CHAPTER 8
LABORATORY SERVICES

INTRODUCTION

Laboratory (lab) services form an essential component of HIV services. It is important to know how to collect specimens and perform tests correctly in order to obtain correct results. Regular quality management is important. The lab space will need to be large enough for all the equipment and staff required for the services. Patients need to be counselled to help them understand what the tests are for, how they will be performed and the meaning of the results. To do all of this, you will need to make sure that you follow steps provided in this chapter. Lab services must be consistent and dependable to correctly assess and manage patients with various illnesses. Without good quality lab services, test results may be wrong, and if they are not currently accurate, consistent, and dependable, every effort should be made to raise them to an acceptable standard.

Good communication is very important in your health centre and also in your lab. Talk regularly with staff working there to make sure that processes are followed correctly and that results are accurate. For lab tests that are not performed at the health centre, it is important that your staff have good communication with the district hospital lab or other referral lab.
This chapter provides the information you will need to set up a lab in your centre, as well as guidelines and steps on how to use various tests, read different test results and assure quality of services. With guidelines on how to build and run a lab, your centre will be able to provide consistent and dependable lab services for your patients. In addition, job aids and standard operating procedures (SOPs) are provided and can be made available to be easy to see and use.

■ A job aid is a simple tool that helps a worker do his or her job (for example, step-by-step instructions on how to do a test, often with pictures). Job aids generally provide quick reference information rather than in-depth training. They are a storage place for information other than your memory that you can use to help you do your job. These should be posted on a wall near where the testing is done.

■ An SOP is a prescribed written procedure to be followed routinely in doing a task. In the case of the lab, these describe in detail what a person doing specimen collection, testing, recording of results or other necessary lab tasks.

The chapter assumes that your lab at the health centre forms part of the national laboratory system. This system includes the district hospital lab that provides those tests not available at your centre. It also assists in quality assurance and sends specimens on to higher level labs at the provincial or national level for more complex lab tests.

Please note that this Operations Manual assumes your centre has some electricity and refrigeration.

8.1. ESSENTIAL LAB SERVICES

Essential lab services are the minimum lab tests that should be done at your centre to offer comprehensive HIV services they are not available directly at your health centre they should be available at your district hospital lab. You may be able to send the specimens you have collected from your patients or you may need to send the patient to the district hospital lab for these tests. This process and type of tests to be sent to level II should be clearly defined with your district hospital lab before you start.
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**ESSENTIAL LAB TESTS AVAILABLE AT HEALTH CENTRE**

- HIV diagnostics
  - Rapid HIV antibody tests (first and second tests)
  - Infant diagnosis; preparation of dried blood spot (DBS) out for virological testing
- Haematology
  - Haemoglobin determination
- Venous whole blood collection and send-out for CD4 cell absolute count and for percentage
- Blood sugar (glucose)
- TB diagnostics:
  - Sputum send-out for smear microscopy (or on-site acid fast bacilli (AFB) smear microscopy)
  - Sputum send-out for culture and drug susceptibility testing
- Malaria diagnostics (if in endemic area):
  - Peripheral blood smear (PBS) preparation and smear microscopy or
  - Rapid test to detect and discriminate between Plasmodium falciparum and mixed Plasmodium species
- Syphilis diagnostics:
  - Rapid syphilis test
  - Rapid plasma regain if refrigeration (RPR)
- Pregnancy test:
  - Rapid test for pregnancy
- Urine dipstick for sugar and protein

**ADDITIONAL ESSENTIAL LAB TESTS THAT CAN BE DONE AT YOUR DISTRICT HOSPITAL**

- HIV diagnostics
  - Rapid HIV antibody tests (first, second and third tests)
  - CD4 absolute count and percentage
- Full blood count with differential
- TB diagnostics:
  - Acid fast bacilli (AFB) smear microscopy
  - Sputum send-out for culture and drug susceptibility testing
- Serum alanine aminotransferase (ALT)
- Serum creatinine and blood urea nitrogen
- Gram stain
- Syphilis - rapid plasma reagin (RPR) and TPHA
- Basic cerebrospinal fluid (CSF) and urine microscopy
- Bilirubin determination for neonates
- Blood and sputum cultures (send out)
- Cryptococcal antigen and/or India ink
- Lactic acid
- Type and cross-match for transfusion
- Pulse oximetry
- Chest X-ray
8.2. LAB SAFETY

Your health centre staff will need to know how to safely use lab supplies and collect, test, and transport specimens. See chapter 9 the Human Resources for information on standard lab precautions, injection safety, post-exposure procedures, and TB infection control. See chapter 5 the Infrastructure for information on safe water, sanitation, hygiene, waste management, and power.

Bio-safety guidelines:

■ Treat all specimens (blood, urine, sputum, etc) as potentially infectious.

■ Wear protective gloves and a lab gown while drawing blood and handling specimens.

■ Do not, eat, drink, or smoke in the lab.

■ Do not keep food in the lab refrigerator.

■ Do not wear open toe footwear in the lab.

■ Clean up spills with an appropriate disinfectant, e.g. 1% bleach.

■ Decontaminate all instruments and materials with an appropriate disinfectant.

■ Dispose of all waste, including test kits, in a biohazard container.

Phlebotomy safety
Injuries may occur when drawing blood or using a finger stick or heel stick to obtain a blood specimen, or testing with sharps (blood collection needles, lancets, cutting blades, glass pipettes or slides, broken plastic or glass, etc.). When possible, use single-use vacuum blood collection tubes with safety needles rather than a syringe and needle. This also reduces the amount of biological waste.
Sharps disposal
All sharps should be placed in a puncture-resistant, leak proof, sharps disposal container. Follow the disposal instructions in chapter 5 Infrastructure. The chapter 9 Human Resources also has instructions on safe handling of needles and syringes, and what to do if a needle stick injury occurs.

■ If possible, only use vacuum tubes and a needle to draw blood (instead of syringe and needle).

■ DO NOT recap, bend, break, or manipulate needles by hand. Throw these items away intact.

■ After you use sharps, put them in a puncture-resistant, leak-proof trash container right away. DO NOT place sharps in regular trash containers.

■ Report all injuries involving sharps to the (person in charge of safety) at your centre or at the district level.

Post-exposure prophylaxis (PEP)
PEP is the use of ARV drugs to reduce the risk of HIV infection following accidental exposure. PEP should be available for all staff members following exposure of non-intact skin (through percutaneous sharps injury or skin abrasion) or mucous membranes (through sexual exposure or splashes to the eyes, nose or oral cavity) to a potentially infected body fluid from a source that is HIV-positive or has unknown HIV status

PEP includes:

■ a staff person trained to provide prompt clinical advice; and

■ access to antiretrovirals (drugs to prevent HIV infection) as soon as possible after exposure and within 72 hours.

See chapter 9, the Human Resources for more details.
8.3 LAB TESTING

Specimen testing has three parts:

PART 1: Before performing the test

■ Specimen collection: Collect the specimen or give clear instructions when the patient is to collect the specimen themselves (urine and sputum).

■ Record keeping: Review the requisition forms to ensure that all necessary information is recorded. Enter the required information from the requisition form into the lab logbook. Fill out lab worksheets for the tests that will be run that day. If specimens are to be sent to another lab for testing, store the specimens properly until they are sent-out. Pack the specimens properly and fill out and include the requisition forms and specimen shipping inventory.

■ Equipment set-up: Make sure that any equipment you need to run the test is available and in good condition. Also ensure that regular maintenance is done at the right times.

■ Test-related preparation: Make sure that you have all the supplies and reagents (substances used for detecting or measuring another substance, such as chemical stains for acid fast bacteria) for doing the test. Make sure that you have a clean area in the lab to do the testing.

■ Perform quality control for tests or reagents. For example, test the chemical stain on a slide with a known positive sputum before doing the test each day.

PART 2: Testing

Test the specimen following SOP for the test. You should have SOPs for each test performed in your lab. These should include all the information needed to correctly perform the test.

For rapid tests, ensure that the control line is present before reporting results.

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1 Logbook: to avoid confusion between logbook and report form, it is advised that a logbook is a document which is kept in the lab in which the lab technicians record all information related to specimen including test results. A report form is a form on which test results are filled by the lab technicians and sent to the clinician who requested the test.
PART 3: After performing the test

- Record keeping: Record the test results in the proper lab logbook.

- Reporting results: Fill in the test results on the report form (may be part of the requisition form). If possible, have another person check to make sure that the correct patient’s results are put on the right form. If another person is not available, recheck this yourself. Send the report forms to the clinical staff to be put into the patient’s medical records. If results are coming to you from testing at another lab, make sure that these results are also sent to the clinical staff who will write them in the patient's medical records.

- Interpreting results: If there are any questions about the test results, be prepared to answer them. If you do not know the answer, consult with lab staff at the district level.

8.4 SPECIMEN LABELLING AND LOGGING

All specimens need to be labelled with the following information using a waterproof pen:

- specimen ID number;

- patient’s first and last name (may be excluded in some cases where protecting the patient’s privacy is a concern);

- patient’s date of birth (if known);

- date and time of collection;

- collector’s initials.

