

SELECTIVE AND DIFFERENTIAL MEDIA

Selective and differential media are used to isolate or identify particular organisms. **Selective** media allow certain types of organisms to grow, and inhibit the growth of other organisms. The selectivity is accomplished in several ways. For example, organisms that can utilize a given sugar are easily screened by making that sugar the only carbon source in the medium. On the other hand, selective inhibition of some types of microorganisms can be achieved by adding dyes, antibiotics, salts or specific inhibitors which affect the metabolism or enzyme systems of the organisms. For example, media containing potassium tellurite, sodium azide or thallium acetate (at concentrations of 0.1 - 0.5 g/l) will inhibit the growth of Gram-negative bacteria. Media supplemented with penicillin (5-50 units/ml) or crystal violet (2 mg/l) will inhibit the growth of Gram-positive bacteria. Tellurite agar, therefore, is used to select **for** Gram-positive organisms, and nutrient agar supplemented with penicillin can be used to select **for** Gram-negative organisms.

Differential media are used to differentiate closely related organisms or groups of organisms. Owing to the presence of certain dyes or chemicals in the media, the organisms will produce characteristic changes or growth patterns that are used for identification or differentiation. A variety of selective and differential media are used in medical, diagnostic and water pollution laboratories, and in food and dairy laboratories. Three of the more common selective and differential media are described below and will be used in the laboratory exercise.

MANNITOL SALT AGAR (MSA)

Mannitol salt agar is a **selective** medium used for the isolation of pathogenic staphylococci. The medium contains mannitol, a phenol red indicator, and 7.5% sodium chloride. The high salt concentration inhibits the growth of most bacteria other than staphylococci. On MSA, pathogenic *Staphylococcus aureus* produces small colonies surrounded by yellow zones. The reason for this change in color is that *S. aureus* ferments the mannitol, producing an acid, which, in turn, changes the indicator from red to yellow. The growth of other types of bacteria is generally inhibited.

EOSIN METHYLENE BLUE AGAR (EMB agar)

Eosin methylene blue agar is a **differential** medium used for the detection and isolation of Gram-negative intestinal pathogens. A combination of eosin and methylene blue is used as an indicator and allows differentiation between organisms that ferment lactose and those that do not. Saccharose is also included in the medium because certain members of the Enterobacteria or coliform group ferment saccharose more readily than they ferment lactose. In addition, methylene blue acts as an inhibitor to Gram-positive organisms.

Colonies of *E. coli* normally have a dark center and a greenish metallic sheen, whereas the pinkish colonies of *Enterobacter aerogenes* are usually mucoid and much larger than colonies of *E. coli*. Other organisms, such as *Salmonella* (one of the causative agents of food poisoning), do not ferment lactose or saccharose and produce colonies that are noncolored.

MacCONKEY'S AGAR

MacConkey's agar is a **differential** plating medium used in the detection and isolation of all types of dysentery, typhoid and paratyphoid organisms. It is generally used for differentiating strains of *Salmonella typhosa* from members of the coliform group; however, the medium supports the growth of all *Salmonella* and *Shigella* strains and gives good differentiation between these enteric pathogens and the coliform group. When grown on MacConkey's medium, colonies of coliform bacteria are brick-red in color and are surrounded by a zone of precipitated bile. These reactions are due to the acid produced by the fermentation of lactose. The acid end-products act on bile salts, and neutral red is absorbed by the precipitated salts. Dysentery, typhoid and paratyphoid bacilli do not ferment lactose but give an alkaline reaction when grown on the medium. Colonies of these organisms are noncolored and transparent. The growth of Gram-positive organisms is inhibited because of the crystal violet and bile salts in the medium.

FIRST PERIOD

Material:

1. Cultures of: *Staphylococcus aureus*
Staphylococcus epidermidis
Escherichia coli
Enterobacter aerogenes
Salmonella enteritidis
2. Petri dishes of Mannitol Salt agar, EMB agar, MacConkey's agar and nutrient agar

Procedure: (work in pairs)

1. Divide each plate of MSA and MacConkey's agar into two sections. Divide the EMB agar plate into three sections.
2. Inoculate the MSA plate with *S. aureus* and *S. epidermidis*.
3. Inoculate MacConkey's agar with *E. coli* and *Salmonella enteritidis*.
4. Inoculate the 3-sectional EMB agar plate with *E. coli*, *Enterobacter aerogenes*, and *Salmonella enteritidis*.
5. Divide one plate of nutrient agar into two sections and one into three sections. Inoculate the 2-sectional plate with the two species of *Staphylococcus*; inoculate the 3-sectional plate with the remaining three organisms.
6. Incubate all plates at 37°C for 18 to 24 hours, then observe colonies. Pull plates and place in refrigerator (4°C) till next lab period.

