

Special Stains

OBJECTIVES

- To understand identification of cellular structure in special stains, including acid-fast, flagella, capsule, and spore stains
- To observe flagella and acid-fast staining
- To perform capsule and spore staining

BACKGROUND

Some bacteria have characteristic surface structures (capsules or flagella) or internal components (endospores) that are of value in identifying these organisms. Special stains are available for these cell components, as well as for other cell components to aid in the study of microbes.

The acid-fast stain is a differential stain for *Mycobacterium* and related bacteria. *Mycobacterium* is genus that includes the causative agents of leprosy and tuberculosis, so it is useful clinically to have a stain for rapid identification of these organisms in sputum or skin scrapings.

Mycobacterium are unusual in that the cell wall has a mixture of waxy lipids called mycolic acids, that prevent the bacterium from staining by simple and gram stains. They also prevent drying of the microbe. In the acid-fast stain, a red stain (carbol-fuchsin) is cooked into the waxy cell wall, and cannot be removed with acidified alcohol. That is, it is acid-fast. Other bacterium and tissue background will lose this red stain when washed with acid-alcohol, becoming colorless. They then are stained blue to see them, with a counterstain (methylene blue), just as in the Gram stain.

The genus *Mycobacterium* contains some pathogens and many saprophytic species, found in soil and water, and also on human skin and mucous membranes. Laboratory diagnosis of mycobacterial diseases is made by identifying the organism in acid-fast smears, and by culture of patient specimens. *Mycobacterium* grows very slowly in the laboratory on a complex media. Often it will take 4-8 weeks for visible growth in culture. Some strains, such as *M. leprae*, cannot yet be grown in culture. Thus the acid-fast stain is important for quick diagnosis. Other staining methods have also been developed.

Flagella are tiny hairlike organelles for locomotion. Their fine protein structure requires special staining techniques to allow resolution in a light microscope. The number of flagella and their arrangement (monotrichous, amphitrichous, lophotrichous, peritrichous) may be useful in identifying a particular organism. A stain and mordant are used to react with the flagella, increasing its diameter to allow viewing in the microscope.

Capsules are found on some bacteria (and fungi) and the capsules may actually relate to pathogenicity in some strains, such as *Streptococcus pneumoniae*, *Klebsiella pneumoniae*, and *Clostridium perfringens*. In these, the large capsule can protect the microbe from host defense mechanisms, especially phagocytosis. These capsules are often identified immunologically, but can also be visualized with a simple negative stain. Material such as India ink or Congo red is used to coat the slide but not penetrate the cell or capsule. A stain can be used to color the cells, and then the capsule is seen as a clear region surrounding the cells against the dark background. There are also direct methods of staining capsules.

Endospore formation is most characteristic of two genera of bacteria, *Bacillus* and *Clostridium*. These endospores provide a resistant form for survival of the organism in unfavorable conditions. Because of the tough spore coating, they are not readily stained and may appear as empty “holes” in simple or gram-stained bacteria. A special staining technique must be used to drive dye into these resistant spores. Usually malachite green is heated to stain the spores, and then the vegetative bacteria are counterstained with safranin. In this case, spores appear green in red bacterial cells. This stain will be performed on *Bacillus* cultures.

MATERIALS

Prepared slides:

Acid-fast stain of *Mycobacterium*

Flagella stains

Culture (72 hrs) of *Bacillus cereus* or other *Bacillus* species

Culture of *Klebsiella*

Malachite green and Safranin

Ring stand and beaker of water

Microscope

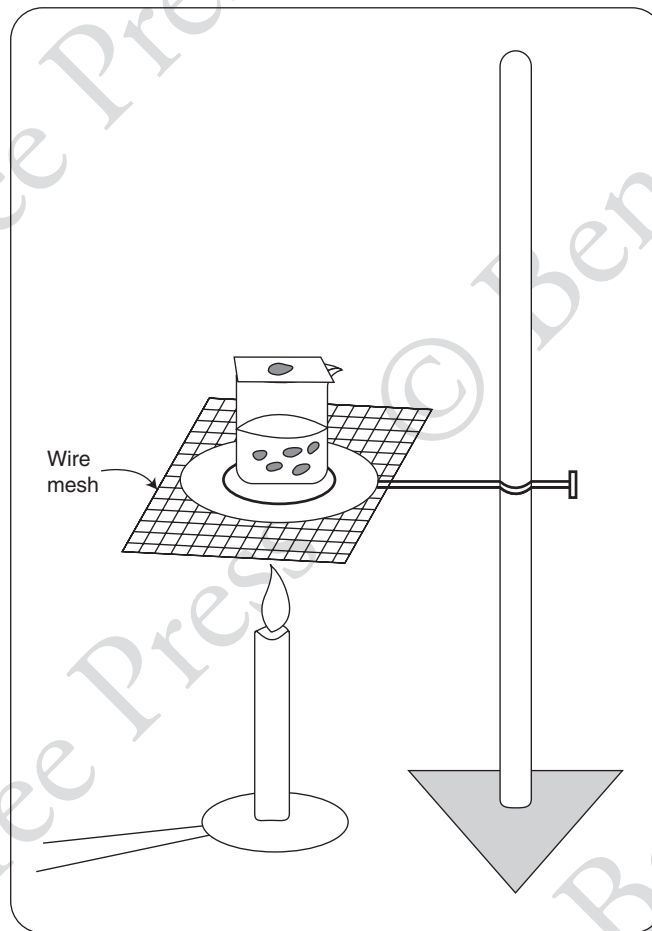
Immersion oil

PROCEDURES

View the prepared slides of acid-fast, and flagella stains. Be sure to identify the particular organism or component of the organism in each slide.

Spore Stain:

1. Make a heat-fixed smear of a *Bacillus* culture. Cover the smear with a small piece of paper towel, not hanging over the edges of the slide.
2. Set up the ring stand over the Bunsen burner as shown in the diagram. Heat about 2 inches of water in a beaker on the stand until boiling. Place the slide on top of the beaker of boiling water, being certain that the slide is horizontal. Observe caution around flame, boiling water, and stain on ring stand.
3. Cover the smear and paper towel with malachite green, and steam gently for 15-20 minutes. Do not start timing until water is boiling under the slide. Don't let the slide get dry—keep adding stain if it approaches dryness. This is cooking the malachite green into the resistant endospore wall.



4. Remove the slide from the beaker, turn off the flame, and carefully dispose of the green paper in the wastebasket, using your forceps.
5. Wash the slide gently in running water for about 20 seconds, getting all the extra green off the slide. Running water washes the green stain out of the vegetative cells and sporangia, and they become colorless. The counterstain then dyes the vegetative cells red.
6. Counterstain with safranin for one minute, at the staining sink. Gently rinse with water and shake off excess water.
7. Gently blot the slide dry and let it air dry. Focus on smear and examine with the oil immersion objective. Observe the red vegetative cells and sporangia, and green endospores and free spores.

Capsule Stain: (Negative stain)

1. Place a drop of congo red on a clean slide. Mix a loop of *Klebsiella* culture into the stain drop.
2. Using a second slide, spread the drop of stain and microbe across the first slide, making a smear and feathering the end.
3. Allow slide to air dry, and then view under oil immersion.

RESULTS

1. Label appropriately colored drawings of all observed microbes from your spore stain, capsule stain, and the prepared slides.
2. What is the clinical value of an acid-fast stain?
3. Why is a capsule stain useful in the lab?
4. Describe the different arrangements of flagella. What is their importance?
5. Why must the spore stain include a heating step? What would happen if it were omitted?
6. Why is staining bacterial components useful in strain identification?

BACTERIAL CELL CHARACTERISTICS

