National Department of Health

Title: Urine Analysis, Microscopy and Culture

ID: G_90_SOP_11_A

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Changes to the last authorized version:

Version	Date	Amendments
G_90_SOP_11_A	6/5/22	New version
G_90_SOP_11_A	13/9/22	Novobiocin as identification for S. saphrophyticus added

Certification of printed copy:

Version	
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Date	

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1. Purpose & Scope

This manual documents procedures for urine analysis, microscopy and culture to screen for detection and evaluation of renal and urinary tract disorders as well as other systemic diseases. It includes the interpretation and reporting of results.

Urine cultures are performed on symptomatic patients to detect the causative agent of UTI. It is important that clinicians do not routinely request urine culture from asymptomatic patients, but will only do so if the patients are of high risk.

Antenatal screening for bacteriuria is of relevance for pregnant women – when detected, bacteriuria is treated with antibiotics to prevent pyelonephritis occurring later in the pregnancy.

2. Principle & Clinical application

Urine is usually a sterile body fluid that is easily contaminated by urogenital and intestinal flora, especially when a patient is catheterised. Presence of pyuria (urine WBC count >100 x 10^9/L) and/or bacteriuria (presence of uropathogenic bacteria in the urine) is a non-specific finding - it may be present in the absence of symptomatic UTI – most commonly in older patients or patients with indwelling urinary catheters.

Urinary tract infections (UTI) may involve the kidneys, ureters, bladder, and urethra. UTI symptoms include dysuria, urinary frequency, fever, loin pain and when severe, rigors with vomiting. Pyelonephritis (renal infection) may be associated with bloodstream infection and severe sepsis.

Common uropathogens include but are not limited to:

- *Escherichia coli*, by far the most common (80%)
- *Klebsiella* spp, *Proteus* spp, and *Enterobacter* spp
- *Staphylococcus saphrophyticus* (adult pre-menopausal women)
- *Streptococcus agalactiae* (group B strep)
- *Enterococcus* spp (especially catheterized patients but frequently a contaminant)
- *Pseudomonas aeruginosa* (especially catheterized patients)
- *Staphylococcus aureus* (especially catheterized patients but may indicate systemic bacteraemic infection)

3. Responsibilities

Role	**Responsibility**
Medical Lab bench scientist/technician	Setup, reading and data entry Identify and document AMR phenotypes that require storage/ referral
On duty senior scientist	Checking of positive culture results Addition of interpretative comments on the LIMS for the report

4. Specimen

Urine should be transported to the laboratory immediately. If there is a delay in transport, refrigerate the specimen so that the colony count will be accurate. If not refrigerated, the bacteria will increase in number and the colony count will not be accurate.

If there is going to be a delay in examination of > 1 hour, refrigerate the urine or put in a pan of ice.

Urine can be collected in several ways:

Mid-Stream Urine Specimen

The most common method is the clean catch or mid-stream collection. Men should retract the foreskin of the penis and women should part the vaginal labia with their fingers while collecting the sample. The patient should be instructed to start urinating and allow the first part of the stream to go as waste, and collect the middle portion of the stream, and then discard the last portion. A clean-catch specimen from a child is also acceptable.

Catheter Specimen (indwelling or in-out catheter specimen)

In-out specimen is collected via a straight catheter through the urethra into the bladder.

Suprapubic Aspirate Specimen (usually infant or neonate)

After careful skin disinfection, a 25 gauge needle aspirate of the bladder is used do access urine for examination. This approach rules out the possibility of urethral contamination.

5. Safety

Handling of the primary specimen should be performed with gloves as per Standard Precautions.

For other safety aspects, please review this document G_10_Info_3 Laboratory Biosafety.

