

Module 7: Microscopy

Purpose To provide participants with the knowledge and skills to accurately read AFB smears for the diagnosis of TB.

Pre-requisite Modules None

Module Time 3 hours

Learning Objectives At the end of this module, participants will be able to

- Describe the method for observing 100 high power fields
- Correctly use microscope objectives
- Recognize the appearance of AFB in a stained smear
- Appropriately quantify report results in the Laboratory Register
- Describe the procedure for storing slides for External Quality Assurance (EQA).

Module Overview

Step	Time	Activity / Method	Content	Resources Needed
1	10 min	Presentation	Introduction to Module	Slides 1–4
2	30 min	Presentation	Overview of Microscopy	Slides 5-17
3	10 min	Presentation	Storage of smears	Slides 18-19
4	10 min	Presentation	Summary	Slide 20
5	2 hrs	Laboratory Practical	Laboratory Practical Session #5: Demonstration of microscope and Microscopy	One microscope per two participants

Material and Equipment Checklists

- PowerPoint slides
- Overhead projector or computer with LCD projector
- Microscopy laboratory with at least one microscope per two participants
- Supplies of immersion oil and lens tissue
- A result recording sheet per participant (Use or adapt from Appendix 2)

Teaching Guide

Slide Number	Teaching Points
1	<p><u>Module 7 Microscopy</u></p> <p>DISPLAY this slide before you begin the module.</p>
2	<p><u>Learning Objectives</u></p> <p>STATE the objectives on the slide.</p>
<p>3</p> <p>Flipchart</p> 	<p><u>Content Overview</u></p> <p>(Suggested format)</p> <p>WRITE the content outline before beginning this session.</p> <p>REFER to flipchart frequently to orient participants to where they are in the module.</p> <p>EXPLAIN that these are the topics that will be covered in this module.</p>
4	<p><u>Required Materials</u></p> <p>STATE each item in the checklist.</p> <p>ASK participants why they should use immersion oil. EXPLAIN immersion oil is used with the 100X objective in order to closely examine the smear.</p> <p>ASK participants why they should not use solvents to clean microscope objectives. EXPLAIN that the solvents damage the microscopes by destroying the cement which holds the lens in place. Lens paper or toilet tissue is adequate for cleaning the objectives at the end of each day</p> <p>ASK participants why a red ink pen should be used. EXPLAIN that marking the register with a red pen makes it easier to count all positive smears when reporting cases.</p>

Slide Number	Teaching Points
5	<p><u>Overview of Microscopy</u></p> <p>EXPLAIN the first step in examining any smear is to use a good quality binocular microscope.</p> <ul style="list-style-type: none"> • The microscope must be equipped with an electrical light source or mirror, 100X objective, 8–10X eyepieces, and a mechanical stage. <p>ADD the following points:</p> <ul style="list-style-type: none"> ▪ At least 100 high power fields (HPF) should be examined before reporting a negative result. Examining fewer than 100 HPF may create a false negative result. • Follow guidelines for grading and interpreting smear results <p>EMPHASIZE that all smears should be stored after reading for EQA purposes.</p>
6	<p><u>Parts of Microscope</u></p> <p>FAMILIARIZE with the parts of microscope</p> <p>EXPLAIN the function of each part</p>
7	<p><u>Area for Microscopy</u></p> <p>EMPHASIZE that the microscope should be placed on a table free of vibration, and away from wet areas.</p> <ul style="list-style-type: none"> • Subdued lighting is preferable for reading slides. • If no electricity is available, sunlight can be used as a light source by placing the microscope in front of a window.
8	<p><u>Application of Oil</u></p> <p>EMPHASIZE the importance of checking the smear is facing upwards when placing onto the mechanical stage.</p> <ul style="list-style-type: none"> • Put one drop of immersion oil on the stained smear. • Let it fall freely onto the slide. <p>EMPHASIZE that the oil applicator should never touch the slide. The oil could become contaminated with AFB and could transfer AFBs to a negative smear.</p>

Slide Number	Teaching Points
9	<p><u>Systematic Examination of Smears</u></p> <p>EMPHASIZE that smear examination needs to be performed systematically using the 100x objective</p> <ul style="list-style-type: none"> • Scan smears by moving across the smear in a horizontal direction. • Stop and observe each field before moving onto the next field • Read at least 100 HPF before reporting a negative result. <p>EXPLAIN that for a well made smear approximately 2cm in length in a single pass will be equivalent to 100 HPF. Additional passes maybe required if there is uneven distribution of the smear material or low number of bacilli load.</p>
10	<p><u>Stained Smear-1</u></p> <p>ASK participants if this smear is acceptable or not.</p> <p>HIGHLIGHT that the polymorphs are numerous and evenly distributed across the smear. No squamous epithelial cells are present. These are the features of a properly prepared smear from purulent sputum.</p>
11	<p><u>Stained Smear-2</u></p> <p>ASK participants if this smear is acceptable or not.</p> <p>HIGHLIGHT that the polymorphs are absent and that numerous squamous epithelial cells are present. These are the features of a smear from a poor quality specimen or saliva.</p>
12	<p><u>AFB in Single Arrangement</u></p> <p>EXPLAIN the following points:</p> <ul style="list-style-type: none"> • AFB resemble fine red rods standing out against the blue background. They may be slightly curved, granular, and may occur singly, in pairs, or groups. <p>POINT OUT the single AFB in the slide</p>
13	<p><u>AFB- in Various Arrangements</u></p> <p>EXPLAIN that AFB may be arranged in different ways</p> <p>POINT OUT the “V” forms circled on the slide and also the single AFBs.</p>