Each specimen should have a lab requisition form.

The information on the specimen label should match the information on the lab requisition form. Each time the lab takes a specimen it should be logged into the lab logbook.
Lab testing requires supervision and training for quality assurance. Every centre offering lab services will need:

**Initial and ongoing staff training in**

- specimen collection
- testing techniques
- quality lab management
- quality assessment
- specimen packaging – for send-out tests
- lab recordkeeping

**Supervisory visits by district level lab staff**

- To observe and review lab processes including:
  - arrangement of workspace
  - preparation for testing
  - collection of specimens
  - testing procedures
  - recordkeeping.

- To provide training as needed.
Quality assessment

- quality control (QC) – ensure the use of internal quality control specimens (if on hand);

- monitoring results – proportion of follow-up AFB smears positive;

- external quality assessment (EQA) – participation in a programme (if on hand);
  
  - testing a coded panel of specimens (also known as proficiency testing);
  or

  - blinded rechecking; or

  - supervisory visits (see above).

- SOPs and job aids;

- standardized record forms;
  
  - requisition forms;

  - specimen logbooks;

  - lab worksheets;

  - report forms;

  - forms for reviewing the status of lab equipment;
    
    - temperature logs for refrigerators (if present);

    - maintenance log (documenting routine maintenance of microscope (if present);

- Forms for ordering reagents and supplies;

- QC logbook for recording QC results (see section - “Quality control” below).
Organization and management

- Make sure that there is a clear organization of staff involved in the lab in order to ensure that standardized procedures can be implemented and followed by all staff.

- There should be one person with overall responsibility for the coordination of the lab services at the site.

Purchasing and inventory

- Have a clear plan for maintaining a supply of test kits and other consumables so that stock-outs do not occur.

- Use the FEFO principles – see chapter 7 “Supply Management”.

Documentation

- Ensure that documents and records are well-kept and accessible by staff.

- Have a standardized lab logbook for entering all testing results, batch number and expiry dates of test kits, etc.

Standard operating procedures

- Have concise, clear SOPs in your local language for those trained to perform the test.

- Include SOPs for specimen collection procedures, test performance, and interpretation of overall testing results and reporting, etc.

- See the instructions and job aids throughout this chapter.
Quality control

- Use internal quality control specimens, if included with the test kit, for each testing session or daily—AND, if available use an external quality control specimen (provided by your district hospital lab or national reference lab). These specimens should have known results. This is particularly true for rapid tests.

- Store these controls appropriately. Label the vial with the date when first used, test before expiry date, and take care not to contaminate the control material. Make sure that you have a regular and ongoing supply of controls (as part of your purchasing and inventory system).

- External quality control specimens should be used at the following times:
  - once a week;
  - when you receive a new shipment of control materials and test kits;
  - at the beginning of a new lot number of test kits;
  - whenever you suspect that the test kits may not be in good working order;
  - when a new operator performs testing (a newly trained staff member or a staff member who has not performed testing for a while).

- Your standardized lab logbook should contain space for recording QC results. These results should then be transferred to a QC logbook for quick review of data.

For specifics on rapid tests and malaria and AFB smears, please see section -s...?????

External quality assessment (EQA)

- Proficiency testing
  - Periodically, you will receive a panel of specimens to assess how well you are doing at providing testing results. This panel will come from the district hospital
lab or national reference lab and it measures the performance of the tests and of the operator performing testing. You will test the panel of specimens and report the results back to the panel provider. Your performance on testing this panel will be compared with that of other testing sites. You will receive feedback on how well you are doing at performing the testing.

- Onsite evaluation and monitoring (also called audits, assessments, or supervisory visits);
  - Periodically, your lab will be visited by staff from the district hospital lab. They will observe how you are doing the testing. They will give you feedback on this to ensure testing quality. This is part of every lab quality system.

- EQA may identify problems. If so, corrective actions will be recommended to correct the problem or deficiency.

For specifics on rapid tests and smears, please see the relevant sections

Training and certification

- Ensure that all individuals who will be doing collection and testing, whether lab staff or others, have received appropriate training in:
  - specimen collection, quality assessment and packaging
  - testing techniques and quality lab management
  - lab recordkeeping and communication of results

- Have a programme for training of all new staff and for re-training of staff who have not performed testing in a while.

- Test any new staff who will be performing testing with a proficiency testing panel of at least 10 specimens. This panel can be provided by your district hospital lab or the national reference lab. Make sure that the operator has a proficiency score which is acceptable to your national laboratory programme before they begin to do testing.
8.6 INSTRUCTIONS FOR THE COLLECTION OF BLOOD SAMPLES

Please also use Vacuum which is a generic name instead of Vacutainer + several other specific amendments in the various figures are needed but I cannot access the original.

Instructions for collecting blood by venipuncture (adult)
For use with syringe

A. Syringe with needle

1. Choose method:
   A. Syringe and needle

2. Collect supplies.

3. Wash hands.

4. Glove hands.

5. Position patient in a chair with a back.

6. Put tourniquet on client about 4-5 finger widths above venipuncture site

7. Have client form a fist so veins are more prominent.

8. Clean collection site with 70% isopropyl alcohol. Allow to dry.

9. Remove cap from needle and place on table.

10. Move syringe plunger up and down to break seal.

11. Use your thumb to draw skin tight about 2-3 finger widths below the venipuncture site.
12. Insert the needle, bevel side up, into the vein. Establishment of blood flow is indicated by spurt of blood into the syringe. Have the client open their fist.

13. Pull back on syringe plunger so blood will flow into syringe.

14. Fill the syringe until the desired amount of blood has been collected.

15. After the desired amount of blood has been collected, release the tourniquet.

16. Check to make sure client has opened their hand, place dry gauze over the site without applying pressure.

17. Slowly remove the needle and then apply firm pressure to the pad.

18. Have client continue applying mild pressure until bleeding has stopped. Put on an adhesive bandage if necessary.

19. Place the cap on a flat surface.

20. With one hand use the needle to scoop up the cap.

21. Use the other hand to secure the cap.
22. Twist off, carefully remove needle from syringe.
23. Discard the capped needle into sharps container.
24. Remove rubber stopper from tube.
25. Slowly inject blood into the tube.
26. Carefully restopper the tube.
27. Shake by inverting tube back and forth 5-10 times.
28. Properly dispose of all contaminated supplies.
29. Label tube with the client identification number, date and collector's initials.
Instructions for collecting blood by venipuncture (pediatric)
For use with butterfly and vacuum tubes

1. Vacuum tube and butterfly needle.
2. Collect supplies.
3. Wash hands.
4. Glove hands.
5. Restrain the child by either a) lying down or b) having them sit upright on a parent’s lap. The parent should wrap their arm around the child and over the arm that is not being used.
6. Put tourniquet on client about 2 finger widths above venipuncture site.
7. Attach the end of the winged infusion set to the end of the vacuum tube.
8. Insert the collection tube into the holder until the tube reaches the needle.
<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>12.</td>
<td>Remove plastic sleeve from end of butterfly.</td>
</tr>
<tr>
<td>13.</td>
<td>Clean collection site with 70% isopropyl alcohol. Allow to dry.</td>
</tr>
<tr>
<td>14.</td>
<td>Use your thumb to draw skin tight about 2 finger widths below the venipuncture site.</td>
</tr>
<tr>
<td>15.</td>
<td>Bend the wings and insert the needle, bevel side up, into the vein. Establishment of blood flow is indicated by a spurt of blood into the tubing.</td>
</tr>
<tr>
<td>16.</td>
<td>Push the vacuum tube completely onto the needle. Blood should begin to flow into the tube.</td>
</tr>
<tr>
<td>17.</td>
<td>Fill the tube until it is full or until vacuum is exhausted. If you are filling multiple tubes, carefully remove the full tube and replace with another tube. Try not to move the needle in the vein. Shake by inverting removed tube back and forth 5-10 times.</td>
</tr>
<tr>
<td>18.</td>
<td>After the desired amount of blood has been collected, releases the tourniquet. (place tube)</td>
</tr>
<tr>
<td>19.</td>
<td>Release patient's hand, place dry gauze over the venipuncture site and slowly withdraw needle.</td>
</tr>
<tr>
<td>Step</td>
<td>Instruction</td>
</tr>
<tr>
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</tr>
<tr>
<td>20.</td>
<td>Have mother continue applying mild pressure.</td>
</tr>
<tr>
<td>21.</td>
<td>Remove butterfly from vacuum tube holder and dispose in sharps container.</td>
</tr>
<tr>
<td>22.</td>
<td>Properly dispose of all contaminated supplies.</td>
</tr>
<tr>
<td>23.</td>
<td>Label tube with the client identification number, date and</td>
</tr>
<tr>
<td>24.</td>
<td>Put on an adhesive bandage if necessary.</td>
</tr>
</tbody>
</table>
Instructions for collecting blood by finger prick

1. Collect supplies.
2. Position hand palm-side up. Choose whichever finger is least calloused.
3. Apply intermittent pressure to the finger to help the blood to flow.
4. Clean the fingertip with alcohol. Start in the middle and work outward to prevent contaminating the area. Allow the area to dry.
5. Hold the finger and firmly place a new sterile lancet off-center on the fingertip.
6. Firmly press the lancet to puncture the fingertip.
7. Wipe away the first drop of blood with a sterile gauze pad or cotton ball.
8. Collect the specimen. Blood may flow best if the finger is held lower than the elbow.
9. Apply a gauze pad or cotton ball to the puncture site until the bleeding stops.
10. Properly dispose of all contaminated supplies.
8.7 RAPID TESTS ON BLOOD – COMMON INSTRUCTIONS

Rapid tests are tests that can be done in a short period of time so that the results can be given to the patient while they are still at the centre. Rapid tests can be performed for HIV, syphilis and malaria according to national guidelines.