6. Equipment & Materials

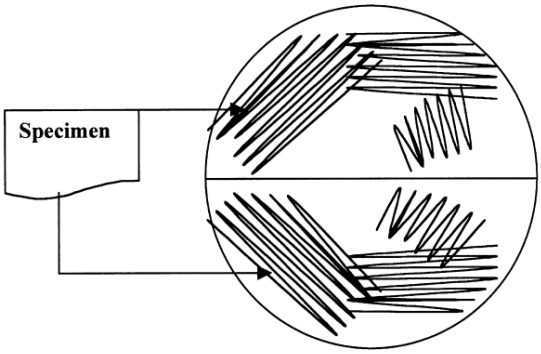
- Disposable loops (1ul)
- Urinalysis dipsticks
- Blood and MacConkey agar (1/2 plates) or Blood and GNR-Chromagar (1/2 plates)
- Capillary tubes
- Coverslips
- Improved Neubauer counting chamber
- Kova slides (if used)
- Refrigerator or pan of ice to keep the urine container in or on to maintain cold temperature until cultured

6 Procedure

6.1 Urine Specimen Culture

- Record the macroscopic appearance of the urine sample.
- Label the plates with the time of incubation, to ensure that they receive a full 16-hour incubation
- Using calibrated loop, streak 1uL of urine over the surface of each of the BA /MAC half plate.
- Incubate in the 35° C incubator in for 18-24 hours.

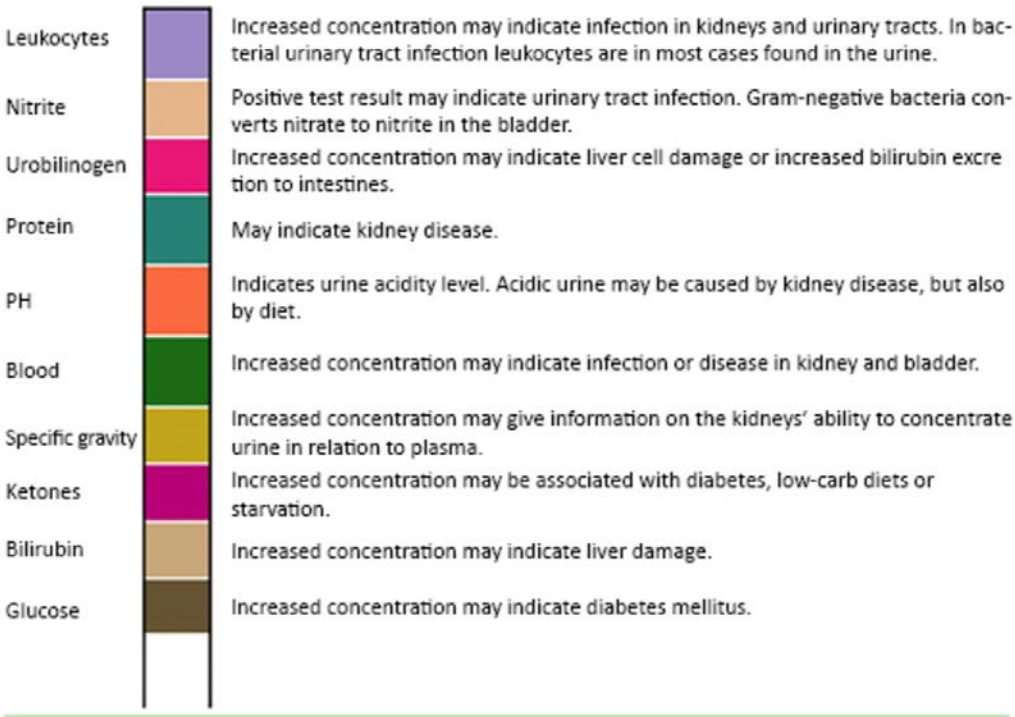
Urine Half Plate Streak pattern



6.2 **Urinalysis**

- Dip urine test strip into urine for 1 second ensuring all reagent pads are wet.
- Drain off excess urine on the side of the rim of the urine bottle.
- Compare reaction colour on urine test strip against the chart on the bottle after 60 seconds. Leukocytes will take 120 seconds.
- Record results in the LIMS as negative, trace, 1, 2, 3+.
- Urines showing >=1 positive reactions are passed on for Microscopy and Culture.
- Urines showing Negative detection for Leukocytes, Nitrite, Protein & Blood are reported as "Urinalysis screen negative - culture not performed". **No microscopy or culture is needed for these urines unless the patient is known to be neutropenic. Use the appropriate negative urinalysis comment in the LIMS.**

Figure 1: How to interpret a urine dipstick



The figure briefly describes the meaning of the various reagents.

