PURPOSE: To determine the presence or absence of bacterial pathogens in urine specimens.

SAMPLE INFORMATION:

Early-morning specimens are preferable – allowing urine to remain in the bladder for at least 4 hours will decrease the number of false-negative results.

Type	Urine • Fresh urine collected in sterile container • Fresh urine collected in urine transport tube	
Source	Voided urine (non-sterile)	• Midstream urine (MSU) • Neonatal bagged urine • Indwelling catheter (Foley) urine • Ileal conduit urine
	Aseptically collected urine (sterile)	• Straight or "in and out" catheter • Nephrostomy urine • Cystoscopy urine • Suprapubic bladder aspirate
Stability	• Fresh urine collected in sterile container is acceptable for 24 hours, if refrigerated. • Fresh urine collected in urine transport tube is acceptable for 72 hours (refrigeration not necessary).	
Storage Requirements	• Fresh urine without preservative should be refrigerated until processing. • Fresh urine collected in urine transport tube can be kept at room temperature.	
Criteria for rejection and follow up action	1. Fresh urine specimens (orange top) > 24 hours old. 2. Blue top urines > 72 hours old. 3. Unlabeled/mislabeled specimen. 4. Specimen container label does not match patient identification on requisition. 5. 24 hour urine collections. 6. Duplicate specimen within 24 hours. 7. Foley catheter tips. 8. Leaking specimens. 9. Improperly collected, labeled, transported or handled aseptically collected specimens should be processed. Waver of responsibility form SCM40110 needs to be filled out by the responsible nurse.	

REAGENTS and/or MEDIA:

- Blood agar (BA) and MacConkey agar (MAC)
- Identification reagents: catalase, oxidase, Staph latex test, Strep latex test, etc.

SUPPLIES:

- 1 µL loops
- Biosafety cabinet
- 35° ambient air incubator
- Vitek 2 and supplies

SPECIAL SAFETY PRECAUTIONS:

Containment Level 2 facilities, equipment, and operational practices for work involving infectious or potentially infectious materials or cultures.

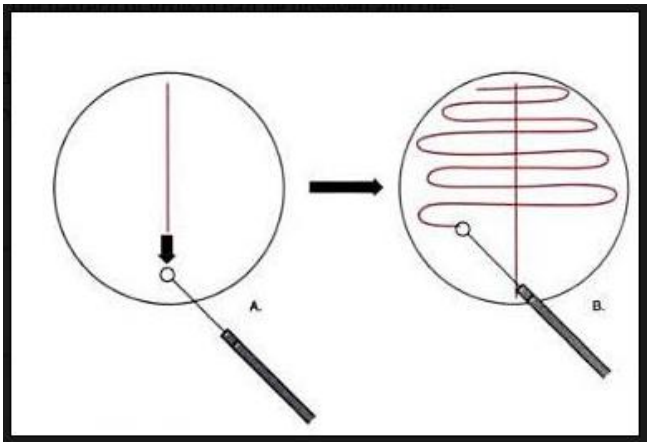
- Lab gown must be worn when performing activities with potential pathogens.
- Gloves must be worn when direct skin contact with infected materials is unavoidable.
- Eye protection must be used where there is a known or potential risk of exposure to splashes.
- All procedures that may produce aerosols, or involve high concentrations or large volumes should be conducted in a biological safety cabinet (BSC).
- The use of needles, syringes, and other sharp objects should be strictly limited.

All patient specimens are assumed to be potentially infectious. Universal precautions must be followed. Since viable micro-organisms are used, all cultures must be handled with appropriate precautions. All equipment in contact with cultures should be decontaminated by appropriate methods.

QUALITY CONTROL:

- Refer to Test Manual for reagent quality control procedures.

PROCEDURE INSTRUCTIONS:

Step	Action	
Processing specimens for urine culture		
1	Hold a 1 µL loop vertically and immerse just below the surface of a well-mixed urine specimen.	
2	Deliver a loopful of urine onto the Blood agar and MacConkey agar.	
3	Make a straight line down the center of the plate.	
4	Streak the urine by making a series of passes at 90° angles through the inoculum:  	
5	The same loop can be used for each plate per patient sample. The loop must be re-dipped for every plate.	
6	**IF**	**THEN**
	<u>Voided urines (non-sterile):</u> • Midstream urine (MSU) • Neonatal bagged urine • Indwelling catheter (Foley) urine • Ileal conduit urine	• Inoculate BA and MAC. • Incubate plates for 18-24 hours at 35° in the O_2 incubator.
	<u>Aseptically collected urines (sterile):</u> • Straight, intermittent or ”in and out” catheter • Nephrostomy urine • Cystoscopy urine • Suprapubic bladder aspirate	• Inoculate BA and MAC. • Incubate plates for 48 hours at 35° in the O_2 incubator.

INTERPRETATION OF RESULTS:

- Using a 1 µL loop, 1 colony equals 1 X 10^6 CFU/L.
- Determine the colony count and extent of the work-up required for each morphotype on the plates.
- Record all observations in the LIS.