Test kit preparation (applicable to all test kits)

■ Follow all storage procedures. Some kits that do not require refrigeration should still be kept in a cool place. (If you lack refrigeration, make sure that the tests you use do not need it.) If kept in a cool place, remove the number of tests and reagents that you expect to use that day and let them stand for at least 20-30 minutes to reach room temperature (20-25°C). The use of cold test kits may lead to false-negative results. Close the pouch that the test comes in properly before storing.

■ Check expiry date to make sure the kit has not gone bad. Do not use the kit beyond that date.

■ If a desiccant (a chemical that absorbs water to keep the package dry) is included in the package, do not use the kit if it has changed colour.

■ Once opened and brought to room temperature, a test kit should be used immediately.

■ Prepare your lab logbook: write down the test batch number (test kits are made in large quantities by manufacturers and each is labelled with a number) and expiry date; write the name of the person performing the test and date. Clearly write specimen number and record the results right away.

■ Validate the test kit using the manufacturer’s directions and the positive and negative controls provided. Controls are used to ensure that a test is working properly; giving positive results for positives and negative results for negatives. This is the process of internal quality control. Preferably, run the controls prior to the beginning of each day’s testing, whenever a new kit lot is introduced and whenever you are concerned with storage conditions.
Different lab staff members should alternate running the controls on different days. For kits that do not contain controls, controls may be provided from your district hospital lab. These controls should be stored appropriately. This is in addition to the internal control which is built into the test kit (making sure that a control line is seen to ensure that the specimen was added, and that the test was done properly). Record results of control tests on the lab worksheet and in the QC logbook.

- Write the specimen number on the lab logbook.
- Remove the test device from its protective wrapping.
- Write the specimen number on the test device. Always label specimens and test devices clearly.
- Follow all the manufacturer’s instructions, including the full waiting time until the test should be read for results. Do not read tests early, even if the control line is visible. Failure to wait the full waiting time can lead to false negative results, and do not read past the specified end point time.
- Do not use reagents from one kit with another kit.
Job aid for rapid HIV and syphilis tests on whole blood. This is only an illustrative figure

Always follow the manufactures’ instructions
8.8 RAPID HIV ANTIBODY TESTS

HIV antibody testing is done with rapid HIV tests. Rapid tests give results in less than 30 minutes, allowing you to give the patient results in the same visit. The rapid HIV test kits usually include everything you need to do a test. Some may require a pipette (a narrow, glass or plastic tube into which small amounts of liquid are suctioned for transfer or measurement). Make sure you have all the materials needed for testing before you begin. Testing will be based on the national HIV testing algorithm. All testing should be recorded in the HIV testing logbook (see Annex). The HIV testing logbook can also be used to help prepare periodic reports on test results for higher authorities. This data can also be used for quality assurance purposes.

Below is the WHO recommended HIV rapid testing algorithm. It should be replaced by the national testing algorithm and displayed as a job aid in the lab. It should include testing for both HIV-1 and HIV-2 for countries with HIV-2 prevalence.

<table>
<thead>
<tr>
<th>High HIV prevalence:  &gt; 1% in pregnant women (for country adaptation)</th>
<th>HIV Rapid Testing Algorithm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Do HIV rapid test 1 (all specimens)</td>
<td></td>
</tr>
<tr>
<td>Do HIV rapid test 1 result: Positive</td>
<td>Do HIV rapid test 2</td>
</tr>
<tr>
<td>Do HIV rapid test 2 result: Positive</td>
<td>Report specimen as HIV-positive</td>
</tr>
<tr>
<td>Do HIV rapid test 2 result: Negative</td>
<td>Send for or do rapid test 3</td>
</tr>
<tr>
<td>Do HIV rapid test 1 result: Negative</td>
<td>Report specimen as HIV-negative</td>
</tr>
</tbody>
</table>

- All specimens are given rapid test 1. If the first test is negative, report the specimen as HIV-negative.
- If the rapid test 1 result is positive, do rapid test 2. Be aware that rapid test 2 should be a different type of test kit from rapid test 1. Be aware that rapid tests 1 and 2 can be done from a single finger prick; then interpreted using this algorithm.
- If both rapid test 1 and rapid test 2 results are positive, report the specimen as HIV-positive.
- If rapid test 1 is positive and rapid test 2 is negative, send out for or do rapid test 3 (a different test kit from rapid tests 1 and 2).
Specific quality assurance

Quality Control

- Use internal quality control specimens, if included with the test kit, for each testing session or daily-AND, if available use an external quality control specimen (provided by your district hospital lab or national reference lab). These specimens should have known results - see the quality section at the beginning of the chapter.

External quality assessment (EQA)

- Note that the number of invalid or discordant results you obtain on testing each month should be recorded. If this number suddenly increases, you should look into the integrity of the test kits and/or how the testing is done. The number of discordant results should also be recorded. A change in this number may also indicate a problem with testing.

8.9 RAPID SYPHILIS TESTS

Many health centres do not have access to a consistent power source required for adequate refrigeration (required for storage of reagents for the Rapid Plasma Reagent (RPR) test). Therefore, a rapid syphilis test that does not require refrigeration of reagents is recommended. Clear guidelines should exist on the clinical use of testing results.

Specific quality assurance

Proficiency testing

- Currently, there is no proficiency testing programme that includes primary health centres. WHO is working on a guide for countries on how to produce proficiency testing panels for rapid syphilis tests.
8.10 RAPID MALARIA TESTS

Correct and rapid diagnosis of malaria is crucial and needs to be performed in all patients presenting with symptoms indicating suspected of malaria before the patient leaves the health centre. WHO recommends diagnosis with a blood smear when possible, but centres without reliable electricity or a microscope with a suitable light source should use the rapid test.

Rapid malaria tests may not be the best tool at present for malaria parasite species differentiation as most non P. falciparum tests still have challenges of stability that affect their sensitivity and specificity.

Specific quality assurance

External quality assessment (EQA)

- If possible, prepare a thick and a thin smear from the blood of every 10th patient being tested. Send this for microscopic examination. This should be arranged with your district hospital lab.
How to do the rapid test for malaria

READ THESE INSTRUCTIONS CAREFULLY BEFORE YOU BEGIN.

1. Check the expiry date on the test packet.
2. Put on the gloves. Use new gloves for each patient.
3. Open the packet and remove:
   a. Test
   b. Loop
   c. Crescent sachet
4. While the patient's name on the test....

5. Open the alcohol swab. Grip the 4th finger on the patient's left hand. Clean the finger with the spirit swab. Allow the finger to dry before pricking.
6. Open the lancet. Hold the patient's finger. Get a drop of blood.
7. Discard the lancet in the sharps box immediately after pricking. Do not set the lancet down before discarding it.
8. Use the loop to collect the drop of blood.
9. Use the loop to put the drop of blood into the square hole marked "A."
10. Discard the loop in the sharps box.
11. Put six (6) drops of buffer into the round hole marked "B."
12. Wait 15 minutes after adding buffer.
13. Read test results:
   (NOTE: Do Not read the test sooner than 15 minutes after adding the buffer. You may get FALSE results.)

14. How to read the test results:
   **POSITIVE**
   One red line in window "C" and one red line in window "T" means the patient **DOES** have Plasmodium falciparum.
   The test is **POSITIVE** even if the red line in window "T" is faint.

15. Dispose of the gloves, spirit swab, lancet, sachet and packaging in a non-sharps waste container.

16. Record the test results in your CTH register. Dispose of cassette in non-sharps waste container.

**INVALID RESULT**
   NO LINE in window "C" means the test is damaged.
   A line in window "T" and NO LINE in window "C" also means the test is damaged. Results are **INVALID**.

**NOTE:** Each test can be used ONLY ONE TIME. Do not try to use the test more than once.

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CHAPTER 8 | LABORATORY SERVICES
8.11 INFANT HIV DIAGNOSIS

Virological testing for infant HIV diagnosis is usually done in a national or regional reference lab. It is extremely important to follow infants from PMTCT programmes and to test them as early as possible.

The specimen collected from the infant is capillary blood from a heel, big toe, or finger prick that is put onto a filter paper (dried blood spot (DBS)).

