PROVIDENCE
Sacred Heart
Medical Center &
Children's Hospital

Urine Culture Procedure

Department of Microbiology

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1.0 Principle

Etiologic agents of urinary tract infections are generally limited to the patient's own intestinal microbiota, with *Escherichia coli, Enterococcus, Klebsiella-Enterobacter* spp., and *Proteus* spp. representing a majority of isolates from both hospitalized patients and outpatients. The media used for urine cultures is chosen to recover and differentiate these types of organisms.

BBL™ CHROMagar™ Orientation medium is a nonselective medium for the isolation, differentiation, and enumeration of urinary tract pathogens. Some of the organisms encountered in UTIs produce enzymes either for the metabolism of lactose or glucosides or both. Other organisms produce none of these enzymes. For example, *E. coli* contains enzymes for lactose metabolism but is β-glucosidase negative. Some members of the family *Enterobacteriaceae* are β-glucosidase positive but do not contain enzymes necessary for lactose fermentation; others may contain both types of enzymes or none of them. β-glucosidases are also found in gram-positive cocci, such as *S. agalactiae* and the enterococci. Tryptophan deaminase (TDA) is an enzyme characteristically found in the *Proteus-Morganella-Providencia* group. Specially selected peptones supply the nutrients in CHROMagar™ Orientation Medium. The chromogen mix consists of artificial substrates (chromogens), which release differently colored compounds upon degradation by specific microbial enzymes, thus assuring the differentiation of certain species or the detection of certain groups of organisms, with only minimal confirmatory tests. *Proteus* swarming is partially to completely inhibited. CHROMagar™ Orientation medium allows for the differentiation and identification of *E. coli* and *Enterococcus* without confirmatory testing. Combining CHROMagar™ Orientation with Trypticase Soy Agar w/5% Sheep Blood in a split plate format allows for the visualization of colony morphology and hemolytic reactions of urinary pathogens.

Each side of the CHROMagar™ Orientation/TSA Blood split plate is inoculated with 1 µL or 10 µL of urine, depending on the type of specimen collection, in order to provide quantitative culture results. Colony counts are used to aid in interpreting and determining the significance of culture isolates.

2.0 Clinical Significance

Urinary tract infections (UTI) encompass diseases that affect body sites ranging from the kidney to the urethra, with the urethra and bladder most commonly affected. The infection may occur in the kidney (pyelonephritis), the bladder (cystitis), the prostate (prostatitis), and the urethra (urethritis).

Proper management of UTIs requires an understanding of the number and kinds of organisms present. Generally, UTI are associated with only a single organism (i.e. *E. coli*). Polymicrobic infections are atypical. The collection method used to obtain urine is critical to establish a definitive diagnosis. Contamination from the urethra, vagina, labia, and skin can be reduced by using the clean catch method of collection, but this method has limitations.

Guidelines based on clinical correlation studies allow the microbiologist to differentiate between "significant" bacteriuria and probable contamination. Early studies showed the value of quantitative urine culture in discriminating between true urinary tract infection and contaminated urine specimens. Bacterial counts of 10^5 colony-forming units (CFU) per milliliter or higher in midstream urine cultures were predictive of bladder bacteriuria in asymptomatic women and women with pyelonephritis, whereas lower counts were more likely to be associated with contamination. However, more recent studies have shown that women with symptoms of cystitis often have lower colony counts, especially with Gram-negative rods, such as *E. coli.*

3.0 Scope

This procedure is classified under CLIA as Highly Complex. It should be carried out by technical personnel familiarized and trained to identify and quantitate urinary pathogens. Testing includes but is not limited to: morphologic recognition, confirmatory testing, and Quality Control testing of media and reagents.

4.0 Safety - Personal Protective Equipment

Performance of this procedure will expose testing personnel to biohazardous material. All specimens must be handled as potentially infectious material as outlined in the Microbiology Safety Guidelines.

