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	The urine specimens submitted to the laboratory for culture are either: • Fresh urine collected in sterile container. • Fresh urine collected in urine transport tube.	
Source	Voided Urines (non-sterile)	• Midstream urine (MSU) • Neonatal bagged urine • Indwelling catheter (Foley) urine • Ileal conduit urine
	Aseptically collected Urines (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	• Unrefrigerated fresh urine received longer than 2 hours after collection. • Refrigerated fresh urine specimens received longer than 24 hours after collection. • 24 hour urine collections. • Duplicate specimens obtained with same collection method within 48 hours. • Foley catheter tips. • Urine from the bag of a catheterized patient. • Specimens in leaking container and unlabeled specimens.	

REAGENTS and/or MEDIA:

- Blood Agar and MacConkey Agar
- Identification reagents: catalase, oxidase, spot indole, PYR, tube coagulase, etc.

SUPPLIES:

- 0.001 mL loops
- Vitek 2 Compact and supplies
- Biosafety cabinet
- 35° ambient air incubator

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.

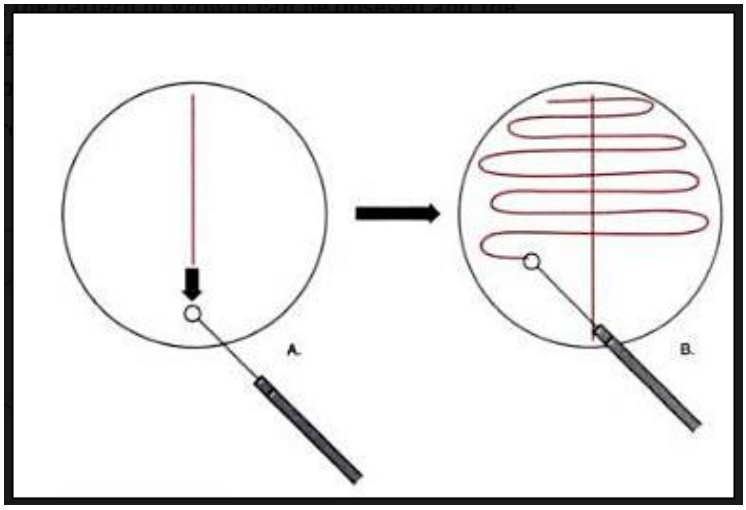
QUALITY CONTROL:

Refer to Quality Control procedures as required for individual identification and susceptibility procedures used in evaluating and testing.

PROCEDURE INSTRUCTIONS:

<table>
<tr><th>Step</th><th colspan="2">Action</th></tr>
<tr><td colspan="3">Follow the steps in the table below to set-up the culture for a urine specimen</td></tr>
<tr><td>1</td><td colspan="2">

Hold a 0.001 mL loop vertically and immerse just below the surface of a well-mixed urine specimen.
Deliver a loopful of urine onto appropriate agar plates.
Make a straight line down the center of the plate.
Streak the urine by making a series of passes at 90° angles through the inoculum.



***Note: the same loop can be used for each plate per patient sample. The loop must be re-dipped for every plate.
</td></tr>
<tr><td rowspan="3">2</td><td>If:</td><td>Then:</td></tr>
<tr><td><u>Voided Urines (non-sterile):</u>
• Midstream urine (MSU)
• Neonatal bagged urine
• Indwelling catheter (Foley) urine
• Ileal conduit urine</td><td>• Inoculate BAP and MAC
• Incubate plates for 18-24 hours at 35° in the ambient air incubator</td></tr>
<tr><td><u>Aseptically collected Urines (sterile):</u>
• Straight, intermittent or ”in and out” catheter
• Nephrostomy urine
• Cystoscopy urine</td><td>• Inoculate BAP and MAC
• Incubate plates for 48 hours at 35° in the ambient air incubator</td></tr>
</table>

	• Suprapubic bladder aspirate	

INTERPRETATION OF RESULTS:

Reporting Results:

- Using a 0.001 mL loop, 1 colony equals 1 X E6 cfu/L.
- Determine the colony count and extent of the work-up required for each morphotype on the plates.

