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Effective: September 9, 2016
Revised: March 21, 2017

# URINARY TRACT CULTURE PROTOCOLS

## I. Principle

Urinary tract infections are amongst the most common infections of humans. Infections of the urinary tract may involve various sites including the urethra, bladder, kidneys, and communicating glands (e.g. prostate). They may also spread to adjacent tissues and may be complicated by bacteremia.

Urinary tract infections can be due to an ascending infection by microorganisms from the lower tract (urethra and bladder) into the upper tract (ureters and kidneys). Reflux of urine into the ureters and up into the renal pelvis is probably an important mechanism for introducing organisms into the upper tract. Infections can also occur due to hematogenous spread and is more common with certain organisms such as Staphylococcus aureus, Candida sp., and Mycobacterium sp The ultimate reservoir for many infecting organisms is the patient's own feces. These organisms are thought to colonize the introital or periurethral areas, the urethra, and ultimately the bladder. The reservoir for Staph saprophyticus, the urinary tract pathogen that is most often found among young women and elderly men, has not been defined but is likely the skin.

The normal urethral flora of women varies with age and state of health. In healthy menarcheal females, the flora consist primarily of lactobacilli, coagulase negative Staph, and Streptococci. The flora of women of reproductive age is similar except lactobacilli predominate. The normal urethral flora of post-menopausal women is significant for the increase in anaerobes, particularly Bacteroides melaninogenicus. Mycoplasmas and low densities of enteric Gram negative rods can also be recovered from the urethra of healthy women. Because of this colonization which can be as high as $10^3$ to $10^4$ CFU/ml in the first voided 10 ml of urine, specimens can be easily contaminated when both midstream and catheter specimens are collected improperly. In men, there is generally less contamination of urine by the normal flora. The organisms known to colonize the urethra and to be present in low numbers in urine of healthy men include: coagulase negative Staphylococci, group D Streptococci, coryneforms, and mycoplasmas.

In both men and women, colonization of the urethra with "Uropathogens" frequently leads to infections because these organisms can grow in urine. These organisms include the Enterobacteriaceae, Pseudomonas, Enterococci, Staph saprophyticus, and yeast. Incidence of infection is higher in females than males although infections can occur in both sexes at all ages. Reasons for this higher incidence of infections include:

A. Shorter urethra in females.
B. Genetic differences in tissue receptors may favor the colonization of the periurethral and urethral sites of certain high-risk women.
C. Men have bactericidal substances in prostatic fluid which discourages urethral

colonization.

In all individuals the risk of urinary tract infection may be increased due to:

A. Congenital or acquired structural abnormalities of the urinary tract.
B. Conditions such as pregnancy.
C. Tumors
D. Foreign bodies (e.g. indwelling catheters or stones)
E. Colonization by organisms which are more pathogenic (e.g. some strains of E. coli)

Urinary tract infections may be asymptomatic or symptomatic. Asymptomatic infections are often seen in pregnant and diabetic individuals. Symptomatic syndromes involving lower urinary tract infection include clinically: cystitis (bladder infection), hemorrhagic cystitis, and the acute urethral syndrome.

## II. Clinical Significance

Urine cultures aid in the diagnosis of urinary tract infection.

## III. Specimen

Bio-Safety Level 2 – All specimens are processed within a biological safety cabinet.

Refer to procedure **Specimen Collection and Transport** and **Initial Processing, Inoculation, and Incubation of Bacteriology Specimens**

## IV. Reagents

A. Refer to procedure **Initial Processing, Inoculation, and Incubation of Bacteriology Specimens**
B. Refer to the reagent section of specific test being performed on positive cultures or suspected isolates.