Instructions for collecting dried blood spots (DBS) from infants for virological Testing:

1. Gather necessary supplies:
   - gloves
   - blood collection card (filter paper)
   - lancet (2mm)
   - 70% isopropyl alcohol
   - gauze or cotton wool
   - pen.

2. Complete all necessary paperwork:
   - infant diagnosis registration form
   - clinic register
   - laboratory request/report form

3. Wash hands.

4. Glove hands.
6. Ask the mother to warm this area.

7. Position the baby with the foot or hand down, then clean the spot to be pricked with 70% isopropyl alcohol, and allow to dry for 30 seconds.

8. Gently squeeze and release the area to be pricked until it is ready to bleed, and then prick the infant in the selected spot with the 2mm lancet.

9. Wipe away the first spot of blood, and then allow a large drop of blood to collect.

10. Touch the filter paper gently against the large drop and allow it to completely fill the circle. Collect at least three good drops.
11. Clean area; no bandage is needed.

12. Fill out DBS card.

13. Dispose of lancet.

Two examples of valid DBS specimens
3 good specimens

Invalid DBS specimen
A. May have been soaked with syringe
B. Drops too small
C. “spots” that are streaky
D. Clotted/layered
E. Yellow serum rings around blood drops.
Diagnosis of HIV Infection in Sick Infants and Children Under 18 Months Where Viral Testing is Not Available

**Sick infant <18 months of age**

- with unknown HIV exposure and signs and symptoms suggestive of HIV infection

  **Confirm exposure:**
  - Check mother’s HIV status

  - **Mother HIV-negative**
    - Treat infant’s medical conditions

  - **Mother HIV-positive**
    - **Maternal testing not possible**

  **Recommend HIV antibody testing for infant**

  **Negative**

  - Assume infant is uninfected and/or unexposed

    - Treat infant’s medical conditions

    - Regular and periodic clinical monitoring

    - Repeat HIV testing for infant
      - known to be exposed
      - continuing to breastfeed

  **Positive**

  - **Infant is exposed and may be infected**

    - Infant has symptoms that meet clinical criteria for presumptive severe HIV infection
      - **NO**
        - • Monitor infant’s clinical condition frequently and confirm HIV status at 18 months of age or if viral testing is available
      - **YES**
        - • Manage as if infant is HIV infected.
        - • Seek CD4 testing.
        - • Consider starting ART
        - • Confirm HIV status at 18 months of age or if viral testing is available
Establishing Presence of HIV Infection in HIV-exposed Children, Aged Under 18 Months, In Resource-limited Setting to Facilitate ART and HIV Care
See drying and packing instructions for DBS (Section 8-19).

- See Annex for an example of an infant virological test lab requisition form and a logbook for DBS testing.

**External quality assessment (EQA)**

**On-site evaluation and monitoring**

- Periodically, your lab will be visited by staff from the district hospital lab. They will observe how you are collecting and processing DBS specimens. They will give you feedback to ensure quality collection, processing, and shipping.

### 8.12 ESTIMATING HAEMOGLOBIN

The WHO Haemoglobin Colour Scale is an inexpensive, rapid, and simple to use tool that can be used to screen for anaemia. It gives an estimate of the amount of haemoglobin in a blood sample. Good training is essential to do this test. If a low haemoglobin < 10g/dl is obtained on the Colour Scale, more accuracy can be obtained with a second test, for example, using a haemoglobinometer. If your centre has a second test available, test the patient again so that you can more accurate results.
Haemoglobin colour scale: instructions for use

Preparation

1. Find a well-lit place, inside, under a veranda or under a tree

2. Lay the colour scale flat on a table in front of you.

3. Put the colour scale at an angle with light passing over your shoulder.

4. Avoid direct light.

How to use colour scale

5. Put a drop of blood on a test strip (about 3 mm in diameter).

6. Allow to dry for 30 seconds. DO NOT LEAVE FOR MORE THAN 2 MINUTES.

7. Match test strip to colour on the scale.
8.13 URINE DIPSTICK FOR SUGAR AND PROTEIN

Specimen collection

You should have a space with privacy for the patient to collect a urine specimen for testing. Give the patient clear instructions (see below) on how to collect a good urine specimen.

Instructions for collecting urine - for women

- Label a clean container with the patient name, DOB, and date and time of collection.
Give the woman the clean container and tell her where she can urinate. Teach her how to collect a clean-catch urine sample. Ask her to:

- spread labia with fingers;
- clean vulva with water, going from front to back;
- urinate while keeping labia spread (urine should not touch the vulva. If urine touches the vulva, the specimen may be contaminated);
- catch middle part of the stream in the cup;
- remove container before urine stops.

**Instructions for collecting urine - for men**

- Label a clean container with the patient’s name, DOB, date and time of collection.
- Give the man the clean container and explain where he can urinate.
- Teach the man how to collect a clean-catch urine sample. Ask him to:

  - Pull back foreskin with fingers (if uncircumcised);
  - Clean head of penis with water;
  - Urinate while keeping foreskin pulled back (urine should not touch foreskin because the sample may become contaminated);
  - Catch middle part of the stream in the cup;
  - Remove container before urine stops.

**Analyse urine using dipstick method**

- Dip coated end of paper dipstick in urine sample, and shake off excess by tapping against side of container.
- Wait the recommended amount of time (see dipstick package instructions).
8.14 PREGNANCY TESTS

Pregnancy testing is included in the essential lab tests at your centre because of the importance of excluding pregnancy before starting a woman on efavirenz.

(Insert instructions/job aid for rapid pregnancy test used locally in the adaptation process)

See the sample pregnancy testing logbook in Annex 8.6.

8.15 MALARIA SMEAR AND MICROSCOPY

Malaria smear microscopy

Malaria smear microscopy is the preferred test for diagnosing malaria. The rapid malaria test should be used if this is not available (see above section 8.10).

Preparing Blood Smears: Blood for testing is usually collected by finger prick directly onto a clean glass slide. If you are using venous blood, blood smears should be prepared as soon as possible after collection (delay can result in changes in the malaria parasite’s shape and staining characteristics).
<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Thick smears</th>
<th>Thin smears</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Characteristics</strong></td>
<td>Thick smears allow a more efficient detection of parasites (increased sensitivity). However, they are often not good enough to identify the species of malaria parasites as they do not permit an optimal review of parasite shape. If the thick smear is positive for malaria parasites, the thin smear should be used to identify the species.</td>
<td>Thin smears consist of blood spread in a layer so that the thickness decreases progressively towards the feathered edge. In the feathered edge, the cells should be in a monolayer, not touching one another.</td>
</tr>
</tbody>
</table>

**Slide preparation**
Prepare at least two smears per patient!

- Note: If slides are scarce, prepare both a thick and a thin smear on the same slide. This can work well if you make sure that of the two smears; only the thin smear is fixed.

**STEP 1:**
- Place a small drop of blood in the centre of the pre-cleaned, labelled slide. Be careful of using anticoagulated blood.
- Place a small drop of blood on the pre-cleaned, labelled slide, near its frosted end.

**STEP 2:**
- Using the corner of another slide or an applicator stick, spread the drop in a circular pattern until it is the size of a fingernail (1.9 cm across).
- Bring another slide at a 30-45° angle up to the drop, allowing the drop to spread along the contact line of the two slides. Quickly push the upper (spreader) slide towards the unfrosted end of the lower slide.
### STEP 3:
A thick smear of proper density is one which, if placed (wet) over newsprint, allows you to barely read the words.

Make sure that the smears have a good feathered edge (A thin, sharp edge with the cells not touching one another). You can do this by using the correct amount of blood and spreading method.

### STEP 4:
Lay the slides flat and allow the smears to dry thoroughly (protect from dust and insects). Insufficiently dried smears (and/or smears that are too thick) can detach from the slides during staining. At room temperature, drying can take several hours; 30 minutes is the minimum; in the latter case, handle the smear very delicately during staining. Protect thick smears from hot temperatures to prevent heat-fixing them. Heat fixing can prevent the breakdown of the red blood cells.

Allow the thin smears to air dry. They dry much faster than the thick smears, and are less likely to come off the slide because they will be fixed.

### STEP 5:
Do not fix thick smears with methanol or heat. If there will be a delay in staining smears, dip the thick smear briefly in water to haemolyse (break down) the red blood cells.

Fix the smears by dipping in absolute methanol.

---

**Giemsa staining**

Giemsa stain (a mixture of eosin and methylene blue) is often used for staining blood films.

### STEP 1:
Use prepared Giemsa stain or prepare a 3% solution by adding 3 ml of Giemsa stock solution to 97 ml of buffered water.

### STEP 2:
Pour the stain gently into the trough until the slides are totally covered. Do not pour the stain directly on the thick films.

### STEP 3:
Leave the slides in the stain for 30-45 minutes.

### STEP 4:
Pour clean water gently into the trough to float off the scum on the surface of the stain. While pouring water, do not disturb the thick films.