6.3 Urine Microscopy

A. Using Kova Slide – 10 wells; each well has a grid as shown

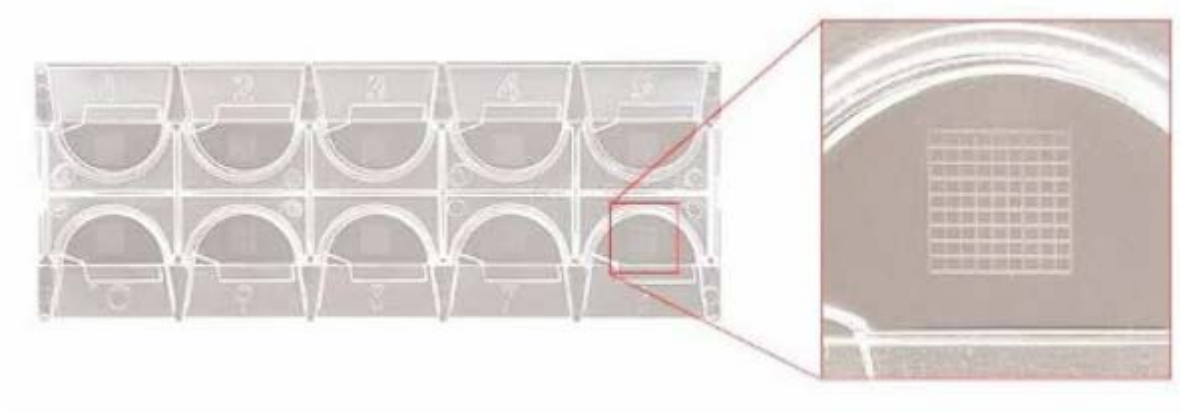


Figure 2: Kova Slide

- Gently swirl to mix each sample immediately before filling the counting chamber
- Using well-mixed specimen, fill a section of a Kova-slide counting chamber using a micro-haematocrit tube; allow to settle for 1-2 minutes
- Using phase contrast microscopy, initially examine each sample for epithelial cells on low power (X10), taking note of whether there are <50 or >50 present in the grid.
- If > 20 cells on the entire grid area (as shown above), count 1 large square and multiply the count by 10. This gives the number of cells x10^6/litre.

  i.e. $\frac{\text{total cells counted}}{\text{Number of squares counted}}$ X 10 = cells x 10^6/litre

- Turn the objective to high power (X40) and examine the well for WBCs, RBCs and organisms, in addition to any casts if present.

** If epithelial cells other than squamous are seen (e.g., cuboidal, columnar) add comment e.g. "Renal epithelial cells seen"

