**PROCEDURE: URINE CULTURES**

1. **PRINCIPLE**

Urinary tract infections (UTIs) account for 8 million visits to physicians’ offices and over 100,000 hospital admissions per year. The etiologic agents of uncomplicated UTIs are generally limited to the patient’s own intestinal bacteria with *Escherichia coli* causing the majority of UTIs and *Enterococcus* spp., *Klebsiella* spp., *Enterobacter* spp., and *Proteus* spp. representing most of the remainder of isolates from both hospitalized patients and outpatients.

Urine is easily contaminated with bacteria from the perineum, prostate, urethra, or vagina. Due to this probable contamination for most specimens, colony count is critical to determine significance.

1. **AVAILABILITY**

Submission of specimen: 1st, 2nd & 3rd shift at RIH, TMH & NH

1. **TEST CODE**

CXURN – Urine Culture

CXURG – Urine Culture and Gram Stain for patients <6 months old from Hasbro ED

1. **SPECIMEN**
   1. Urine Sources

|  |  |
| --- | --- |
| **POTENTIALLY CONTAMINATED** | **INVASIVELY COLLECTED** |
| Clean Catch | Straight Catheter |
| Foley | Cystoscopy |
| Ileal Loop | PCN-Percutaneous Needle Asp |
| Urostomy | Suprapubic Needle Aspirate |
| Drain, Nephrostomy |
| Pedi Bag |
| Suprapubic Indwelling Catheter |

* 1. Collection Instructions
     1. Collection source MUST be specified
     2. Early morning specimens are preferred
     3. A sterile container must be used
     4. Clean catch urine should be a clean voided and midstream specimen
     5. Collection of urine during menses is not recommended
  2. Refer to *Planting Procedure* for specimen processing

1. Plates belonging to invasively collected urine and potentially contaminated urine sources should be filtered and separated into separate racks. Invasively collected urine sources should be placed into a rack labelled “**Special Procedures**”
2. Plates must be incubated ambiently in 35-37°C incubator for 18-24 hours before the first read
   1. Rejection Criteria
      1. Improperly labeled specimen
      2. Urine collected from a bedpan or urinal
      3. Urine that has been obviously contaminated with stool
      4. Urine collected from a catheter bag
      5. Urine from a diaper
      6. Unpreserved urine >2 hours old
      7. Preserved urine >48 hours old
      8. Urine collected over a 24-hour period
      9. Duplicate specimens from the same source and obtained within 24-hours
      10. Urine in a leaky container
          1. Urine that has not been handled aseptically by another department prior to arriving to the microbiology lab
3. **MATERIALS AND EQUIPMENT**
   1. *Trypticase*Soy Agar with 5% Sheep Blood (BAP)
   2. MacConkey II Agar (MAC)
   3. Disposable calibrated **GREEN** loop (delivers a volume of 1µL (.001))
   4. Disposable calibrated **BLUE** loop (delivers a volume of 10µL (.01))
   5. 35-37°C ambient incubator
   6. Gram stain reagents
   7. BD Vacutainer Plus C&S Preservative tube
   8. WASP – Walk Away Specimen Processor
4. **STORAGE AND HANDLING**
   1. Specimen Storage: DO NOT FREEZE
   2. Transport to laboratory within 2 hours of collection (w/o preservative)
   3. Specimens unable to be processed by the microbiology laboratory should be immediately refrigerated. Ideally, a urine culture tube containing a preservative should be used
   4. Specimens in BDVacutainer® Plus C&S Boric Acid Sodium Borate/Formate tube may be held at room temperature for up to 48 hours
   5. Aseptic techniques are always required
5. **QUALITY CONTROL**
   1. Media and Reagents are QC’d according to guidelines. Refer to *IQCP* *Procedure*
   2. Disposable loops used for manual planting the culture plates are calibrated and quality assured by the manufacturer. Loops are visually checked before use to ensure the loop is not grossly defective
   3. WASP colony count accuracy and carry-over test is performed weekly. Refer to *QC Procedure*
6. **TEST PROCEDURE**
   1. Examination of Media
      1. Colony counts should be performed from growth on the BAP planted with the 1µL [.001] loop
         1. The BAP planted with the 10µL [.01] loop should be only evaluated when there is lack of growth on the plates planted with the 1µL [.001] loop
            1. This plate is utilized only to evaluate very low concentration of uropathogens in invasively collected urine sources
   2. Invasively Collected Urine Sources
      1. 1st read (approx. 18-24hrs)
         1. Cultures with no growth are set to Prelim status: ***~No growth to date, ~Culture in progress***
            1. The BAP planted with the10µL [.01] loopmust be evaluated at this time
            2. Plates are re-incubated for an additional day
         2. Cultures determined to be a mixed culture may be set to Final status. Mixed urogenital flora is semi-quantitated in the test comment field. Refer to *Table MUL/MUH CC* for more details
         3. Cultures with growth are worked up and reported according to *Table 1*
         4. If the plates planted with the 1µL [.001] loop have growth, the additional BAP planted with the10µL [.01] loopmay be discarded at this time
      2. 2nd read(approx. 48 hours)
         1. Cultures with no growth are set to Final status: ***No Growth***
            1. Plates can be discarded
         2. Cultures determined to be a mixed culture may be set to Final status. Mixed urogenital flora is semi-quantitated in the test comment field. Refer to *Table MUL/MUH CC* for more details
         3. Cultures with growth
            1. Cultures may be set to Final status when work-up is complete
            2. Finalized cultures, which have an organism(s) reported that do not have a complete ID/AST need to be saved in urine 7-day rack
   3. Potentially Contaminated Urines Sources
      1. 1st read (18-24hrs)
         1. Cultures with no growth may be set to Final status: ***No Growth***
            1. Plates can be discarded
         2. Cultures determined to be a mixed culture may be set to Final status. Mixed urogenital flora is semi-quantitated in the test comment field. Refer to *Table MUL/MUH CC* for more details
         3. Cultures with growth should be worked up and reported according to *Table 1*
            1. Primary culture plates are left on the bench-top until finalized
            2. All completed cultures that have an organism(s) that does not have a complete ID/AST need to be saved in urine 7-day save pile
      2. 48hrs (2nd read)
         1. Cultures with growth may be set to Final status when work up is complete
         2. All completed cultures that have an organism(s) reported that does not have a complete ID/AST need to be saved in urine 7-day rack
   4. Semi-quantitation/ Colony Count (CC) of Pathogens & MIXGU
      1. Determined from growth on the BAP planted with1µL [.001] loop whenever possible
         1. 1µL [.001] loop
            1. One colony = 1,000 Colony Forming Units (cfu) /mL

