

LABORATORY

Bacterial Types and Morphology

OBJECTIVES

- To recognize and identify colony morphology
- To perform gram stains of bacteria
- To discuss *Mycoplasma*, *Chlamydia*, and *Rickettsiae* and special handling that is required for their culture in the laboratory

BACKGROUND

One of the most important steps in identifying a new bacteria or one isolated from a patient's specimen is noting colony morphology. This is how the colony or clone of cells looks on the agar surface. It varies between microbes. Many have very characteristic appearances. It is often used for identification of unknown microbes in classes. Therefore, it is important to make careful observations and take good notes that may assist with later exercises.

Some types of bacteria are discussed in this lab, even though we will not be working with them. This is either because they are pathogens, or because the conditions to grow them are unavailable. These include *Mycobacterium*, *Mycoplasma*, *Chlamydia*, and *Rickettsia*.

Mycoplamas, *Rickettsiae*, and *Chlamydiae* are classified as true bacteria, but they are extremely small, and for differing reasons, cannot be cultured by ordinary methods. They will be discussed here, although not cultured in this lab, since many are pathogens.

Species of *Mycoplasma* can cause pneumonia, urogenital and other infections. Many strains are found in normal mucous membrane microflora, but can be opportunistic pathogens. *Mycoplasma* are pleomorphic because they have no cell wall. They can pass through sterilizing filters, and do not stain with ordinary stains. You might guess that they are resistant to antibiotics that act by inhibiting cell wall synthesis.

Rickettsiae are very small bacteria that can only grow and multiply intracellularly, as they are obligate parasites. They do have a cell wall that can be stained. In the lab, they must be grown in cell culture or intact animals. Most *Rickettsiae* are transmitted by arthropods, and there are several pathogens in this group, including the causative agents for typhus and Rocky Mountain spotted fever.

Chlamydiae are nonmotile, coccoid, obligate intracellular parasites. They have two life forms, or stages, known as the elementary body and the reticulate body. They must grow and multiply

inside another cell; then they are released to infect new cells. There are two species responsible for human disease: *C. psittaci* (parrot fever) and *C. trachomatis* (STD, trachoma, LGV). These also can only be studied in cultured cells or intact animals.

MATERIALS

Plates of various microbes
Gram stain reagents
dissecting microscopes

PROCEDURE

1. Observe plates for growth, especially noting the areas where there are isolated colonies. Also observe other students' plates in order to see what all the microbes look like.
2. Make notations about how the colony appears to you. The recognition of colony morphology is an individual accomplishment. The characteristics to observe will be pointed out, but you must learn how to recognize them. Any characteristic that allows you to differentiate and recognize colony types may be used. This can include size, color, shape, topography, consistency, margin, and edge. See figure for examples.
3. After observation of the colonies, perform gram stains of the organisms, so that notations can also be made about the cellular morphology of each strain used. Be sure to observe the gram stains of other students, also.

