**MICRO.CULT.3.0 URINE CULTURE**

**PRINCIPLE**

Urinary tract infections account for seven million visits to physician offices and over one million hospital admissions per year. Laboratory detection of urinary tract infections is accomplished through the use of quantitative cultures of urine specimens. Urine is normally a sterile fluid; however, unless the specimen is collected properly, it can become contaminated with microbial flora from the perineum, prostate, urethra, or vagina. In order to effectively utilize resources and to prevent the unnecessary prescribing of antibiotics, the laboratory must use specific workup guidelines to differentiate contaminating organisms from urinary pathogens. Potential urinary pathogens include: enteric gram negative rods, Beta *streptocccus*, *Enterococcus* species, *Pseudomonas aeruginosa* and other non-fermenters, *Staph aureus*, *Staph saprophyticus*, and yeast. *Gardnerella vaginalis*, a probable vaginal pathogen, is also reported. Anaerobic cultures should be performed only on suprapubic bladder aspirations.

**SPECIMEN**

Patient Preparation: Prior to collection of the specimen, the periurethral area should be thoroughly cleaned.

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| **Collection** |  |
| Specimen Type | Urine |
| Collection Containers | B-D Gray top boric acid urine collection tubes (or boricon tubes)  Sterile container  Uricult paddles |
| Sources | Clean catch midstream (CCMS) urine (first morning specimen is preferred); catheterized urine (in and out or indwelling); cystoscopy (bladder) urine; ileal conduit urine; kidney urine; suprapubic aspiration (SPA) |
| Volume | A urine volume of at least 4 ml is required to avoid an inhibitory effect by boric acid in urine collection tubes. |
| Stability and Storage Requirements | Urine should be processed within 2 hours of collection if unrefrigerated and up to 24 hours if refrigerated. The B-D collection tubes require no refrigeration and may be used up to 72 hours after collection. Transportation of Uricult paddles should not exceed 48 hours at 7-25°C. |
| Unacceptable Specimens | Foley cath tips; red and yellow cap urinalysis collection tubes |

**MATERIALS**

1. BAP-Blood Agar Plate
2. MAC-MacConkey Agar Plate
3. ABAP—CDC anaerobic BAP (Suprapubic aspirate or kidney urine only)
4. Columbia CNA- (Uricult only)
5. BM—Blood Agar/MacConkey Agar biplate
6. 1 µl plastic disposable loops
7. 10 µl plastic disposable loops
8. Anaerobe jar
9. Indicator strips
10. Anaerobic generator packs
11. 37° non-CO2 incubator
12. Inoculab

**CALIBRATION**

Disposable loops do not require calibration.

**QUALITY CONTROL**

BAP, MAC, CDC anaerobic BAP and CNA are exempt from QC beyond that performed by the manufacturer. Each new shipment of BAP, MAC, CDC anaerobic BAP and CNA is checked for appearance.

**PROCEDURE**

Specimen Processing –Manual plating

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| **Step** | **Action** |
| 1 | Label plates. |

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| **If** | **Then** |
| The specimen is CCMS or catheterized urine | Use a sterile, disposable 0.001 ml calibrated loop to inoculate BAP and MAC. |
| The specimen is bladder or ileal conduit urine | Use a sterile, disposable 0.01 ml and 0.001 mL calibrated loop to inoculate sets of BAP and MAC. Mark the plates as 0.01 mL and 0.001 mL |
| The specimen is kidney or suprapubic aspiration | Use a sterile, disposable 0.01 mL and 0.001 mL calibrated loop to inoculate two sets of BAP, MAC and ABAP. Mark the plates as 0.01 mL and 0.001 mL |
| The specimen is a Uricult paddle | The lab assistant should deliver the Uricult paddle directly to a technologist for workup. The technologist will calculate the colony and then use a sterile loop to sample both sides of the paddle. Using the inoculated loop, streak for isolation on BAP, MAC and CNA plates. |

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| 2 | Gently invert the sample 2-3 times to mix the specimen. |
| 3 | Immerse the appropriate calibrated loop vertically just below the surface of the urine. Be careful to avoid bubbles. |
| 4 | Deliver a loopful of specimen onto the BAP by making a straight line down the center of the plate. |
| 5 | Streak the specimen by making a series of passes at 90° angles through the primary inoculum. |
| 6. | Repeat steps 2-5 for the MAC, CNA and ABAP (if required). |
| 7. | Incubate MAC, BAP and CNA at 37°C in a non-CO2 incubator. Incubate ABAP at 37°C in the anaerobic chamber. |

Specimen Processing—WASP automated plating instrument (used only with CCMS or catheterized urine at Regional Microbiology)