|  |  |
| --- | --- |
| **Number of colonies** | **CC to be used:** |
| 1-9 | <10,000 cfu/mL |
| 10-49 | 10,000-50,000 cfu/mL |
| 50-99 | 50,000-100,000 cfu/mL |
| >100 | >100,000 cfu/mL |

* + - 1. 10µL [.01] loop
         1. One colony = 100 cfu/mL

|  |  |
| --- | --- |
| **Number of colonies** | **CC to be used:** |
| 1-99 | <10,000 cfu/mL |

* 1. Common Urinary Pathogens
     1. Gram negative rods
     2. *Enterococcus* sp.
     3. Beta-hemolytic *Streptococcus* Group A or B
     4. *Staphylococcus aureus*
     5. *Staphylococcus saprophyticus*
     6. Yeast
     7. *Aerococcus urinae*
  2. Common Urogenital/Skin Flora (MIXGU)
     1. When present with a reported pathogen, MIXGU is semi-quantitated in the test comment field. Refer to *Table MUL/MUH CC* for more details
        1. Alpha-hemolytic *Streptococcus*
        2. *Corynebacterium* sp. (Refer to *Table 1)*
        3. *Bacillus* sp.
        4. Coagulate-negative *Staphylococcus* (not *S. saprophyticus*) (Refer to *Table 1*)
        5. *Gardnerella* sp.
        6. Beta-hemolytic *Streptococcus* (not A or B group)
        7. *Lactobacillus* sp.
        8. Mixture of MIXGU and pathogens in low numbers (Refer to *Table 1)*
  3. Work-up of Significant Organisms
     1. Probable Pathogens:
        1. Gram-negative rod
        2. *Enterococcus* sp.
        3. Beta-hemolytic *Streptococcus* Group A or B,
        4. *S. aureus*
        5. *S. saprophyticus*
     2. Consult *Table 1* for reportable pathogens
     3. If pathogen is not fully ID/AST, only minimal identification is needed
     4. If pathogen is completely worked-up, presumptive identification is required until full identification is complete. Spot tests and Gram-stain should be utilized
     5. Refer to *ORGANISM ID & AST Procedure* as a guide for colony morphology, test results, and susceptibilities
  4. Organisms with special considerations
     1. Alpha hemolytic strep (AHS)
        1. If PURE & ≥100k cfu/mL growth
           1. GPC suggestive of strep, perform PYR and LAP

