Acid Fast Stain
(Ziehl-Neelsen method)

1. Prepare a heat-fixed bacterial smear as usual.

2. Place the slide over a beaker of rapidly boiling water and cover the smear with a piece of bibulous paper no larger than the smear.

   **NOTE:** If the piece of bibulous paper is too large or hangs off the slide you will have a real mess on your hands when you carry out the next step.

3. Add **Carbolfuschin** to completely saturate the paper covering the smear and leave for 5 minutes over the boiling water being sure to keep it moist with stain.

   **NOTE:** Periodically add drops of **Carbolfuschin** to ensure the paper-covered smear is moist throughout the 5 minute staining period.

4. Rinse the slide briefly with water to remove the **Carbolfuschin** and piece of paper.

5. Decolorize with acid alcohol for no more than 2 or 3 seconds (at room temperature).

6. Rinse briefly with water to remove the acid alcohol.

7. Counterstain the slide with **Methylene Blue** for 1 minute (at room temperature).

8. Rinse with water, blot dry with bibulous paper and observe at 1000X.