PROCESSING AND INTERPRETATION OF URINARY TRACT SPECIMENS

Technical Procedure

PURPOSE

This procedure establishes a standard format for processing and interpretation of urinary tract specimens.

PRINCIPLE

Urine specimens are submitted for culture from patients with symptoms of urinary tract infections (UTI) and from asymptomatic patients with a high risk of infection. The number of microorganisms per milliliter, recovered on urine culture, can aid in the differential diagnosis of UTI. Calibrated inoculating loops are used to deliver a known volume of urine, enabling the microbiologist to estimate numbers of organisms in the original specimen based on colony forming units (CFU) of growth on culture. The use of quantitative culture for diagnosis of UTI includes separate criteria which are applied to different specimen types.

SPECIMEN

A. Type (urostomy is a generic term; call clinician for specific source details.
   1. Clean-voided midstream urine
   2. Catheterized, nephrostomy (renal pelvis), cystostomy (bladder),or ureterostomy (ureter)
   3. Suprapubic needle aspiration urine
   4. Special collection urine (e.g. bladder wash or urine localization studies, pre-prostrate and post-prostrate massage specimens)

B. Collection and transport
   Refer to the MICROBIOLOGY SET UP MANUAL for specific collection and transport criteria.

MATERIALS

A. Media and Reagents
   1. Blood agar (BAP)
   2. MacConkey agar (MAC)
   3. Cinnamaldehyde reagent
   4. Oxidase reagent

B. Supplies
   1. Incubator (35 - 37°C ambient)
   2. Sterile inoculating loops – 0.001 ml
   3. MLA pipette – 0.1ml

QUALITY CONTROL

A. Quality control testing (or documentation of commercial testing) of agar media is performed by the QC bench upon receipt of each new lot. Refer to the QUALITY CONTROL PROCEDURE MANUAL for specific set-up procedures.

B. Cinnamaldehyde and oxidase reagent quality control is performed and recorded each day of use. Refer to the QUALITY CONTROL PROCEDURE MANUAL for specific set-up procedures.

PROCEDURE

A. Specimen processing

procedures.

B. Incubation

Incubate the inoculated plates at 35°C in ambient atmosphere for a minimum of 48 hours.

C. Microscopic Examination

1. Gram stain examination of an unspun urine sample is a rapid and reliable method for detecting significant bacteriuria and pyuria. The presence of at least one bacterial cell and one leukocyte per oil immersion field correlates with significant bacteriuria (>10^5 CFU/ml) and pyuria. In addition, the bacterial morphotype observed in positive smears may provide a useful guide in the initial selection of antimicrobial therapy. Gram stain is performed upon physician request.
2. Refer to the GRAM STAIN protocol for complete procedure on the performance of the gram stain.

D. Culture and Isolation

1. Examine all culture plates after overnight (18 – 24 hour) incubation. Reincubate plates and examine after an additional 24 hours. A magnifying light may be needed to to observe pinpoint growth. After final examination, if no growth occurs, discard plates. Save all direct and subculture plates which have growth for one week at room temperature in case further workup is necessary.
2. Reincubate culture plates with tiny or scant colonies that are not discernible before proceeding with workup. Do not workup cultures unless the growth is relatively mature and the culture is pure. This is especially important when working up cultures on day 1. They are often mixed, on day 2, with gram positive flora which was not visible on the initial examination.
3. For positive cultures, examine culture plates for the quantity and morphological type of organisms present. If culture plates produce discrepant colony counts, report the highest colony count calculated.
   a. With 0.001 ml inoculum, one colony equals 1,000 organisms per ml.
   b. With 0.1 ml inoculum, (used for suprapubic needle aspiration, special collection and renal stone only) one colony equals 10 organisms per ml.
4. Semi-quantitate growth and perform organism identification and special processing based on the colony count and method of urine collection as detailed in **Table 1: Interpretation of Cultures of Voided and Unspecified Urine Specimens,** and **Table 2: Interpretation of Cultures of Catheterized Urine Specimens.** Perform all organism identification and susceptibility testing in accordance with routine identification protocols as outlined in MICROBIOLOGY PROCEDURE MANUAL 5: IDENTIFICATION, with the following additions and/or modifications:

   a. Lactose-fermenting gram negative bacilli in pure culture: Perform cinnamaldehyde test in accordance with **Cinnamaldehyde Test** protocol. **NOTE: indole is a diffusable product and, in mixed cultures, an indol negative organism could test falsely positive if it has grown near an indole producer.**
      (1) Report indole positive organisms with flat colony morphology as presumptive *E.coli* (MPEC).
      (2) Report indole negative organisms with mucoid colony morphology as presumptive *Klebsiella/Enterobacter* group (MPKE).
      (3) Any other situation, report as Lactose-fermenting gram negative bacilli (MPLF).