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| **Step** | **Action** |
| 1 | Verify that specimen has a readable barcode label. |
| 2 | Load the specimen onto the WASP as per SOP MICRO.PROC.11.0 |
| 3 | Incubate MAC and BAP at 37°C in a non-CO2 incubator. |

Culture Reading

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| **Step** | **Action** |
| 1 | Read plates. Plates must be held a minimum of 20 hours from the inoculation time indicated on the plate before finaling except in cases exhibiting mixed growth indicative of contamination (MULT1). |

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| **If (Regional Only)** | **Then (Regional Only)** |
| Plates were incubated prior to 1800 the previous day | The culture may be finaled after 1200 the next day or 20 hours from the inoculation time indicated on the plate. |
| Plates were incubated between 1800 and 2400 the previous evening | The cultures may be finaled on the following evening shift. |
| Plates were incubated after 2400 the previous evening | The plates are incubated with the current day’s plates and may be finaled the following day. In Sunquest, enter an ‘A12” comment on hold to document the time of plating on these specimens. |

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| **Step** | **Action** |
| 2 | Calculate the colony count. |

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| **If** | **Then** |
| The culture was set up using a  0.001 ml calibrated loop | The colony count is calculated as the number of colonies x 1000 (CFU/ml). |
| The culture was set up using a 0.01 ml calibrated loop | The colony count is calculated as the number of colonies x 100 (CFU/ml). |
| The culture is submitted as a Uricult | The paddle is compared to the colony density chart to obtain the colony count (see Attachment A). |
| There is a discrepancy with GNR growth between the colony count on BAP, MAC | Use the larger colony count. |
| There is a large quantity of growth on the MAC plate and little or no growth on BAP. | The urine should be replated. If the same after replating, use the larger colony count. |
| Proteus has swarmed over a BAP that appears to have other organisms present. | Have the urine replated to BAP, MAC, and PEA. |

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| **Step** | **Action** |
| 3 | Quantitate the organisms present and proceed according to Tables 1 and 2. |
| 4 | Identify the organisms present using the identification flow charts and appropriate reference material. |
| 5 | Perform susceptibility testing if appropriate. Organisms include: gram-negative rods, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Enterococcus*, etc. |
| 6 | Save the original BAP and purity plate(s). Save the MAC if growth is different than the BAP. |

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| **If** | **Then** |
| The culture was submitted as a Uricult | The subcultured plates are used for organism identification and susceptibility testing if appropriate |

**INTERPRETATION AND REPORTING RESULTS**

1. For isolates of uropathogens, enter the colony count followed by the organism result mnemonic. Refer to Tables 1 and 2 for reporting message codes and guidelines to specimen workup.
2. For all cultures with growth provide a description of growth under the culture workup or on the worklist.

**PROCEDURE NOTES**

1. More than 95% of urinary tract infections (UTIs) are attributed to a single organism and have colony counts >100,000 colonies per ml (>105 CFU/ml of urine). In cases of UTI where more than one organism is present, the predominant organism is usually clinically significant and others are probable urethral or collection contaminants. When multiple organisms are isolated from patients with indwelling catheters, UTI is doubtful and colonization likely.
2. Some organisms commonly found on external and internal genitalia are considered to be contaminants. These organisms may colonize the genitourinary tract area and are usually not recognized as causes of UTI. These include coagulase negative staphylococci (CNS) except for *Staphylococcus saprophyticus*, non-pathogenic *Neisseria spp*., some *Corynebacterium spp*., Lactobacillus spp., and viridans streptococci. A descriptive identification is given followed by a standard code indicating probable contamination. Susceptibility testing is not reported on these organisms including CNS in pure culture at ≥10,000 CFU/ml and significant growth of S. saprophyticus.
3. Studies have shown that in acutely dysuric young women (commonly referred to as “Acute Urethral Syndrome” OR “frequency/dysuric syndrome”) greater than 40% will demonstrate bacteriuria in the range of 102 to 104 CFU/ml. In these cases growth of a single uropathogen at less than 104 CFU/ml may be significant. Optimally, in order to obtain a more accurate estimate of organism load, another specimen should be submitted. Report colony count and descriptive identification for uropathogens in case additional workup is required.
4. *Corynebacterium urealyticum* can be an important but rare cause of UTIs. Therefore, cultures exhibiting growth of *Corynbacterium* species in quantities >100,000 CFU and 10 times greater than that of all other microbiota will be worked up to rule out the presence of this species. Isolates of *Corynebacterium urealyticum* meeting the stated requirements will be reported. Isolates of other *Corynebacterium* species will be viewed as urogenital flora.
5. Any amount of Group B *Streptococcus* in urine specimens obtained from pregnant females is a marker of heavy genital tract colonization, and an indication for intrapartum chemoprophyaxis. Therefore, catalase-negative, slightly beta-hemolytic (in some cases non-hemolytic), flat gray colonies from cultures on female patients between the ages of 15 and 44 must be further identified to rule out Group B *Streptococcus*. Any isolates of Group B Streptococcus from these patients will be reported regardless of colony count or growth of other organisms.
6. Any amount of Group B *Streptococcus i*n urine specimens obtained from patients < 6 months of age will be reported regardless of colony count.
7. Susceptibility testing for beta-hemolytic streptococcus, while indicated for penicillin-allergic patients, will not routinely be done without a physician’s specific request. Add report comment #PENS.
8. The IDU1 (positive culture) charge will be attached to all cultures exhibiting growth. This charge is added after the organisms growing have been evaluated to determine their significance.
9. Non-saprophyticus coagulase negative staphylococcus (NSCNS) can be an important but rare cause of UTIs. Therefore, cultures exhibiting growth of NSCNS in quantities >100,000 CFU and 10 times greater than that of all other microbiota will be provided with susceptibility.