SECOND PERIOD

1. Observe the growth and appearance of colonies on all plates. Notice that nutrient agar is neither a selective nor differential medium.

MICROBES OF THE BODY: GRAM-POSITIVE COCCI

The bodies of mammals, molluscs, insects and other representatives of the “animal kingdom” are natural habitats for microorganisms. Many microorganisms exist in balanced symbiosis with host organisms. That is, the microorganisms grow on the surface of the animal hosts including, of course, the *internal* surfaces of the gastrointestinal, genitourinary, and respiratory tracts. They grow without causing measurable damage to the host. In some cases, the microorganisms actually benefit the host by producing useful compounds such as vitamins, or by preventing access to tissues by pathogenic microorganisms. There are also those microorganisms which can damage the host; they are termed **pathogenic** because of their ability to cause disease.

One of the major morphological groups associated with animal hosts are the cocci. Some of the most important cocci are the Gram-positive cocci belonging to the genera *Staphylococcus* and *Streptococcus* and the Gram-negative cocci belonging to the genus *Neisseria*. One species of the genus *Staphylococcus* is *Staphylococcus epidermidis*, which is commonly found on the skin of humans and which is rarely associated with disease. *Staphylococcus aureus*, on the other hand, is associated with a wide variety of disease states, including impetigo, toxic shock syndrome and food poisoning. *S. aureus* is also found in the “normal flora” of the nasopharynx of about 40% of the human population. This shows that disease does not result simply from the presence of the disease-causing agent, but is also affected by factors such as the physiological state of the host and the virulence (degree of pathogenicity) of the particular strain of organism.

The genus *Streptococcus* also contains both pathogenic and nonpathogenic species. Examples of nonpathogenic streptococci are the lactic acid bacteria which are associated with the “normal flora” of both animals and plants, and generally cause no adverse effects. Other streptococci are pathogenic. For example, *Streptococcus pyogenes* and *Streptococcus pneumoniae* cause a wide range of diseases including “strep throat,” pneumonia, scarlet fever and acute rheumatic fever.

Likewise, both nonpathogenic and pathogenic species occur in the Gram-negative cocci of the genus *Neisseria*. For example, *Neisseria meningitidis* and *Neisseria gonorrhoeae* are the causative agents of meningitis and gonorrhea, respectively.

Differentiating between these groups of cocci is easy. *Neisseria* are oxidase-positive, Gram-negative, kidney bean-shaped cells often found in pairs. *Staphylococcus* are Gram-positive, catalase-positive cocci usually found singly or in clusters. *Streptococcus* are Gram-positive, catalase-negative cocci often found in chains. Differentiating between the species within a genus is more complex, but obviously necessary for proper diagnosis of disease. In this exercise, we will isolate cocci from the human body and identify some of the isolates.

BIOCHEMICAL REACTIONS USED FOR IDENTIFICATION OF GRAM-POSITIVE COCCI

CATALASE

During aerobic respiration, microorganisms produce hydrogen peroxide (H_2O_2). This compound is toxic, and accumulation of the substance will result in death of the organism unless it can be enzymatically degraded to water and oxygen.



Catalase is the enzyme responsible for degradation of hydrogen peroxide. To determine the catalase activity of a bacterial culture, a solution of dilute H_2O_2 is added to a bacterial smear on a glass slide. The formation of bubbles (O_2) is evidence of catalase activity. Catalase is invariably present in aerobes and is also present in some facultative organisms. As a differential test, the catalase test is used to distinguish between streptococci (catalase negative) and staphylococci (catalase positive).

