

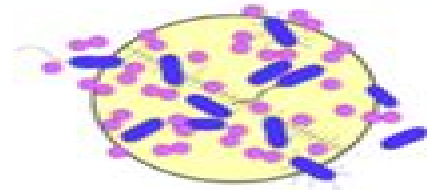


## Exercise 4 - Staining Microbes

### Learning objectives

Following this exercise the student should be able to:

1. prepare a smear properly from broth or solid cultures.
2. list reagents, functions and steps of a Gram stain.
3. evaluate a Gram stain reaction quality and troubleshoot causes of Gram staining problems.
4. describe the Gram stain reaction, cell shape and arrangement of *S. aureus*, *E. coli* and *Bacillus sp.*
5. interpret unknown slides for Gram stain reaction, cell shape and arrangement.
6. explain the information gathered from AFB, endospore, capsule, flagella and inclusion body stains.



Microbes are invisible to the naked eye and difficult to see and identify even when using a microscope. A simple stain **visualizes** the microorganisms; a differential stain **displays the chemical differences** in cellular structures, including the cell wall and cell membrane because the macromolecules within the structure bind to different components of the stain. An example of this differential staining is seen in staining used for blood smears.

Staining white blood cells with a differential stain displays the difference between the five white blood cell types; basophils, eosinophils, neutrophils, monocytes and lymphocytes. The intracellular granules of basophils stain dark blue because of their affinity to basic portion of the stain. Basophil means basic loving. On the other hand, the eosinophil (acid loving) stains red as a result of the intracellular granule's affinity to the acidic portion of the stain. Treatment of microbial diseases depends upon the correct identification of microorganisms and depends upon the ability to read and interpret stained smears. Bacterial cells are commonly stained with a differential stain called the **Gram stain** and protozoal cells with the **Trichrome stain**; this reveals the internal structural differences and aids in identification. Properly preparing slides for staining is important to ensure good results. Remember, you cannot see the material you are working with so you must develop good technique based upon principles.

Always start with clean slides using lens paper to clean them. Slides can be made from direct clinical material (a wound, sputum, knee fluid, the throat etc.), broth cultures and from solid media cultures. **The first principle is that some fluid is needed to emulsify the material if it is dry, however too much fluid may make the microbes hard to find.** Slides from clinical cultures are usually placed directly on the slide without the addition of water, as are slides from broth cultures. Slides from solid media require water to emulsify and separate the individual bacterial cells for better observation, but a very single drop of water is usually adequate. Next the material must be attached to the slide so they don't wash off with the staining process. **The second principle involves fixing the slide using either a chemical fixative or heat.** In this lab a heat-fixing tray will be used.

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