Type of Urine: Non-sterile urine

Colony count	1 uropathogen	2 uropathogens	3 or more uropathogens
≤ 10 (≤ 10 X E6 cfu/L)	No workup; Report: **"No Significant Growth"**	No workup; Report: **"No Significant Growth"**	No workup; Report: **"No Significant Growth"**
11-99 (11-99 X E6 cfu/L)	ID and susceptibility testing	ID and susceptibility testing on both	No workup; Report: **"}CON1 Mixed Culture, Repeat"**
≥ 100 (≥ 100 X E6 cfu/L)	ID and susceptibility testing	ID and susceptibility testing on both	No workup; Report: **"}CON1 Mixed Culture, Repeat"**
Uropathogens >10 XE6 cfu/L and other isolates ≤10 XE6 cfu/L	ID and susceptibility on isolate >10 XE6 cfu/L and ignore isolates ≤ 10 XE6 cfu/L	ID and susceptibility on both isolates >10 XE6 cfu/L and ignore isolates ≤10 XE6 cfu/L	No workup; Report: **"}CON1 Mixed Culture, Repeat"**
>10 XE6 cfu/L Normal skin/urogenital flora	No workup; Report: **"Usual Urogenital Flora"**	No workup; Report: **"Usual Urogenital Flora"**	No workup; Report: **"Usual Urogenital Flora"**

Type of Urine: Sterile urine

Colony Count	Any number of morphotypes
Any growth	Perform ID and susceptibility testing
No growth after 48 hour incubation	"}NG2D"

List of Uropathogens and Non-Uropathogens:

Uropathogens	Non-uropathogens (normal skin/urogenital flora)
Enterobacteriacea	*Lactobacillus*
Pseudomonas aeruginosa	Diptheroids (not *C.urealyticum*)
Other gram negative bacilli	Viridans Streptococci (not *A.urinae*)
Enterococcus species	Bacillus species
Streptococcus pyogenes	*Neisseria spp.*
Streptococcus agalactiae	
Yeast	
*Aerococcus urinae******	
Corynebacterium urealyticum	
Staphylococcus aureus	
Staphylococcus saprophyticus	
Other coagulase negative staphylococcus	

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

ORGANISM	BIOCHEMICAL TESTS & EXPECTED RESULTS	ID & SENS
E.coli	LF, flat, deep pink Spot Indole: Positive Oxidase: Negative PYR: Negative	Vitek AST- N213
Enterobacteriaceae	LF or NLF Spot Indole: Negative/Positive Oxidase: Negative PYR: Positive/Negative	Vitek GN & AST-N213
P.aeruginosa	NLF, typical smell/morphology Spot Indole: Negative Oxidase: Positive	Vitek GN & AST-N213
Non-fermenters	NLF Oxidase: Positive/Negative	Vitek GN & AST-N213
Group A Strep	Beta hemolysis GAS latex: Positive	Add comment: "This organism is generally susceptible to beta lactam antibiotics"
Group B Strep	Small or no zone of beta hemolysis GBS latex: Positive	Add comment: "This organism is generally susceptible to beta-lactam antibiotics. This organism is resistant to TMP-SMX".
S.aureus	Catalase Positive, Slide Coagulase Positive, Tube Coagulase Positive	Vitek AST-GP67
S. saprophyticus	Catalase Positive, Slide Coagulase Negative, Novobiocin Resistant	Add comment: "This organism is generally susceptible to TMP-SMX, nitrofurantoin and urinary quinolones"
Enterococcus	Catalase Negative PYR Positive	Vitek AST-GP67
Yeast	Wet prep = yeast	Vitek YST ID card:
Aerococcus urinae	Resembles viridans Streptococcus Gram stain: GPC, tetrads Catalase: Negative PYR: Negative	Vitek GP Perform susceptibility testing as per DynaLIFE ASTM
Diptheroides	Catalase positive • Rule out uropathogenic Corynebacterium urealyticum (urea+) if numbers are significant.	Urea

Limitations:

- A mixed culture in an uncomplicated outpatient population likely indicates contamination.
- For uncomplicated UTI, culture is usually not indicated.
- 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.

J Obs Gyn Can 2004; 26(9):826-32

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

Invalid signature

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Jennifer G. Daley Bernier, R.T. (CSMLS)
A/ Manager, Laboratory Services
Signed by: Jennifer G. Daley Bernier