack in a clean, dry, protected area until dried completely, for at least 4 hours or overnight.
 - While drying, DBS should not be touched and should be kept out of direct sunlight.
 - If your site collects DBS for both Early Infant Diagnosis (EID)* and HIV viral load (VL) monitoring, then two sets of drying racks are needed. Label one drying rack for EID DBS and the other for VL DBS.
 - You may label each DBS card "EID" or "VL" at the time of specimen collection in order to ensure that it is dried in the correct rack
 - In order to avoid nucleic acid contamination, the DBS for EID and VL should NEVER be dried together on the same rack. EID DBS are used for HIV diagnostic testing, while VL DBS are used for HIV monitoring. This type of contamination can lead to inaccurate test results.

2. Keep lab request forms with DBS cards.

Note:
The term Early Infant Diagnosis (EID) here is used broadly to include all virologic testing for the purpose of HIV diagnosis in infants and children <18 months of age.



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1

Drying and Packaging Dried Blood Spot (DBS) Samples

Packaging

Key Points:

- It is important to package each DBS card separately so that the cards do not contaminate each other.
- Package DBS for EID and VL at different times to prevent contamination.
- Packaging may depend on clinic volume and available supplies.

Follow **Route A (preferred method)** if you are using glassine paper or envelopes to separate DBS.
Follow **Route B** if you are using individual zip closure plastic bags to separate DBS.

Route A (Preferred method of packaging)

1. Place each DBS in a glassine paper or envelope so that DBS cards will not have direct contact with one other.



2. Insert up to 10 individually wrapped DBS cards into the batch bag. Add 10 desiccant packets to the batch bag.



3. Add at least one humidity card per batch bag. Gently press the bag to remove air before sealing.



4. Place the bag of DBS, all the DBS lab forms and the specimen delivery checklist into the shipping envelope.

- Use specimen delivery checklist to verify that you have a lab form for each DBS.
- Label the envelope with:
 - Clinic name
 - "EID" or "VL"
 - Date you are sending the specimens to the lab
- Place the envelope in designated area to be picked up for the laboratory.



5. After the DBS has been prepared for transport, it can be stored at room temperature for up to two weeks.

If DBS specimens cannot be shipped within two weeks, then they should be stored at -70°C until shipment (or -20°C if freezer at -70°C is not available). If a sample has been at the clinic for longer than two weeks without appropriate storage, then a new sample is needed.



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2

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Drying and Packaging Dried Blood Spot (DBS) Samples

Route B (if glassine packaging is not available):

1. Place each DBS in an individual plastic bag. Add 3 desiccant packets to each individual plastic bag.
2. Insert 5 - 10 individually sealed DBS into one zip closure batch bag.
3. Add at least one humidity card per batch bag. Gently press the bag to remove air before sealing.
4. Place the bag of DBS, all the DBS lab forms and the specimen delivery checklist into the shipping envelope.
 - Use specimen delivery checklist to verify that you have a lab form for each DBS.
 - Label the envelope with:
 - Clinic name
 - "EID" or "VL"
 - Date you are sending the specimens to the lab
 - Place the envelope in designated area to be picked up for the laboratory
5. After the DBS has been prepared for transport it can be stored at room temperature for up to two weeks.



If DBS specimens cannot be shipped within two weeks, then they should be stored at -70°C until shipment (or -20°C if freezer at -70°C is not available). If a sample has been at the clinic for longer than two weeks without appropriate storage, then a new sample is needed.



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References



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1. WHO Regional Office for Africa. *Operational guidelines on HIV testing and counseling of infant, children and adolescents for service providers in the African region*. 2014; Available from: <http://apps.who.int/iris/handle/10665/192410>.
 2. WHO. *Consolidated Guidelines on HIV Testing Services*. July 2015; Available from: <http://www.who.int/hiv/pub/guidelines/hiv-testing-services/en/>.
 3. WHO. *Consolidated Guidelines on the Use of Antiretroviral Drugs for Treating and Preventing HIV Infection: Recommendations for a Public Health Approach, Second Edition*. . June 2016; Available from: <http://www.who.int/hiv/pub/arv/arv-2016/en/>.
 4. WHO, *Recommendations on the diagnosis of HIV infection in infants and children: Laboratory methods for diagnosis of HIV infection in infants and children*. 2010.
 5. WHO and CDC, *PMTCT Generic Training Package. Module 5 HIV Testing and Counseling for PMTCT*. 2008.
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