b. Non-lactose fermenting gram negative bacilli in pure culture that exhibit swarming morphology on BAP: Perform cinnamaldehyde test in accordance with **Cinnamaldehyde Test** protocol. Refer to NOTE D.4.a above.
   (1) Report indole negative organisms as presumptive *Proteus mirabilis* (MPPM).
   (2) Report indole positive organisms as presumptive *Proteus vulgaris* (MPPV).

c. Non-lactose fermenting gram negative bacilli that do not exhibit swarming morphology on BAP: Perform oxidase test in accordance with **Oxidase Test** protocol.
   (1) Report oxidase positive organisms with morphology consistent with *Peudomonas* sp. as presumptive *Pseudomonas* species (MPPS).
   (2) Report oxidase positive organisms with morphology not consistent with *Pseudomonas* sp, as non-lactose fermenting gram negative bacilli (MPNLF).
   (3) Report oxidase negative organisms as non-lactose fermenting gram negative bacilli (MPNLF).

d. Coagulase negative staphylococcus: Issue a preliminary report of *Staphylococcus*, coagulase negative (CNS). Perform novobiocin susceptibility test in accordance with **Novobiocin Susceptibility Test** protocol when organism is present in pure culture at greater than 100,000 organisms/ml.
   (1) Report as *Staphylococcus saprophyticus* if resistant to novobiocin. Enter comment regarding susceptibility testing of *S.saprophyticus* (MSSMIC).
   (2) Report as *Staphylococcus*, coagulase negative if sensitive to novobiocin.

e. *Staph aureus*: Issue a preliminary report of *Staph aureus*, MIC to follow, and set up MIC. Perform novobiocin susceptibility test in accordance with **Novobiocin Susceptibility Test** protocol.
   (1) Report MIC's if organism is susceptible to novobiocin.
   (2) Perform coagulase test in accordance with **Coagulase Test** protocol if organism is resistant to novobiocin.
      (a) Report as *Staphylococcus saprophyticus* if coagulase negative. Enter comment "Final identification after further evaluation" (FID) and comment regarding susceptibility testing of *S.saprophyticus* (MSSMIC).
      (b) Report as *S.aureus* if coagulase positive. Repeat MIC's and novobiocin test. Consult with supervisor or specialist if novobiocin is resistant on repeat.

f. *Corynebacterium ureolyticum* and *Corynebacterium glucuronolyticum*: Perform urea test in accordance with **Urea Hydrolysis Test** protocol on any *Corynebacterium* sp. which is to be reported. Note: these organisms can often produce a positive urea test within 5 - 10 minutes.
   (1) Report as *C.ureolyticum* if urea positive and lipophilic (white/gray pinpoint colony after 48 hours)

(2) Perform MUG Disk test in accordance with **MUG Disk Test** protocol on any organism which is urea positive and non-lipophilic (small to medium colony at 48 hours).
  (a) Report as *C.glucuronolyticum* if MUG Disk test positive.
  (b) Report as *Corynebacterium* sp. if MUG Disk test negative.
(3) Report as *Corynebacterium* sp. if urea negative.

g. *Aerococcus* species: Perform a gram stain and catalase test on tiny alpha hemolytic colonies which are pure or predominant. *Aerococcus* sp. stain as gram positive cocci in clusters and are catalase negative. Inoculate a thioglycolate broth if unable to determine morphology on gram stain. Perform PYR disk test in accordance with the **PYR Procedure** protocol.
  (1) On isolates which are PYR negative, subculture to a BAP and add a Vancomycin 30 mg disk. Report as *Aerococcus* sp. organisms which are vancomycin sensitive (any zone of inhibition).
  (2) On isolates which are PYR positive perform a LAP disk test in accordance with the **LAP Procedure** protocol. Report as *Aerococcus* sp. if LAP negative.

