Module 9: Urine Cultures/Gram-negative rods

Objectives:

Upon completion of MM550 lectures, required reading, on-line material and laboratory exercises, the learner will:

Urine Cultures
1. Categorize the following urine specimens as sterile or non-sterile:
   a. Voided
   b. Clean catch
   c. Catheterized
   d. Suprapubic
   e. Nephrostomy

2. Describe the procedure for setting up a urine culture, including proper specimen handling.

3. Explain why a voided urine is not an acceptable specimen for culture.

4. Evaluate urine culture findings, including calculation of colony counts.

Gram-negative Rods
5. Interpret:
   a. Cytochrome oxidase test
   b. Indole test
   c. MacConkey agar reactions
   d. Kligler’s Iron Agar (KIA) reactions
   e. Urea agar reactions
   f. Simmon citrate agar reactions

6. Describe the following characteristics of the Enterobacteriaceae, Pseudomonas aeruginosa, Acinetobacter sp. and Aeromonas hydrophila:
   a. Gram stain reaction and morphology
   b. Oxidase reaction
   c. Glucose fermentation

7. Differentiate the following gram-negative rods based on the characteristics and tests listed in objective #5 and #6.
   a. Escherichia coli
   b. Klebsiella pneumoniae
   c. Proteus mirabilis
   d. Serratia sp.
   e. Acinetobacter sp.
   f. Pseudomonas aeruginosa
   g. Aeromonas hydrophila

8. Evaluate ESBL (extended-spectrum beta-lactamase) testing results, including susceptibility to specific beta-lactamase antibiotics.
I. Introduction

A. Urinary tract infections (UTIs) are among the most common infections of humans. The majority are caused by a limited number of bacteria, and the presence of these microorganisms in urine is termed bacteriuria.

B. Clinical Considerations

1) Infections of the urinary tract may involve various sites including the urethra, bladder, kidneys and communicating glands such as the prostate gland. It is generally accepted that urinary tract infections are usually a consequence of ascending infection by microorganisms from the lower tract (urethra) into the upper tract (ureters and kidneys).

2) Gram-negative rods, which include members of the family Enterobacteriaceae (i.e., Escherichia coli, Klebsiella sp., Enterobacter sp. & Proteus mirabilis) and Pseudomonas aeruginosa, are the most common etiologic agents of UTIs. Other bacteria causing urinary tract infection include Staphylococcus aureus, the coagulase-negative staphylococci (i.e., S.saprophyticus), and Enterococcus sp.

3) The ultimate reservoir for infecting organisms appears to be the patient's normal fecal flora.

4) Urethral normal flora includes:
   a) Anaerobic bacteria
   b) Corynebacterium sp. (diphtheroids)
   c) Alpha and nonhemolytic Streptococci
   d) Lactobacilli
   e) Nonpathogenic Neisseria sp.
   f) Coagulase negative Staphylococci

II. Specimen Collection

A. Urine is the most common specimen collected to diagnose urinary tract infections. Urine under normal circumstances is sterile. It may, however, become contaminated with the flora from the urethra, vagina, or perineum.

B. Specimen Types

1) Voided or random: urine voided into a specimen container without any cleansing of the external genitalia. Urine will be contaminated with normal genital-urinary tract flora and is therefore unacceptable for culture.

2) Clean-catch midstream: the method of collection of a clean-voided midstream urine will vary depending upon age, sex, and the ability of the patient to cooperate. Instructions on proper cleansing of the external genitalia are given to the patient prior to collection. A sterile container is used (as is true with all other collections) and the specimen submitted for culture in a transport medium or refrigerated as rapidly as possible. If contaminating organisms are present, they can rapidly multiply causing a falsely high colony count).
3) Indwelling catheter: the risk of urinary tract infection is increased for patients with indwelling catheters. These infections represent a large proportion of all nosocomial infections in hospitalized patients. Urine for culture is to be collected aseptically by aspiration from the catheter port and not from the draining bag.

4) Straight catheter: obtaining urine by single straight catheterization of the bladder is not routinely recommended because of the risk of introducing bacteria into the bladder from the colonized urethra.

5) Suprapubic aspiration: a sterile urine specimen obtained by suprapubic needle aspiration of the bladder avoids the problem of contamination associated with the collection of voided urine. This is the preferred method for collecting urine from infants, bedridden patients, and for suspected anaerobic infections.

6) Nephrostomy: a sterile urine specimen obtained directly from the nephrons of the kidney.

III. Specimen Handling and Transport

Once the urine specimen has been collected, it should be sent to the laboratory as quickly as possible. The urine should be cultured within 2 hours after collection, refrigerated for up to 24 hours after collection, or submitted in a transport medium, which keeps constant the number of viable organisms in the urine specimen.

IV. Laboratory Examination

A. Direct Microscopic Examination

1) Urinalysis: utilizes numerous biochemical reactions on a plastic strip. Amongst many other parameters, the strip will detect white blood cells (WBCs) in the urine and the reduction of nitrate to nitrite by certain bacteria. Both are indications of a possible urinary tract infection (UTI). Most laboratories will perform a microscopic exam of the urine if any of the screening biochemical tests on the reaction strip are positive. The presence of WBCs and microorganisms are indicative of a UTI.

2) The Gram stain may be useful for detecting significant bacteriuria from uncentrifuged (unconcentrated) urine. However, this procedure lacks sensitivity since levels of bacteria <10^5 cfu/ml may be missed.

B. Culture: Selection of Media

1) Routine urine cultures are plated onto 5% sheep blood agar
2) and a gram-negative selective/differential medium such as MacConkey agar.

