

Module 7: Microscopy

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
- Use the WHO/IUATLD grading scale
- Appropriately quantify in the Laboratory Register
- Describe the procedure for storing slides for EQA

Content Overview

- **Required materials**
- **Reading the smear**
- **Appearance of AFB**
- **WHO/IUATLD grading scale**
- **Cleaning of objectives**
- **Storage of smears**

Required Materials

- **Good quality binocular microscope**
- **Immersion oil**
- **Lens paper or fine tissue paper**
- **Laboratory register**
- **Slide boxes**
- **A red and blue pen**

Overview of Microscopy

- Place smear facing upwards onto stage
- Focus using 10x objective
- Apply immersion oil and focus 100x objective
- Examine at least 100 fields
- Quantitate AFB
- Record and report results
- Store all smears

Parts of Microscope



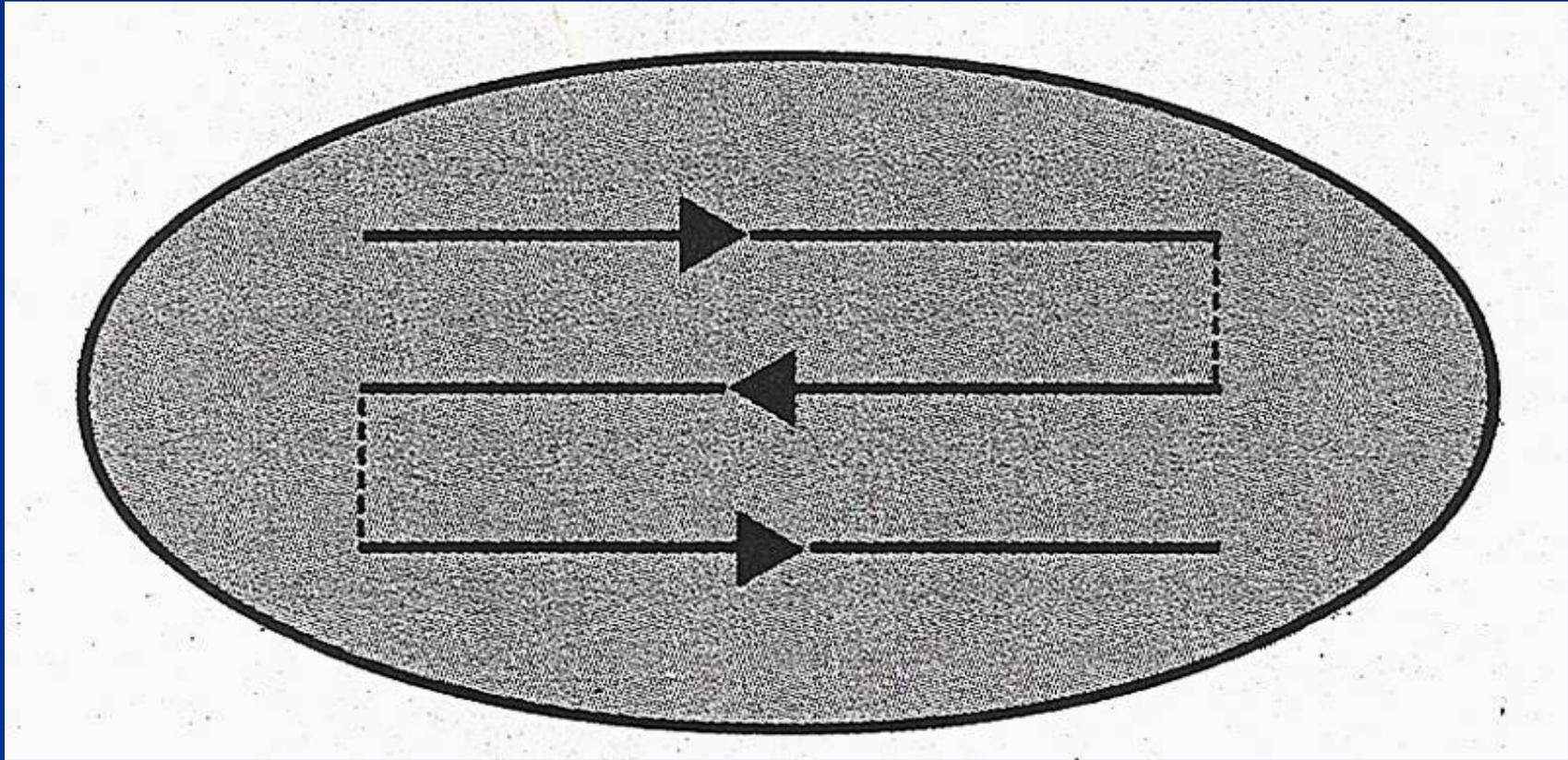
Area for Microscopy

- **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**

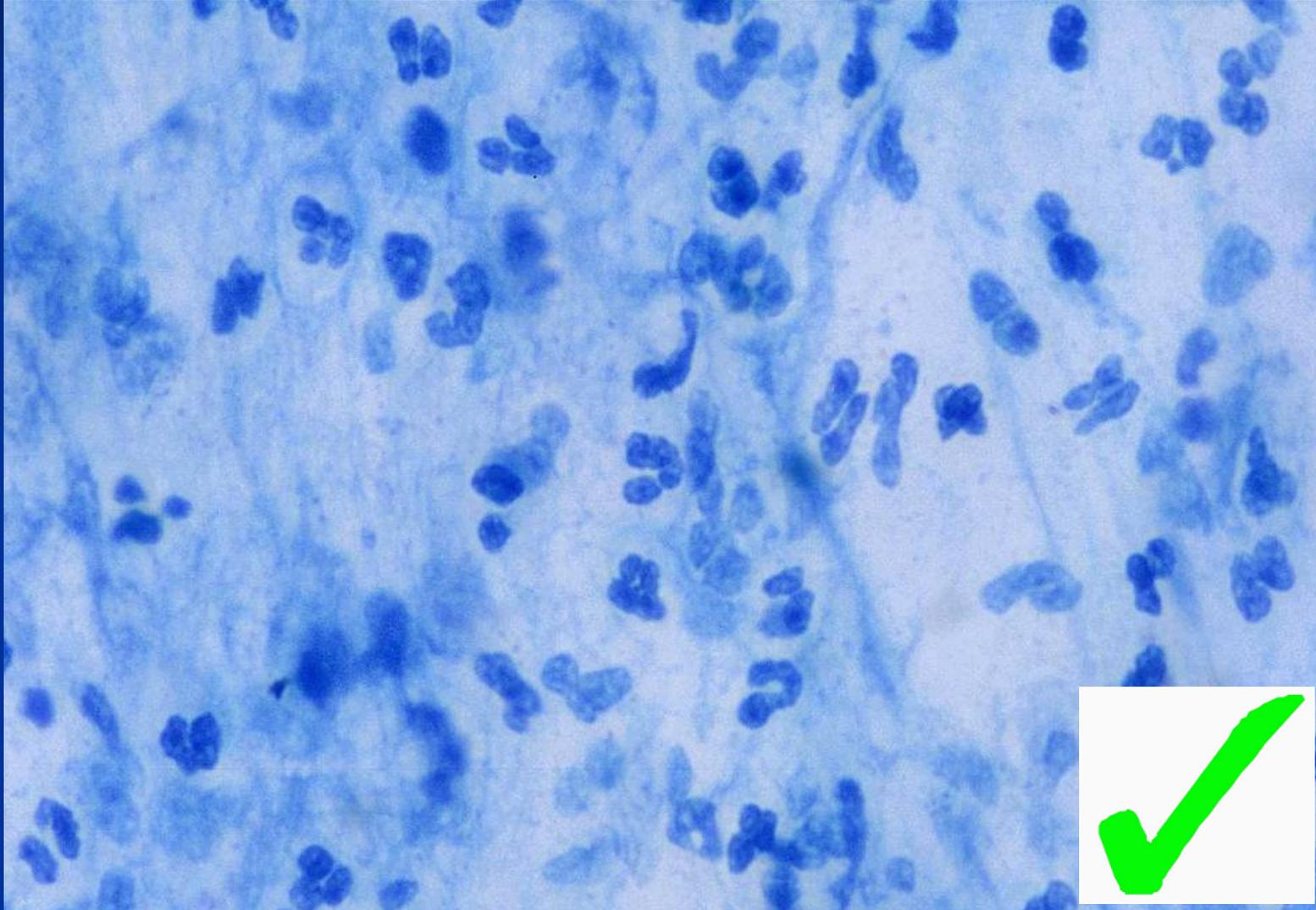
Application of Oil



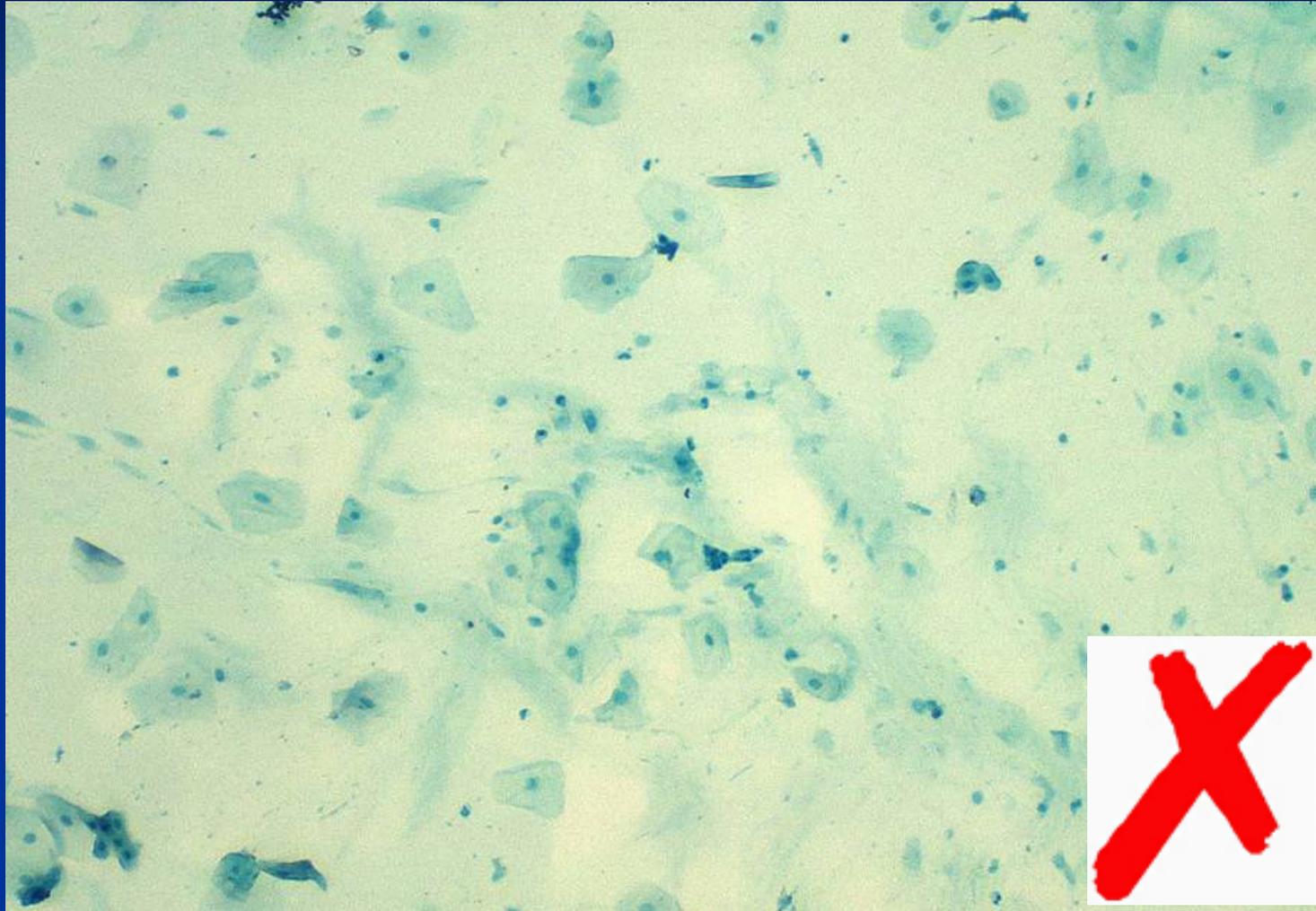
Systematic Examination of Smears