The reagents and/or chemicals that are used in this procedure may be hazardous to your health, if handled incorrectly. A brief listing of precautions for each chemical hazard is included in the reagent section of each respective procedure.

More extensive information concerning the safe handling of the reagents and/or chemicals used in this procedure, as well as other important safety information, may be obtained by consulting the Safety Data Sheet (SDS). Before performing any part of this procedure, the technologist must take any and all precautions and adhere to all prescribed policies.

This procedure may expose you to:

- Bloodborne pathogens
- Hazardous reagents

To perform this procedure, you must use:

- Gloves – must be worn when handling specimens.
- Laboratory Coat – must be worn when handling specimens, cultures, and reagents.
- Biological Safety Cabinet – must be used when processing specimens.

Disinfectant following procedure:

- Bleach dilution sprayers can be used for on demand disinfectant.

Reference for spill/decontamination:

- SDS
- Chemical hygiene plan

5.0 Specimen Collection, Handling and Storage

5.1 Specimen Collection

1. Noninvasive Collection
    - Clean-Voided midstream urine
      Patient preparation required. Refer to the test directory for patient directions.
    - Indwelling catheter urine (Foley)
      Using a sterile needle, urine should be collected through the catheter port, after the port has been cleaned with alcohol. Alternatively, urine can be collected directly into a Vacutainer tube with boric acid, using a Vacutainer holder and needle. Urine should NOT be collected from the bag.
    - Ileal Conduit
      The external device should be removed and the stoma cleansed with 70% alcohol followed by iodine. The iodine should be removed with alcohol. A double catheter should be inserted into the stoma, to a depth beyond the fascial level, and the urine collected.
    - Pediatric bagged urines
      Although collection of urine by in-and-out straight catheterization is the recommended method to obtain an accurate urine culture sample, catheterization is not always possible, particularly in neonates. Collection of urine by pedibag may be the only way to obtain a urine sample in this age group.
2. Invasive Collection
    - Straight catheters
      A straight catheter is used by a trained health care worker to obtain urine directly from the bladder. The catheter is inserted into the bladder via the urethra. The procedure must

be carried out using aseptic technique to avoid introducing microorganisms into the bladder. The initial 15 to 30 mL of urine should be discarded and the next flow of urine used for culture.

- Suprapubic aspiration
  Urine collected by suprapubic needle aspiration directly into the bladder is performed by a physician or trained health care worker. This method is preferred for infants, for patients for whom the interpretation of results of voided urine is difficult, and when anaerobic bacteria are suspected as the cause of infection.
- Multiple urine specimens with prostatic secretion
  Sequential urine specimens, collected before and after prostatic massage, may be used to diagnose the location of a lower urinary tract infection in men. The different urine samples and the prostatic massage specimen should be clearly labeled.
- Cystoscopy
  Cystoscopy is a bilateral ureteral catheterization to determine the site of infection in the urinary tract. This procedure is usually performed in areas such as operating rooms and specialty clinics. The specimens should be labeled with the location from which they were collected (left kidney vs. right kidney).
- Percutaneous Nephrostomy
  Urine is not aspirated but allowed to drain into a sterile container for collection. After the sample is collected, the tube is reconnected to the urine collection bag.

5.2 Timing of Specimen Collection

Early-morning specimens should be collected whenever possible. Allowing the urine to remain in the bladder overnight, or for at least 4 h, will decrease the chance of a false-negative result. Excessive fluid intake will dilute the urine and decrease the colony count of urinary pathogens.

5.3 Minimum Volume, Specimen Transport, and Stability

If the urine cannot be delivered to the laboratory within 2 h after collection, it should be refrigerated or preserved in collection tubes with boric acid. At least 3 mL of urine should be placed into the transport tube to avoid inhibiting microorganisms. Refrigerated samples must be delivered to the lab within 1 day, and preserved samples (refrigerated or at room temperature) within 2 d. Frozen specimens are unacceptable for culture.