**LIMITATIONS**

1. Patients receiving antibiotics or chemotherapy may have false negative results.
2. Failure to add the minimum amount of urine to the boric acid tube will result in rejection of culture request.

**REFERENCE**

1. Pezzlo, Marie, Urine Culture Procedure, Clinical Microbiology Procedures Handbook, American Society for Clinical Microbiology, 2007, 3.12.
2. Clarridge, J.E., Pezzlo, M.J., and Vosti, K.L., Cumitech 2A, “Laboratory Diagnosis of Urinary Tract Infections”, American Society for Microbiology, 1998.
3. Lennette, E.H., Manual of Clinical Microbiology, 5th Edition, p.24, 1991.
4. Miller, J. Michael, A Guide to Specimen Management in Clinical Microbiology, 1996.
5. Package insert, B-D Vacutainer Urine Products, January, 2001.
6. Package insert, Uricult Urine Culture Paddles, Orion Diagnostica, 1992.

**TABLE 1. GENERAL GUIDELINES FOR SPECIMEN WORK UP**

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| --- | --- | --- | --- | --- | --- | --- |
| **Number of Isolates** | **Colony Count (CFU/ml)** | **# of Colonies on Plate** | **Extent of Identification** | **Susceptibility Testing** | **Misys Report** | **QLS Report** |
| No Growth | <1,000 | None | None | No | NG | #X |
| 1 | <10,000 | <10 | None | No | LTT | #LTT |
| 2 or more | <10,000 each | <10 each | None | No | LTTM | #LTTM |
| 1-2 | ≥10,000  ≥10,000 | ≥10 each  ≥10 each | Uropathogen:  Definitive  Probable non-uropathogen: Descriptive | Yes  No | Colony count, genus, species and susceptibility results for each  Colony count, morphologic description and NFC | Colony count, genus, species and susceptibility results for each  Colony count, morphologic description and #NFC |
| 1  and  1 | ≥10,000  <10,000 | ≥10  <10 | Uropathogen:  Definitive  Probable non-uropathogen: Descriptive  None | Yes  No  No | Colony count, genus, species and susceptibility results  Colony count, morphologic description and NFC  and LTTA | Colony count, genus, species and susceptibility results  Colony count, morphologic description and #NFC  and #LTTA |
| 3 or more | Mix of any number with none predominant | | None | No | MULT1 | #MULT1 |
| 3 or more | 1 isolate  > 100,000  and  2 isolates  < 10,000 | > 100  < 10 each | Predominant organism(s) if uropathogen:  Definitive | Yes | Colony count, genus, species, susceptibility results and AMF | Colony count, genus, species, susceptibility results and AMF |
| Any other amount or if predominant organism is a non-uropathogen | | None | No | MULTI | #MULTI |

**TABLE 2. GUIDELINES FOR ORGANISMS THAT DO NOT REQUIRE SUSCEPTIBILITY TESTING IN URINE CULTURES**

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| Organism | Routine Susceptbility | Additional Report Message | |
| Misys | QLS |
| Beta-hemolytic streptococci | No | PENS | #PENS |
| Corynebacterium spp. | No | NFC | #NFC |
| Haemophilus spp. | Beta-lactamase only |  |  |
| G. vaginalis | No | NFC | #NFC |
| Viridans streptococci | No | NFC | #NFC |
| Lactobacillus sp | No | NFC | #NFC |
| Coagulase negative staphylococci | No  (Unless >100,000 CFU **and** 10 times greater than other microbiota) | NSCNS | #NSCNS |
| Staphylococcus saprophyticus | No | NSSAP | #NSSAP |
| Neisseria gonorrohoeae | No |  |  |
| Yeast | No |  |  |