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 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 includes a nutrient agar along with a selective GNR media is used. Urinary tract infections are not usually associated with fastidious organisms.

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 cytoscope.

B. Specimen transport and handling
1. Urine held at room temperature should be cultured within 2 hours of collection. 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. 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)
2. Method
   a. Place a drop of well-mixed urine on a slide and allow to air dry
   b. Stain slide using Gram stain procedure
   c. Evaluate slide under oil immersion for bacteria and PMN’s
3. Report gram stain results as part of urine culture
B. Other screening tests such as a routine urinalysis (nitrite, leukocyte esterase, protein, microscopic examination) may be used to correlate with urine culture.

IV. Culture Setup
A. Media
1. Blood agar plate (BAP)
2. MacConkey agar plate (MAC)
   (or another selective/differential GNR medium such as EMB)
3. Anaerobic BAP (for suprapubic, cystoscopy and nephrostomy specimens) when requested by physician

B. Colony count
1. Mix urine specimen well!
2. Select 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
3. Inoculate media
   a. Dip calibrated loop vertically into well-mixed urine
   b. Quickly make a single streak down the middle of the BAP with the loop containing urine
   c. Streak back and forth across the plate perpendicular to the original inoculum (creating a “lawn”). See diagram below.
   d. With the same calibrated loop, do the same (a-c) with the MAC

C. Incubate media
1. Temperature: 35°C
2. Atmosphere: BAP - either ambient air or CO2, MAC - ambient air
3. Time: 18-24 hours

V. Examination of Culture Media
A. Is there growth?
1. If no growth:
   a. At 24 hours:
      i. Preliminary report: Colony count <1000 cfu/ml (if setup with 0.001 ml loop), No growth at 24 hours
      ii. Reincubate culture plates
   b. At 48 hours:
      i. Final report: Colony count <1000 cfu/ml, No growth at 48 hours
      ii. Discard culture plates
2. If growth, proceed to next question.

B. What media has growth? See part D below for determination of colony count.
1. BAP only – rules out the enteric GNR’s, colonies may be GPC, GPR, GNDC, or a GNR that does not grow on MAC. A gram stain must be done.
2. BAP and MAC both have growth – most likely have an enteric GNR or Pseudomonas. If multiple colony types on the BAP, a gram stain must be done.

C. How many colony types are growing?
1. A single colony type, if not considered a contaminant (see E-2 below), can be indicative of a pathogen (see E-1 below) and should be worked up
2. Growth of three (3) or more different organisms is to be considered a contaminated specimen and work-ups are not done

D. What is the colony count for each organism?
1. Determine colony count for each colony type
   a. Multiply number of colonies on $10^{-2}$ plate (0.01 ml loop) times 100 to arrive at colony count
   b. Multiply number of colonies on $10^{-3}$ plate (0.001 ml loop) times 1000 to arrive at colony count
   c. If number of colonies on $10^{-3}$ plate is >100, report out colony count as >100,000 cfu/ml
      Examples:
      - $10^{-2}$ plate 50 colonies x 100 = 5000 cfu/ml
      - $10^{-3}$ plate 5 colonies x 1000 = 5000 cfu/ml
      - $10^{-2}$ plate no colonies = <100 cfu/ml
      - $10^{-3}$ plate no colonies = <1000 cfu/ml
2. Colony count guidelines
   a. Generally speaking, >100,000 cfu/ml is indicative of a UTI, except when the isolate is one of the contaminants (see E-2 below).
   b. 10,000 – 100,000 cfu/ml may indicate infection especially if there is only one isolate that is a pathogen (see E-1 below).
   c. <10,000 cfu/ml, especially if there are contaminants present, are not worked up.
   d. 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.
3. 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.

E. Is the growth a potential pathogen or a contaminant?
1. Pathogens commonly isolated in urine
   a. Escherichia coli – most common cause of urinary tract infections
   b. Proteus species
   c. Enterobacter, Klebsiella, Citrobacter, Serratia
   d. Enterococcus species
   e. Group B Streptococcus
   f. Staphylococcus saprophyticus – most commonly seen in young females
   g. Staphylococcus aureus
   h. Pseudomonas aeruginosa and other pseudomonads
   i. Corynebacterium jekeium
   j. Acinetobacter species
   k. Yeasts
2. **Contaminants commonly isolated in urine**
   a. Diphtheroids
   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 and cystoscopy specimens should be sterile. Thus any organism growing should be worked up
   b. Number of colony types
   c. Colony counts – see guidelines (see D-2 above)
   d. Patient clinical history (if available):
      i. Age
      ii. Female or male
      iii. Exhibiting symptoms of a UTI
      iv. Previous antibiotic therapy

**VI. Interpreting Culture Results**

A. **Determine Colony count**
   1. >100 colonies/ml from suprapubic, cystoscopy, and nephrostomy require work up (identification and susceptibilities if appropriate) of all species of potential pathogens.
   2. >10,000 colonies/ml of pure culture of potential pathogen from clean catch or catheterized specimen requires workup.
   3. >10,000 colonies/ml of two species of potential pathogens of organism from clean catch or catheterized specimen requires further workup.
   4. >10,000 colonies/ml of three or more species from a clean catch or catheterized specimen require no further workup.
   5. >10,000 but less than 50,000 colonies/ml of coagulase negative *Staphylococcus* requires identification only.

B. **Culture work up**
   1. 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 Micrococcaceae flow chart
                  ▪ Perform susceptibilities testing if appropriate
            • Catalase negative
               o Observe hemolysis pattern
                  ▪ Beta hemolytic (potential pathogens)
                     • Perform PYR (see procedure)
                        o For further tests see Streptococcaceae flowchart
                        o Perform susceptibilities testing if appropriate
- **Alpha hemolytic**
  - Perform PYR (see procedure)
    - Positive
      - Report as Enterococcus
      - Perform Susceptibility testing
    - Negative
      - No further workup
  - **Gamma hemolytic**
  - Perform PYR (see procedure)
    - Positive
      - Report as Enterococcus
      - Perform Susceptibility testing
    - Negative
      - No further work up

b. **Gram negative rods**
   i. Observe MacConkey Growth
      - Lactose fermenter
        - 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
        - Perform oxidase
        - 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 identifications
        - Negative
          - No growth on MacConkey
            - Perform oxidase and OF Glucose
              - Nonfermenter or Oxidizer
                - Growth at 42 degrees, sub clear media for pigment production, Moeller based arginine, ornithine, and lysine
                - Utilize non fermenting gram negative bacilli charts for identifications
      - ii. Perform susceptibility testing if appropriate.
   c. **Yeast**
      i. Perform Germ tube
         - Positive
           - Report as Candida albicans
         - Negative
           - Perform additional identification panels (MicroScan, Vitek or API)
   d. **Gram positive rods**
      i. Pallisading gram positive rods
         - Perform catalase
         - 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)

2. 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

G. After 24 hours, culture plates are reincubated.

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

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