<u>Stability</u>
Room temp – 2 h
Refrigerated – 1 d

<u>Stability</u>
Room temp – 2d
Refrigerated – 2 d

5.4 Specimen Rejection

If a specimen is received and is unacceptable for any reason, consult the physician before processing. Do not discard any specimen until a new specimen is received, or the physician has been consulted. Request a new specimen when:

1. The specimen arrives in a leaky container.
2. There is no evidence of refrigeration, and the specimen is > 2 h old.
3. A refrigerated specimen is > 1d old.
4. The specimen has been collected from a bedpan, ileostomy bag, catheter drainage bag, or a condom catheter.
5. The specimen is a 24-h collection.
6. The specimen has been diluted in broth or transport media.
7. The specimen was submitted in a urinalysis tube with preservative. →

Unacceptable for culture

Foley catheter tips are unacceptable for culture. They are unsuitable for the diagnosis of urinary tract infection. Except for suprapubic bladder aspirates, requests for anaerobic culture are inappropriate.

6.0 Equipment & Materials

- Ambient (non-CO2) incubator at 35 ± 2°C
- Automated plate streaker with calibrated loop or, for manual inoculation, sterile, disposable calibrated inoculation loops (1-µL & 10-µL).
- BBL™ CHROMagar™ Orientation/TSA with 5% Sheep Blood agar bi-plate

7.0 Specimen Processing

7.1 Gram Stain

Gram stains are only performed on urine specimens by request. If requested, order the Gram Stain separately. After mixing the specimen, use a 0.01 mL calibrated loop to transfer the specimen to a pre-etched glass slide. Heat-fix the smear, and perform Gram stain. Refer to the Gram Stain Procedure for interpretation and reporting guidelines for urine specimens.

7.2 Culture

1. Inoculate a CHROMagar Orientation/TSA Blood agar bi-plate using the 1-µL calibrated loop. For invasively collected specimens, also inoculate a BAP using the 10-µL loop.
2. Mix the specimen prior to sampling.
3. Dip the loop straight into the urine. This is important in order to obtain the correct amount of urine in the loop.
4. Make one streak down the blood agar side of the plate (see Fig. 1), and proceed to cross streak making very close lines (see Fig. 2). Using the same loop, dip straight into the urine again, and inoculate and streak the CHROMagar™ Orientation side of the bi-plate (Fig. 3).
5. Incubate the plate at 35 ± 2°C for 18-24 h before 1st reading.

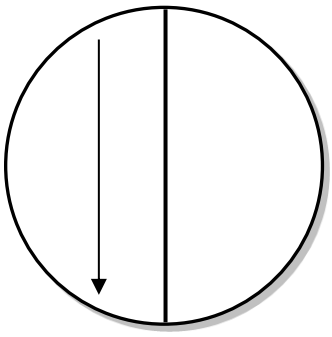


Fig. 1

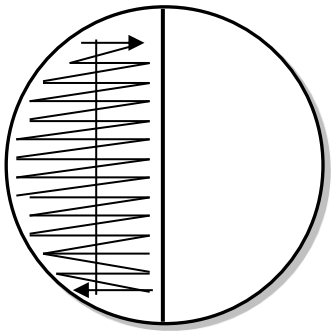


Fig. 2

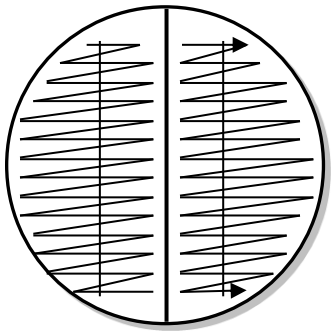


Fig. 3

8.0 Culture Workup Procedure

8.1 Determining Colony Count

After 18 to 24 h incubation, examine both sides of the split plate for growth. For positive cultures, determine the quantity and morphological type(s) of the colonies.

