Department of biology

Microbiology Lab

((Acid Fast Stain))

Lab/8

2 stage

By

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Acid Fast Stain:

is a differential stain used to identify acid-fast organisms such as members of the genus Mycobacterium. Acid-fast microorganisms are characterized by wax-like, nearly impermeable cell walls; they contain mycolic acid and large amounts of fatty acids, waxes, and complex lipids. This type of cell wall is resistant to most compounds, therefore acid-fast microorganisms require a special staining technique.

Acid-fast organisms like Mycobacterium contain large amounts of lipid substances within their cell walls called mycolic acids. These acids resist staining by ordinary methods such as a Gram stain. It can also be used to stain a few other bacteria, such as Nocardia.

Preparation of microscope slide:

1. Clean slide with a Kimwipe and alcohol to remove any fingerprints.
2. Draw two circles with your Sharpie on the **bottom** of the slide.
3. Using your inoculation loop, put two **small** drops of water in each circle.
4. Using aseptic technique, remove a very small amount of bacteria from the culture tube. Make sure you flame the tube before and after you enter.
5. Smear the bacteria in the drop of water on your slide. You may go out of the perimeter of your circles!
6. Let the slide **air dry completely**.

7. Heat-fix the slide by running it through the flame 3-4 times with the ‘smear’ side up. Do not flame the side with the bacteria!
8. Let the slide cool completely and you are ready to stain it.

Principle of Acid-Fast Stain

When the smear is stained with carbol fuchsin, it solubilizes the lipoidal material present in the Mycobacterial cell wall but by the application of heat, carbol fuchsin further penetrates through lipoidal wall and enters into cytoplasm. Then after all cell appears red. Then the smear is decolorized with decolorizing agent (3% HCL in 95% alcohol) but the acid fast cells are resistant due to the presence of large amount of lipoidal material in their cell wall which prevents the penetration of decolorizing solution. The non-acid fast organism lack the lipoidal material in their cell wall due to which they are easily decolorized, leaving the cells colorless. Then the smear is stained with counterstain, methylene blue. Only decolorized cells absorb the counter stain and take its color and appears blue while acid-fast cells retain the red color.

Reagents required –

1. Ziehl Neelson Carbol – fuchsin (primary stain).
2. Acid Fast Decolourisation (acid alcohol)
3. Methylene Blue (secondary stain – 0.3%)

Apparatus required –

1. Acid fast kit (includes above mentioned reagents)
2. Bunsen Burner

3. Slides
4. Glass rods
5. Inoculating loop
6. Mycobacteria culture
7. Microscope (oil immersion objective)

Procedure –

1. Prepare a smear on clean grease free slide.
2. Allow it for air dry and fix it by giving a gentle heat.
3. Flood the slide with Carbol Fuchsin stain until it get spread all over the slide.
4. Heat the slide for 5 minutes on a very low flame (temperature may go up to 60°C.
5. Then the slide is kept undisturbed for 5 minutes and allows it to cool.
6. Rinse the slide with distilled water and wash the stain.
7. Washing is followed by Acid Fast Decolourizer (3% v/v acid alcohol) treatment for about 2 minutes.
8. Remove the Acid-fast Decolourizer with water.
9. Flood the slide with Methylene Blue (counterstain) for about 2 min.
10. Wash the counter stain, allow it dry in air and then observe it under oil immersion objective.

PERFORMANCECHARACTERISTICS

Acid fast bacilli – Red

Background – Green