URINE CULTURES – GENERAL PROCEDURE

I. Principle
Urine cultures are performed to detect organisms that are the causative agents of urinary tract infections. Urinary tract infections are one of the most common bacterial infections. Normally the urinary tract is sterile above the urethra. However, during noninvasive collection techniques urine is potentially contaminated with normal flora of the urethra and genitourinary tract. For this reason, urine cultures utilize a colony count (quantitation of growth) to aid in determining if dealing with contamination, colonization, or infection. Infections are associated with counts of 100,000 (10^5) or more organisms per ml of urine. However, low counts can be clinically significant in symptomatic patients. In properly collected specimen, contamination from external genitalia usually yields counts lower than 1,000 cfu/ml. Selection of media and incubation requirements are based on the potential pathogens isolated. Common pathogens include but are not limited to: Enterobacteriaceae, nonfermenting gram negative rods, Staphylococcus saprophyticus, Enterococcus, Group B Streptococcus and yeast. Based on potential pathogens, in general media used includes a nutrient agar along with a selective GNR medium. Urinary tract infections are not typically associated with fastidious organisms and therefore are not routinely cultured for.

II. Specimen Collection, Transport and Handling
A. Acceptable specimen types and collection: first morning specimen is preferred as this specimen is the most concentrated and will yield the most accurate colony counts. Urine must be collected in a sterile container.
   1. Clean-catch midstream urine – Clean area with soap and water, rinse, and hold labia or retract foreskin, begin to voiding after several ml passed collect midstream.
   2. Straight (quick) catheterized urine – (in and out) clean area with soap and water, and rinse. Insert catheter into bladder and allow first 15 ml to pass; then collect remainder
   3. Indwelling catheterized urine – specimen should be aspirated from the drainage tube with a needle and syringe, rather than sampling the urine from the drainage bag. The urine from the bag may have falsely elevated colony counts due to prolonged standing at room temperature.
   4. Suprapubic urine – collect by passing a needle through the skin directly into the bladder.
   5. Surgically collected specimen – kidney or bladder

B. Unacceptable specimens:
   1. Specimens that do not follow transport and handling guidelines. (see below)
   2. Voided urine
   3. Urine sediment
   4. Twenty-four (24) hour urine specimens are unacceptable for culture due to bacterial overgrowth.
   5. Clean catch and catheterized urine for anaerobic culture as the urethra is colonize with anaerobic streptococcus and Propionibacterium species.

C. Specimen transport and handling:
   1. Urine is an excellent culture media and colony counts will rapidly increase giving falsely elevated results if not processed in timely manner.
      a. Room temperature: Urine may be kept for 30 minutes at room temperature after collection. If it cannot be processed in 30 minutes it must be refrigerated.
      b. Refrigerated: Urine may be held for up to 24 hours prior to culture if refrigerated at 4ºC. The lower temperature inhibits bacterial growth and keeps the colony count stable.
      c. Transport containers: Urine may be placed in an appropriate transport media such as boric acid and held at room temperature for up to 24-48 hours (verify with package insert of media). Prolonged storage may decrease colony count.
III. Reagents, Supplies and Equipment:
   A. BAP = Sheep blood agar, MAC = MacConkey agar
      a. CHOC = chocolate agar, BY SPECIAL REQUEST ONLY
      b. ABAP = anaerobic blood agar (suprapubic urine)
   B. 1:100 and 1:1000 calibrated loops
   C. Gloves
   D. Lab coat
   E. Biological Safety Cabinet
   F. 35°C incubator

IV. Quality Control
   A. See specific identification and susceptibility procedures.

V. Direct Examination
   A. Gram stain
      1. Some laboratories perform a gram stain on the urine specimen (not all labs do this as it is labor intensive). **Gram stains will not be done in student laboratory.**
   B. Other screening tests such as a routine urinalysis (nitrite, leukocyte esterase, protein, microscopic examination) should be used to correlate with urine culture when available.

