
1. **Process Sample**
   - Allow slide to completely cool before staining.

2. **Apply Stain**
   - Use 8-10 drops of F.A.S.T. Auramine O Stain, or until smear is covered. Let stand for approximately 1 minute.

3. **Rinse**
   - Gently rinse slide with water. Be careful to avoid washing specimen off of slide. Carefully shake slide to remove excess water.
   - Note: Tap water may contain chlorine, which can interfere with fluorescence. Avoid using if possible.

4. **Apply Decolorizer**
   - Use 8-10 drops of F.A.S.T. Decolorizer/Quencher, or until smear is covered. Let stand for approximately 1 minute.

5. **Rinse**
   - Rinse slide again with water.

6. **Dry Slide**
   - Allow slide to air dry. If necessary, gently blot excess water with a lint free tissue.

7. **Examine**
   - Examine the slide using the ParaLens Advance™ LED fluorescence microscope attachment, or other fluorescent microscope.
   - See reverse side for examples of AFB stained with F.A.S.T. reagents.
Mycobacterium tuberculosis, viewed at 400x magnification. A dark background helps to clearly observe the fluorescence of the AFB.

The clump of bacteria in the center is magnified at greater detail where single bacilli can be seen even clearer. (Original photo taken at 400x, digitally modified)

Mycobacterial bacilli sometimes fluoresce at varying degrees, as seen in this picture. Note that there are single bacilli as well as clumped bacilli here. (400x)

AFB appearing as a brilliant green against an orange/black background (possibly due to residual stain). While the AFB are clear, it is best to wash the slides completely so that the background remains a contrasting black color. (400x)