Summary of the Gram stain procedure

First fix a smear of the bacterium to the slide as follows:

1. Place a small piece of **tape** at one end of the slide and **label it with the name of the bacterium** you will be placing on that slide

2. Sterilize your inoculating loop and place **one loop full of deionized water from your dropper bottle on your slide**.

3. Using the **edge** of your sterile inoculating loop, aseptically remove a **small amount** of the culture from the agar surface and **gently touch it several times to the drop of water** until the water **becomes visibly cloudy.**

- A good smear with the correct amount of bacteria is essential to Gram staining. **Too many bacteria on the slide could result in under-decolorization; too few could lead to over-decolorization**.

4. **Incinerate the remaining bacteria on the inoculating loop.** If too much culture is added to the water, you will not see stained individual bacteria and you may not have a reliable Gram stain.

5. After the inoculating loop cools, **spread the suspension over the slide** to form a thin film.

6. Allow this thin suspension to **completely air dry**. **The smear must be completely dry before the slide is fixed!**

7. **If your professor instructs you to heat-fix the bacteria to the slide**, pick up the air-dried slide with provided close pin and **hold the bottom of the slide opposite the smear against the opening of the microincinerator for 10 seconds**) as shown in **Fig. 3**. If the slide is not heated enough, all the bacteria will wash off. If it is overheated, the bacteria structural integrity can be damaged.

8. **If your professor instructs you to fix the bacteria to the slide using methanol**, add 2-3 drops of **95% methanol** to the air-dried smear of bacteria and let sit for **2 minutes** or until the methanol evaporates. Let the slide again **air dry** before staining.

b. Stain with Hucker's **crystal violet** for **one minute**. **Gently wash with water**. Shake off the excess water but **do not blot dry between steps**.

c. Stain with **Gram's iodine solution** for **one minute** and **gently wash with water**.

d. **Decolorize by picking up the slide and letting the Gram's decolorizer run down the slide <u>until the purple just stops flowing at the bottom of the slide</u>**.

- **Make sure the entire smear is evenly decolorized and that you are not under-decolorizing or over-decolorizing**.
- **Wash immediately with water**.

e. Stain with **safranin** for **one minute**. **When you wash off the excess safranin, be very careful to wash gently and briefly** as it is possible to wash out some of the safranin in the bacterium.

f. **Blot dry** and observe using oil immersion microscopy.