VI. Specimen Processing
   A. Culture Setup
      1. Use one Sheep Blood agar plate (BAP), and one MacConkey Agar plate (MAC) per specimen (or another selective/differential GNR medium such EMB).
      2. For suprapubic aspiration urine specimens use a BAP, MAC and Anaerobic blood agar plate (ABAP).
      3. Use a chocolate agar when requested by physician.
      4. Gently swirl the urine specimen to mix.
      5. Select a calibrated loop
         a. 0.001 ml calibrated loop for clean-catch midstream and catheterized specimens
         b. 0.01 ml calibrated loop for suprapubic, cystoscopy and nephrostomy specimens
      6. Inoculate media
         a. Vertically immerse the appropriate loop just below the surface of a well-mixed urine sample. (Indicate type of loop used, 0.01 loop = $10^{-2}$ plate)
         b. This process should be done rapidly holding the loop at a straight 90° angle for accurate delivery of volume.
         c. Verify sample is in the loop.
         d. Quickly make a single streak down the center of the BAP. (Always inoculated non-selective media before selective media)
         e. Then with a sweeping motion at right angles to the primary inoculum, streak the entire plate, being careful not to streak over the same area. See diagram below.
         f. With the same calibrated loop, do the same (a-e) with the MAC.

   ![Diagram of streaking method](image)

   7. Incubate media
      a. Temperature: 35°C
      b. Atmosphere: BAP - ambient air, MAC - ambient air, ABAP – anaerobically
      c. Time: minimum of 48 hrs, first read 18-24 hrs. ABAP should be held for 72 hrs.
VII. Examination of Culture Media
A. Examine culture media that has been incubated overnight.
   1. If there is no visible growth:
      a. At 18 to 24 hours:
         i. Preliminary report: Colony count <1000 cfu/ml (if setup with 0.001 ml loop), No growth at 24 hours
         ii. Preliminary report: Colony count <100 cfu/ml (if setup with 0.01 ml loop), No growth at 24 hours
         iii. Re-incubate culture plates for additional 24 hours. ABAP should be kept for 72 hours.
      b. At 48 hours:
         i. Final report: Colony count <1000 cfu/ml, No growth at 48 hours (if setup with 0.001 ml loop)
         ii. Final report: Colony count <100 cfu/ml, No growth at 48 hours (if setup with 0.01 ml loop)
         iii. Discard aerobic culture plates, hold ABAP for 72 hours before reporting as no growth on anaerobes.

   2. If there is visible growth, determine colony count for each morphological type of organism present. See part B below for determination of colony count.

B. Colony Count Determination for each morphological type of organism present.
1. With the 0.001 loop (10^-3), one colony equals 1,000 cfu/ml.
   a. Multiply number of colonies on 10^-3 plate (0.001 ml loop) times 1000 to arrive at colony count
   b. If number of colonies on 10^-3 plate is >100, report out colony count as >100,000 cfu/ml
   c. Confluent growth of bacteria, covering most of the inoculated surface area of the plates to be read as >100,000 cfu/ml.
2. With the 0.01 loop (10^-2), one colony equals 100 cfu/ml.
   a. Multiply number of colonies on 10^-2 plate (0.01 ml loop) times 100 to arrive at colony count
3. Examples:
   
<table>
<thead>
<tr>
<th>Plate</th>
<th>Colonies</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>10^-2</td>
<td>50</td>
<td>5000 cfu/ml</td>
</tr>
<tr>
<td>10^-3</td>
<td>5</td>
<td>5000 cfu/ml</td>
</tr>
</tbody>
</table>
4. Colony count discrepancies
   a. The MAC plate is used to estimate gram-negative rod growth only.
   b. If there is a large difference in colony counts between the two plates (for the same organism), the larger count should be reported.

VIII. Interpreting Culture results
A. Common pathogens, contaminants, and reporting and work up of results
1. Pathogens commonly isolated in urine
   a. Escherichia coli – most common cause of urinary tract infections
   b. Proteus species
   c. Other Enterobacteriaceae including: Enterobacter, Klebsiella, Citrobacter, Serratia
   d. Enterococcus species
   e. Beta-hemolytic streptococcus including Streptococcus agalactiae and Streptococcus pyogenes
   f. Staphylococcus saprophyticus – most commonly seen in young females
   g. Staphylococcus aureus
   h. Staphylococcus epidermidis (hospitalized patients)
   i. Pseudomonas aeruginosa, Acinetobacter and other non-fermenting gram negative rods
   j. Candida sp. (Yeasts)
2. **Potential contaminants commonly isolated in urine**
   a. Diphtheroids (>100,000 cfu/ml if in pure culture may be considered a pathogen)
   b. Coagulase-negative staphylococci other than *Staphylococcus saprophyticus* (>100,000 cfu/ml if in pure culture may be considered a pathogen)
   c. Alpha hemolytic and non-hemolytic streptococci (i.e., *viridans* group)
   d. *Lactobacillus* species
   e. *Escherichia coli* and other "coliforms" – especially when mixed and isolated with other contaminants
   f. *Bacillus* species
   g. Non-pathogenic *Neisseria* species

