Exercise 3.5 – Acid-Fast Stain: Kinyoun Method

LEARNING OUTCOMES

1. Discuss the clinical importance of acid-fast bacteria.
2. Explain why the cell wall structure of acid-fast bacteria is unique.
3. List the steps in preparing an acid-fast smear by the cold Kinyoun method.

The acid-fast stain is a differential stain used to identify organisms that are members of the genera *Mycobacterium* and *Nocardia*. The cell walls of these bacteria contain a waxy component known as *mycolic acid* (Figure 3.10). The presence of mycolic acid makes acid-fast bacteria highly resistant to staining, antibiotics, disinfectants, and environmental factors such as desiccation or drying. Acid-fast bacteria are ubiquitous in soil and water, and they include medically important species that cause diseases such as tuberculosis and leprosy.

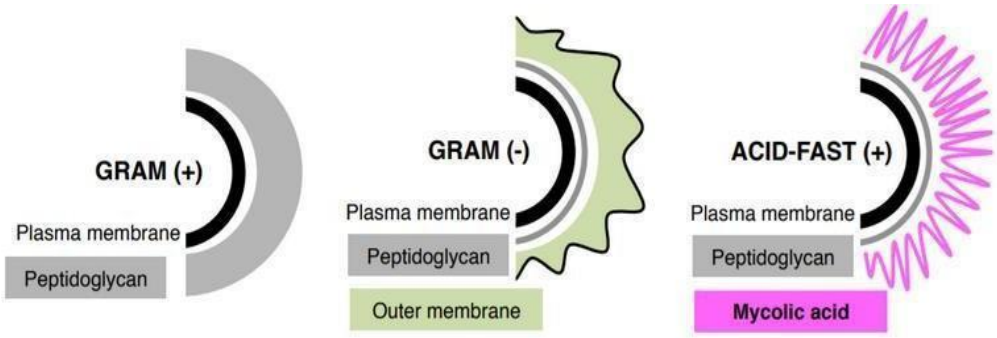

Figure 3.10: A comparison between the cell envelopes of Gram-positive, Gram-negative, and acid- fast bacteria.

Mycobacteria often takes weeks to months to cultivate on media. When a patient is suspected of having tuberculosis, an acid-fast stain prepared from sputum or other specimen confirms that mycobacteria are present. While these bacteria contain peptidoglycan, they Gram stain poorly because mycolic acid prevents dyes from entering their cell walls. The Ziehl-Neelsen and cold Kinyoun procedures are special staining techniques that penetrate this waxy component.

The Ziehl-Neelsen method uses heat to soften cell walls prior to application of the primary dye carbol fuchsin. Carbol fuchsin imparts a bright pink or fuchsia color to all cells. When slides are removed from the heat and permitted to cool, carbol fuchsin becomes trapped within the bacterial cell walls. Next, acid-alcohol is applied to decolorize non- acid-fast cells. Bacteria that retain the carbol fuchsin and do not lose their primary color are said to be acid fast (i.e., color-fast) despite decolorization. Finally, the counterstain methylene blue is applied to stain non-acid fast cells (Figure 3.11).

A cold acid-fast staining technique is the Kinyoun procedure. This method uses the same three reagents, but rather than heating cells to melt mycolic acid, additional phenol is added to the carbol fuchsin which disrupts lipids. The microscopic appearance of cells stained by the Kinyoun or Ziehl-Neelsen methods is the same (Figure 3.12).