## V. Instrumentation/Equipment

Ambient Incubator 35°C

## VI. Quality Control

All medias and reagents are quality controlled by the manufacturer and by lot # or shipment when received in our laboratory. (exception: exempt media)

## VII. Procedure

A. Urine Gram Stain Procedure – Urine Gram stains are not performed routinely. When specifically requested by a physician perform as follows:
   1. Mix unspun urine well.
   2. Obtain a microscope frosted end slide and mark a circle in the middle of the clear section of slide using a black China marker or red Gram stain marker. Label frosted end with media label.
   3. Pipet one drop of urine inside the marked area on the slide.
   4. Allow the drop to dry without spreading.
   5. Fix smear for 1 minute with methanol or heat fix on slide warmer for 5 minutes.
   6. Stain following Gram Stain Procedure.
   7. Scan smear using the 100X oil immersion objecting.
   8. Report findings.

B. Culture procedure
   1. Plates are incubated for 18 – 24 hours prior to observing for growth.
   2. Interpretations are based on the type of culture (catheterized, voided, cysto, suprapubic). Refer to the Tables 1 and 2.
   3. Work up low counts and nonpathogenic organisms when isolated from patients of Peoria Urological; or on immunocompromised patients including bone marrow transplant patients.
   4. All specimens are held for 2 days before discarding plates and reporting as "No growth". If significant contamination is present at Day 1, the culture need not be held any longer and can be finalized Day 1.
   5. Yeast may be referred for susceptibility testing if requested. Physician must indicate anti-fungal agent to be tested.
   6. All yeast isolates are screened with a germ tube, including bladder urines. Positive germ tube isolates are reported as Candida albicans/cannot rule out Candida dubliniensis and negative germ tube isolates reported as Yeast not Candida albicans.

**Table. 1 Voided/Catheterized Specimen Interpretations:**

| # of Isolates | Colony Count | Interpretations |
|---|---|---|
| 1 | >10,000 to >100,000 CFU | Contaminant e.g. Lactobacillus or Diphtheroids. Report descriptive ID only |
| 1 | 10,000 to >100,000 CFU | Definitive I.D. and Susceptibility if potential pathogen. If alpha strep won't identify or grow for suscept on PID or POS COMBO, report: alpha strep, unable to identify or unable to perform susceptibility |

| # of Isolates | Colony Count | Interpretations |
|---|---|---|
| | | testing due to fastidious nature of organism. |
| 1 | 100 to 10,000 CFU | If Potential Pathogen and one organism only. Gram stain and descriptive report. No workup<br>(i.e. <10,000 GNB) |
| 1 | <10,000 CFU | Contaminant only (SCN, diphtheroids, lactobacillus, alpha strep not enterococcus) Report as No Growth |
| 2 | >100,000 and >100,000 CFU or<br>>100,000 and >10,000 CFU or<br>>10,000 and >10,000 CFU (if possible pathogen) | Definitive I.D. and Susceptibility. of potential pathogen(s). If 2 isolates of lactobacillus, diphtheroids, etc, then report probable contaminant urogenital flora. If coag negative staph is clearly predominant and is from women under 45 or men over 65,rule out Staph saprophyticus with a PID.before calling PCUF. |
| 2 | One organism >/=10,000 CFU and one clearly predominant organism (i.e. at least 10-fold more than other) | Definitive I.D. and Susceptibility of predominant organism only. Descriptive report and count of other organism if looks like a contaminant. If alpha strep and PYR negative, report alpha strep not enterococcus.<br>Suscept is not needed on the alpha strep. |
| 2 | <10,000 CFU | If potential pathogen descriptive report only. If organisms are suggestive of contamination (e.g. Lactobacilli, diphtheroids, etc., report probable contaminant urogenital flora. |

| # of Isolates | Colony Count | Interpretations |
|---|---|---|
| 2 or more | A possible pathogen such as E. coli or S. saprophyticus at > / = 10,000 CFU and others at about the same level | WBCs present from urinalysis – Definite I.D. on possible pathogen and list the other isolates or may be reported as "probable contaminant urogenital flora" |
| 3 or more | One organism at >10,000 CFU and clearly predominant | Definitive I.D. and Susceptibility on predominant organism only. List others descriptively with count or report as probable contaminant urogenital flora .Add comment URREC. |
| 3 or more | No predominant organism | Report PCUF. Add comment URREC |

