SUPPLEMENTAL EXERCISE #2
MICROBIOLOGICAL ANALYSIS OF URINE SPECIMENS

LEARNING OBJECTIVES
After completing this exercise, the student will:

- Be familiar with the microbial species that most often cause infections of the human urinary system.
- Understand and be able to use a standard laboratory method for the detection of bacteriuria.
- Understand and be able to use laboratory tests that can distinguish between the bacterial species that most often infect the human urinary system.

INTRODUCTION AND BACKGROUND
Some parts of the human urinary system, especially the external genitalia and the lower portions of the urethra, are normally occupied by a wide variety of microorganisms. In contrast, the rest of the urinary-system tissues (i.e., the bladder, ureters, and kidneys) are normally sterile, as is the urine that passes through these tissues. Urinary infections occur when pathogenic microorganisms find their way into the normally sterile parts of the human urinary system and start growing there.

Urinary infections may be caused by an unusually diverse group of microorganisms that includes bacteria, fungi, protozoa, and viruses. This exercise will focus on the bacterial causes of urinary infections, the most significant of which include *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Streptococcus faecalis*.

Urinary tract infections are among the most frequently occurring problems in clinical practice, resulting in more than three million visits to doctors each year in the United States. These infections occur in several forms, including urethritis (inflammation of the urethra), cystitis (inflammation of the bladder), urethrocytitis (inflammation of both the urethra and the bladder), and, in males, prostatitis (inflammation of the prostate gland). Urinary infections that originate in one area sometimes spread to other organs and tissues of the urinary tract. Such infections usually start in the lower urethra and then "ascend" to cause problems like pyelonephritis (inflammation of the kidneys) and glomerulonephritis (inflammation of the glomeruli of the kidneys).

Laboratory examination of a urine specimen is always the first step toward diagnosis when a patient is suspected of having a urinary infection. A midstream urine specimen must be collected in a sterile container after cleansing the patient's external genitalia. The specimen must then be cultured immediately (in the clinical laboratory) because some of the microorganisms that normally inhabit the lower urethra will be collected in the specimen along with any pathogens that are present. The naturally occurring microorganisms can grow much faster than most pathogens. If the specimen is not processed quickly, then, the naturally occurring microbes will eventually outnumber the pathogens to such a large extent that the pathogens are not detected by the laboratory tests. The final result will be an incorrect diagnosis. In situations where specimens cannot be processed quickly in the laboratory, this problem can be avoided to some extent by refrigerating the urine specimen immediately after collecting it.

To clinically evaluate a urine specimen, it is first necessary to determine the number of microorganisms in it. Urine containing up to 1,000 (10^3) microbial cells per ml is considered normal. Cell counts greater than 100,000 (10^5) per ml are considered indicative of a significant urinary infection, whereas counts between 1,000 (10^3) and 100,000 (10^5) cells per ml are marginal (i.e., they may or may not be indicative of an infection). Cell counts are most often obtained by spreading a measured volume of the urine specimen (usually 0.1 ml) over the surface of an appropriate nutrient medium in a petri plate. The number of colonies appearing on the plate after incubation will then indicate the number of microbial cells that were present in the specimen. To obtain accurate counts with urine specimens that contain large numbers of microorganisms (e.g., specimens that are visibly turbid), it is often necessary to dilute the specimens (as in Exercise 5) before spreading them on the plates.

The hospital laboratory can begin to identify the microorganisms (and look for the presence of pathogens) in a urine specimen while awaiting the results of that specimen's microbial cell count (above). Many different methods are now available for this purpose. A scheme for differentiating some of the major bacterial species that may be present in a urine sample is shown in Figure 1.

In this exercise, you will enumerate the microorganisms that are present in a specimen of your own urine. You will also attempt to identify these organisms by means of the scheme shown in Figure 1. Most of the media and/or tests employed in this scheme have been described in previous exercises (blood agar in Exercise 8; EMB agar, TSI slants, and urease broth in Exercise 11). The scheme in Figure 1 also makes use of the catalase test, a
simple test for the ability to produce catalase. Catalase is an enzyme that catalyzes the conversion of hydrogen peroxide (H₂O₂) to water and oxygen. To perform a catalase test, one simply places a drop of dilute hydrogen peroxide on top of a colony. The subsequent appearance of bubbles (oxygen) indicates that the test organism produces the enzyme.

