URINE CULTURES

I. OBJECTIVES
Upon completion of this exercise the student shall be able to:
1. Isolate organisms from a simulated urine specimen using the urine streaking technique.
2. Interpret results using the colony count and variety of organisms present.
3. Recognize bacteria as Gram positive or Gram negative based on culture results; further speciate organisms as Enterococcus, S. aureus, S. epidermidis, Streptococci, P. aeruginosa, Proteus, and E. coli.

II. PRINCIPLE
Chronic or acute infections of the urinary tract may involve the kidneys, ureters, bladder, or urethra. Such infections may cause high blood pressure, kidney damage or uremia. In some instances the infections are inapparent and go unnoticed for some time. Most infections of this tract enter by way of the urethra; very few originate in the blood. The presence of bacteria in the urine is called bacteriuria.

A multitude of organisms can cause urinary infections. The most common cause of such infections in women of child bearing age is Escherichia coli. In order of frequency after E. coli is Enterococcus faecalis (a streptococcus), other related Gram negative rods, yeast and staphylococci.

The sequence of steps in performing a complete study of microorganisms in urine includes:
1. aseptic collection (clean catch)
2. culture
3. quantitative evaluation
4. isolation of the pathogen
5. identification
6. antimicrobial sensitivity testing

III. MATERIALS
1 BAP plate     1 MSA plate
1 MacConkey agar plate (MAC)   1 CNA
4 sterile calibrated
   inoculating loops (0.001 ml)

CULTURES:
Each student will receive a simulated urine specimen containing an organism to be cultured and identified.

IV. PROCEDURE
Day One
1. Label your four plates with your name, seat #, and specimen #.
2. Obtain the urine specimen and gently rotate it so as to mix it thoroughly. Remove lid; place it facing up on the bench top.
3. Aseptically remove a loop from the package. Dip one disposable 0.001 ml calibrated loop vertically into the urine so it goes just below the surface. Withdraw the loop.
4. Examine the loop to be sure that it is full.

5. Touch the loop to the BAP plate at the top edge of the medium and draw the loop down the center of the plate. This deposits the drop of urine on the plate. Streak back and forth as shown in figure 15.1. Dispose of the loop in a biohazard bag.

6. Repeat this procedure with the MSA, CNA, and MAC plates using a NEW loop for each plate.

7. Incubate all plates at 37°C for 24 hours.

**Day Two**

1. Examine all the plates carefully. Identify the number of colony types on each plate; count the number of colonies on each plate. Record this on WORKSHEET 15.1.

   Interpret the results of the bacterial counts (see interpretation chart). Remember that each colony grew from one bacterial cell, therefore each colony that you see represents one bacterial cell in the original urine specimen and is reported as CFU's (Colony Forming Units).

2. Since colony counts are reported as the number of bacterial cells present in one milliliter of urine, the volume of urine that was actually deposited on the plate must be multiplied by a factor that converts it to 1 ml.

   The loop was calibrated to deliver 1/1000 milliliter (0.001 ml). To get 1 ml, multiply the amount of fluid in the loop by a factor of 1000.

   To determine the number of bacteria in 1 ml of specimen, multiply the number of colonies growing by a factor of 1000.

**V. INTERPRETATION OF GROWTH ON URINE CULTURES**

It is important that the number of organisms that are present in urine at the time that it is collected be determined. Because urine itself is a good culture medium, any organisms present in a specimen will multiply unless the specimen is handled properly. Urine specimens should be stored at 2 - 8°C if processing cannot take place within one-half hour of collection.

In the clinical laboratory, the number of organisms in a clean catch voided urine specimen is determined by using loops that deliver an exact volume to streak specimens over the entire surface of plates. When the plates are observed for growth, the number of colony types is noted first, and if only one, or at the most two are seen, the number of colonies is determined and the following interpretive guideline is used.

* Colony Count (1 or 2 types) | Interpretation
---|---
A. < 10,000 CFU's/ml. | Normal flora, skin contamination
B. 10,000 - 99,000 CFU's/ml. | Specimen stored improperly; or infection just beginning
C. 100,000 = 10^5 CFU's/ml. | Significant
D. > 100,000 = > 10^5 CFU's/ml. | Significant

* NOTE: These are suggested guidelines only. Some persons may have a significant infection with a colony count of only 10,000 CFU's/ml. Good judgment and the results of a routine urinalysis are required when evaluating urine culture results. Considerations include the patient's medical condition, underlying disease, and if the patient took antibiotics before the culture was collected. For our lab exercise we will use these criteria in order to interpret results.