### STEP 5:
Pour off the remaining stain gently and rinse again in clean water for a few seconds. Pour off the water.

### STEP 6:
Remove the slides one by one and place them, film side downwards, in a drying rack to drain and dry, making sure that the thick film does not touch the edge of the rack.
**Microscopic examination of the film**

**Microscopic examination**

Since it takes almost 10 times as long to examine a thin film as it does to examine a thick film, examine the thick film first. The thin film is examined only when the thick film becomes autofixed (by being exposed to heat), or when it is necessary to confirm the identification of a species (the type of malaria parasite).

<table>
<thead>
<tr>
<th>STEP 1:</th>
<th>Using the 40x objective, select a part of the film that is well stained, free of staining debris, and is well populated with white blood cells.</th>
<th>Place the slide on the mechanical stage and position the 100x oil immersion objective over the edge of the middle of the film.</th>
</tr>
</thead>
<tbody>
<tr>
<td>STEP 2:</td>
<td>Place a drop of immersion oil on the thick film.</td>
<td>Place a drop of immersion oil on the edge of the middle of the film.</td>
</tr>
<tr>
<td>STEP 3:</td>
<td>Lower the 100x oil immersion objective over the selected portion of the blood film, so that it touches the immersion oil.</td>
<td>Lower the oil immersion objective until it touches the immersion oil.</td>
</tr>
<tr>
<td>STEP 4:</td>
<td>Confirm that the portion of the film is acceptable and examine the slide for 100 oil immersion fields by moving along its width.</td>
<td>Examine the blood film by moving along the edge of the thin film, then moving the slide inwards by one field, returning in a lateral movement, and so on.</td>
</tr>
<tr>
<td>STEP 5:</td>
<td>Examine at least 100 good fields before a slide pronouncing negative for malaria.</td>
<td></td>
</tr>
<tr>
<td>STEP 6:</td>
<td>Record your findings on the proper form.</td>
<td></td>
</tr>
</tbody>
</table>

**Quantifying parasites GRADING (parasites per field) on thick smear only**

<table>
<thead>
<tr>
<th>Parasite count:</th>
<th>Grade:</th>
<th>Not done</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 to 9 malaria parasites per 100 fields</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>10 to 99 malaria parasites per 100 fields</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>1 to 9 parasites per field</td>
<td>+++</td>
<td></td>
</tr>
<tr>
<td>10 to 100 parasites per field</td>
<td>++++</td>
<td></td>
</tr>
</tbody>
</table>

**Specifics of Quality Assurance**

- Check the quality of your stain by staining one thin and one thick blood smear and assessing the quality of red cell staining to control buffer quality, stain white cells and parasite chromatin for nuclear staining, and stain white blood cell granules and parasite inclusions.
- Do this on each day that you do the test or weekly, if larger volume lab.
- Prepare extra positive slides periodically to use for this purpose.
| Quality control | • Use external quality control specimens (thick and thin smears from both positives and negatives).  
• Store these controls appropriately.  
• Your standardized lab change with logbook should contain space for recording QC results. These results should then be transferred to a QC logbook for quick review of data. |
|-----------------|-------------------------------------------------------------------------------------------------|
| External quality assessment. (EQA) | • Proficiency testing:  
   • Periodically, you will receive a panel of slides to assess how well you are doing the test. This consists of a minimum slide set including 20 slides, including negative slides and positive slides with different malaria species, and different counts. A combination of stained and unstained slides will allow the testing of staining capability. This will come from the district hospital lab or the national reference lab. You will test the panel of slides and report the results. Your performance on testing this panel will be compared to that of other testing sites. You will receive feedback on how well you are doing the test.  
   • Slide validation at a higher level by random rechecking:  
     • If your national malaria programme uses this quality assessment method, save your positive slides and some of your negative slides as instructed.  
     • Store these in secure slide boxes protected from excess heat and/or humidity.  
     • Send these, when asked, to be blindly rechecked by a higher level lab. |

The bench aid for the diagnosis of malaria infections, 2nd ed. Geneva, World Health Organization, 2000 should be available in the centre’s lab for staff to use if smear microscopy is being performed.
8.16 TB SMEAR AND MICROSCOPY

This section covers TB sputum collection and transport for smear microscopy or culture and drug susceptibility testing elsewhere or for on site smear microscopy.

Tuberculosis (TB) is diagnosed by detecting the TB acid fast bacillus in the sputum. Rapid identification and treatment of people who are becoming infected is important to reduce the risk of death or severe illness associated with the disease, as well to protect health workers, community members and other patients from becoming infected.

All health centres should be able to handle sputum for AFB microscopy. Some may be able to do AFB smear microscopy onsite, but if not, sputum sample collection can be done onsite and the sample sent to the district hospital lab for testing. TB is highly infectious and thus proper training in specimen collection is important to protect the staff and patients. Specimens also need to be correctly packaged for shipment to prevent any leakage that would be dangerous during transport and might also compromise results. Post clear instructions (text and pictures) for centre staff, lab staff and patients about safe methods for specimen collection, the number of specimens to collect and when to collect them.

In addition to AFB smear microscopy, sputum specimens may also need to be referred to the district or higher level lab for TB culture and drug susceptibility testing (DST). TB culture and sensitivity testing done at a higher level lab will help detect whether the TB is resistant to first-line anti-TB drugs.

Culture and sensitivity of TB is particularly important:

- in HIV-infected persons who may have AFB smear-negative TB; and

- when a patient’s clinical course may suggest resistance to the first-line TB drug regimen. Decisions on when a patient’s sputum specimen should be referred for culture and DST are usually made by the district TB clinician.
INSTRUCTIONS FOR SPUTUM COLLECTION

STEP 1: Be sure to list the TB suspect’s name and address in the register of TB suspects (see Annex).

Suitable specimen containers
Use clean, wide-mouthed, leak-proof specimen containers. Single-use disposable plastic containers (50 ml capacity) are best. One type of preferred container is a rigid, wide-mouthed screw-capped container made of unbreakable transparent plastic that is easy to dispose of by burning. Its screw cap can be tightly sealed to prevent leakage and drying of the sample. Another type of container is a screw-capped, heavy glass container, such as a Universal bottle. This type of container can be used again after it is disinfected in an autoclave for 30 minutes at 121°C and cleaned carefully. On the side of the container write your centre identification number or code, the TB suspect or patient’s name or identification number. Do not write this information on the lid, but on the side of the container.

The number and timing of sputum specimen collection
To ensure the best detection of the TB germ in sputum, collect and process at least two sputum specimens. Insert the country’s national tuberculosis programme’s specific guidelines here.

■ For outpatients, collect one sample when the person first visits your centre with signs or symptoms of illness. This is known as the “spot” specimen.

■ Give the patient a second sputum container for collection the next morning at home. This “early morning” specimen should be collected by the patient as soon as they wake up. Tell the patient to bring the morning specimen to the lab the same day they collect it. Early morning specimens have the highest yield of AFB. If the patient cannot return the next day, collect the second specimen during the patient first visit.

■ If a third specimen is to be collected, it should be done as a spot specimen when the patient delivers their early morning specimen.
**Explain to each TB patient:**

- the importance of checking sputum to diagnose TB or to follow-up on treatment;
- how to open and close the containers;
- how to produce good sputum: breathing in deeply and breathing out, followed by cough from as deep inside the chest as possible - it is important to collect sputum and not saliva;
- how to keep the outside of the container clean: carefully spitting sputum in the container and then closing it;
- the importance of collecting the sputum sample outside in the open air or in a well-ventilated, private place;
- how to collect and safely deliver the morning sputum to the centre lab;
- the need to collect at least two samples to obtain a correct diagnosis.

When the TB suspect returns with the sputum sample, take a good look at it. A good specimen should be about 3–5 ml. If there is not enough sputum, ask the TB suspect to add some more. It is usually thick and mucoid (like mucus). It may be fluid and contain pieces of purulent (pus) material. Colour varies from opaque white to green. Bloody specimens will appear reddish or brown. Clear saliva or nasal discharge is not a good TB specimen.

- When the second (or third) sample is collected, inform the patient when to come back for results.
- Check that the lid is tight, put each sputum container in its own plastic bag or wrap it in newspaper.
- Store the sample in a cool place.
- Wash your hands.
- Complete the Request for Sputum Smear Microscopy Examination form (see Annex).
Sputum collection for follow-up of treatment
For patients on treatment, collect follow-up specimens at intervals specified by the national tuberculosis programme (NTP). This usually includes one sputum collection at the end of the intensive phase of treatment, one during the continuation phase, and one at the end of treatment. Early morning sputum is the best specimen.

Safety precautions during sputum specimen collection
TB suspects should be identified early in triage and then sent directly for sputum collection in a well ventilated area. Lab staff are at particularly high risk of contracting TB.

- Never collect sputum in the lab, waiting area, toilets, or reception area.

- All lab staff should be trained in TB infection control – see chapter 5 Infrastructure chapter.

You can take some simple steps to lower TB risk at your centre:

STEP 1: You and other centre staff must tell patients to cover their mouths when coughing before teaching them how to produce sputum.