COAGULASE

Production of coagulase is indicative of a pathogenic staphylococcal species (*Staphylococcus aureus*). Coagulase acts by a thrombinase-like action. In normal blood clotting, the following reactions occur:



Coagulase acts within host tissues to convert fibrinogen to fibrin. It is theorized that the fibrin meshwork that is formed surrounds the bacterial cells or infected tissues, protecting the organism from nonspecific host defense mechanisms such as phagocytosis.

A coagulase-positive strain of *Staphylococcus* will cause fibrin formation, i.e., will clot plasma. Clot formation within 4 hours is interpreted as a positive result and indicates a virulent *S. aureus* strain.

HEMOLYSINS

Hemolysins are extracellular enzymes that can be detected by their ability to lyse red blood cells. Hemolysins are produced by various pathogenic bacteria and are believed to play a role in the virulence of the organism. The streptococci are classified by their hemolytic activity. Three types of hemolytic reactions can be observed on blood agar plates (nutrient agar supplemented with a 5% concentration of sheep blood). **Alpha** hemolysins partially lyse the red blood cells and reduce the hemoglobin to methemoglobin which produces a green zone around the colony. **Beta** hemolysins cause complete lysis of the red blood cells and there is a clearing of the hemoglobin around the colony. The term **gamma** hemolysis is used to designate no hemolysis or change in the red blood cells.

FIRST PERIOD

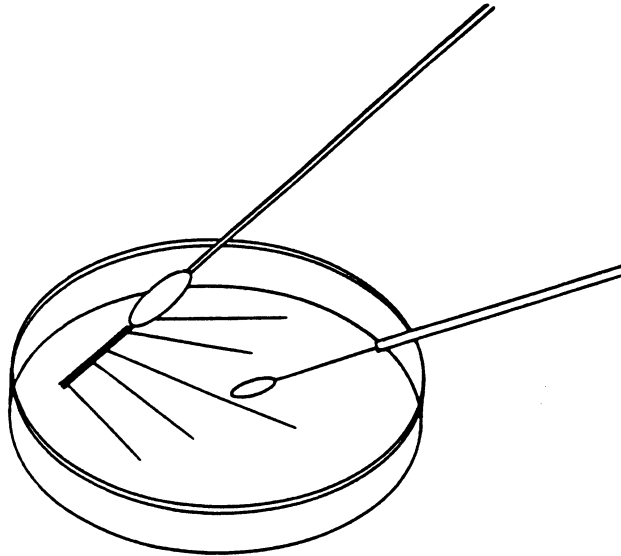
Material:

1. One mannitol salt agar (MSA) plate
2. Two blood agar plates
3. Overnight broth cultures of *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterococcus faecalis* (from plate) and *Streptococcus pyogenes* (from plate)
4. Sterile swabs and sterile toothpicks
5. Rabbit plasma
6. 1.0 and 0.1 ml pipets
7. 3% Hydrogen peroxide solution

Procedure: (work in pairs)

1. Perform coagulase test on *S. aureus* and *S. epidermidis*. Add 0.5 ml of diluted rabbit plasma to small sterile tube. Add 0.5 ml of *S. aureus* or *S. epidermidis* to the tube of plasma. Incubate in the 37°C waterbath, and examine at the end of the lab period.
2. Swab an area of the face (e.g., cheek or forehead) and streak a mannitol salt agar plate. Incubate the plate at 37°C for 18 to 24 hours, then observe colonies. Pull plates and place in refrigerator (4°C) till next lab period.
3. Perform the catalase test on *S. aureus* and *E. faecalis*. Take a loop full of colonies from a **plate** of each bacterium and place inoculum separately on a glass microscope slide. Add a drop of 3% hydrogen peroxide to each. Immediate vigorous oxygen bubbles indicates catalase-positive bacteria.

4. Use a sterile toothpick to scrape tooth enamel. Using the toothpick, make a single short streak on a blood agar plate. Then, using your inoculating loop, streak out the toothpick streaks as illustrated below. Alternatively, you can use a sterile swap to do a back of the throat swap for strep-throat. Incubate the plate in anaerobic jar at 37°C for 48 hours.



5. Divide the second blood agar plate into two sections. Inoculate with *Enterococcus faecalis* and *Streptococcus pyogenes*. One is a negative control and the other a positive control for beta-hemolysis. Incubate the plate in anaerobic jar at 37°C for 48 hours.