Sterile urine collection such as straight catheterization must be indicated on the lab requisition since any colony count (in pure culture) is considered significant.
Figure 15.1
PLATING A URINE SPECIMEN
(STREAKING PATTERN)
**WORKSHEET 15.1**

**VOIDED URINE SPECIMENS**

Name ______________________________    Seat # _____________    Date ______________

Fill in the following table:

<table>
<thead>
<tr>
<th>Unknown Specimen #: ______________</th>
<th>Unknown Identification: ______________</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Medium</th>
<th>Number of Colonies on Plate</th>
<th>Final Count CFU's/ml</th>
<th>How Many Types of Bacteria are Present?</th>
<th>Organisms(s) Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAP</td>
<td></td>
<td></td>
<td>(Include Type of Hemolysis)</td>
<td></td>
</tr>
<tr>
<td>MAC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAN</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MSA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Refer to flow charts in Labs 11 and 12. A Gram stain may be performed to help with the identification.

1. **Draw a flow chart on the back of this page to show how you identified the organisms.**

2. **Complete Worksheets 15.2 and 15.3.**
FLOW CHART

Gram Stain

[+] Cocci  Bacilli

[−] Cocci  Bacilli

Complete only the portion of the flow chart appropriate for your Gram stain results. Refer to Appendix B.
**WORKSHEET 15.2**

Name ______________________________    Seat # _____________    Date ______________

1. Urine Culture Unknown # ___________________________

2. Gram Stain Results ________________________________

3. Choose only the appropriate ones and NOT every single one. Indicate which ones were performed with a √.

<table>
<thead>
<tr>
<th>Performed</th>
<th>Results ( + or –)</th>
</tr>
</thead>
</table>

### Gram Positive Tests

<table>
<thead>
<tr>
<th></th>
<th>Performed</th>
<th>Results ( + or –)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A.</td>
<td>Catalase</td>
<td></td>
</tr>
<tr>
<td>B.</td>
<td>Hemolysis (BAP)</td>
<td></td>
</tr>
<tr>
<td>C.</td>
<td>Coagulase</td>
<td></td>
</tr>
<tr>
<td>D.</td>
<td>“A” Disc</td>
<td></td>
</tr>
<tr>
<td>E.</td>
<td>Bile Esculin Agar</td>
<td></td>
</tr>
</tbody>
</table>

### Gram Negative Tests

<table>
<thead>
<tr>
<th></th>
<th>Performed</th>
<th>Results ( + or –)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F.</td>
<td>Lactose Fermentation</td>
<td></td>
</tr>
<tr>
<td>G.</td>
<td>Glucose Fermentation</td>
<td></td>
</tr>
<tr>
<td>H.</td>
<td>Urea Agar Slant</td>
<td></td>
</tr>
<tr>
<td>I.</td>
<td>Indole Production</td>
<td></td>
</tr>
<tr>
<td>J.</td>
<td>Hemolysis (BAP)</td>
<td></td>
</tr>
<tr>
<td>K.</td>
<td>Swarming</td>
<td></td>
</tr>
<tr>
<td>L.</td>
<td>Odor</td>
<td></td>
</tr>
</tbody>
</table>

4. **Growth on Agar**

<table>
<thead>
<tr>
<th></th>
<th>Performed</th>
<th>Results ( + or –)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A.</td>
<td>Blood Agar</td>
<td></td>
</tr>
<tr>
<td>B.</td>
<td>MacConkey’s Agar</td>
<td></td>
</tr>
<tr>
<td>C.</td>
<td>Colistin Naldixic Agar</td>
<td></td>
</tr>
<tr>
<td>D.</td>
<td>Mannitol Salt Agar</td>
<td></td>
</tr>
</tbody>
</table>
WORKSHEET 15.3
VOIDED URINE SPECIMENS

1. Does your patient have a “significant” colony count? Explain.

2. What is the treatment for urinary tract infections?

3. List 5 organisms that are commonly isolated from patients with urinary tract infections (U.T.I.s).

4. Describe the proper collection technique for urine cultures.
   a. Patient instructions.
   b. Type of container to be used. ________________________________
   c. What information should be on the container? ____________________________
      Lab request form? ________________________________
   d. If there is a delay in transport to the laboratory, how should the specimen be stored?
      ________________________________
      Why? ________________________________