If identification is an AHS add isolate comment *&CONS* “Consult Required for Further Workup” to final report

Save a plate in the urine 7-day rack

* + 1. *Aerococcus* *urinae*
       1. If ≥50k cfu/mL OR pure work-up
       2. Gram-positive cocci suggestive of *Aerococcus* (clusters), perform MALDI/Vitek identification
       3. If identified as *Aerococcus urinae*, add isolate comment *&AERU*“Isolates are typically susceptible to Beta-lactam antibiotics.” to final report.
    2. Yeast
       1. If ≥100k cfu/mL
          1. If “feet” are present, report by semi-quantitating with “Probable” as *Candida albicans*
          2. If no “feet” present, identify by MALDI
          3. If identification is unsuccessful send isolate to the Mycology laboratory for further identification
          4. Culture is complete when identification is made. Save plates in the 7-day rack
       2. If <100k cfu/mL
          1. List yeast generically, if it is appropriate to site as potential pathogen (refer to Table 1). Also add *&NFW* *“No Further Work-up”* to the final report
          2. Save a plate in the urine 7-day rack
    3. Beta-hemolytic Group B *Streptococcus*
       1. For male patients, treat as a pathogen using *Table 1*. Add *&GBS* comment to final report
       2. For female patients, if ***≥10,000*** cfu/mL: identify and add *&UGRB* and *&GBS* comment to final report. Refer to *Table 1* for guidance
       3. Save plate in urine 7-day rack
    4. *Corynebacterium* species
       1. If PURE & ≥100k cfu/mL growth
          1. Set up MALDI/Vitek ANC card for identification
          2. If identification is *C. urealyticum*, add isolate comment *&NFW “No Further Work up”* to final report
          3. All other identifications should be brought up on ROUNDS
       2. Save plate in urine 7-day rack
    5. Coagulase-negative *Staphylococcus* species
       1. Perform MALDI to rule out *Staphylococcus saprophyticus*
          1. If *S. saprophyticus,* report if necessary by referring to *Table 1*. Add *&SAP* to the final report
          2. If different CNS work up only if PURE & >100k cfu/mL growth. Do not speciate the CNS
       2. Save plate in urine 7-day rack
    6. Any other organisms that could be considered urogenital/skin flora (MIXGU), that is PURE & ≥100k cfu/mL growth bring up on ROUNDS
    7. If the colony count of an organism does not fit into the criteria of *Table 1*, check for a Urinalysis (UA) result and bring up on ROUNDS
       1. A positive UA is determined if either of the following is present:
          1. Positive Leukocyte Esterase
          2. White Blood Cells
  1. **Evaluation and Work-up of Urine Cultures After 18-24 Hours Incubation**

**TABLE1**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  |  | **Colony Count of**  **PATHOGEN cfu/mL** | **WORK-UP**  **PATHOGENS ONLY** | **Test Comment** |
| **Number of PROBABLE PATHOGENS** | **1** | **<10,000**  (pure) | **List** w/ minimal ID, with **&LOW** | N/A |
| **<10,000**  (with <10,000 *MIXGU*) | N/A | **}NSG** |
| **10,000 – 50,000**  (*MIXGU* ≥ pathogen) | N/A | **Semi-quant. }MUL(H)**  *(add $CCNT in media)* |
| **10,000 - 100,000**  (pure) OR(pathogen > *MIXGU*) | **ID/AST** | **Semi-quant. }MIXGU** |
| **50,000 – 100,000**  (*MIXGU* ≥ pathogen) | If UA is POSITIVE or No UA;**ID/AST** and  **Semi-quant }MIXGU** | If UA is negative,  **Semi-quant. }MUL(H)**  *(add $CCNT in media)* |
| **>100,000**  (with or without *MIXGU*) | **ID/AST** | **Semi-quant. }MIXGU** |
| **2** | **<50,000**  (NO *MIXGU*) | **List** w/ minimal ID **&LOW** | N/A |
| **10,000 – 50,000**  (pathogen > *MIXGU*) | **List** w/ minimal ID **&LOW** | **Semi-quant. }MIXGU** |
| **10,000 – 50,000**  (*MIXGU* ≥ pathogen) | N/A | **Semi-quant. }MUL(H)**  *(add $CCNT in media)* |
| **50,000 – >100,000**  (NO *MIXGU*) OR (pathogen > *MIXGU*) | **ID/AST** any pathogen >50,000;  IF 2nd path <50k but > *MIXGU*: **List 2nd**w/ minimal ID **&LOW**  *(2nd pathogen is part of MIXGU IF pathogen ≤ MIXGU)* | **Semi-quant. }MIXGU** |
| **≥3** | **Any Amount**  (NO *MIXGU*) | **List** w/ minimal ID  **&MULP** | N/A |
| **Any Amount**  *MIXGU* is present | N/A | **Semi-quant. }MUL(H)**  *(add $CCNT in media)* |