3. **Work up of organism, including identification and sensitivity, is based on the correlation of all of the following:**
   a. **Specimen type**
      i. Clean-catch midstream urine can contain contaminants usually in low numbers if collected properly
      ii. Catheterized urine may contain contaminants but in very low numbers
      iii. Suprapubic specimens should be sterile. Thus any organism growing should be identified and sensitivities performed if appropriate.

   b. **Number of colony types**
      i. A single colony type of probable pathogens, can be indicative of an infection if the organism is present in high enough numbers. See below.
      ii. Growth of two species of probable pathogens, can be indicative of an infection if present in high enough numbers. See below.
      iii. Growth of three (3) or more different organisms is to be considered a contaminated specimen and work-ups are not done unless specimen is a suprapubic, nephrostomy or cystoscopy sample or a suspected pathogen comprises 80% of the total growth (predominant).
      iv. Unless you have a distinct colony morphology growing on your BAP or MAC, gram stains must be performed to identify colonial growth.

      **Note:** When determining colony types present, remember that what is growing on your MAC will most likely be present on your BAP, gram stains may need to be done to determine which colony morphology on your BAP matches growth on your MAC.

   c. **Colony counts**
      i. Generally speaking, >100,000 cfu/ml is indicative of a UTI, except when the isolate is one of the contaminants.
      ii. 10,000 – 100,000 cfu/ml may indicate infection especially if there is only one isolate that is a pathogen.
      iii. <10,000 for catheterized or clean catch urine, especially if there are contaminants present, are not worked up.
      iv. Persistence of the same organism on repeat urine cultures will increase the likelihood that it is a pathogen even if the colony counts are low (i.e. <10,000 cfu/ml). This is especially true if the patient has symptoms of a UTI.

   d. **Patient clinical history (if available):**
      i. Age
      ii. Female or male
      iii. Exhibiting symptoms of a UTI
      iv. Previous antibiotic therapy
e. **Overview of interpretation of results:**
   i. ≥100 cfu/ml from suprapubic, cystoscopy, and nephrostomy require work up (identification and susceptibilities if appropriate) of all species of potential pathogens.
   ii. ≥10,000 cfu/ml of pure culture of potential pathogen from clean catch or catheterized specimen requires workup.
   iii. ≥10,000 cfu/ml of two species of potential pathogens of organism from clean catch or catheterized specimen requires further workup
   iv. >10,000 cfu/ml of three or more species from a clean catch or catheterized specimen requires no further workup unless on organism comprises 80% of the total growth. When there is growth of three or more organisms, and suspected pathogen comprises 80% of the total growth (predominant), perform identification and sensitivities on the predominant organism.

4. **Culture work up – Perform gram stain if necessary.**
   a. Gram positive cocci- Perform Catalase (see catalase procedure)
      i. Catalase positive
         o For identification see Staphylococcal Flowchart, stopping when pathogens are identified or ruled out.
         o Perform susceptibilities testing if appropriate
      ii. Catalase negative
         o Observe hemolysis pattern
         o For identification see Streptococccaceae Flowchart, stopping when pathogens are identified or ruled out.
         o Perform susceptibilities testing if appropriate
   b. Gram Negative rods
      i. Observe MacConkey growth and perform oxidase (see oxidase procedure)
      ii. Perform GNR rapid identification testing of full identification panel (e.g. Micoscan, Vitek, or API)
      iii. Perform susceptibility testing if appropriate
   c. Yeast
      i. Perform Germ tube
         o Positive – report as *C. albicans/dubliensi* if organism is a budding yeast
         o Negative – perform Rapid trehalose and gram stain
            o Small yeast positive for rapid trehalose – report as *C. glabrata*
            o Rapid trehalose negative – perform additional identification panels
   d. Gram Positive rods- if >100,000 cfu/ml and pure culture
      i. Pallisading GPR
         o Perform catalase
            o Positive- perform rapid urease
               ▪ Rapid urease positive – identification panel to screen for *Corynebacterium urealyticum*
               ▪ Rapid urease negative – no further testing report as diphteroids
      ii. Long thin gram positive rods
         o Alpha hemolysis – probable *Lactobacillus*, no further work-up
5. **Report results**
   a. Correlate all information – Does it make sense?