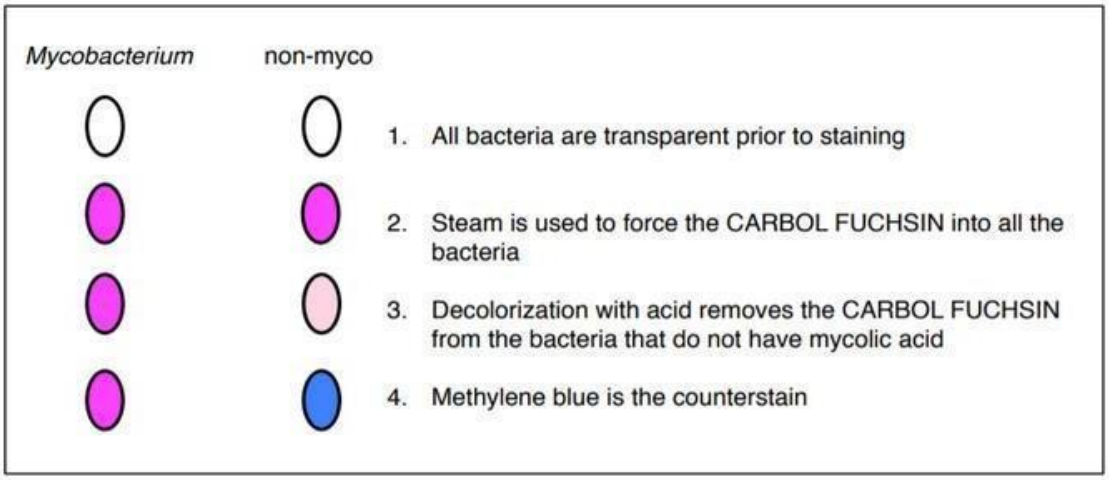

Figure 3.11. Acid-fast mycobacterial cells retain the bright pink primary dye carbol fuchsin despite decolorization with acid alcohol (hence, they are "colorfast"). Non-acid-fast bacteria are stained by the methylene blue counterstain following decolorization.

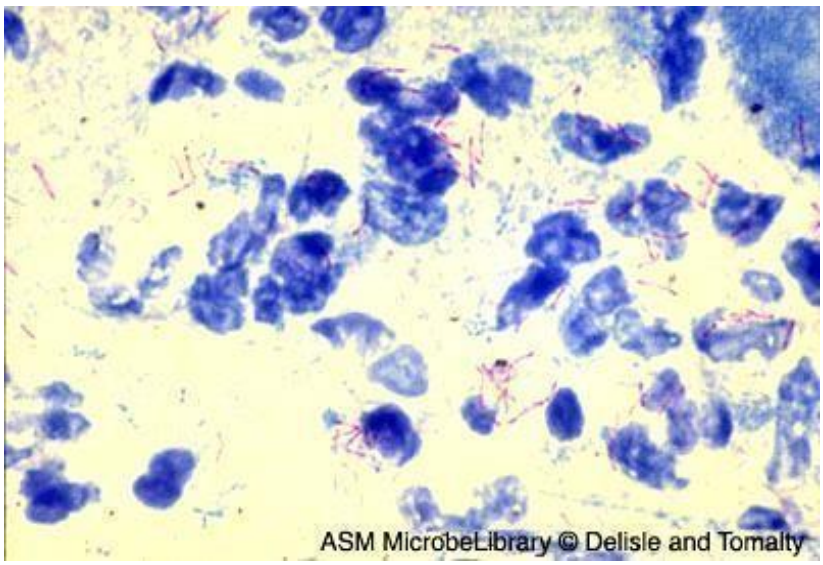

Figure 3.12: Acid-fast Stain of Mycobacterium tuberculosis in sputum. Note the reddish acid-fast bacilli among blue microbiota and white blood cells in the sputum which are not acid-fast.

Exercise 3.5 – Acid-Fast Stain: Cold Kinyoun Method

OBJECTIVE

Stain cells by the cold Kinyoun method to determine the presence of mycolic acid.

MATERIALS

- SLIDES: *Mycobacterium smegmatis* and *Staphylococcus aureus* heat-fixed smears
- SOLUTIONS: Acid-fast kit (carbol fuchsin, acid alcohol, methylene blue)
- EQUIPMENT: Stain pan, rack, wash bottle, bibulous paper, clothespin/slide holder

PROCEDURE – STUDENTS WORK IN PAIRS

IMPORTANT: If your staining pan becomes full, empty it into the sink at the center of your bench.
Never carry a full pan across the room*!*

1. Place the heat-fixed smears of *M. smegmatis* and *S. aureus* on the rack of the staining pan.
2. Cover both smears entirely with carbol fuchsin and let stand for three minutes.
3. Using the clothespin or slide holder, rinse both sides of the slides with water. Do not blot.
4. Lifting one slide at a time, apply acid alcohol until observing the color just beginning to run (10-20 seconds)
5. Immediately rinse the slides with water to stop the action of the decolorizer. Do not blot.
6. Cover both smears entirely with methylene blue and let stand for two minutes.
7. Using the clothespin or slide holder, rinse both sides of the slides with water.
8. Blot the slides gently in the bibulous paper booklet and put the slides aside.
9. Dispose of the pan water in the bench sink.
10. View the stained slides microscopically under oil immersion and complete the lab report.