h. Yeast: Perform gram stain. See **Mycology Referral** protocol for identification criteria.
  (1) Yeasts that do not require complete identification are reported as "Yeast, not further identified "(YNFI).
  (2) Yeasts that require further identification are reported as "Yeast, identification to follow" (MYITF). A copy of the urine culture report and a subculture of the organism on Sabouraud agar is sent to Mycology for further work-up.

i. ~~Group B *Streptococcus*: Report, in any quantity, if present in females of child-bearing age (approximately 11 - 60 years old).~~ deleted 1-18-12 LK
j. Occasionally urine specimens are contaminated with genital flora, such as *Gardnerella vaginalis*. These organisms are tiny gram variable bacilli. Consult the **Genital Bench** protocol for further instructions on identifying these organisms if they are predominant.
k. Consult **Table 3: Identification of Gram Negative Bacilli on Urine Bench** and **Table 4: Identification of Gram Positive Cocci on the Urine Bench** for additional instructions on organism identification.
l. If specific organisms are requested by the physician, identify and report in any amount.
m. If there is a question as to whether mixed morphotypes represent the same organism (i.e. round and spready NLF's., mucoid and flat LF's, NLF and LF in same culture), subculture **1 colony** of each morphotype to a BAP/MAC biplate. Perform oxidase and cinnamaldehyde tests **first** to determine if subcultures represent different organisms. If these tests cannot determine if the organisms are different, identify and perform susceptibility testing on each.
n. Cultures which grow several strains (different susceptibilities) of the same organism are not considered mixed cultures. Report identification and susceptibility results for each strain.

o. Specimens which appear mixed and are overgrown with swarming *Proteus* sp. after 18 to 24 hours incubation, should be replated to a Rose agar to rule out predominant gram positive organisms. Incubate this plate for a minimum of 48 hours.

REPORTING

A. Preliminary Report
1. Report "No growth to date" (NG) if the culture has no growth after 24 hours.
2. Report "Culture growth too young; needs additional incubation prior to work-up" (NGPY) if the culture has not incubated sufficiently to distinguish colony types or predominant organism.
3. Report the quantity and any preliminary information on organisms with identification in progress, in decreasing order of predominance. Quantitate and report mixed flora.
4. Cultures which are growing >100,000 organisms/ml mixed flora after 24 hours incubation, and which will not have further workup, can be finalized on day 1.
5. Update the culture report each day as necessary until all work is complete.

B Final Report
1. Report "No growth after 2 days" (NG) if the culture has no growth after 48 hours incubation.
2. For cultures with growth, report all organisms identified and any applicable susceptibilities, in decreasing order of predominance. Quantitate and report mixed flora.
3. If a preliminary report has indicated that an organism identification is in progress, yet upon further incubation no further work-up is indicated according to protocol, quantitate and report mixed flora, and add the comment "No predominant organism upon further incubation" (MNP).
4. If the preliminary report has indicated an identification which upon further testing is not correct, report the correct identification and add the comment "Final identification upon further testing" (MFID).
5. If specific organisms requested by the physician have been ruled out, report "No (organism) isolated".

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PROCEDURE NOTES: URINE CULTURE IF INDICATED

Upon physician request, urine cultures will be performed selectively when indicated by abnormal urinalysis results. Urine specimens, with urinalysis results in the normal range, will not be cultured. The culture is canceled in Chemistry. No further work is required in Microbiology. Urine specimens with any of the following abnormal urinalysis results, will be sent to Microbiology and set up for routine culture as outlined above. Specimens received at the end of the evening shift may not have completed urinalysis results. These specimens will be set up. The following morning the CLS assigned to the Urine Bench will check any urinalysis results, pending from the previous night, to see which cultures have been canceled. Place all plates set up on canceled cultures in the room temperature rack for that day.

NITRATE:	≥trace or positive
LE:	≥trace or positive
WBC's:	>3/hpf on microscopic exam
BACTERIA:	moderate to many/hpf on microscopic exam
YEAST:	any on microscopic exam

REFERENCES

Clarridge, Jill E., Johnson James R., and Pezzlo, Marie T., 1998, Laboratory Diagnosis of Urinary Tract Infections, *Cumitech 2B*, American Society of Microbiology, Washington,D.C.

