SOP

Acid Fast Staining Using Ziehl–Neelsen Technique

Department of Microbiology

Faculty of Medicine

UWUSL

Standard Operating Procedure (SOP)
Acid Fast Staining Using Ziehl–Neelsen Technique

Title:
Identification of Acid-Fast Bacilli Using the Ziehl–Neelsen Staining Technique

Issued By:
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(1) Purpose

To establish a standardized procedure for performing Ziehl–Neelsen staining in microbiology practical sessions to identify acid-fast bacilli (AFB), particularly Mycobacterium species, based on their ability to retain carbol fuchsin after acid-alcohol decolorization.

(2) Scope

This SOP applies to all medical students, academic staff, and laboratory personnel involved in microbiology practical sessions, including:

- Preparation of bacterial smears
- Performing Ziehl–Neelsen staining
- Microscopic identification of acid-fast bacilli

(3) Responsibilities

(3.1) Students

- Follow correct staining procedures
- Handle reagents and equipment safely
- Accurately observe and record findings

(3.2) Demonstrators / Lecturers

- Supervise staining procedure
- Ensure correct technique is followed
- Assist in interpretation of results

(3.3) Laboratory Staff

- Provide reagents and materials
- Ensure proper functioning of equipment
- Maintain laboratory safety standards

(4) Principle

- Acid-fast bacteria contain a high concentration of mycolic acids in their cell walls, making them impenetrable by ordinary aqueous-based staining solutions and difficult to stain by Gram's method, although they are structurally Gram-positive.
- The lipid-rich cell wall of acid-fast organisms stains with carbol fuchsin and resists decolorization with acid-alcohol. Therefore, these organisms retain the red/pink dye even after treatment with acids and alcohol.
- Only Mycobacteria with their thick waxy coats resist decolorization and remain stained with carbol fuchsin. These organisms are referred to as "acid-fast bacilli (AFB)" or "acid-alcohol-fast bacilli."
- The smear is counterstained with methylene blue, which stains the background and non–acid-fast organisms blue.

(5) Equipment and Materials

- Sterile inoculating loop
- Strong carbol fuchsin
- Acid-alcohol decolorizer
- Methylene blue
- Distilled water
- Bunsen burner
- Glass slides
- Staining rack
- Compound microscope
- Immersion oil

(6) Procedure

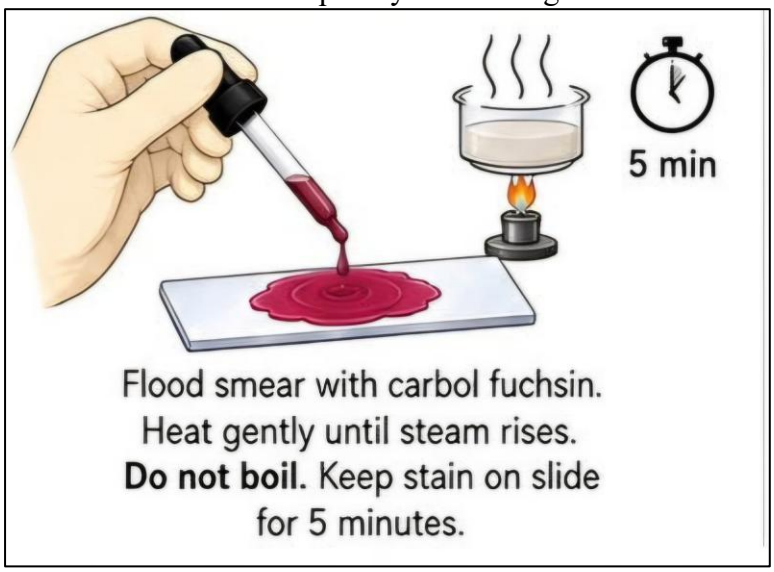
Step 1: Smear Preparation & Heat Fixing

Label one end of a clean, dry glass slide using a grease pencil to ensure proper identification. Prepare a thin smear of the specimen on the slide and allow it to air dry completely.

Pass the slide briefly through a flame 2–3 times to heat-fix.
Explanation: Heat fixing kills microorganisms, helps them adhere to the slide, and preserves cellular morphology during staining.