Slide Number	Teaching Points
14	<p><u>AFB in Clumps</u></p> <p>EXPLAIN that when AFBs are present in large numbers, they can align with one another to form small clumps or rope like patterns also known as cords.</p> <p>POINT OUT the clumps of tubercle bacilli on the slide.</p>
15	<p>WHO/IUATLD Grading Scale</p> <p>EMPHASIZE the need to be consistent in the use of the reporting scale.</p> <p>EXPLAIN each category of the reporting scale</p>
16	<p><u>Cleaning the Objectives</u></p> <p>STATE the importance of cleaning of oil immersion lens and the possibility of transferring AFB from one slide to another</p> <p>EMPHASIZE that these organic solvents must not be used since they damage objectives permanently.</p> <p>EMPHASIZE the use of soft lens paper or fine tissue paper to avoid damage to the objectives</p>
17	<p><u>Protect the Microscope</u></p> <p>STATE the messages on the slide.</p>
18	<p><u>Remove Oil From Smears</u></p> <p>ASK the participants how they remove the oil from the smears.</p> <p>EXPLAIN that oil can be removed from smears by either gently placing face down on toilet tissue or wrapping in the tissue paper and leaving overnight.</p> <p>EMPHASIZE that the slides should not be blotted or wiped as this may remove the smear.</p>
19	<p><u>Storage of Smears</u></p> <p>EMPHASIZE the need to store all smears following examination for EQA purposes.</p> <ul style="list-style-type: none"> • Store smears in slide boxes in the order they were recorded in the laboratory register. • Ensure that three slide spaces a left for each new diagnostic case

Slide Number	Teaching Points
20	<p><u>Summary</u></p> <p>ASK participants to answer the questions on the slide.</p> <ul style="list-style-type: none"> • How many AFBs are required for a 1+, 2+ and 3+ smear? • How many fields need to be examined? • Which smears must be stored after examination? • When and how are microscope objectives cleaned? <p>ANSWER any questions participants may have.</p>
	<p>Laboratory Practical Session # 5: Demonstration of microscope and Microscopy</p> <p><u>Transition to Laboratory and Microscopy</u></p> <p>EXPLAIN participants will go to the laboratory for a practical session on demonstration and microscopy of smears</p> <p>INSTRUCT the participants to bring their slides from Laboratory Practical session #4</p> <p>Note: It is important that participants use smears that they have prepared themselves in practical session # 3 and stained in practical session # 4.</p> <p>Panel slides stained in practical session # 4 will be examined in module 8.</p> <p>DEMONSTRATE the use of microscope and show appearance of AFB under the microscope</p> <p>INSTRUCT the participants to view their slides and help them in identifying AFB</p> <p>EMPHASIZE that every one must view smears</p> <p>PROVIDE sufficient time so that all the participants can view their own stained smears</p> <p>DEBRIEF the session and answer all the queries or doubts</p>

Slide Number	Teaching Points
 <p>TIPS</p>	<p>Tips for Demonstration</p> <ul style="list-style-type: none"> • Make sure everyone can see the demonstration • Show each step slowly and methodically. Move slowly enough so participants can follow what you are doing – this is slower than normal. • Point to each part of microscope and talk out loud name of the part. • Review the function of each part. • While demonstrating the AFB, it is better to refer the position of AFB to position of a clock. • Point out commonly made mistakes and teach participants how to avoid them. • Repeat steps as necessary • If you repeat the procedure, do exactly the same thing each time.

Laboratory Practical Session # 5: Demonstration of Microscope and Microscopy

Materials and Equipment (per pair of participants)

- One microscope per two participants
- Immersion oil
- Lens or tissue paper
- Prepared and stained smears from laboratory practical session # 4
- Result sheet
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Procedure

1. SHOW the various parts of the microscope and their respective functions
2. DEMONSTRATE the correct way of applying oil
3. DEMONSTRATE the correct way of focusing the smear
4. DEMONSTRATE the correct usage of microscope
5. REVEAL the appearance of AFB
6. SHOW how to write the appearance of AFB in the result sheet
7. DEMONSTRATE the removal of smear from the stage and storage
8. DEMONSTRATE the proper cleaning of oil immersion lens
9. DEMONSTRATE the proper storage of microscope
10. ASK participants to view their smears
11. PROVIDE enough time to view smears
12. ENCOURAGE participants to write their findings in the recording sheet
13. ANSWER any question or confusion over appearance of AFB
14. DEBRIEF at the end of session