1-µL loop: 1 colony equals 1,000 CFU/mL	10-µL loop: 1 colony equals 100 CFU/mL
< 10 colonies is 1,000 (10^3) CFU/mL	< 10 colonies is 100 (10^2) CFU/mL
10-99 colonies is 10,000 (10^4) CFU/mL	10-99 colonies is 1,000 (10^3) CFU/mL
≥ 100 colonies is ≥ 100,000 (10^5) CFU/mL	≥ 100 colonies is ≥ 10,000 (10^4) CFU/mL

8.2 Urinary Pathogens vs. Microbial Flora

The workup of microorganisms in urine cultures is dependent on quantity, mixture, and types of organisms present. Certain microorganisms are commonly associated with urinary tract infections while other organisms are more likely to represent contamination of the specimen with urogenital flora. Table 1 provides guidelines for workup and reporting of pathogens.

Potential Pathogens

- *Aerococcus* spp.
- *Corynebacterium urealyticum*
- *Enterococcus*
- Gram-negative rods
- Beta-hemolytic strep - always report Group B from potentially pregnant females (≤ 50 yo), regardless of numbers or mixture of organisms. In mixed urines with ≥ 3 organisms, the GBS should be included in a list of potential pathogens. It is not necessary to list the GBS on a separate line and attach the susceptibility comment.
- *Neisseria gonorrhoeae*
- *Staphylococcus aureus*
- *Staphylococcus saprophyticus*
- Yeast (ID with germ tube and report as *C. albicans* or *Candida* spp. not *albicans*)

Urogenital Microbial Flora

These organisms commonly colonize the urogenital areas of the human body. They nearly always represent specimen contamination and are not considered clinically significant. The presence of urogenital flora does not affect the workup of Gram-negative rod (GNR) pathogens. Urogenital flora that is at least 10-fold less than non-GNR pathogens is ignored for purposes of workup. If microbial flora is greater than or equal to non-GNR pathogens, the culture is considered contaminated and reported as "Mixed flora." These organisms should only be treated as potential pathogens when they are recovered in quantities ≥ 10^5 CFU/mL, with no other organisms > 10^3 CFU/mL.

- Coagulase-negative staph (other than *Staphylococcus saprophyticus*)
- *Corynebacterium* spp. (other than *C. urealyticum*)
- *Gardnerella vaginalis*
- *Lactobacillus* spp.
- Viridans strep

9.0 Interpretation of CHROMagar™ Orientation

Table 2 provides guidelines for identifying and differentiating frequently encountered pathogens. CHROMagar™ Orientation medium allows for the differentiation and identification of *E. coli* and *Enterococcus* without confirmatory testing, based on criteria for identification established by CLSI standard M35-A, “Abbreviated Identification of Bacteria and Yeast; Approved Guideline.” Atypical or infrequent isolates require subculture and additional testing for identification.