Step 2: Application of Strong Carbol Fuchsin

- Flood the smear completely with strong carbol fuchsin



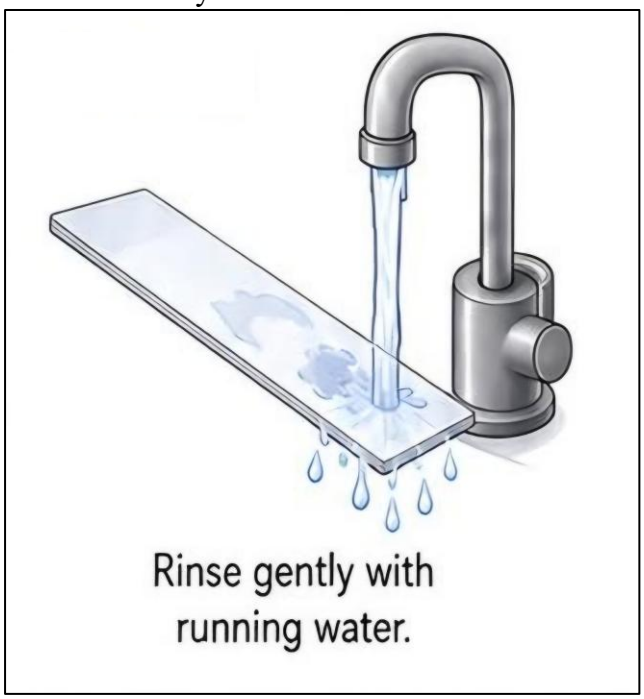
- Heat intermittently to keep the slide steaming for approximately 5 minutes. Do not boil. Add more stain if necessary to prevent the slide from drying.
- Wash the slide gently with water.

- Explanation: Heating facilitates penetration of carbol fuchsin through the thick waxy lipid-rich cell wall containing mycolic acids.

Step 3: Decolorization with Acid-Alcohol

- Wash the slide with acid-alcohol until the color disappears from the smear.

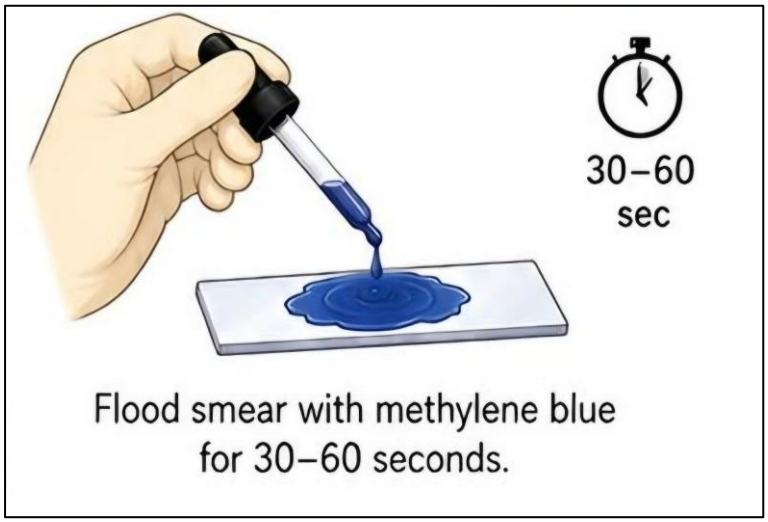
- Immediately rinse with water.



- Explanation: Acid-alcohol removes the stain from non–acid-fast organisms, while acid-fast organisms resist decolorization and retain the red/pink stain.

Step 4: Counterstaining with Methylene Blue

- Flood the smear with methylene blue for 30 seconds.

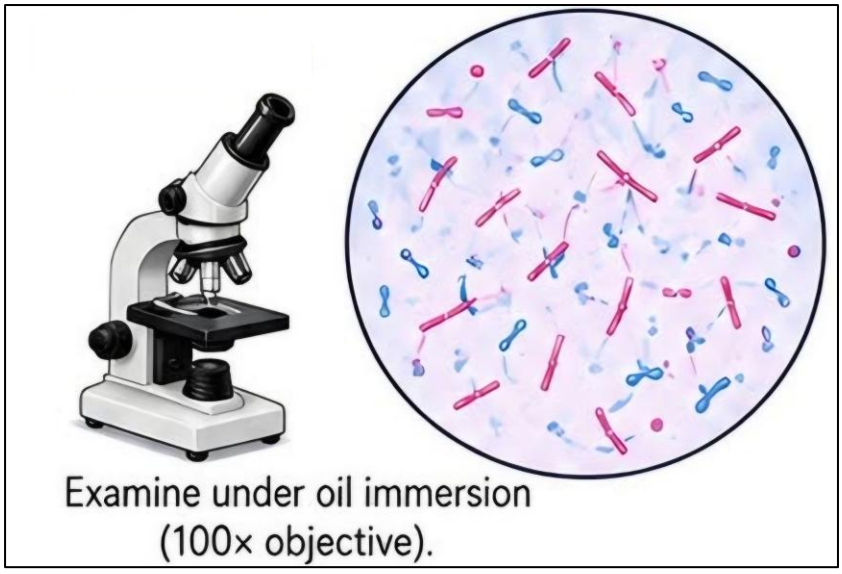


- Wash with water and allow the slide to air dry.

