Background
Differential Staining: The Acid Fast Stain

The acid fast stain is one of the most medically important stains, second only to Gram staining. This is due to the highly pathogenic nature of certain members of the genus *Mycobacterium*- *M. tuberculosis*. Because of a high concentration of mycolic acid in their cell wall, all members of this genus will not completely destain during the Gram stain process, producing that second example of Gram positive irregular. Additionally, this waxy material allows for these bacteria to be identified easily in the acid fast stain. During the acid fast stain, heat is used as a mordant to allow the primary stain to penetrate the waxy mycolic acid layer. The heat will prevent the cells from being destained using acid-alcohol. Because these cells hold fast to the primary stain with acid alcohol treatment, they are termed acid fast positive. All other cells will easily be destained by the addition of acid alcohol and are termed non-acid fast. These non-acid fast cells are counterstained with methylene blue.

Introduction

The method you will be using for your acid fast stain, the Ziehl-Neelsen method, uses carbolfuchsin mixed with phenol as a mordant. The waxy mycolic acid of acid fast bacteria is quite sticky and makes preparing a thin smear difficult. Keep this in mind as you are preparing your acid fast stain.

Objectives

1. Perform a successful acid fast stain.
2. Be able to distinguish acid fast from non-acid fast cells under oil immersion.
3. Be able to explain the steps in the AF stain.
4. Explain why AF bacteria hold the stain when decolorized with acid-alcohol.
5. Give examples of AF bacteria.

Acid Fast Staining Protocol

<table>
<thead>
<tr>
<th>Table supplies</th>
<th>Individual supplies</th>
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</thead>
<tbody>
<tr>
<td>Culture of <em>Mycobacterium smegmatis</em></td>
<td>Acid Fast staining kit (carbolfuchsin, acid alcohol, methylene blue)</td>
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<tr>
<td>Culture of <em>Staphylococcus aureus</em></td>
<td>Microscope slides</td>
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<tr>
<td></td>
<td>Staining tray</td>
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<tr>
<td></td>
<td>Water bottle</td>
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<td>Bibulous paper</td>
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1. Fill your staining can 2/3 full with water.
2. Place it on your hot plate. Turn the heat on the hot plate on.
3. Prepare your smear a mixed smear of *M. smegmatis* and *S. aureus*.
4. Allow smear to air dry and heat fix.
5. Once the water in your staining can is steaming, place slide onto your staining can. (Be careful not to burn yourself or drop slide into the can.)
6. Immediately flood your smear with **carbolfuchsine**.
7. Be sure to keep the surface of the slide flooded with carbolfuchsine, incubate your smear on the staining can for 5 full minutes of steaming time. **Do not allow carbolfuchsine to dry, replenish as needed.**
8. Carefully remove your slide.
9. Rinse slide with a gentle stream of water, remember which side of the slide has the smear.
9. Decolorize with a dropper full of **acid alcohol**. About 5 seconds of one dropper full running over smear.
10. Rinse with water.
11. Counterstain by flooding the smear with **methylene blue** for 30 seconds.
12. Wash with water to remove excess dye.

Blot dry with bibulous paper.

Examine your slide(s) microscopically.
Data Collection & Analysis

What property of the acid fast cell allows it to retain the primary stain?

What is the decolorizer in the Acid Fast stain protocol?

Name two important species of acid fast organisms that are serious medical concerns.

Draw your view of your acid fast stain mix. Label the two bacterial species in view.