

Smear

1. Clean slide
2. Label: my name, organism, date, medium

Broth Smear

1. Agitate broth unless otherwise stated
2. FLAME, 1 loopful in center of slide spread to dime-size.
3. FLAME, 2nd loopful in center of slide spread to dime-size.
4. FLAME

Solid Smear

1. FLAME, 1 loopful water (or drop)
2. FLAME, 2nd loopful water
3. FLAME 1 loopful specimen and mix well in water.
4. FLAME 2nd loopful specimen if needed and mix well in water.
5. FLAME
6. Air Dry
7. **Heat Fix**
 - a. Use clothespin.
 - b. Pass slide's underside over the flame (coolest part of flame)
 - c. 3 passes

Simple Stain (Methylene blue, Crystal Violet, Carbol Fuchsin)

1. Smear and heat fix.

2. Flood stain

Carbol Fuchsin, 15-30 sec

Crystal Violet, 20-60 sec

Meth. Blue, 1-2 min

3. Rinse with Di Water

4. Bibulous blot, Do not wipe

Gram Stain

1. Flood with Crystal Violet

2. Wait 1 min

3. Flush with Di water

4. Flood with Gram's Iodine mordant

5. Wait 1 min

6. Flush with Di water

7. Decolorize

8. Flush immediately

9. Counterstain Safranin

10. Wait 45 sec

11. Flush with Di water

12. Bibulous Blot

Acid-Fast Staining

1. Smear and heat fix.
2. Flood smears with carbol fuchsin
3. Place over a beaker of water on a warm hot plate
4. **Steam 5 minutes** (do NOT allow stain to evaporate, replenish as needed)

OR

Flood smear with carbol fuchsin WITH TERGITOL for **5-10 minutes**

5. If slides are heated, allow to cool.
6. Flush with Di water
7. Decolorize with acid-alcohol
8. Flush with Di water
9. Counterstain with **Meth. Blue for 2 min**
10. Flush with Di water
11. Bibulous blot.

Spore Staining

1. Smear and heat fix
2. Flood with **malachite green**
3. Place on beaker of water sitting on hot plate
4. **Steam for 2-3 minutes** (do NOT allow stain to evaporate, replenish as needed)
5. Remove from hot plate
6. Cool
7. Flush with Di water (this is the decolorizing step)
8. Counterstain **Safranin for 30 seconds**
9. Flush with Di water
10. Bibulous blot