Acid-Fast (Ziehl-Neelsen) Stain

Objective/ purpose/ learning goals:

After performing this lab exercise, students will be able to

a. explain principles of acid-fast stain
b. perform acid-fast staining procedure
c. differentiate bacteria into acid-fast and non-acid-fast groups based on their appearance under microscope

Key terms

Carbol fuchsin, acid-fast strain, non-acid-fast strain, mycolic acid

Introduction / theory/overview:

The Acid-Fast stain is a differential staining technique that was first developed by Paul Ehrlich in 1882 and later modified by Franz Ziehl and Friedrich Neelsen in 1883. Therefore the acid-fast stain is also called the ***Ziehl-Neelsen stain***. The acid-fast stain differentiates bacteria into acid-fast and non-acid-fast groups. This method is used for members of the genus *Mycobacterium* and *Nocardia. Acid-fast* microorganisms are resistant to simple or Gram staining methods.

Principle of Acid-Fast Stain:

As the dye carbol fuchsin is applied to a bacterial smear, it solubilizes the lipid material present in the bacterial cell wall. With application of heat, carbol fuchsin further penetrates through the lipid-wall and enters into the cytoplasm. At this point all cells appear red. When these red cells are decolorized with acid-alcohol decolorizing agent (3% HCL in 95% alcohol), the acid-fast cells are resistant to decolonization because of the presence of large amounts of mycolic acid (a specific kind of lipid) in their cell wall which prevents the penetration of decolorizing solution. The non-acid-fast bacteria lack mycolic acid in their cell walls such that they are easily penetrated by the decolorizing agent and thus decolorized. This results in colorless cells. The smear is then counterstained with methylene blue. Only decolorized cells will absorb the counterstain, taking up its color and appear blue. Acid-fast cells do not absorb methylene blue and retain the red color.

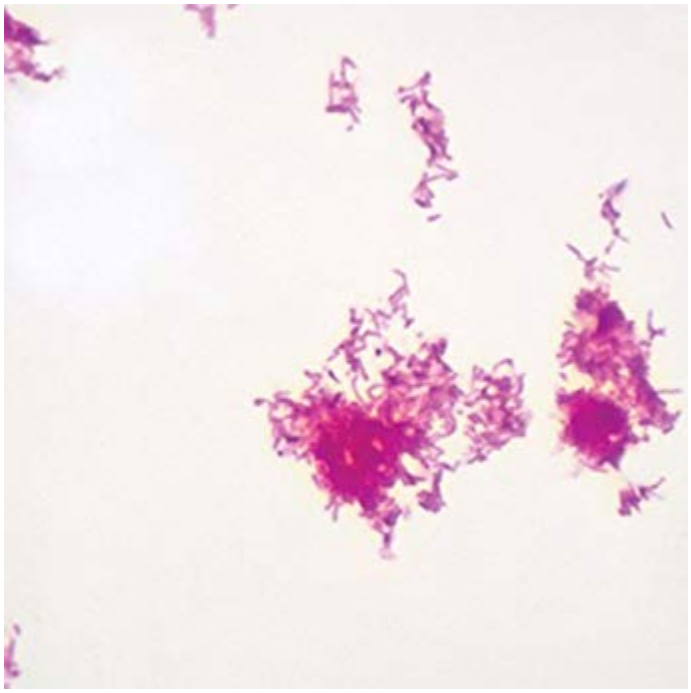
Materials, equipment and organisms (per student/ per group):

a. Live Organisms: *Mycobacterium pheli, E.coli*
b. Two slides per student for smears

c. Standard materials used for making, drying and heat-fixing smears,
d. Staining hot plate, **Carbol fuchsin dye, acid alcohol decolorizing solution, and filter paper.**

Procedure:

a. Obtain a slide and make two smears, one for ***Mycobacterium pheli*** and one for ***E. coli*** on the slide. Take time to break the clumps of *Mycobacterium* when making smears before drying your smear on designated hotplate. Heat-fix the smears. Each student will make at least two slides, each with smears for both organisms side by side.
b. Find the fume hood where acid-fast staining materials are located
c. Put heat fixed smears onto staining hot plate in the fume hood.
d. Place filter paper(s) on the heat fixed smear and use the dropper to completely saturate the filter paper with **carbol fuchsin** dye.
e. Allow dye to stain your smear for at least 5 minutes.
f. Wipe off the bottom of the slide with a wet paper towel and rinse off the dye with tap water.
g. Decolorize carefully with acid alcohol (3-5 drops) for a minute and rinse your slide with water.
h. Counterstain with methylene blue for a minute.
i. Rinse off the counter stain, blot dry (and place coverslip if required by your instructor).
j. View your slide at 100X under oil
k. You may wipe the **BOTTOM** of the slide with lens cleaner to remove excess carbol fuchsin. **NEVER** wipe the top of the smear!



Safety and disposal:

Follow standard lab safety procedure.
Dispose used slides into the glass disposal container, as the organisms are heat-fixed and killed.

Observations/ interpretation/ Results:

Record your observation below

Take home Lesson:

You need to know the reagents and their function in the staining procedure. Why do we use the acid-fast stain? What kind of organisms are acid-fast positive?