   b. Preliminary report – include:
      i. Colony count
      ii. As much information about organism identification as possible
         Example:
         1. >100,000 cfu/ml Lactose-fermenting GNR, ID and sensitivity pending
         2. 50,000 cfu/ml coagulase negative *Staphylococcus*, ID pending
         3. 50,000 cfu/ml Mixed Gram positive flora
         4. 80,000 cfu/ml Lactose-fermenting GNR, ID and sensitivity pending
            30,000 cfu/ml Mixed Gram positive flora

c. Final report – include:
   i. Colony count
   ii. Organism identification (if pathogen) or organism descriptor (if contaminant)
   iii. Organism susceptibility results or comment that they are pending (if pathogen) or comment that they are not performed.
      Example:
      1. >100,000 cfu/ml *Escherichia coli*, MIC pending or results
      2. 50,000 cfu/ml *Staphylococcus saprophyticus* (Routine antimicrobial susceptibility testing of *Staphylococcus saprophyticus* is not recommended by CLSI as in vitro testing does not correlate with in vivo response. Urinary tract infections respond favorably to concentrations achieved in urine of antimicrobial agents commonly used to treat acute, uncomplicated urinary tract infections)
      3. >100,000 cfu/ml Mixed Gram negative and Gram positive flora
         Three or more colony types indicate contamination. If clinically indicated, please submit a new specimen
      4. 80,000 cfu/ml *Escherichia coli*, MIC pending or results
         30,000 cfu/ml Mixed Gram positive flora

B. After 24 hours, culture plates are reincubated.

C. After 48 hours, culture plates are discarded (if all organism workups are completed).
Rapid Testing may be performed to identify the following organisms from urine culture:

<table>
<thead>
<tr>
<th>Organism</th>
<th>Observation</th>
<th>Additional Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>Non spreading on BAP, Lactose fermenting GNR on MAC</td>
<td>Spot indole positive, Oxidase negative, Beta hemolytic or Nonhemolytic and PYR-negative</td>
</tr>
<tr>
<td><em>Proteus mirabilis</em></td>
<td>Swarming colony on BAP, Non-lactose fermenting GNR</td>
<td>Indole-negative, Ampicillin-sensitive</td>
</tr>
<tr>
<td><em>Proteus vulgaris</em></td>
<td>Swarming colony on BAP, Non-lactose fermenting GNR</td>
<td>Indole-positive</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>Metallic or mucoid colony, Non-lactose fermenting GNR, Grape or tortilla odor</td>
<td>Oxidase positive, Indole-negative</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>White to yellow opaque colonies, Beta-hemolytic</td>
<td>Catalase Positive, Staph Latex-positive or Tube coagulase positive</td>
</tr>
<tr>
<td><em>Enterococcus sp.</em></td>
<td>Non-hemolytic strep on BAP, &gt;1 mm diameter colony, GPC in chains (no clusters)</td>
<td>Catalase – negative, PYR-positive</td>
</tr>
<tr>
<td><em>Candida albicans/dubliniensis</em></td>
<td>Oval shaped budding yeast in smear</td>
<td>Stellating in less than 48 hours; Or Germ tube positive</td>
</tr>
<tr>
<td><em>Candida glabrata</em></td>
<td>Small yeast with no hyphae in smear, Better growth on chocolate than Sheep blood agar</td>
<td>Germ tube negative, Rapid trehalose positive at 42°C</td>
</tr>
</tbody>
</table>

IX. References


C. Bailey & Scott’s Diagnostic Microbiology, Forbes, 11th edition, Chapter 60, pages 927-938.