List of Uropathogens and Non-Uropathogens:

Uropathogens	Potential Uropathogens	Non-uropathogens (skin/urogenital flora)
Enterobacteriaceae *Pseudomonas aeruginosa* Other gram negative bacilli *Enterococcus* spp. *Streptococcus pyogenes* *Streptococcus agalactiae* *Aerococcus urinae** *Corynebacterium urealyticum* *Staphylococcus aureus* *Staphylococcus saprophyticus*: (Females, aged 13-55yrs) Yeast spp.	Coagulase negative *Staphylococcus* (Not *Staphylococcus saprophyticus*) **NOTE:** Only considered significant if: ✓ The patient is symptomatic (indicated in clinical history) **AND** ✓ The organism is pure	*Lactobacillus* spp. Diptheroids: (not *C.urealyticum*) Viridans *Streptococci*: (not *A.urinae*) *Bacillus* spp. *Neisseria* spp.

***** Considered a uropathogen only if colony count is 10 times greater than that of all other microbiota.

REPORTING OF RESULTS: Non-sterile urine

NOTE: If a potential uropathogen is determined to be significant, treat as uropathogen below.

If a potential uropathogen is not determined to be significant, treat as non-uropathogen.

No. of colonies Colony count	1 isolate (uropathogen or non-uropathogen)	2 isolates (uropathogens or non-uropathogens)	3 or more isolates (uropathogens or non-uropathogens)
≤ 10 colonies (≤ 10 X 10^6 CFU/L)	No workup; Report: **"No Significant Growth"**	No workup; Report: **"No Significant Growth"**	No workup; Report: **"No Significant Growth"**

No. of colonies Colony count	1 uropathogen	2 uropathogens	3 or more uropathogens
11 - 99 colonies (11 - 99 X 10^6 CFU/L)	ID and susceptibility	ID and susceptibility testing on both	No workup; Report: **"}CON1 Mixed Culture, Repeat"**
≥ 100 colonies (≥ 100 X 10^6 CFU/L)	ID and susceptibility	ID and susceptibility testing on both	No workup; Report: **"}CON1 Mixed Culture, Repeat"**

No. of colonies	1 uropathogen and 1 or more isolates ≤10 colonies (uropathogen or non-uropathogen)	2 uropathogens and 1 or more isolates ≤10 colonies (uropathogen or non-uropathogen)	3 uropathogens and 1 or more isolates ≤10 colonies (uropathogen or non-uropathogen)
• Uropathogens: >10 • Other isolate(s): ≤10	• ID and susceptibility on uropathogen • Ignore isolate(s) ≤10	• ID and susceptibility on uropathogens • Ignore isolate(s) ≤10	No workup; Report: **"}CON1 Mixed Culture, Repeat"**

No. of colonies Colony count	1 non-uropathogen	2 non-uropathogens	3 or more non-uropathogens
≤ 10 colonies (≤ 10 X 10^6 CFU/L)	No workup; Report: **"No Significant Growth"**	No workup; Report: **"No Significant Growth"**	No workup; Report: **"No Significant Growth"**
11 - 99 colonies (11 - 99 X 10^6 CFU/L)	No workup; Report: **"No Significant Growth"**	No workup; Report: **"No Significant Growth"**	No workup; Report: **"No Significant Growth"**
≥ 100 colonies (≥ 100 X 10^6 CFU/L)	No workup; Report: **"No Significant Growth"**	No workup; Report: **"No Significant Growth"**	No workup; Report: **"No Significant Growth"**

*** Perform susceptibility testing as per ASTM.**

REPORTING OF RESULTS: Sterile urine

Colony Count	Any number of morphotypes
Any growth	Perform ID and susceptibility testing
No growth after 48 hours incubation	Report: **"}NG2D"**

LIMITATIONS:

1. A mixed culture in an uncomplicated outpatient population likely indicates contamination.
2. For uncomplicated UTI, culture is usually not indicated.
3. False-negative results may be due to interfering substances, diluted urine, low urine pH and subjective interpretation of the criteria for further workup of the culture.

REFERENCES:

- Clinical Microbiology Procedures Handbook, 4th edition, ASM Press, 2016
- Jorgensen J.H., Pfaller M.A., Carroll K.C., Funke G., Landry M.L., Richter S.S., Warnock D.W. 2015. Manual of Clinical Microbiology, 11th edition, ASM Press, Washington, D.C.

REVISION HISTORY:

REVISION	DATE	Description of Change	REQUESTED BY
1.0	24-Nov-10	Initial Release	M-L Dufresne
2.0	23-Dec-16	Updated to new template; Procedure updated to remove UriCult; Computer detailschanged to reflect practice for SoftMic SCC SoftComputer.	L. Steven
3.0	28 Apr 2017	Updated number; Changed Logo	JGD Bernier
4.0	30 Nov 2018	Updated to include new Vitek 2 instrument	L. Steven