STEP 2: Have them collect a sputum specimen outside to allow aerosols to be diluted and exposed to the ultraviolet radiation of direct sunlight. Sputum collection involves the greatest risk of infection to lab staff as well as other patients, and must be done in the open air and away from other people.

Storage and transport of sputum specimens
After specimen collection, make sure that the container lid is closed tight and store all the sputum specimens in a cool, dry place. If your centre does not offer sputum smear microscopy, all the sputum specimens should be sent to the district hospital lab as soon as is possible depending on your shipping arrangements. Each sputum specimen should be kept in a separate plastic bag or wrapped in newspaper. You should include a Request for Sputum Smear Microscopy Examination form (see Annex 8.3) for each specimen and a list of all the specimens contained in the transport box.
Before you deliver the specimens to the district hospital lab, make sure that:
- The total number of sputum containers in the box corresponds to your list and the Request for Sputum Smear Microscopy Examination forms.
- The identification number on each sputum container corresponds to that on the accompanying list and to the Request for Sputum Smear Microscopy Examination forms.
- The accompanying Request for Sputum Smear Microscopy Examination forms contain the requested information for each of the TB suspects.
- Date the list of specimens.
- Put the list and Request for Sputum Smear Microscopy Examination forms in an envelope which will be attached to the outside of the transport box.
- If screw-capped heavy glass containers are used for sputum collection, use custom-made boxes made of metal, wood, or styrofoam to send them. These are built to keep the containers from breaking when you send them.
- Sputum specimens should be delivered to the district hospital lab within three-four days of collection. If possible specimens should be refrigerated, before you deliver them. Contaminating bacteria do not affect the acid-fastness of mycobacteria, but may make the sputum more liquid, making smear preparation difficult and reading of slides unreliable.

**Sputum collection for culture and drug susceptibility testing**
Sputum that is sent to a district hospital lab for culture and drug susceptibility testing should be packed correctly, refrigerated if possible, and sent to the lab immediately. Be careful and follow the safety tips when packing and delivering specimens to the district hospital lab as they may contain drug-resistant TB.

**SMEAR PREPARATION AND STAINING**

The quality of work in AFB diagnostic microscopy depends on a number of factors. These include specimen collection, the quality of reagents, the staining technique, the reading of the smear, the reporting and recording of results, and the training of the technician. However, collecting a good quality specimen and obtaining a good smear are critical, since the quality of the rest of the procedure depends upon these two factors. Smear preparation must be done carefully and with attention to detail.
Preparing sputum smears

Numbering the slides

- Select new, clean, grease-free, unscratched slides that have no fingerprints on them.
- Using a pencil, record the patient identification number in the lab register and order number of the sputum specimen on the frosted end of the slide. If plain unfrosted slides need to be used, labelling is best done using a diamond pencil.
- Ensure that the number on each slide corresponds to the number on the specimen container.

Sputum smearing

**STEP 1**: Using the end of an applicator stick or wire loop, select and pick up sputum.

**STEP 2**: Prepare the smear in an oval shape in the centre of the slide. The smear size should be 2–3 cm in length and 1–2 cm wide, which will allow 100–150 fields to be counted in one smear length.

**STEP 3**: For good spreading of sputum, firmly press the stick perpendicular to the slide, and move in small concentric circles or coil-like patterns.

**STEP 4**: Throw away the used stick in a trash container with a disinfectant. Also be sure to:

- Use a new stick for each specimen.
- If a wire loop is used instead of a broken stick, dip the wire loop in a sand-alcohol bottle. Remove the excess sputum from the wire loop by moving it up and down. After each smear is completed, heat the wire loop in a flame until red-hot.
- Thorough spreading of the sputum is very important; it should be not too thick or too thin. Prior to staining, hold the smear about 4-5 cm over a piece of printed paper. If letters cannot be read, it is too thick.
■ Air drying of smear

■ Allow the smear to air dry completely at room temperature, and do not dry smears in direct sunlight or over a flame.

■ Heat fix smear

■ After the slide is completely dry, use forceps to hold the slide upwards and pass it over the flame two–three times for about two–three seconds each time. Do not heat the slide for too long or keep it stationary over the flame, or else the slide will be scorched. Allow the smear to air dry completely at room temperature, and do not dry smears.

**Staining with Ziehl-Neelsen carbol fuchsin solution - on AFB smear training (see the job aid below)**

**STEP 1:** Arrange the slides in serial order on the staining bridge, with the smear side up.

**STEP 2:** Flood the slides completely with filtered carbol fuchsin stain (consider substituting 1% basic fuchsin stain).

**STEP 3:** Gently heat for five to 10 minutes or more (as long as the stain does not dry on the smear).

**STEP 4:** Rinse with water (preferable distilled water since tap water may contain environmental mycobacteria) and drain.

**STEP 5:** Put on decolourizing solution for three minutes (25% sulphuric acid or acid alcohol (more costly)).

**STEP 6:** Rinse with water and drain.

**STEP 7:** Put on 0.1% methylene blue counter stain for NOT MORE THAN one minute.

**STEP 8:** Rinse slides with water and drain (rinsing water must be clean, and, if re-staining is required for quality assessment the water must be as free of environmental mycobacteria as possible). Use clean water from a beaker that can be thoroughly cleaned.

**STEP 9:** Air dry the slides on a slide rack.
Evaluating smears
Spend time looking at good and bad smears. Bad smears can lead to false results. A good stained smear using ZN shows strong red AFB against a weak blue background.

See the bench aid for quality issues of AFB smear preparation and staining techniques

Report qualitative and semi-quantitative results
The information on the number of bacilli found is very important because it relates to how infected the patient is, as well as to the severity of the infection. For this reason, the report of the results of sputum smear microscopy must be not only qualitative (whether AFB are present or not), but also semi-quantitative (give some indication of the number of AFB present). You should take at least five minutes to read 100 fields (10 minutes is optimal).

International Union Against Tuberculosis and Lung Disease (IUATLD - recommended grading (AFB per field)

<table>
<thead>
<tr>
<th>AFB count:</th>
<th>Recording/reporting:</th>
</tr>
</thead>
<tbody>
<tr>
<td>No AFB in at least 100 fields*</td>
<td>0/negative</td>
</tr>
<tr>
<td>1 to 9 AFB per 100 fields†</td>
<td>Specify the actual number of AFB per 100 fields†</td>
</tr>
<tr>
<td>10 to 99 AFB per 100 fields‡</td>
<td>+</td>
</tr>
<tr>
<td>1 to 10 AFB per field in at least 50 fields†</td>
<td>++</td>
</tr>
<tr>
<td>&gt;10 AFB per field in at least 20 fields‡</td>
<td>+++</td>
</tr>
</tbody>
</table>

* A finding of 1 to 3 bacilli in 100 fields does not correlate well with culture positivity. The interpretation of the significance of this result should be left to the NTP and not to the microscopist. It is recommended that a new smear be prepared from the same sputum specimen and be re-examined.
† The reporting of actual AFB counts is recommended to allow a competent authority to determine whether the number fits the TB case definition of the NTP.
‡ In practice most microscopists read a few fields and confirm the finding by a quick visual scan of the remaining fields.

Specific quality assurance issues

Internal quality assurance: checking quality of the stain

- Check the quality of your stain by staining one positive sputum smear and assessing the quality of slide.
Do this on each day that you do the test.

Prepare extra slides of positive slides periodically to use for this purpose.

**Quality control**

- Use external quality control specimen (smears from both positives and negatives).

- Store these controls appropriately.

- Your standardized lab logbook should contain space for recording QC results. These should then be transferred to a QC logbook for quick review of data.

**External quality assessment. (EQA)**

- Proficiency testing

  - Periodically, you will receive a panel of slides to assess how well you are reading and counting. This will usually be a minimum of 20 slides, including negative slides, positive slides, and slides with different counts. A combination of stained and unstained slides will allow the testing of staining capability. The panel of slides will come from the district hospital lab or national reference lab. You will test the panel of specimens and report the results. Your performance on testing this panel will be compared to that of other testing sites, and you will receive feedback on how well you are doing the test.

- Slide validation at a higher level by random rechecking

  - If your national TB programme uses this quality assessment method, save your positive slides and some of your negative slides as instructed.

  - Store these in secure slide boxes protected from excess heat and/or humidity.

  - When asked send these to be blindly rechecked by a higher level lab.
AFB SMear STaining

1. Always use new, grease-free, and clean slides. Correctly label slides with stylus or lead pencil.
2. Fish out yellowish portion from sputum container and place on slides with the rough end of the stick.
3. Spread material evenly in an approximate area of 2mm X 2mm so that newsprint is noticeable on drying.
4. Air dry smear completely and then heat fix smear in a flame.
5. Place slides on the staining rack without touching each other. Always add Positive and Negative control slides.
6. Cover slides with freshly filtered carbol fuchsin.
7. Heat gently with a torch until steam rises from the slides. Steam for five minutes.
8. Wash gently with water.
9. Drain the water.
10. Wash thoroughly with water. If slide is not decolorized properly repeat step 10 for additional 1-3 minutes. Rinse thoroughly with water.
11. Drain the water.
12. Cover slides with decolorizing solution for three minutes.
13. Cover with counter stain: Methylene blue for one minute.
14. Drain the counter stain.
15. Wash with water. Wipe the back side of slides with tissue paper.
16. Air dry the slides in a rack.
17. View the smear under oil immersion. AFB: Fine, red rods against blue background.

