



1. Grasp the inoculating loop like a pencil.
2. Flaming. Heat the **entire wire** in the upper portion of the flame until it is red hot.
3. Place a small amount of growth in a **loopful of water** already on the slide, mix, spread over a small area, and allow to air **DRY**. (Omit the water for broth cultures.)
4. Flame the loop.
5. Heat fixing. When completely dry, pass the smear through the hot portion of the flame three times.
6. Cover the smear with crystal violet for 30 seconds.
7. Gently wash off the crystal violet by squirting water so it runs through the smear.
8. Cover the smear with Gram's iodine for 10 seconds.
9. Gently wash the smear with water.
10. Decolorize is with acetone-ethanol for 10-20 second.
11. Gently wash the smear with water.
12. Cover the smear with safranin for 30 seconds.
13. Gently wash the smear with water.
14. Dry the slide by placing it between pieces of paper towel. Don't rub.
15. Observe the slide under oil immersion (1000×), it does not need a cover slip.