**PROBABLE PATHOGENS:** Gram-negative rod, *Enterococcus spp*, Beta-hemolytic *Streptococcus* Group A or B,

*S. aureus*, *S. saprophyticus*

**SPECIAL CONSIDERATIONS:** Refer to *Section H* above*,* under Test Procedure for further details

* Suprapubic Needle Aspirates
* Yeast
* *Aerococcus urinae*
* *C. urealyticum*; AHS; and CNS (not *S. saprophyticus*)
* Any MIXGU organism that is PURE & >100K cfu/mL
* BHSB ALWAYS listed if >10k cfu/mL in females

1. **REPORTING RESULTS-** performed in SoftMic
   1. All clinically significant isolates worked up on pediatric patient (<2 months old) should be called to a provider. If the Gram stain result has already been called, a duplicate phone call is not necessary. Critical Notification Policy must always be followed
   2. Refer to *Gram Stain Procedure* on appropriate Gram-stain reporting
   3. Growth results
      1. *Isolates*
         1. Pathogens
            1. Significant pathogens are reported according to *Table 1*
      2. *Test Comments*
         1. **}MUL/ }MUH** (media: $CCNT must be added to work card) (example below)

|  |
| --- |
| ***10,000 - <100,000 cfu/mL Multiple Organisms Isolated; suggestive of normal urogenital or fecal contamination. Recollect specimen for repeat culture if clinically indicated. Transport to the microbiology lab should be within 2 hours of collection and refrigeration is necessary if a delay is expected.*** |

* + - * 1. Reported when there is growth of predominant skin/urogenital and pathogens in lower number
        2. Semi-quantitate according to *TABLE: MUL/MUH CC*:

***TABLE: MUL/MUH CC***

|  |
| --- |
| **Semi-Quantitation of Mixed cultures (*Table 1*)** |
| 10,000 - <100,000 cfu/mL = **}MUL** |
| ≥100,000 cfu/mL = **}MUH** |

* + - 1. **}NSG** (media: $CCNT must be added to work card)
         1. ***No Significant Growth (<10,000 cfu/mL mixed urogenital flora)***
         2. <10,000 growth of MIXGU in cultures **}NSG**
      2. **}MXGU** – mixed urogenital flora (example below)
         1. ***10,000 – 50,000 cfu/mL mixed urogenital flora***
         2. If skin/urogenital flora is present along with the reported pathogens, report MIXGU as “Test Comment” using the keypad
         3. “MIXGU” may include low numbers of potential pathogens when mixed with skin/urogenital flora
         4. Include semi-quantitation before “MIXGU”
         5. MIXGU is reported according to *Table 1*
    1. Urine Culture Comments