Table 1: Protocol for workup of urine cultures

Specimen Type	1 pathogen	2 pathogens	≥ 3 pathogens
Noninvasive collection: • Clean catch voided • Indwelling catheter (Foley) • Pediatric bag • Ileal-conduit	**Gram-Negative Rod, avg. ≥ 5 colonies:** Perform & report ID/CC/AST. Report any amount of flora as Mixed flora. **GNR, avg. < 5 colonies, pure culture:** Perform & report ID/CC/AST. **GNR, avg. < 5 colonies, with mixed flora:** Report as Mixed flora.	**Gram-Negative Rod(s), avg. ≥ 5 colonies:** Perform & report ID/CC/AST. Report any amount of flora as Mixed flora. **GNRs, avg. < 5 colonies, pure culture:** Perform & report ID/CC/AST. **GNRs, avg. < 5 colonies, mixed culture:** Report as Mixed flora.	**≤ 2 types Gram-Negative Rod(s):** • **For each GNR, avg. ≥ 5 colonies**: Perform & report ID/CC/AST. Report any flora as Mixed flora. • **For each GNR, avg. < 5 colonies**: Report as Mixed flora. **>2 types of GNR, any colony count**: Perform minimal ID. Report "Mixed flora including:" and list each ID/CC (no AST). Add URCONT comment.
	Or	**and/or**	**and/or**
	Non-GNR pathogen ≥ 10^4 CFU/mL: Perform & report ID/CC/AST. **Non-GNR pathogen 10^3 CFU/mL**: Perform minimal ID and report with CC and no AST. Add NOTINF comment. **Note:** Flora 10-fold less than the non-GNR pathogen can be reported as Mixed flora. If flora is ≥ pathogen, report the non-GNR pathogen as "Mixed flora including:" ID/CC (no AST), & add URCONT comment.	**Non-GNR pathogens ≥ 10^4 CFU/mL**: Perform & report ID/CC/AST for each. **Non-GNR pathogens 10^3 CFU/mL:** Report as "Mixed flora." **Note:** Flora 10-fold less than the non-GNR pathogen(s) can be reported as Mixed flora. If flora is ≥ pathogens, report the non-GNR pathogens as "Mixed flora including:" ID/CC (no AST). Add URCONT comment if all potential pathogens are listed.	**For each non-GNR pathogen ≥ 10^4 CFU/mL**: Perform minimal ID. Report "Mixed flora including:" and list each ID/CC (no AST). Add URCONT comment if all potential pathogens are listed. **For each non-GNR pathogen 10^3 CFU/mL:** Report as "Mixed flora."
Invasive collection: • Straight catheter • Cystoscopy-kidney • Suprapubic aspirate • VB1, VB2, VB3 prostatic urines • Prostatic secretion • Cystoscopy-bladder • Percutaneous nephrostomy	**Gram-Negative Rod, any colony count:** Perform & report ID/CC/AST. Report any amount of flora as Mixed flora.	**Gram-Negative Rods, any colony count:** Perform & report ID/CC/AST for each GNR. Report any amount of flora as Mixed flora.	**≤ 2 types GNR, any colony count**: Perform & report ID/CC/AST. Report any amount of flora as Mixed flora. **>2 types GNR, any colony count**: Perform minimal ID. Report "Mixed flora including:" and list each ID/CC (no AST). Add URCONT comment.
	or	**and/or**	**and/or**
	Non-GNR pathogen ≥ 10^3 CFU/mL: Perform & report ID/CC/AST. **Non-GNR pathogen 10^2 CFU/mL**: Perform minimal ID and report with CC and no AST. Add NOTINF comment. **Note:** Flora 10-fold less than the non-GNR pathogen can be reported as mixed flora. If flora is ≥ pathogen, report the non-GNR pathogen as "Mixed flora including:" ID/CC (no AST), & add URCONT comment.	**Non-GNR pathogens ≥ 10^3 CFU/mL**: Perform & report ID/CC/AST for each. **Non-GNR pathogens 10^2 CFU/mL:** Report as "Mixed flora." **Note:** Flora 10-fold less than the non-GNR pathogen(s) can be reported as mixed flora. If flora is ≥ pathogens, report the non-GNR pathogens as "Mixed flora including:" ID/CC (no AST). Add URCONT comment if all potential pathogens are listed.	**For each non-GNR pathogen ≥ 10^3 CFU/mL**: Perform minimal ID. Report "Mixed flora including:" and list each ID/CC (no AST). Add URCONT comment if all potential pathogens are listed. **For each non-GNR pathogen 10^2 CFU/mL:** Report as "Mixed flora."