Joint effort of: IUATLD; WHO; CDC; RIT.
8.17 CD4: BLOOD COLLECTION AND SEND-OUT

Blood Collection

- Label the tube correctly with the date of birth (DOB).

- Collect in a vacuum tube containing spray coated K2 ethylenediamine tetraacetic acid (EDTA—an anticoagulant and preservative plastic) or K3 EDTA (glass) or a CD4 stabilization tube (if the time to testing will be >72 hours).

- Draw this specimen last if drawing multiple tubes. Fill the tube until no additional blood can be drawn in.

- Use paediatric tubes for collecting specimens from infants and young children.

- Mix the tube well right after collection to stop blood clots from forming.

- Keep the tube at room temperature (20-25°C) until it is transported to the testing lab.

Shipment

Set up a schedule with the district lab staff on how and when you can send these specimens to the district hospital lab. Remember that testing should be done within 48 hours (preferred), but no later than 72 hours after drawing. Transport the specimen to the testing lab at room temperature (20-25°C).

- See Annex for CD4 Request Form and CD4 logbook

Specific quality assurance issues

Standard operating procedures

- Have concise, clear standard operating procedures (SOPs) in your local language or for those trained to collect and ship CD4 specimens, and interpretation of overall testing results and reporting, etc.
8.18 FULL BLOOD COUNT AND DIFFERENTIAL: BLOOD COLLECTION AND SEND-OUT

See section - on CD4 above.
Testing should be done within 48 hours of collection.

8.19 SPECIMEN TRANSPORT

How to pack and send specimens
Specimens tested at a higher level lab need to be sent in a way that protects them from high or low temperatures and/or humidity. They should be packed to protect both the specimens and the people transporting them. (See SOP, and instructions for specimen transport below.)

Specimen collection and referral for testing off-site
The dried blood spots (DBS) for infant diagnosis, whole blood CD4 counts, full blood counts and differentials, and TB sputum specimens usually need to be sent out for testing.

Specimens should be collected and sent to the district hospital lab on certain days of the week; post a list of dates at your centre. Some specimens can be collected daily and then sent together to the district hospital lab days later. Other specimens need to be collected and brought to the lab on the same day. For specimens that need to be taken to the lab immediately patients should be scheduled to give specimens on the same day of the week that specimens are delivered to the district hospital lab. Scheduling specimen shipments helps reduce the costs and prevents the district hospital lab from receiving too many specimens on any given day.

Complete the table on p.220 with your centre’s information on “Days for collection” and “Days to send and how.” Remember that, in some cases, patients may need to be sent to the district hospital lab for testing.
MANAGEMENT OF SPECIMENS FOR CD4 AND HAEMATOLOGY – REFERRAL TESTING

Purpose
To provide steps to ensure that samples for transport are packaged appropriately to maintain specimen identification, integrity, and biosafety standards.

General

■ Special care must be taken to protect samples from the effects of extreme temperatures and fluctuations.

■ Packaging of specimens for shipment must be designed to minimize breakage.

■ Rough handling of blood specimens may cause haemolysis and compromise test results.

■ Transfer of specimens to the laboratory should occur within as short a time period as possible.

Biosafety

■ Wear gloves and lab coat when handling specimens.

Specimen identification and labelling

■ All specimens sent to a laboratory should be identified with the following:

■ Patient’s first and last name (may be excluded in some cases where protecting the patient’s privacy is a concern)

■ Patient’s medical record or other identification number

■ Patient’s date of birth if known

■ Date and time of collection

■ Collector’s initials
Requisition forms (see samples in Annex 8)

- Information that is identical to that on the sample tube should be on the requisition form. In addition, other information should be included on the form, such as:
  - Requesting physician or other clinical staff’s name
  - Centre name
  - Type of specimen
  - Specific tests being requested.

Keep shipping documents separate from the inner box containing the specimens in case of leaks from breakage or spills

Primary containers

- EDTA anticoagulated specimens drawn for haematology and CD4 testing should never be centrifuged.

- If smears are to be included as part of the requested testing, two unstained whole blood smears should be prepared within one hour of sample collection.
<table>
<thead>
<tr>
<th>Test</th>
<th>Specimen</th>
<th>Optimal temperature</th>
<th>Optimal time to be tested</th>
<th>Packing requirements</th>
<th>Health centre to fill in</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4 count and/or percentage</td>
<td>1 mL EDTA whole blood or CD4 stabilization tube, minimum of 250μL-500μL (paediatric sample)</td>
<td>20-25°C</td>
<td>0-48-72 hours (using flow cytometry)</td>
<td>Maintain a temperature of 20-25°C</td>
<td></td>
</tr>
<tr>
<td>TB sputum</td>
<td></td>
<td>4°C</td>
<td>0-4 days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DBS for infant diagnosis</td>
<td>Dried whole blood spots</td>
<td>Dried whole blood spots</td>
<td>0-4 weeks</td>
<td>Pack in an airtight ziplock bag with desiccant (silica sachet) and humidity indicator card</td>
<td></td>
</tr>
<tr>
<td>Full blood count and differential</td>
<td>1 mL EDTA whole blood, minimum of 250μL-500μL (paediatric sample)</td>
<td>20-25°C</td>
<td>0-24-48 hours*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Full blood count and differential</td>
<td>1 mL EDTA whole blood, minimum of 250μL-500μL (paediatric sample)</td>
<td>20-25°C</td>
<td>0-24-48 hours*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Dependent on the haematology instrument used.
Outer Shipping Container

Materials
1. **Figure- 1: Transport Container Option A**
   - Recycled Styrofoam or molded foam lined corrugated cardboard box

2. **Figure- 2: Transport Container Option B**
   - Plastic picnic type cooler

3. **Figure- 3: Transport Container Option C**
   - In-house made insulated transport container

The foam lined outer shipping container used for option A above can be obtained by recycling various transport boxes used by commercial suppliers to ship refrigerated or frozen items. These containers generally provide a high degree of insulating capability and are usually the best choice of the three listed options.

4. **Figure-4:**
   - Hard plastic containers filled with water and frozen (“ice packs”), or 8-10 lbs cubed melting wet ice in plastic bags to cover the bottom of the box.

5. **Figure- 5:**
   - Styrofoam sheets, soft foam, or newspapers.

6. **Figure- 6:**
   - Thick gauge sealed plastic bags (e.g., 6 mil polypropylene bags) approx 8 X 8” filled with water

The thick gauge sealed plastic bags, or unused blood collection bags from blood banks are examples of other items that can be filled with water and frozen and used as ice packs in place of the hard plastic containers. Likewise, these can be filled with room temperature water and used for room temperature transport of specimens as described below. These bags can be used repeatedly once they are made.

7. Thin gauge (e.g., 0.5 mil or thicker) plastic garbage type bags
8. Absorbent material such as paper towels.
9. **Figure- 7:**
    - Perforated cardboard tray
10. **Figure- 8:**
    - Internal bin box
Assembly of In-house Made Shipping Container

1. Obtain a medium sized sturdy corrugated cardboard box (e.g., approximately 16"L X 14"W X 13"D or 41cm X 36cm X 33cm)

2. Figure-9:
   - Find or make an appropriately sized inner box, or "bin" box (e.g., approximately 12"L X 10"W X 11"D or 31cm X 25cm X 28cm). Insert the bin box inside the outer cardboard box, so that a rectangular channel approximately 1.5" (4cm) wide or wider is formed between the two boxes.

3. Fill the channel between the boxes with:
   a) Styrofoam sheets cut to fit (preferred choice)
   b) Soft foam (second choice)
   c) Crumpled newspaper. (third choice)

4. Figure-10:
   - If using newspaper, ensure that the crumpled paper completely fills the channel, but is not packed too tightly. The idea is to pack the newspaper firmly but to allow air cavities within the channel to allow for better insulation capabilities.

5. Figure-10:
   - Cover the bottom of the bin box with approximately 2" (5cm) of newspaper.
Packing CD4 and haematology specimens for room temperature (20-25°C) transport

Obtain transport container Option A, Option B, or Option C

Figure 21
Place 4-6 thick gauge sealed plastic bags, or unused blood collection bags filled with water that has been allowed to stabilize to 20-25°C inside the bottom of the container. The water-filled bags will serve as a heat sink to help stabilize the interior temperature of the container to the desired 20-25°C

If the ambient temperature is > 25°C, then it is desirable to have the temperature of the water filled bags closer to 20°C; if the ambient temperature is below 20°C, then it is desirable that they closer to 25°C.