***TABLE: COMMENTS***

|  |  |  |
| --- | --- | --- |
| **Soft Code** | **Isolate Comment** | **Use when:** |
| **&AERU** | Isolates are typically susceptible to Beta-lactam antibiotics. | *Isolate comment for* ***Aerococcus urinae*** |
| **&LOW** | Low number of organisms isolated may represent urogenital flora, unless pyuria is present. Consult with Micro required for further workup. | Isolate comment when Colony count is low according to ***Table 1*** |
| **&SAP** | Routine susceptibility testing is not performed. Infections respond to concentrations achieved in urine of antimicrobial agents commonly used to treat acute uncomplicated urinary tract infections. | *Isolate comment for* ***Staph. saprophyticus*** |
| **&GBS** | Susceptibility testing not routinely performed. Group B *Streptococci* are predictably susceptible to ampicillin and penicillin. Call laboratory for further testing if patient is allergic to penicillin. | Isolate comment for all reported BHSB |
| **}MUH** | ≥100,000 cfu/mL of growth suggestive of normal urogenital flora or fecal contamination. Recollect specimen for repeat culture if clinically indicated. Transport to the microbiology lab should be within 2 hours of collection and refrigeration is necessary if a delay is expected. | Test comment when indicated in ***Table 1***  **Or when only MIXGU is present** |
| **}MUL** | 10,000 - <100,000 cfu/mL of growth suggestive of normal urogenital flora or fecal contamination. Recollect specimen for repeat culture if clinically indicated. Transport to the microbiology lab should be within 2 hours of collection and refrigeration is necessary if a delay is expected. | Test comment when indicated in ***Table 1***  **Or when only MIXGU is present** |
| **&MULT** | Multiple Organisms Isolated. No Further Work up. | Isolate comment when indicated on ***Table 1*** |
| **“A” on CXURN Keypad** | <10,000 cfu/mL mixed urogenital flora | Test comment when indicated in ***Table 1*** |
| **“B” on CXURN Keypad** | 10,000 – 50,000 cfu/mL mixed urogenital flora | Test comment when indicated in ***Table 1*** |
| **“C” on CXURN Keypad** | 50,000 – 100,000 cfu/mL mixed urogenital flora | Test comment when indicated in ***Table 1*** |
| **“D” on CXURN Keypad** | >100,000 cfu/mL mixed urogenital flora | Test comment when indicated in ***Table 1*** |
| **&NFW** | No further workup | Isolate comment When indicated in ***Table1*** |
| **}NSG** | No Significant Growth (<10,000 cfu/mL mixed urogenital flora) | Test comment when:  <10k growth of cutaneous/urogenital flora or a mixture of this flora with a potential uropathogen (***Table 1)*** |
| **&NOSU** | Susceptibilities not routinely performed. | Isolate comment when there are CLSI guidelines but we do not perform AST routinely. |
| **&UGRB** | Colonization of the genital tract with Group B *Streptococcus* is common and may result in contamination of the urine specimen. Correlate results with clinical presentation. | Isolate comment for BHSB in female patient |

1. **LIMITATIONS**
   1. Anaerobes or organisms requiring CO2 are not isolated in a routine urine culture
   2. The significance of the culture is dependent on appropriate specimen collection
   3. False negatives may occur if specimen has an interfering substance or has been diluted or was frozen
   4. Swarming *Proteus* spp. may make colony count unreliable
   5. Antimicrobial inhibition may make colony count unreliable
   6. Incorrect source of specimen may make colony count & work-up protocol unreliable
   7. Not all colony count/culture scenarios can be explained in a protocol
2. **NOTES**
   1. Discard saved plates on day 7, KB plates and VT2 purity wedges at 72 hours
   2. Check Vitek wedges for purity before reporting results
   3. Confirm any discordant VT2 ESBL test/interpretations with KB ESBL test
   4. At the end of the day, disinfect bench and document
   5. Do not change results (example: pathogen reported🡪MUL/MUH without correcting the report and calling the doctor/nurse.
   6. Do not re-incubate positive culture plates from potentially contaminated urines (planted only withDisposable calibrated 1µL [.001] loop after the initial 18-24-hour incubation
   7. *E. coli* should be identified by a positive spot Indole test when a typical colony morphology (dry, flat, lactose-fermenting usually with umbilicus on MacConkey
   8. Full identification by Vitek if necessary. Refer to *Table 1*
   9. Any questionable or odd results and cultures are brought up on ROUNDS
   10. Suprapubic needle aspirate specimens growing more than one organism are brought up on ROUNDS
3. **TECHNICAL SUPPORT**

Copan – 1-877-927-7457 WASP Serial# 086 050 0154

1. **REFERENCES**
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   2. IDSA Guidelines. International Clinical Practice Guidelines for the Treatment of Acute Uncomplicated Cystitis and Pyelonephritis in Women: A 2010 Update by the Infectious Diseases Society of America and the European Society for Microbiology and Infectious Diseases. (Deemed current 07/2013)
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