Table 2: Guidelines for identification of urinary pathogens on CHROMagar Orientation/TSA biplate

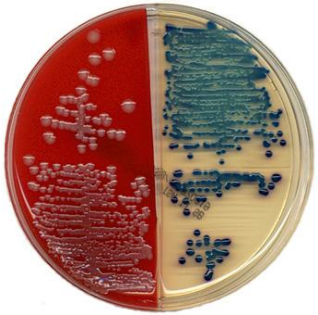
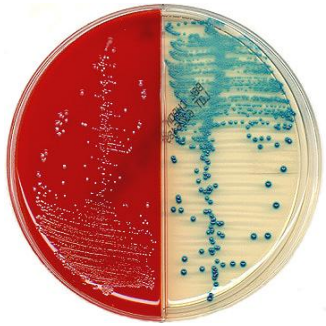
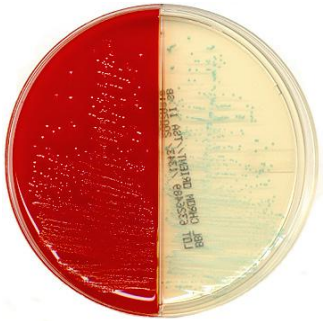
Organism	Colony Pigment	Additional Char.	Example	Confirmatory Tests
E. coli	Pink, transparent, with or w/out halos in surrounding medium	Medium to large size colonies		None
<u>KESC Group</u> *Klebsiella* *Enterobacter* *Serratia* *Citrobacter*	Medium-blue to dark blue, ± pink halos	Medium to large size colonies		Phoenix NID
<u>PMP Group</u> *Proteus* *Morganella* *Providencia*	Pale to beige colonies surrounded by brown halos	About 50% of *P. vulgaris* strains produce blue colonies on a brownish halo		<u>Swarming</u>: Perform spot indole Indole + = *P. vulgaris* Indole - = *Proteus* spp. <u>Nonswarming</u>: Phoenix NID
P. aeruginosa	Transparent, beige or yellow/green (color may darken with age)	Serrated edge		Oxidase + and metallic sheen or grape-like odor on BAP

Table 2: Guidelines for identification of urinary pathogens on CHROMagar Orientation/TSA biplate

Organism	Colony Pigment	Additional Char.	Example	Confirmatory Tests
Enterococcus	Blue-green (turquoise)	Small, alpha or non-hemolytic gray colonies on BAP		None If morphology is atypical verify PYR +
S. agalacticae (Group B)	Light blue-green to light blue, pinpoint to small colonies, with or without halos	Soft beta hemolysis on BAP		Streptex Group B +
S. saprophyticus	Light pink to rose, small opaque colonies, with or without halos	Medium, white to cream colored, butyrous colonies on BAP		Catalase + Coag -
<u>Staph species</u> *Staph aureus* Coag Neg Staph	Golden opaque, white	White to cream colored with variable hemolysis on BAP		Catalase + Slide coag + is *S. auresus* Slide coag - is Coag-Neg Staph Wet prep if necessary to r/o yeast or diphtheroids

10.0 Reporting

10.1 Cultures with No Growth

Preliminary Report (< 24 h incubation)

1. Enter preliminary report: **No growth to date.**
2. Reincubate the culture for the following day.

Final Report (≥ 24 h incubation)

1. Enter a final report:
   No growth or < 1,000 CFU/mL (non-invasive specimens set with 0.001 mL loop)
   No growth or < 100 CFU/mL (invasive specimens set with 0.01 mL loop)
2. Finalize report and discard culture plate.

10.2 Cultures with Growth

With Urinary Pathogens

1. Refer to Table 1 for workup protocols.
2. When appropriate, antimicrobial susceptibility testing (AST) is performed and reported according to the criteria listed in the **Isolate Work-up Charts**.
3. Report the colony count of the organism followed by the organism identification.
4. Review and file AST results. *Enterococcus* isolates tested on the Phoenix system will not have a result for nitrofurantoin (instrument limitation). A canned comment **[ENCSFM]** should be used to describe regional susceptibility of *Enterococcus* isolates to nitrofurantoin. If an *Enterococcus* isolate is not accessible for AST due to overgrowth by another organism, a canned comment can be used to describe the expected antibiogram. **[ENTSE]** Save the plate in the storage cupboards in case follow-up isolation and testing is requested.
5. If a single non-GNR pathogen is recovered in low numbers (10^3 from noninvasively collected specimens and 10^2 from invasively collected specimens), add the following comment:

