

SOP: SP035.2

Modified: 9/1//2023 RAS

Kinyoun Cold Acid Fast Staining

Materials and Reagents:

1. One dried and heat fixed glass microscope slide per sample
2. Staining tray
3. Difco TB carbofuchsin KF stain (note 1)
4. Difco TB brilliant green K or methylene blue
5. Difco TB Decolorizer (1:60:140 mixture of HCl/ddH₂O/methanol)
6. Bibulous paper
7. Clothespin
8. Paper towels
9. Sharps glass disposal bin

Protocol:

1. ____ Cover your thoroughly dried, heat fixed, and cooled slides with Difco TB carbofuchsin KF stain. Leave on for four minutes. Do not heat.
2. ____ Gently wash off the stain with a stream of running water until the water runs clear. Be careful to spray around the smear, not directly on it, or the smear may rinse off. (note 2)
3. ____ Using a clothespin, hold the slide at an angle over the staining pan. Slowly dispense a few drops of decolorizer at the upper end of the slide above the smear (not on the smear itself) so that the decolorizer runs down over the smear. Blot the decolorizer that accumulates in the lower corner of the slide on a paper towel. The blotted decolorizer will be pink in color at first. Apply more decolorizer, as above, just until the blotted decolorizer is colorless.
4. ____ Gently wash off the decolorizer with a stream of running water.
5. ____ Shake off any excess water, cover the smears with Difco TB brilliant green K or TB methylene blue, and leave on for thirty seconds.
6. ____ Gently wash off the stain with a stream of running water until the water runs clear.
7. ____ Gently blot the slides dry, using bibulous paper. Do not rub or push down on the slide which will wipe off the smear. Wipe the back of the slide with a small piece of paper towel dampened with 2-3 drops of decolorizer.
8. ____ Examine the slide under a microscope, then discard the glass slides in a proper sharps disposal bin.

Notes:

1. Contains phenol.
2. Collect all stains, decolorizer, and rinse water in the staining tray, and dispose of as hazardous waste.