URINE CULTURES – GENERAL PROCEDURE

I. Principle
Urinary tract infections 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. 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. Specimen types and collection
1. Clean-catch midstream urine – first morning specimen is preferred as this specimen is the most concentrated and will yield the most accurate colony counts. 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. Cystoscopy urine – bladder urine collected by insertion of a cystoscope.

B. Specimen transport and handling
1. Urine held at room temperature should be cultured within 30 minutes of collection. If it cannot be processed immediately, it must be refrigerated because urine is an excellent culture media and colony counts will rapidly increase giving falsely elevated results.
2. 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.
3. Urine may be placed in an appropriate transport media and held at room temperature for up to 24-72 hours (verify with package insert of media). Prolonged storage may decrease colony count.
4. Twenty-four (24) hour urine specimens are unacceptable for culture due to bacterial overgrowth.

III. Reagents, Supplies and Equipment:
A. BAP = Sheep blood agar, MAC = MacConkey agar (Choc = chocolate agar, ABAP = anaerobic blood agar as needed)
B. 1:100 and 1:1000 calibrated loops
C. Gloves
D. Lab coat
E. Biological Safety Cabinet
F. 35°C incubator
IV. 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) may be used to correlate with urine culture.

V. 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 done 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 enter 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 urine streaking on agar plates]

      7. Incubate media
         a. Temperature: 35°C
         b. Atmosphere: BAP - either ambient air or CO2, MAC - ambient air, ABAP – anaerobically
         c. Time: minimum of 48 hrs, first read 18-24 hrs. ABAP for 72 hrs.

VI. 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. Reincubate 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:
      \[10^{-2} \text{ plate } 50 \text{ colonies } \times 100 = 5000 \text{ cfu/ml}\]
      \[10^{-3} \text{ plate } 5 \text{ colonies } \times 1000 = 5000 \text{ cfu/ml}\]
   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.

VII. Interpreting Culture results

A. Common pathogens, contaminants, and reporting and work up of results
   1. Pathogens commonly isolated in urine (page 1016-1017, 3rd edition Mahon)
      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. Contaminants commonly isolated in urine (page 1016-1017, 3rd edition Mahon)
      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
      h. Anaerobic streptococci
      i. Propionibacterium 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, nephrostomy, and cystoscopy 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).
         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.
      iv. Unless you have a distinct colony morphology growing on your BAP or MAC, gram stains must be performed to identify colonial growth.
   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 colonies/ml from suprapubic, cystoscopy, and nephrostomy require work up (identification and susceptibilities if appropriate) of all species of potential pathogens.
      ii. >10,000 colonies/ml of pure culture of potential pathogen from clean catch or catheterized specimen requires workup.
      iii. >10,000 colonies/ml of two species of potential pathogens of organism from clean catch or catheterized specimen requires further workup
      iv. >10,000 colonies/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 (pairs, chains and clusters)
      i. Perform catalase (see catalase procedure)
         • Catalase positive
            o Perform Coagulase testing (see procedure)
               ▪ For identification and further tests see Staphylococcal flow chart
               ▪ Perform susceptibilities testing if appropriate (not performed in student lab)
            • Catalase negative
               o Observe hemolysis pattern
                  ▪ Beta hemolytic (potential pathogens)
                     • See Streptococcaceae flowchart for identification
                     • Susceptibilities not routinely performed.
                  ▪ Alpha hemolytic
                     • Perform PYR (see procedure)
                        o Positive
                           ▪ Report as Enterococcus
                           ▪ Perform Susceptibility testing (not performed in student lab)
                        o Negative
                           ▪ No further workup
                  ▪ Gamma hemolytic
                     • Perform PYR (see procedure)
                        o Positive
                           ▪ Report as Enterococcus
                           ▪ Perform Susceptibility testing (not performed in student lab)
                        o Negative
                           ▪ No further work up
   b. Gram negative rods
      i. Observe MacConkey Growth
         • Lactose fermenter
            o Perform oxidase
               ▪ Negative
                  • Perform OF glucose, KIA, LIA, ODC, motility, nitrate, indole, citrate, and
                    urease (see procedures)
                  • Utilize Enterobacteriaceae biochemical tables for identification
            • Non lactose fermenter
               o Perform oxidase
                  ▪ Negative
                     • Perform OF glucose, KIA, LIA, ODC, motility, nitrate, indole, citrate, and
                       urease (see procedures)
                     • Utilize Enterobacteriaceae biochemical tables for identification
                       IF organism is a glucose fermenter otherwise see below.
                  ▪ Positive
                     • Perform OF glucose, Growth at 42 degrees, sub clear media for pigment
                       production, Moeller based arginine, ornithine, and lysine
                     • Utilize non fermenting gram negative bacilli charts for identification
      ii. Perform susceptibility testing if appropriate. (not performed in student lab)
c. Yeast
   i. Perform Germ tube
      • Positive
         o Report as Candida albicans
      • Negative
         o Perform additional identification panels (MicroScan, Vitek or API)

d. Gram positive rods
   i. Pallisading gram positive rods
      • Perform catalase
         o Positive- Diptheroids no further work up.
   ii. Long thin gram positive rods
      • Alpha hemolysis – probable lactobacillus, no further work up.

e. The above is used for identification in student lab. Your clinical site may utilize any of the following for identification of pathogens.
   i. Gram stain (if necessary)
   ii. Rapid (screening) tests
   iii. Identification panel (i.e., MicroScan, Vitek)

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:
         >100,000 cfu/ml Lactose-fermenting GNR, ID and sensitivity pending
         10,000 cfu/ml coagulase negative Staphylococcus, ID pending
   c. Final report – include:
      i. Colony count
      ii. Organism identification (if pathogen) or organism descriptor (if contaminant)
      iii. Organism susceptibility results (if pathogen)
         Example:
         >100,000 cfu/ml *Escherichia coli*, MIC results
         10,000 cfu/ml coagulase negative *Staph. not Staphylococcus saprophyticus*

B. After 24 hours, culture plates are reincubated.

C. After 48 hours, culture plates are discarded (if all organism workups are completed).

VI. References
A. Textbook of Diagnostic Microbiology, Mahon & Manuselis, 3rd edition, Chapter 37, pages 1010-1030.


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