   Organisms isolated are in low numbers and may not relate to infection. [NOTINF]
6. If the culture is too mixed, as indicated in Table 1, potential pathogens are identified with minimal testing based on morphologic identification (Gram stain and colony morphology) and same-day biochemical or serological tests. The potential pathogens are reported as, **Mixed flora including,** with each potential pathogen listed with a colony count. The following comment should be added to the report:

   This is a mixed culture suggesting the probability of contamination or colonization not related to infection. Further work-up of these organisms may result in clinically misleading information due to the low numbers and/or mixture of organisms present. Collection of another specimen is suggested, avoiding superficial sources of contamination. [URCONT]

With Urogenital Flora

1. If only urogenital flora is observed, report **Mixed flora**. Organism identifications and colony counts are only reported by request.
2. If urogenital flora is present and at least 10-fold less than any pathogens, report the pathogens according to Table 1, and report the urogenital flora as **Mixed flora**.
3. If an organism normally considered urogenital flora is recovered in quantities $\geq 10^5$ CFU/mL, with no other organisms > 10^3 CFU/mL, report the organism identification, colony count, and AST, if appropriate.

11.0 Quality Control

Check performance of each lot and/or shipment by inoculating a representative sample of plates with the control organisms listed below.

1. Make a suspension of each isolate directly from growth of a fresh agar plate, and adjust the turbidity to a 0.5 McFarland standard.
2. Dilute each suspension 1:100 in normal saline.
3. Using a 10 µL loop (large urine loop), inoculate the agar and streak for isolation.
4. More than one suspension can be inoculated on the same plate to reduce the number of plates used for QC testing (e.g., plate 1: *E. coli* + *E. cloacae*, plate 2: *Enterococcus* + *S. aureus*, and plate 3: *P. mirabilis* + *S. saprophyticus*)
5. Incubate media in ambient atmosphere at 35 ± 2°C for 18-24 h, and examine for expected results on the CHROMagar™.

Control Organism	Expected Results
Enterobacter cloacae ATCC 13047	Growth; medium sized, dark blue to medium-blue colonies with or without violet halos in the surrounding medium
Enterococcus faecalis ATCC 29212	Growth; small sized, blue-green colonies
Escherichia coli ATCC 25922	Growth; medium to large sized, transparent, dark rose to pink colonies with or without halos
Proteus mirabilis ATCC 35659	Growth; medium sized, transparent, pale beige to brown colonies, surrounded by a brown halo. Swarming is partially to completely inhibited.
Staph saprophyticus ATCC 15305	Growth; small to medium sized, light pink to rose, small opaque colonies with or without halos.
Staph aureus ATCC 25923	Growth; small to medium sized, white to cream (natural pigmentation).

12.0 Limitations

1. False-negative results may be due to interfering substances, diluted urine, and low urine pH.
2. CHROMagar™ Orientation is nonselective, other UTI pathogens will grow. Colonies that show their natural color and do not react with the chromogenic substrates must be further differentiated with appropriate biochemical or serological tests to confirm identification.
3. *E. coli* colonies that are dark rose to pink but are pinpoint or small in size require additional confirmatory tests such as spot indole.
4. Gram-negative organisms other than those belonging to the KESC group may produce large blue colonies and thus require other biochemical tests for identification.
5. Very rarely, isolates of *Aeromonas hydrophila* may produce rose colonies. They may be differentiated from *E. coli* by the oxidase test.