**Table 2. Cysto, Suprapubic, and Ileal conduits Specimen Interpretations:**

| # of Isolates | Colony Count | Interpretation |
|---|---|---|
| 1 | 100 to >/= 100,000 CFU | Definitive I.D. and Susceptibility |
| 2 | Each >/= 100 CFU | Definitive I.D. and Susceptibility |
| 2 or more | A possible pathogen such as E. coli at >/=100 CFU and others at same level | Definitive I.D. of pathogen and Susceptibility<br>Definitive ID of others, (lactobacillus, diphtheroids, alpha strep not enterococcus) hold for consult. |

URREC: Isolation of 3 or more uropathogens can indicate a poorly collected or improperly transported specimen. Recollection is strongly suggested.

## VIII. Calculations

A. Colony counts for voided and catheterized urines are calculated by multiplying the number of colonies on the plate by 1000 and expressing in scientific notation.
B. Colony counts for cystoscopy and suprapubic urines are calculated by multiplying the number of colonies on the plate by 100 and expressing in scientific notation.

# IX. Reporting Results

**A. Reporting Gram stain results**

1. Record the number of bacteria observed per oil immersion field along with the morphology and Gram reaction. Numbers over ten should be reported as > 10 bacteria/oif. (e.g. 3 Gram negative bacilli/oif; > 10 Gram positive cocci/oif)
2. Report the number of WBCs observed per oil immersion field. Numbers over ten should be reported as >10 WBCs/oif.

**B. Reporting cultures**

1. Colony counts are reported on every positive urine culture. The lowest count is 100 CFU/ml for cysto, supra, ileal conduit, and the highest count is >100,000 CFU/ml depending on size loop used.
2. No growth urines are have a prelim at Day 1 and finalized at Day 2. Use Phrase "NG"- no growth.
3. Cultures with >100,000 CFU/mL probable contaminant urogenital flora on Day 1 can be finalized as such on Day 1.
4. Cultures with mixtures of organisms resembling those found colonizing the skin, vaginal, and periurethral area should be reported as: "Probable contaminant urogenital flora."
5. When reporting Lactobacillus species LACBPH – "Isolates of these organisms are rarely found as significant urinary tract pathogens even when isolated in high concentration and in pure growth"

# X. Procedural Notes/Problem-Solving Tips

A. Immunocompromised Patients: Urine cultures should be screened for low colony counts ($10^2$). Organisms are identified and susceptibilities performed.

B. If a physician designates specimens as VB1, VB2, VB3, order specimens as a Voided Urine (3509) with a Test Comment indicating VB1, VB2, or VB3. Specimens are set up with a .001 loop and interpretation of the culture results is performed by the physician ordering the test. VB1=voided urine #1, the first 5-10mls voided (urethral colonizer washout), VB2= the midstream aliquot, VB3=first 5-10 ml of urine voided immediately after prostatic massage

# XI. References

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3. Baren, E.J. Finegold, S.M., Bailey and Scott's Diagnostic Microbiology, 7th Edition. Mashy, 1986.
4. Smith, J.W., The Role of Clinical Microbiology in Cost Effective Health Care. College of American Pathologists, 1985.
5. Murray, Patrick R., Editor in Chief, Manual of Clinical Microbiology 9th Edition. 2007.

| ***POLICY CREATION :*** | | |
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| **REVISION HISTORY (began tracking 2011)** | | | |
|---|---|---|---|
| **Rev** | **Description of Change** | **Author** | **Effective Date** |
| 1 | Minor changes added reference 6 | T Smith | 6/2/11 |
| 2 | Removed duplicate table rows from formatting issue. Removed cysto culture from specimen section, minor updates to reporting cultures (deleted step 4 and 9 of previous version) | B Pestien | 4/4/14 |
| 3 | Removed HLAB order numbers and updated LIS result entry | T Nuese | 2/22/16 |
| 4 | Specimen interpretations updated | T. Nuese/L Rasca | 4/21/17 |
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| | | Teresa Nuese | 4/5/16 | L Racca DO | 4/12/16 |
| | | Teresa Nuese | 4/17/17 | L Racca DO | 4/21/17 |
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