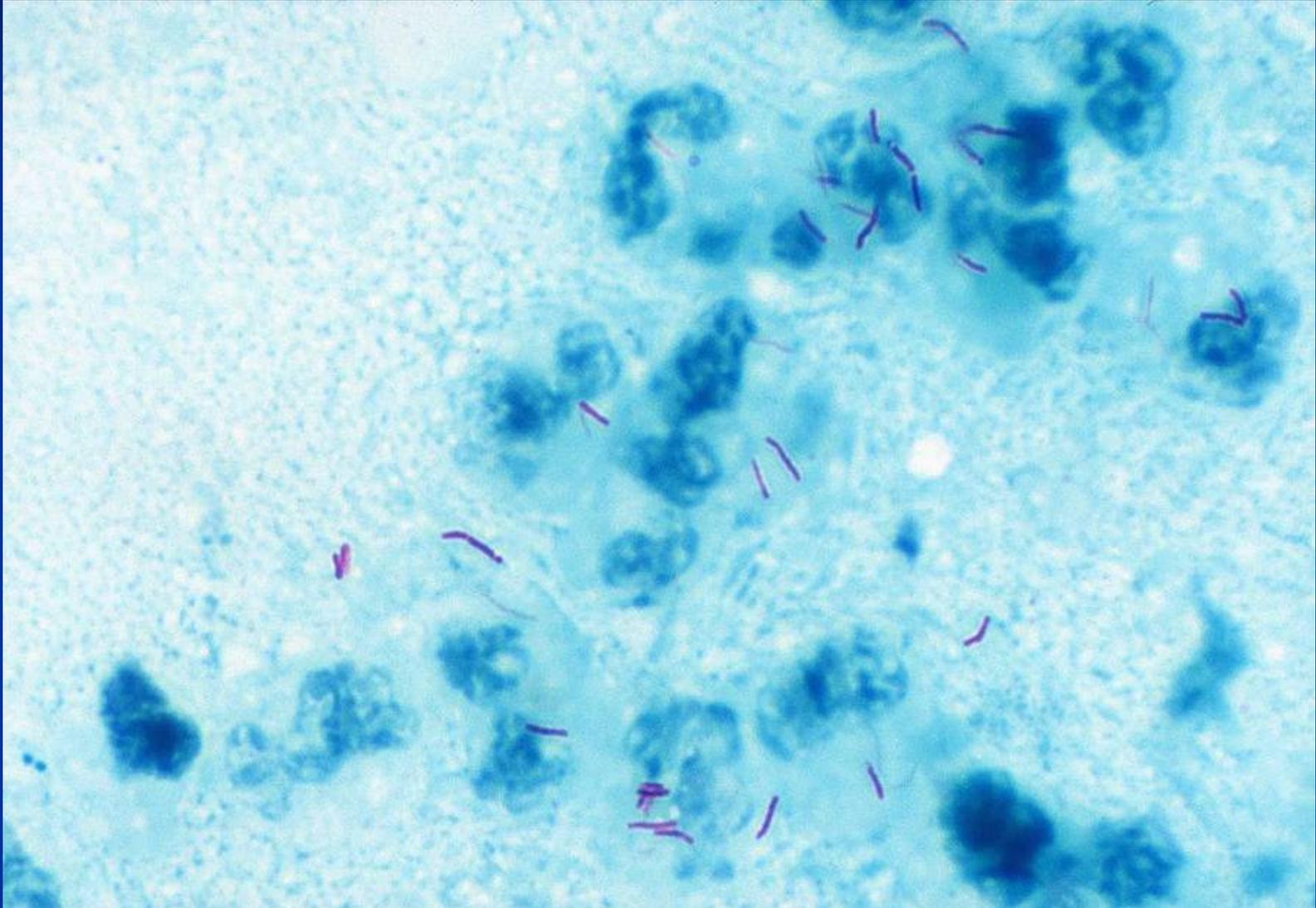
Stained Smear - 1



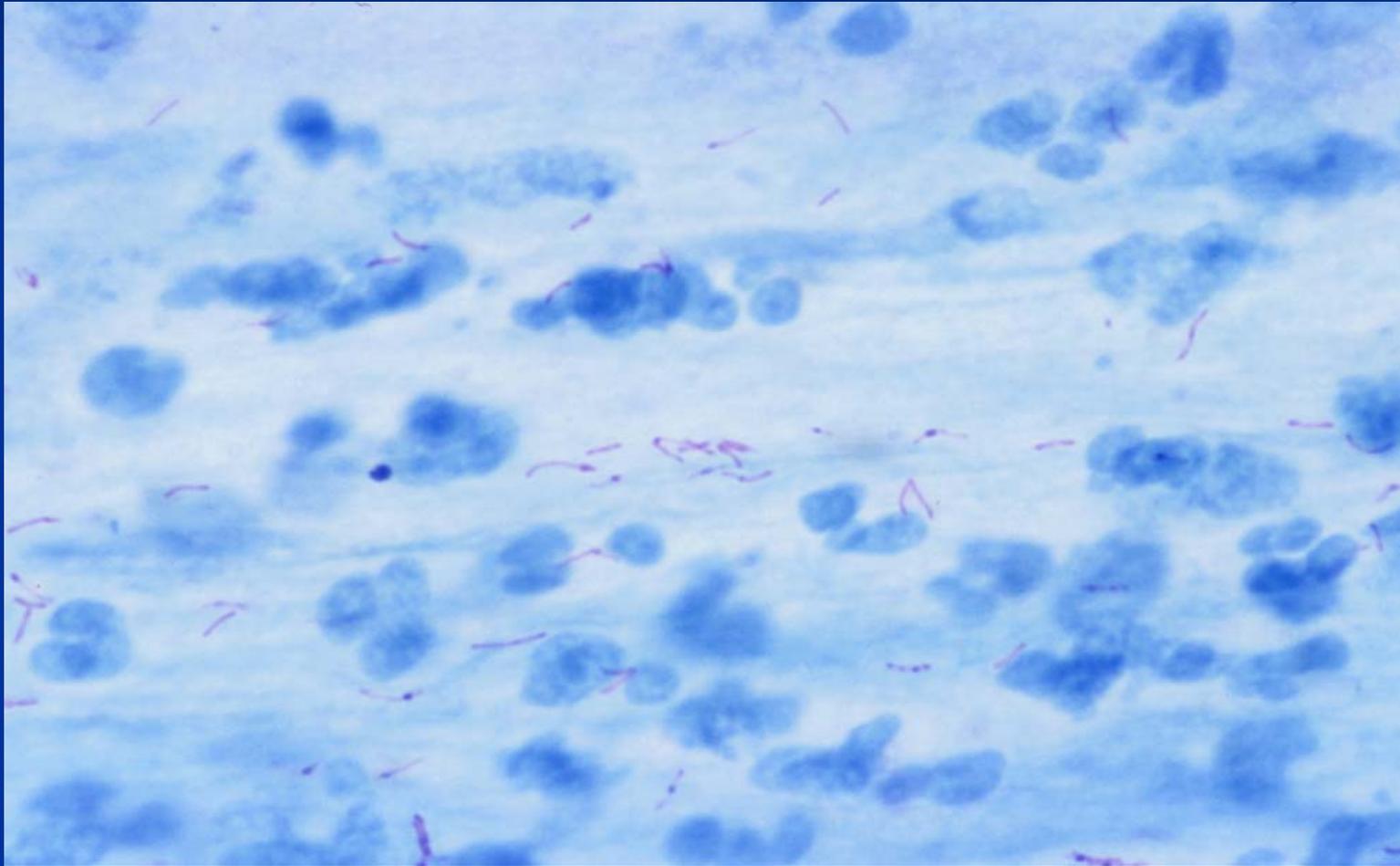
Stained Smear-2



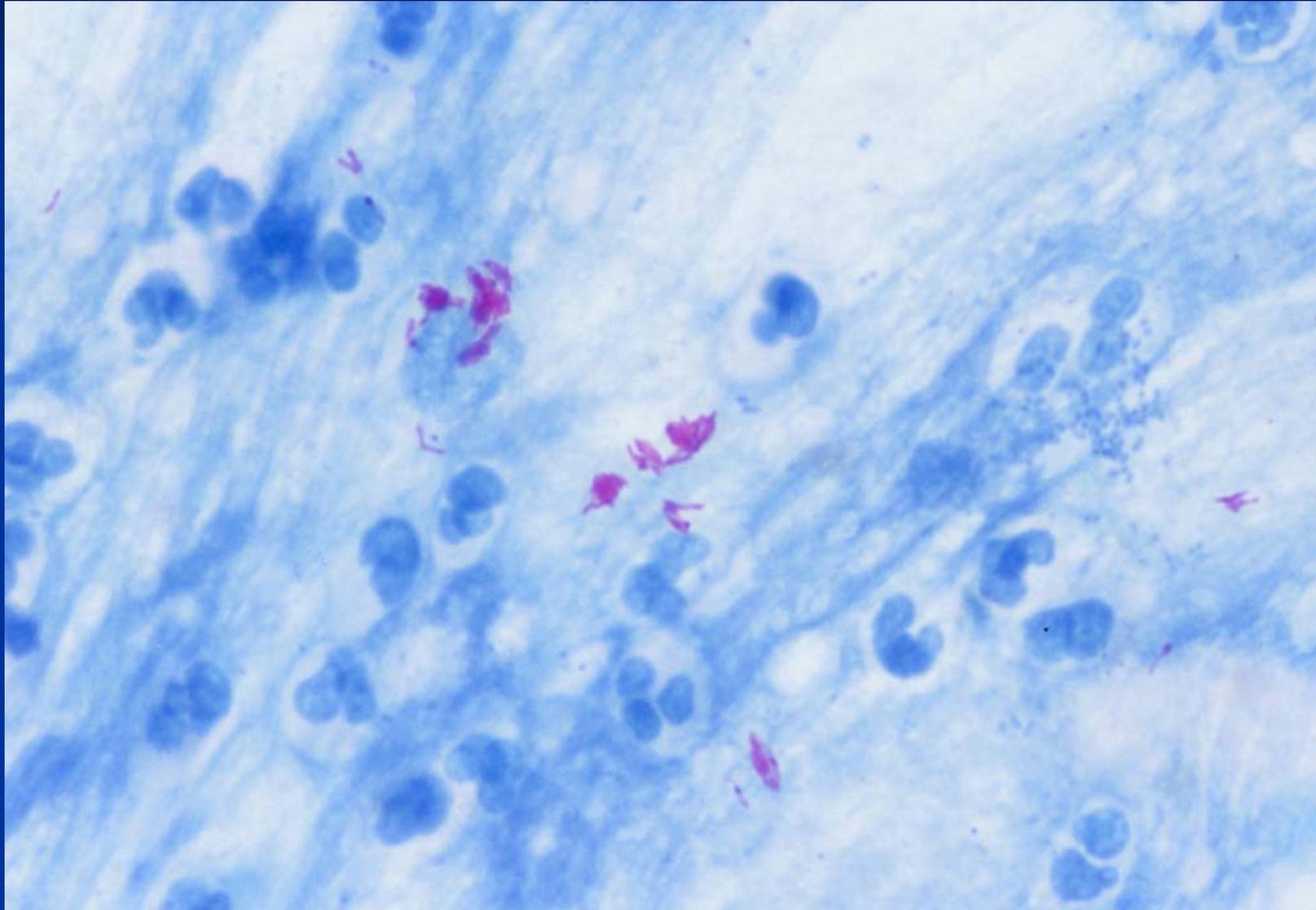
AFB in Single Arrangement



AFB - in Various Arrangements



AFB in Clumps



WHO/IUATLD Grading Scale

Report	AFBs seen
Negative	Number of AFB seen in at least 100 fields
Actual number	1–9 AFB / 100 fields
1+	10–99 AFB / 100 fields
2+	1–10 AFB / field in at least 50 fields
3+	More than 10 AFB / field in at least 20 fields

Cleaning the Objectives

- After reading the smears, gently wipe the objective lens with lens paper or fine tissue paper.
- Organic solvents damage microscope objectives permanently.
- **Xylene, petroleum, benzene or acetone must NEVER be used to clean objective lenses.**

Protect the Microscope

- Humidity and dust damage microscopes.
- In humid climates, the microscope needs to be protected from fungus.
- In dry climates, cover the microscope when not in use with a vinyl or cotton cloth to protect from dust.



Remove Oil from Smears





Storage of Smears



Summary

- 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?