13.0 Verification of CHROMagar™ Orientation

A total of 197 cultures were tested using routine urine culture set-up consisting of MAC, CNA, and BAP. These specimens were also inoculated onto a CHROMagar Orientation/TSA Blood agar split plate at the same time. Cultures were examined at 12-24 h and then again at 48 h incubation. Isolates were worked-up on routine media by current methods, including spot tests, Micro-ID, and susceptibility testing. Of the 195 cultures, 61 (31%) were no growth by both methods; 71 (36%) of the cultures yielded one or two significant isolates; 63 (32%) of the cultures were either mixed (≥ 3 organisms) or the colony count was too low to merit work up (≤ 1,000 CFU/mL).

Discrepancies were considered minor if there were <2 $\log_{10}$ differences in colony counts or types of isolates that were not clinically significant (mixed cultures). There were a total of 21 (11%) minor discrepancies, including 6 for which the conventional culture was incorrect and 15 for which the CHROMagar was incorrect. However, 14 of the 15 (93%) of the minor discrepancies against CHROMagar were cultures that contained mixed Gram-positive flora on the CNA plate in the conventional culture. These organisms were not seen on the CHROMagar/TSA plate which, in effect, decreased time spent working up clinically insignificant organisms.

Discrepancies that could potentially impact patient care were categorized as major. These discrepancies occurred in cultures for which the other method was able to detect significant

isolates that would have otherwise been missed. There were a total of 6 major discrepancies. All of the major discrepancies involved mixed cultures. Clinically significant isolates were not isolated in 4 (2%) of the conventional cultures and 2 (1%) of the CHROMagar/TSA cultures.

In February, 2010 additional validation studies were performed for the identification of *Staphylococcus saprophyticus* on CHROMagar Orientation. A total of 26 isolates were tested. All of these isolates produced light pink to rose-colored, small to medium sized, opaque colonies on the CHROMagar Orientation medium. The isolates produced medium-sized, white to cream-colored, butyrous colonies on TSA w/5% sheep blood. All isolates were catalase positive and coagulase negative. Isolates were identified as *S. saprophyticus* by disk diffusion testing with a 5 μg novobiocin (NB) disk on Mueller Hinton agar. After overnight incubation in ambient air at 35 ±2 °C, the plates were examined for zones of susceptibility. All 26 (100%) of the isolates grew to the edge of the NB disks. This study verifies that coagulase negative staph isolates that produce pink to rose-colored colonies on CHROMagar Orientation may be identified as *Staphylococcus saprophyticus* without the confirmatory testing of NB disk diffusion.

14.0 References

1. Clinical Microbiology Procedures Handbook, 3rd ed. and 2007 update, Vol. 2. Garcia, L.S., editor in chief. ASM Press, Washington, D.C.
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15.0 Document Control History

Microbiology Director Approval: Dr. Ann Robinson 03/01/2010, 02/27/2014

Medical Director Approval: J. Schappert 03/10/10

Microbiology Supervisor Reviews: Jerry Claridge 03/01/201, 03/11/2011, 03/2013, 03/2014

Procedure Changes: 12/29/2010 Guidelines were added for reporting organisms not listed as potential pathogens (*Gardnerella, Lactobacillus,* Viridans strep). 01/20/2011 Added coagulase-negative staph, other than *S. saprophyticus,* to the previous list. 03/19/2011 Updated workup for Group 1 (invasively collected) specimens. Previously ID/AST was performed on any colony count… updated to only ≥ 10^3 CFU/mL. If 10^2 CFU/mL, report with low number comment. 06/17/2011 Added instructions for yeast identification by germ tube. 02/25/2014 Added safety section, details about each specimen type, collection timing, minimum volume, stability, rejection for 24-h specimens, rejection for leaky containers, specimen processing for Gram stains, description for urogenital flora vs. urinary pathogens, workup criteria for urinary pathogens changed with lower colony counts of GNR, ignore urogenital flora if at least 10 times less than non-GNR urinary pathogens, ignore any urogenital flora when working up GNR, combined CHROMagar Orientation procedure into Urine Culture Procedure, added lower limit of colony count detection for reporting no growth cultures. 3/27/14 Modified threshold for working of GNR to an average of 5 colonies for non-invasive specimens.