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 (BAP) and MacConkey agar (MAC)
- Identification reagents: catalase, oxidase, rapid Staph, rapid Strep, etc.

SUPPLIES:

- 0.001 mL 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.

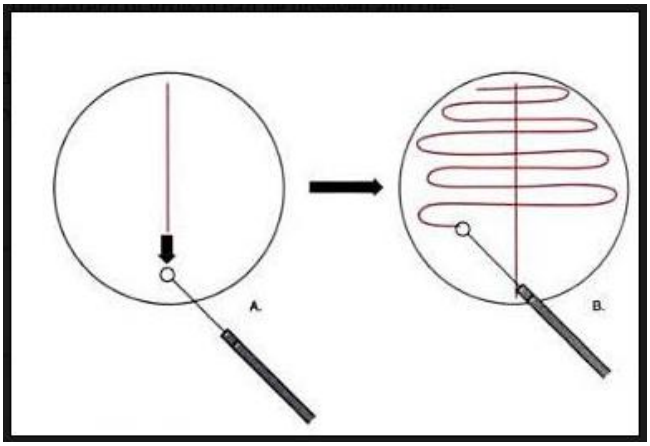
QUALITY CONTROL:

Refer to Quality Control manual for reagent quality control procedures.

PROCEDURE INSTRUCTIONS:

<table>
<tr><th>Step</th><th colspan="2">Action</th></tr>
<tr><td colspan="3">Processing urine for culture</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="2">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><td><u>Aseptically collected Urines (sterile):</u>
• Straight, intermittent or ”in and out” catheter
• Nephrostomy urine
• Cystoscopy urine
• Suprapubic bladder aspirate</td><td>• Inoculate BAP and MAC
• Incubate plates for 48 hours at 35° in the ambient air incubator</td></tr>
</table>

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.
- Record all observations in the LIS.

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	*Coagulase negative staphylococcus* (Not *Staphylococcus saprophyticus*)
Yeast	
*Aerococcus urinae******	
Corynebacterium urealyticum	
Staphylococcus aureus	
Staphylococcus saprophyticus (Females, aged 13-55yrs)	

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

REPORTING OF RESULTS: Non-sterile urine

Colony count	1 isolate (uropathogen or non-uropathogen)	2 isolates (uropathogens or non-uropathogens)	3 or more isolates (uropathogens or non-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"**

Colony count	1 uropathogen	2 uropathogens	3 or more uropathogens
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"**

Colony Count	1 uropathogen and 2 or more isolates ≤10 XE6 (uropathogen or non-uropathogen)	2 uropathogens and 2 or more isolates ≤10 XE6 (uropathogen or non-uropathogen)	3 uropathogens and 2 or more isolates ≤10 XE6 (uropathogen or non-uropathogen)
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"**

Colony Count	1 non-uropathogen	2 non-uropathogens	3 or more non-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)	No workup; Report: **"No Significant Growth"**	No workup; Report: **"No Significant Growth"**	No workup; Report: **"No Significant Growth"**
≥ 100 (≥ 100 X E6 cfu/L)	No workup; Report: **"No Significant Growth"**	No workup; Report: **"No Significant Growth"**	No workup; Report: **"No Significant Growth"**

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"**

ORGANISM	BIOCHEMICAL TESTS & EXPECTED RESULTS	ID & SENS
Enterobacteriaceae (including E.coli)	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 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
4.0	25 Apr 2018	Change to reflect new Vitek 2 instrument	L. Steven