C. Quantitation of Cultures

1) Quantitative cultures for the diagnosis of UTI are used to discriminate between contamination, colonization, and infection.

Calibrated loop method: the calibrated loop involves dipping a calibrated loop (0.01 or 0.001 ml) into the urine and plating the amount of urine picked up by the loop
V. Laboratory Work - Day 1

NOTE: The procedures outlined here are designed to provide maximum information in a minimum amount of time. Because of the time scheduled for this laboratory, it is necessary to do on day one some procedures which would ordinarily not be done until 24-48 hours after the initial culture was set up.

A. Supplies Per Student
   1) **Simulated urine specimen** (for culture set up)
   2) **Organism unknown grown up in T-soy broth** (for biochemical set up)
   3) Blood agar (1 plate)
   4) MacConkey agar (1 plate)
   5) Kligler’s Iron agar (1 tube)
   6) Simmon’s citrate agar (1 tube)
   7) Indole test (1 tube)
   8) Urea agar (1 tube)
   9) Calibrated 0.001 disposable loop (1)

B. Set-Up of Urine Culture & Colony Count - using unknown urine specimen
   1) Appropriately label a sheep blood agar and MacConkey agar plate.
   2) To mix simulated **urine specimen**, gently swirl or tap the test tube containing the specimen
   3) A disposable inoculating loop, calibrated to contain 0.001 ml of liquid, is vertically immersed into the urine sample. (Process should be done rapidly for accurate delivery of volume). When removing the inoculated calibrated loop from the container, care must be taken to avoid touching the sides of the container.
   4) Using the calibrated disposable loop, a single streak is made down the center of the blood agar plate. Using the same loop (without reinoculating the loop) use a sweeping motion at right angles to the primary inoculum to streak the entire plate, being careful not to streak over the same area (see illustration).
   5) Using the same calibrated disposable loop, repeat steps 3 and 4 to inoculate the MacConkey agar plate
   6) Incubate plates, agar side up, in the wire basket located on your bench.

Continue to next page
C. Identification - **USING THE BROTH LABELED “ISOLATED ORGANISM SUSPENSION”**

1. Gram stain the isolated organism suspension. Record results on laboratory record sheet at the end of this module.

2. Inoculate the following biochemicals using one loopful (use a nichrome loop, NOT a plastic calibrated loop) of the “isolated organism suspension” broth per biochemical tube:

   - **KIA:** stab butt (to bottom of tube) and streak slant
   - **Indole:** mix in broth
   - **Simmon’s citrate:** streak slant
   - **Urea:** streak slant

   After each inoculation, loosely recap each of the above biochemical tubes to allow for air exchange.

3. Rubber band the tubes together and place in a cup or place in a test tube rack in the wire basket located on your bench.

D. Look at laboratory demos
VI. Laboratory Work - Day 2

A. Interpretation of Urine Culture/Colony Count

1) Colony Count: the number bacteria in the urine sample is estimated by counting the number of colonies that appear on the surface of the blood agar plate for EACH colony type and multiplying by the dilution factor (i.e., if 0.001 ml loop utilized, hen dilution factor is 1000). If >100 colonies are on the blood agar plate, the colony count is reported as >100,000 cfu/ml.

2) Record the colony count on the laboratory record sheet at the end of this module.

Growth of >10,000 cfu/ml (i.e., >10,000 colony forming units per ml of urine) of a single organism is considered to be a probable urinary tract infection and appropriate identification and susceptibilities are to be performed.

B. Identification of Organism

1) On the laboratory record sheet record:
   a) Colony morphology on sheep blood agar
   b) If there was growth, colony morphology on MacConkey agar (i.e., pink colonies = lactose fermenter and clear colonies = non-lactose fermenter)

2) Perform an oxidase test (see appendix 3) on your organism. Use an isolated colony on your sheep blood agar. Necessary supplies are located at the end of your bench. Record results on your laboratory record sheet.

3) Add 3 drops of Kovac’s reagent (located at the end of your bench) to the indole tube. Gently shake the tube.

4) On your laboratory record sheet, record the results of the four biochemical tests you inoculated on Wednesday. (See appendix 3).

5) Using the flow charts located in appendix 4, identify your organism and record the result on your laboratory record sheet.

C. Using the demos around the room, find the appropriate susceptibility test performed on your organism. (We have set these up for you due to time constraints).

1) Record the susceptibility results on your laboratory record sheet.

2) Indicate on your laboratory record sheet if this susceptibility result is acceptable (use your textbook, antibiogram data, and page 6 of module 4).
Module 9 Urine Culture: Laboratory Record Sheet

Day 1:
Urine culture #____________
Gram stain result (from isolated organism suspension broth): ___________________________

Day 2:
Colony Count = ___________________________

Is this colony count indicative of a urinary tract infection? Why?____________________

Colony morphology on sheep blood agar: _____________________________________________
Colony morphology on MacConkey agar: ______________________________________________

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<tr>
<th>Test</th>
<th>Result</th>
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<tbody>
<tr>
<td>Oxidase</td>
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<td>Kligler’s Iron Agar (KIA)</td>
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<td>Indole</td>
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<td>Simmon’s citrate</td>
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<td>Urea</td>
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Organism identification = ___________________________________
Susceptibility Test:

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<tr>
<th>Antibiotic Tested</th>
<th>Result (i.e., zone or MIC)</th>
<th>Interpretation (i.e., sensitive, intermediate, resistant)</th>
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Are these susceptibility test results acceptable? Explain.