- Explanation: Methylene blue stains non–acid-fast organisms and the background blue, providing contrast for easier visualization of acid-fast bacilli.

Step 5: Microscopic Observation

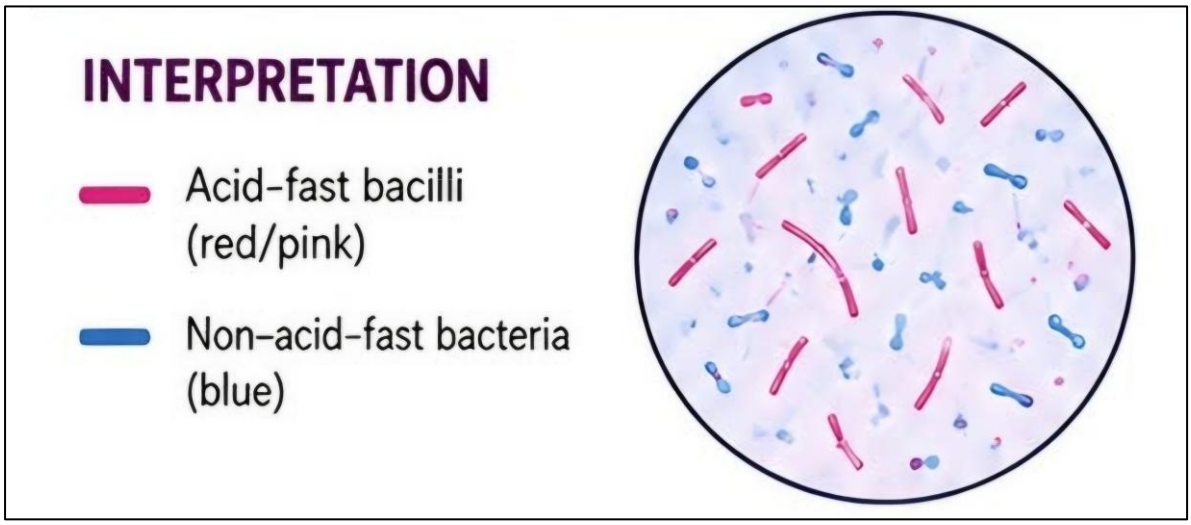
- Place the stained slide on the microscope stage.
- Focus under low power (10x) to locate the smear.
- Switch to high power (40x).
- Add a drop of immersion oil and examine under oil immersion (100x).



Observe and record:

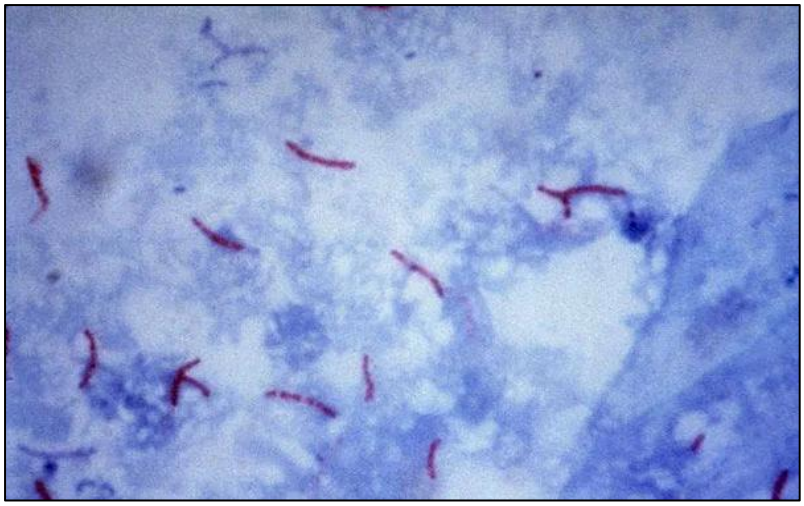
- Presence or absence of AFB
- Morphology and staining characteristics

(7) Results Interpretation



Morphology of AFB

- Pink bright red bacilli
- Slightly curved appearance
- Beaded appearance
- Blue background



(8) Precautions

- Use a thin smear for proper staining
- Do not boil during heating
- Prevent stain from drying during steaming
- Do not over-decolorize
- Use clean slides and fresh reagents
- Follow aseptic technique
- Handle flame carefully

(9) Post Practical Procedure

- Dispose of slides safely
- Clean microscope stage
- Turn off light source
- Clean work area
- Dispose of contaminated materials according to laboratory safety guidelines

(10) Records and Documentation

- Practical number and title
- Date of experiment
- Observations and interpretation
- Student name and signature
- Demonstrator verification

(11) Reference

Source: National Institutes of Health (.gov) https://share.google/2jy5Fi3oxBEIUk64q

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