Forbes, Betty A., Sahm, Daniel F., and Weissfeld, Alice S., 2002, *Bailey and Scott's Diagnostic Microbiology*, 11th ed., p. 927 – 938, Mosby-Year Book, Inc., St. Louis, MO.

Isenberg, Henry D., Ed. in Chief, 1994, Urine Culture Procedure, p.1.17.1-1.17.15, *Clinical Microbiology Procedures Handbook*, Vol.1, American Society for Microbiology, Washington, D.C.

Murray, Patrick R., Ed. in Chief, *Manual of Clinical Microbiology* 8th Edition, 2003, p. 436-440, 481-483, 489-492, ASM Press, Washington, D.C.

Pezzlo, Marie, 1988, Detection of Urinary Tract Infections by Rapid Methods, *Clin. Microbiol. Rev.* 1:268-280

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PROCEDURE HISTORY

Date	Written/ Issued by	Revision/ Annual Review	Approved Date	Approved By
7/85	K. Folkins	New	7/85	J.R.Carlson, PhD.
11/96	S. Andersen	Revision	11/96	J.R.Carlson, PhD.
		Review	02/98	S. Andersen
		Review	11-98	J.R.Carlson, PhD.
		Review	12-99	J.R.Carlson, PhD.
		Review	12/00	J.R.Carlson, PhD.
		Review	01/02	Tom Watson
		Review	11/02	J.R.Carlson, PhD.
		Review	03/04	J.R.Carlson, PhD.
03/2005	L. Lenhart	Revision	5/26/05	Jay Schmidt
		Review	1/06	
		Annual Review	11/06	
		"	10/07	
		"	9/08	
		"	11/09	
		"	3/2010	
		Review	9/2012	Anna Romanelli

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TABLE 1: Interpretation of Cultures of VOIDED and UNSPECIFIED Urine Specimens

# of morphotypes	Quantitation (organisms/ml)	Procedure[a]	Report[b]
Both plates No growth		No further work	No growth (NG)
1	$<10^4$	enumerate	$<10^4$ org/ml
1	$>10^4$	ID, MIC	(B) or (C) org/ml (organism)
2	both $<10^5$	enumerate	(A), (B), or (C) org/ml mixed flora
2	1 $>10^5$ 1 $<10^5$	ID, MIC $>10^5$ enumerate $<10^5$	$>10^5$ org/ml (organism) (A) or (B) org/ml mixed flora
2	both $>10^5$	ID, MIC both	$>10^5$ org/ml (organism) and (organism)
≥3	all $<10^5$	enumerate all	(A), (B), or (C) org/ml mixed flora
≥3	1 $>10^5$ ≥2 at $<10^4$ **each**	ID, MIC $>10^5$ enumerate all others	$>10^5$ org/ml (organism) (A) or (B) org/ml mixed flora
≥3	2 $>10^5$ ≥1 at $<10^4$ **each**	ID, MIC both $>10^5$ enumerate all at $<10^4$	$>10^5$ org/ml (organism) and (organism) (A) or (B) org/ml mixed flora
≥3	any other situation	enumerate all	$>10^5$ org/ml mixed flora quantitate and describe all morphotypes on worksheet

a. ID = identification MIC = antimicrobial susceptibility testing as appropriate

b. Report quantitation as:
(A) <10,000 organisms per ml
(B) 10,000 to 100,000 organisms per ml
(C) >100,000 organisms per ml