**NOTE:** *Detailed instructions and a sterile container for collecting the urine specimen will be provided by your laboratory instructor. To obtain meaningful results in this exercise, you must follow the instructions for collecting the sample very carefully.*

**MATERIALS**

**Media:**
- 1 trypticase soy agar plate
- 1 EMB agar plate
- 1 blood agar plate

**Other Materials:**
- Felt-tip marker
- Sterile container for collecting a urine specimen
- Bunsen burner
- Flint striker
- Sterile pipettes
- Pipette pump
- Glass spreader
- Alcohol
- Catalase test reagent (dilute hydrogen peroxide)

**PROCEDURE (FIRST LAB PERIOD)**

1. Aseptically inoculate the trypticase soy agar plate with the urine specimen as follows:
   a. Label the plate with the date, the exercise number, your name and your section number.
   b. Aseptically pipette 0.1 ml of the urine sample into the plate. You will use this pipette three times so take care to keep it sterile by placing it back in the wrapper or laying it down so that the tip and lower half are not in contact with any nonsterile surface.
   c. Sterilize a glass spreader by dipping it in alcohol and then flaming it (as described in Exercise 5).
   d. Using the sterile spreader, spread the sample evenly over the surface of the agar in the plate.
   e. Resterilize the spreader before putting it down.

2. Inoculate the blood agar plate with the urine specimen, as described in steps 1-a through 1-e using the same pipette.

3. Inoculate the EMB agar plate with the urine specimen, as described in steps 1-a through 1-e using the same pipette.

4. Invert the plates and incubate them at 37°C until the next lab period.

**PROCEDURE (SECOND LAB PERIOD)**

1. Examine the trypticase soy agar plate and calculate the number of microbial cells per ml in the urine sample as directed in the Observations and Results section at the end of this exercise.

2. Examine the blood agar and EMB agar plates and record your findings as directed in the Observations and Results section at the end of this exercise.

3. Select two examples of the numerically predominant colony type appearing on the blood agar plate. (Ask your instructor for help if you cannot decide which colonies to select.)

4. Prepare a Gram stain (Exercise 3) from one of the colonies that you selected in step 3 and examine the stain with an oil immersion lens.

5. Perform the catalase test on the other colony that you selected in step 3, as follows:
   a. Using a sterile loop, take a small amount of the colony and rub it on a clean slide
   b. Place a drop of hydrogen peroxide (H₂O₂) on the dry smear.
   c. Look for the formation of small gas bubbles, which indicate that the test organism is positive for catalase.

6. Select three examples of the numerically predominant colony type appearing on the EMB agar plate. (Ask your instructor for help if you cannot decide which colonies to select.)

7. Prepare a Gram stain (Exercise 3) from one of the colonies that you selected in step 6 and examine the stain with an oil immersion lens.

8. Record your results from steps 4 through 7 in the Observations and Results section at the back of this exercise.
SECOND LAB PERIOD

1. Count the colonies on the trypticase soy agar plate and calculate the number of microorganisms per ml of urine. (Since you put only 0.1 ml of urine on the plate, the number of microorganisms per ml of urine is equal to 10 times the number of colonies on the plate.) Record the number of microorganisms per ml of urine here:

   ___________ / ml

2. Do you consider the number of microorganisms found in your specimen to be "normal"? Why or why not?

3. Examine the blood agar plate carefully. How many different types of colonies are present? Describe the numerically predominant type (i.e., the type that is present in the highest numbers):

4. Examine the EMB agar plate carefully. How many different types of colonies are present? Describe the numerically predominant type:

5. Examine the Gram stain made from the blood agar plate and record your observations here:
   Gram reaction: _____  Cell morphology: ______________________

6. Record the result of the catalase test that you did on the blood agar plate here:
   Catalase reaction (+ or -): _____

7. Examine the Gram stain made from the EMB agar plate and record your observations here:
   Gram reaction: _____  Cell morphology: ______________________
Figure 1. Scheme for differentiation of bacteria that commonly cause urinary tract infections.

Freshly collected midstream urine sample

Spread measured volume of sample on differential media

Blood agar plate

Pinpoint-to-small colonies

Large colonies

Gram stain

Gram-positive cocci

Catalase test

(-)

Streptococcus sp.

(+)

Staphylococcus sp.

Gram stain

Gram-negative rods

Gram-positive cocci

Catalase test

(-)

Streptococcus sp.

(+)

Staphylococcus sp.

Acid slant

& acid butt

H$_2$S -

E. coli

Klebsiella sp.

Urease test

(-)

E. coli

(+)

Klebsiella sp.

Acid slant

& acid butt

H$_2$S +

E. coli

Klebsiella sp.

TSI agar slant

(-)                      (+)

Acid slant           Acid slant

& acid butt         & acid butt

E. coli            Proteus sp.

Klebsiella pneumoniae    Pseudomonas aeruginosa

Proteus vulgaris

No change

Pseudomonas sp.