B. Using Neubauer Counting Chamber

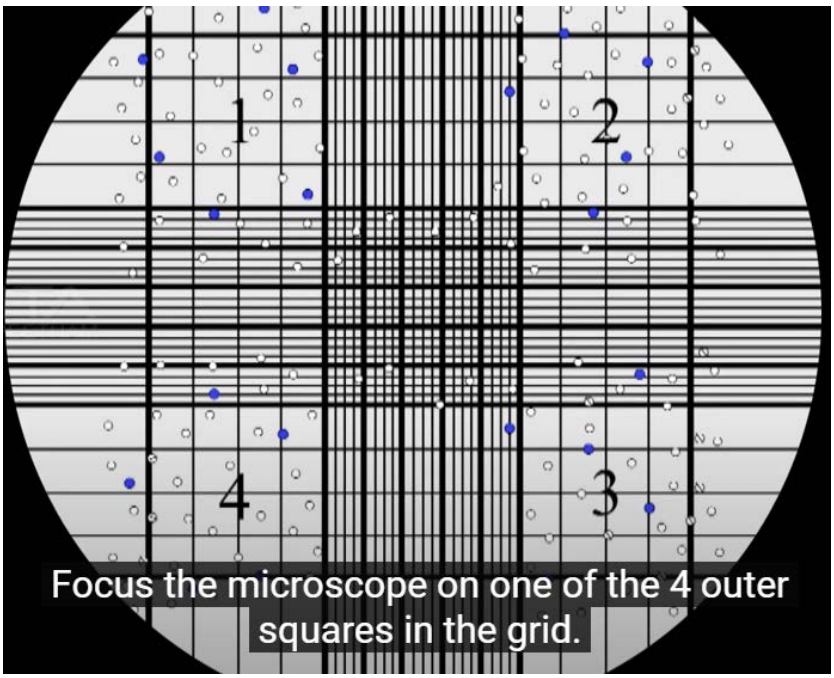


Figure 3: Improved Neubauer counting chamber

- Clean the counting chamber with 70% alcohol.
- Put the coverslip onto the counting chamber.
- Mix the urine for the even distribution of the cells.
- Touch one side of the coverslip with the urine filled capillary tube so that the urine flows slowly and fills the chamber. Do not overflow.
- Focus with 10X objective and then to 40X.
- With the 40X objective, count the number of cells in one large square (labeled 1 to 4 in figure 3 above)
- Read as stated below:

If cells are <10 in one square (WCC) count 5 squares x 2/cmm

- If cell are >10 or <20 in one square count 2 squares x 5/cmm
- If cells are >20 in one square count 1 square x 10/cmm
- If cells are >100 in one square Report as >100/cmm.
- Normal: 1-10 cells/cmm.
- No Cells Report as Nil

** Record presence of bacteria, casts, crystals and yeasts as +, ++ or +++.

- The reporting ranges are:

Nil	0-10 cells/cmm	10-100 /cmm	>100 cells/cmm

Also report presence of bacteria, casts, crystals and yeasts as +, ++ or +++.

This table shows the urine sediment components and their clinical significance.

Urine sediment component	Clinical significance
Bacteria	Urinary tract infection, asymptomatic bacteriuria
Erythrocytes	Most renal disorders, menstruation (contamination), strenuous exercise, indwelling catheter
Leukocytes	Most renal disorders, urinary tract infection, pyelonephritis, asymptomatic bacteriuria, indwelling catheter
Squamous epithelial cells	Normal (i.e. squamous metaplasia present in bladder) or peri-urethral contamination
Casts:	
1.Broad casts	Formation occurs in collecting tubules, serious kidney disorder.
2. Epithelial (renal) cells	Tubular degeneration/inflammation e.g. from drug reaction/toxicity or ischaemia
3. Fatty casts	Nephrotic syndrome
4. Granular or waxy casts	Renal parenchymal disease
5. Hyaline casts	Acid urine, high salt content
6. Red blood cells casts	Acute glomerulonephritis
7. White cells casts	Pyelonephritis

6 Examination of Culture

6.1 Plate reading

- Examine the plates and count the number of colonies of each type of bacteria for each plate.
- Use the following table to report colony counts when using a 1ul loop:

< 10 colonies	< 10^4 CFU/mL
10-100 colonies	10^4 – 10^5 CFU/mL
> 100 colonies	> 10^5 CFU/mL

- Identify the bacteria that have a colony count of at least 10^4 CFU/ml (Usually, a significant urinary tract infection will have greater than 10^5 CFU/ml.)
- Work up ≤ 2 bacteria ONLY. If 3 or more types of bacteria grow, do not work-up.
- Perform Antibiotic Susceptibility Testing (AST) according to G_90_SOP_6_A, AST Testing
- Urines from women of child bearing age with leucocytes and predominant growth of coagulase negative staphylococcus: set up the isolate against the STAPH panel with a novobiocin 5ug disc replacing the CMP disc. Only isolates resistant to novobiocin should be reported; resistance is defined as a zone size of 16mm or less. Report such isolates as *S. saphrophyticus.*
- If no visible growth and cell count does not suggest a UTI, report as “No Growth after 24 hours of incubation”.

- For cultures with no growth but the cell count is suggestive of UTI, reincubate for another 24 hours, if still no growth report as "No growth after 48 hours of incubation".

