Gram Stain Kit

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Kit Contents

- Crystal violet stain
- Gram iodine stain
- Ethyl alcohol solvent
- Safranin O counterstain
- Plain microscope slides
- Medicine dropper
- Coverslips

Background

In 1884 Hans Christian Gram, a Danish bacteriologist, attempted to find a universal stain that would work with all bacteria. In the process, he discovered that bacteria could be divided into two different groups -- one that retained a stain, called "gram-positive," and one that didn't, called "gram-negative." His unique method for identifying these two groups became the first step in any bacterial identification process. Even the simple determination that a bacteria specimen is gram-positive or gram- negative can direct a doctor in diagnosis, as different bacteria cause different diseases. For example, the bacteria that causes scarlet fever is gram-positive, while that which causes typhoid or cholera is gram- negative. In addition, this classification process can help a doctor determine proper treatment, as some gram-negative bacteria are able to resist many common antibiotics.

Gram's method of staining has several steps: the bacteria is stained and then "fixed" with iodine. The slide is then washed in a solvent to "decolorize" the first stain. Gram-positive cells will retain the stain even after being washed in the solvent, but the stain will be removed from the gram-negative cells. This is because the cell walls of gram-negative cells contain more lipids (fatty substances) than gram-positive cells. The solvent dissolves the lipid layer, allowing the color to be drawn from the cell. In contrast, the solvent causes the gram-positive cell wall to dehydrate, closing the pores and trapping the stain inside the cell. In the final step, the gram-negative cells are stained a different color, so that they can be seen and contrasted from the gram-positive cells.

This table illustrates the progression:

Step	**Gram-Positive Cells**	**Gram-Negative Cells**
Specimen before stain	No color	No color
Crystal violet stain	Blue	Blue
Iodine stain	Purple	Purple
Solvent	Purple	No color
Counter stain	Purple	Red

Procedure

Some of the steps of the gram stain process are hard to carry out perfectly. To practice, it is a good idea to make a "control" slide. Try collecting some bacteria from between your teeth (using a toothpick) and placing it on a slide with a drop of water. If the Gram staining procedure is done correctly, your slide should have a mixture of gram-negative and gram-positive cells as well as some neutrophils (white blood cells) with pink nuclei. After you have tried that, test bacteria from the soil or some that you grow in a petri dish.

1. Make a specimen smear by placing a small amount of bacteria on a clean glass slide. Take another slide and use its edge to scrape or "smear" the specimen into a very thin film of material.
2. Let the specimen on the slide air dry, and then heat fix it by passing the slide through a candle flame 3-4 times. (The slide shouldn't get too hot to touch, and it should never stop as it passes through the flame.)
3. Cover the specimen with 1-2 drops of the **crystal violet** stain for 60 seconds and then gently wash it off with very slow running water from the tap or a few gentle squirts from a wash bottle. (If the water is running too fast and hits the slide with too much force, the specimen will be washed off!)
4. Cover the specimen with a few drops of **Gram's iodine** for 60 seconds, and then gently wash the specimen again as in step 3.
5. Use **ethyl alcohol** as the solvent. This is the most sensitive step, because if the ethyl alcohol is left on the specimen too long, it will decolorize the gram-positive cells as well as the gram-negative. Tilt the slide slightly and apply the alcohol drop by drop onto the slide above the specimen, so that the alcohol runs down over the entire specimen. Stop applying the alcohol when the fluid flowing off the edge of the slide is no longer colored. The thinnest parts of the smear should be colorless. This will take about 5 seconds. Wash the slide gently again. Note that the gram-positive cells will retain some of the violet coloring, but the majority of the stain will be rinsed away by the solvent.
6. Cover the specimen with a few drops of **safranin** stain as the counter stain for 60 seconds and then gently wash once more.

7. Blot the slide with absorbent paper (a paper towel will work if you have nothing else), but do not rub the specimen smear. Put a coverslip over the smear.
8. Now you are ready to examine your slide under a microscope at each magnification level. As you do so, look for cells that are purple in color. These are gram positive cells that retained the crystal violet stain. Cells that are pink or red in color are gram negative cells. In these cells, the crystal violet was washed away by the ethyl alcohol and replaced with the safranin.

Safety

Often the chemicals used to prepare slides may be toxic, corrosive or have other related hazards. Always carefully read the entire label before using a chemical. Be sure you understand the hazards involved, the proper safety equipment to wear, and what you will do in case of a spill or contact with your skin. The stains included will discolor clothing and skin.

Basic safety equipment that you should wear includes:

Safety goggles (splash type)
Chemically resistant gloves
Chemically resistant lab apron

Work in a clean, well ventilated, uncluttered area where you can quickly wipe up spills.

Always keep chemical bottles tightly capped except for the short period of time you are measuring the chemical.

The bacteria you work with can also be hazardous. Always wash your hands thoroughly before and after handling the bacteria cultures. Washing them before will minimize contamination of the bacteria cultures you are growing. Washing them afterwards will minimize your exposure to harmful bacteria that may be growing in your cultures. When you have finished studying a culture, pour enough household bleach into the culture to cover the bottom of the dish. Then cover the culture, seal it in a plastic bag, and throw it away.