TABLE 2: Interpretation of Culture of CATHETER Urine Specimens

# of morphotypes	Quantitation (organisms/ml)	Procedure[a]	Report[b]
both plates no growth		no further work	No growth
1	any amount	ID, MIC	(A), (B), or (C) org/ml (organism)
2	both $<10^4$	enumerate	$<10^4$ org/ml mixed flora
2	1 $>10^4$ 1 $<10^4$	ID, MIC $>10^4$ enumerate $<10^4$	(B) or (C) org/ml (organism) $<10^4$ org/ml mixed flora
2	both $>10^4$	ID, MIC both	(B) or (C) org/ml (organism) and (organism)
≥3	all $<10^5$	enumerate all	(A), (B), or (C) org/ml mixed flora
≥3	1 $>10^5$ ≥2 at $<10^5$ **each**	ID, MIC $>10^5$ enumerate all others	$>10^5$ org/ml (organism) (A) or (B) org/ml mixed flora
≥3	2 at $>10^5$ ≥1 at $<10^4$ **each**	ID, MIC both $>10^5$ enumerate all at $<10^4$	$>10^5$ org/ml (organism) and (organism) (A) or (B) org/ml mixed flora
≥3	any other situation	enumerate all	$>10^5$ org/ml mixed flora quantitate and describe all morphotypes on worksheet
Suprapubic aspirate, special collection, renal stone[c]			
any number	any amount	ID, MIC	report actual count of each isolate

a ID = identification MIC = antimicrobial susceptibility testing as appropriate

b Report quantitation as:
(A) <10,000 organisms per ml
(B) 10,000 to 100,000 organisms per ml
(C) >100,000 organisms per ml

c 0.1 ml and 0.001 ml dilutions plated to BAP and MAC

Table 3: Identification of Gram Negative Bacilli on the Urine Bench

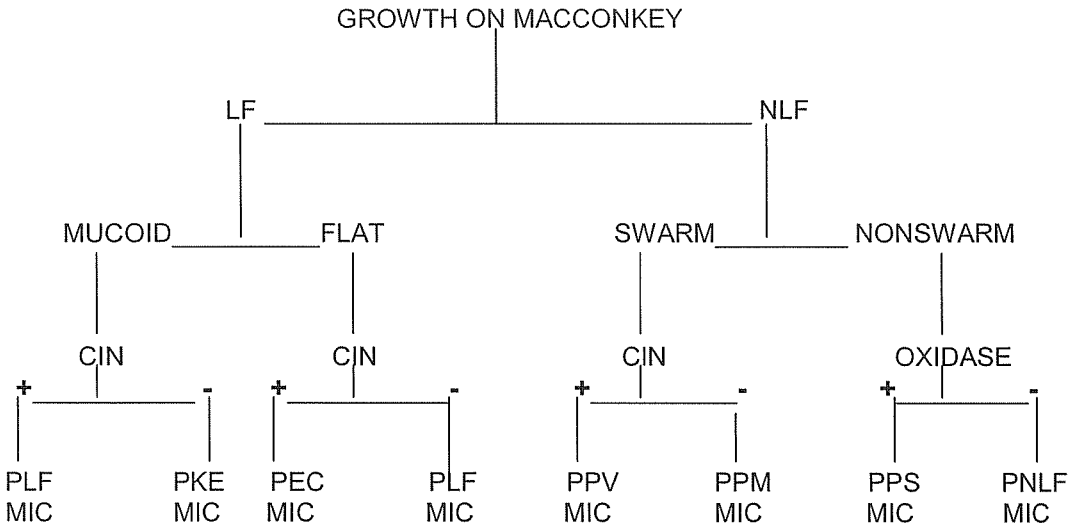
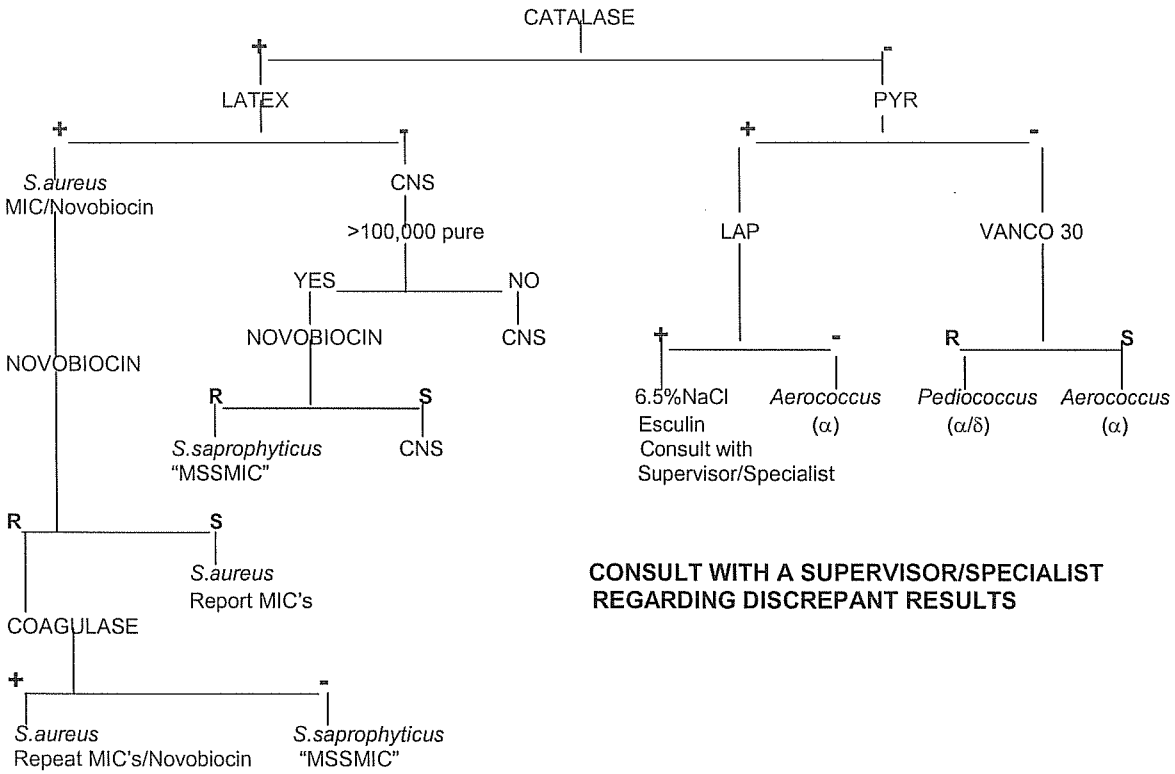