Figure 22
If an insulated cover is available, use this to close off the interior of the container; alternatively, insert a large foam plug, or lay newspaper on top of the interior of the box to form an insulating barrier.

Allow the interior of the container to cool for approximately 30 minutes

Place the rack in two plastic bags; one inside the other. Lay sufficient paper towels over the top of the tubes to absorb potential spills. Secure the double bags around the rack and tie securely.

Figures 23 & 24
Figures 25 & 26:
Open lid of transport container, and remove the insulated cover (if available), foam plug or newspaper.
Remove half of the water-filled bags from the interior of the container.
Insert bagged samples into container on top of the water-filled bags.

Figures 27 & 28:
Replace the remaining water-filled bags in a way that ensures that they surround the bagged samples. This insulates the specimens and keeps them in the interior of the container to secure them for transport.
Place requisition slips and any other shipping documents in a sealed plastic bag, and place this into the container.

Figure 29:
Insert the foam plug or enough newspapers to form an insulating barrier. Top off interior of box with an insulated cover (if available), or additional newspapers for added insulation. Close the outer container.
Packing CD4 and Hematology Specimens for Room Temperature (20-25°C) Transport

1. Obtain transport container Option A, Option B, or Option C
2. Figure- 20:
   - Place 4-6 thick gauge sealed plastic bags, or unused blood collection bags filled with water that has been allowed to stabilize to 20-25°C inside the bottom of the container. The water filled bags will serve as a heat sink to help stabilize the interior temperature of the container to the desired 20-25°C

3. If the ambient temperature is > 25°C, then it is desirable to have the temperature of the water filled bags closer to 20°C; If the ambient temperature is below 20°C, then it is desirable that they are closer to 25°C.
4. Figure- 13:
   - If an insulated cover is available, use this to close off the interior of the container; alternatively, insert a large foam plug or lay newspaper on top of the interior of the box to form an insulating barrier.

5. Allow the interior of the container to cool for approximately 30 minutes
6. Figures 21 & 16:
   - Place sample tubes to be shipped in a test tube rack. Double bag the rack containing the test tubes with plastic garbage type bags, and lay sufficient paper towels over the top of the tubes to absorb potential spills. Secure the double bags around the rack containing the specimens, and tie off.
8. **Figure- 16-18:**

- Insert bagged samples into container on top of cardboard tray. Wedge firmly with soft foam, bubble plastic, or newspapers to secure contents during transport.

9. Place requisition slips and any other shipping documents in a sealed plastic bag, and place into the container.

10. **Figure- 13 and 19:**

Insert foam plug or enough newspapers to form an insulating barrier. Top off interior of box with insulated cover (if available), or additional newspapers for added insulation. Close outer container.
Drying and Packaging Dried Blood Spot (DBS) Samples

1. Leave DBS on a drying rack in a clean, dry, protected area for at least 4 hours or overnight. Keep lab request forms with DBS cards.

2. Wrap the individual DBS card with a glassine paper so that DBS cards will not have direct contact with each other. Insert up to 10 wrapped cards into a special sealable plastic bag.

3. Add 10 desiccant packets to each bag.

4. Add at least one humidity card per bag. Gently press the bag to remove most of the air before sealing.

5. Use the specimen delivery checklist to check if you have a lab form for each DBS.
   - Place the bag of DBS, all the DBS DNA PCR lab forms and the specimen delivery checklist into a large envelope.
   - Label the envelope with:
     - Name of collection site (clinic)
     - Name of person delivering specimen
     - Date you are sending samples
   - Place the envelope in designated area to be picked up for the laboratory.
8.20 HOW TO SET UP A LAB

Any lab that does clinical testing needs a certain amount of space. Your lab space should be at least three meters by three meters (nine square meters). This does not include space for urine collection and TB sputum collection (both should be located outside of the lab). See the table below to create a lab space.

<table>
<thead>
<tr>
<th>Minimum amount of lab space required</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Function of area</strong></td>
</tr>
<tr>
<td>Blood collection</td>
</tr>
<tr>
<td>Finger or heel prick</td>
</tr>
<tr>
<td>Urine collection</td>
</tr>
<tr>
<td>Sputum collection for TB</td>
</tr>
<tr>
<td>Specimen labelling, preparation, results</td>
</tr>
<tr>
<td>Analysis of specimens process onsite</td>
</tr>
<tr>
<td>-------------------------------------</td>
</tr>
<tr>
<td>Store (storage area)</td>
</tr>
</tbody>
</table>

**SAMPLE PLAN OF LAB SPACE**

![Sample Plan of Lab Space Diagram]
8.21 HUMAN RESOURCES

Your centre staff will do their own lab testing and also prepare specimens to send out to the district hospital lab for testing.

Lab testing done by lab staff (centre staff who are trained to do lab work, testing, and specimen collection) requires supervision and training in quality assurance. Lab staff will need training in how to do all of the tests correctly, while monitoring the testing results and direct observation by a knowledgeable person is also necessary. Lab staff from a larger centre lab or a district hospital lab may be a source for this supervision. Supervision should be done in a supportive manner, and viewed as an opportunity to promote good lab practices.

Lab testing at your centre using simple tests can be done by a nurse, a staff person, by a person living with HIV (PLHIV) who is also a centre staff member, or other person trained to do tests. Some tests that are more complex or require more experience to be done correctly (such as TB or malaria smear microscopy), may need staff with special training. In a large centre, it is best to assign specific staff to work in the lab and to do all the testing.

You also need to make sure that you have plans for initial training and certification of centre staff, ongoing training, supervision, job progression, and incentives for retention. – (see also Chapter 9).
8.22 EQUIPMENT MAINTENANCE

Microscope
A microscope that functions very well is necessary for quality TB smear or malaria microscopy. Proper handling and maintenance of the microscope is essential to prolong its useful life. The following points should be observed:

- Use a high-quality microscope with an electric light source (if electricity is available). Microscope mirrors for use with daylight to provide lighting may still be needed, even if electricity is available part of the time.

- Binocular microscopes (with two ocular lenses) are best, but monocular microscopes (with only one ocular lens) will work fine if you have very few smears to read.

- Store the microscope in a dry, dust free place where it will not be shaken or moved when you are not using it. Ensure that all openings meant to hold objective lenses or eyepieces are closed (with a lens cap, a plastic plug, or a piece of tape). In dry countries, store the microscope under a dust cover or in its special carrying box when you are not using it.

- If theft is a problem, keep the microscope in a strong cupboard with a lock.

- In humid climates, dry the lenses daily. You can do this at night by mounting a 20-40 Watt bulb in the cupboard or compartment where the microscope is kept. You should put a few small holes near the bottom of the compartment and put others diagonally opposite at the top to allow air to circulate. Do not use the dust cover in this case. If you do not have electricity at night, you will need to use silica gel or some other drying agent. You should keep the microscope in as small and enclosed space as possible. This can be its box or under a well-sealed cover. Put a small amount of the gel in an open container on the stage of the microscope before putting it away. Usually the silica gel will be saturated after only one night. You have to replace the silica gel daily. You can regenerate the gel by heating it in an oven or pan.

- Avoid exposing the microscope to direct sunlight, moisture, and humidity.

- Clean the microscope with lens paper before and after use. Gently wipe the objective at the end of each reading session with soft tissue paper or lens
cleaning paper to remove excess oil. For a more thorough cleaning, use manufacturer-recommended fluids or a mixture of ethyl ether and alcohol (80/20). Never use xylene to clean any part of the microscope.

■ Wipe the surface of the oil immersion lens with a piece of clean cotton before and after use. Do not use alcohol for cleaning lenses.

■ For oil immersion lens, use a non-drying synthetic oil of medium viscosity (refractive index > 1.5) to ensure long life for the objective lens. Do not use cedar wood or xylene-diluted oils.

■ Never touch the oil immersion lens to the smear.

■ Use the fine focusing knob only while using the oil immersion lens.

■ Keep at least one spare bulb at your centre. Other spare parts are kept at the higher level lab.

■ Keep a record of any maintenance that you do on your microscope in a maintenance log.

■ Microscope troubleshooting: If you have a loose stage or stage-clamp, follow manufacturer’s directions to fix it or contact higher level lab staff for advice.

If the view is dark or unclear: A clear view can be obtained while the light is good and all parts are properly adjusted. Inspect the eyepiece tube(s) for dirt and/or fungus. Take the 100X objective and the eyepieces off. Align the empty objective opening over the lighted field. Look down the tube and check the prisms inside the tube for fungal masses or filaments or other dirt. If these are absolutely clean, inspect the objective and eyepieces by holding them reversed and against the light. If nothing is obvious, reinsert the objective and look down the tube again. This may show more clearly any dirt in it. Clean away any external dirt with a microscope cleaning solution.
8.23. TRAINING MATERIALS

This listing is primarily of WHO developed or adapted training materials. Additional materials will also be available.


- How to use a malaria rapid diagnostic test (RDT): A guide for training CHWs and other health workers. 2006. The Quality Assurance Project (QAP) and the World Health Organization (WHO), Bethesda, MD, and Geneva.