6.2 Identification of significant bacterial isolates and AST

- When workup required (above), determine the presumptive ID using a simplified approach to presumptive phenotypic identification (by either manual biochemical tests and/or chromagar) as per the bacterial identification SOP, G_90_SOP_15. In most circumstances, a precise identification is NOT required– no need for either API or Phoenix testing.
- When available at PMGH, MALDI-TOF identification can replace the manual identification methods for Gram negatives.
- Staphylococcal isolates: report predominant >=10^4/L *S. aureus or S. saphrophyticus*. Don't report other coagulase negative staphylococci.
- Disc AST to be done as per the G_90_SOP_6, AST testing. Incubate AST plates at 35°C for 18 hours.
- Extended AST (Phoenix) required in Goroka for ceftriaxone or meropenem-resistant Gram negatives that are to be reported.
- All labs need to store isolates at -20deg C specified in G_90_J_10 and refer them to the NRL for MALDI identification +/- Phoenix AST if not already done.

7 Results reporting

7.1 Record onto specimen worksheet

7.2 Report the colony count and identification of each bacteria type.

7.3 Report antibiotics according to the AST Guideline for each bacteria.

7.4 For urines with other result (microscopy and culture) combinations, consult table below and add indicated comment(s)

7.5 Record final results into the LIMS (when available) and/or Urine logbook and into soft copy format.

8 Interpretation

8.1 Use the following table to report colony counts when using a 1ul loop

< 10 colonies	$< 10^4$ cfu/mL
10-100 colonies	10^4 - 10^5 cfu/mL
> 100 colonies	$> 10^5$ cfu/mL

8.2 Use the following table to assist with culture interpretation.

8.3 Consult with supervisor to discuss culture interpretation when microscopy and culture findings are unclear

Table: Culture interpretation and reporting

Number of isolates	Colony Count (cfu/mL)	WBC/mm^3 (x 40)	Identification & AST	Comment below)
1	$\geq 10^4$	> 10	YES	*Consistent with UTI*
2	$\geq 10^4$ PP	> 10	YES, workup both	*Consistent with UTI*
2	$\geq 10^4$ one predom. PP	> 10	YES Predom. PP only	*Consistent with UTI*
2	$\geq 10^4$ both PP (no predom.) or 1 predom.	< 10	2 PP – NO (ID only) 1 predominant PP - YES	*Bacteriuria without pyuria usually represents bladder colonization, contamination during collection or delayed transport.*
1 predom, PP	> 10^5 cfu/mL	< 10	YES Pregnancy only	*Significant antenatal bacteriuria that may require treatment as per acute cystitis regardless of symptoms.*
≥ 3	Any	Any	NO	*These isolates most likely represent contaminating perineal flora*
0,1,2	< 10^4	< 10 > 10	N/A i.e. "sterile pyuria"	*No significant growth* *Presence of white cells without significant growth may be caused by antibiotics, tuberculosis, renal tubular damage, recent/current catheterization or urethritis (e.g gonococcal infection)*

PP = Potential Pathogen

Other report Comments

Comment criterion	Comment text
Urine with negative urinalysis	*The urinalysis screen was negative for Leukocytes, Nitrite, Protein & Blood; urinary tract infection excluded. This sample has not been cultured.*
Group B streptococcus isolates	*This organism is susceptible to penicillin.*
Coagulase negative staph isolates from & <100 WBC/ mm^3	*In the absence of urinary tract abnormality or instrumentation, these isolates most likely represent contaminating perineal flora.*
Candida from urine	*Candida from urine usually represents colonisation or contamination. If an IDC is present, it should be removed or replaced.*

9 Quality Control

9.1 Media QC is to be done and recorded for each batch of agar

9.2 AST QC is to be done weekly and subject to documented management review.

9.3 Other test QC is to be performed and recorded daily on Bench Reagent QC Worksheet

7. Related Documents

For access, refer to https://path-png.org/microbiology-sops-fleming-fund/

Specimen Collection Transport and Rejection	G_90_Info_5
Antibiotic disc susceptibility testing	G_90_SOP_6
Bench Reagent QC Worksheet	G_90_WS_1
Bacterial isolate Referral List	G_90_J_10
Urine microscopy Neubauer chamber	G_90_J_16
GNR/GPC/GNC/GPB Organism Identification	G_90_SOP_15 – pends development

8. References

- Previous PMGH SOP
- DMDP Urine Culture SOP
- Urine Analysis PROC.5-444.005 (Pathology North, NSW, 2022)
- The Global Health Laboratories, Urine Culture, September 2005. Derived from https://globalhealthlaboratories.tghn.org/articles/microbiology-clinical-laboratory-sops/