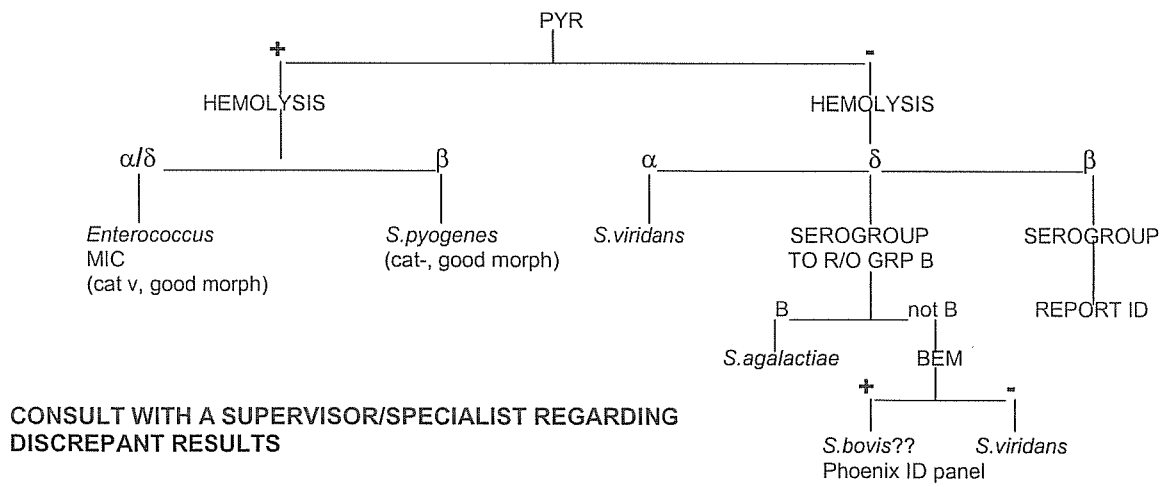
Table 4: Identification of Gram Positive Cocci on the Urine Bench

GRAM POSITIVE COCCI IN CLUSTERS



CONSULT WITH A SUPERVISOR/SPECIALIST REGARDING DISCREPANT RESULTS

GRAM POSITIVE COCCI IN PAIRS/CHAINS



CONSULT WITH A SUPERVISOR/SPECIALIST REGARDING DISCREPANT RESULTS