Ministry of Health

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Contents Table:

Acknowledgment

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Acronyms:

BA	Blood agar
CLED	Cystine-Lactose-Electrolyte-Deficient
H&S	Health and Safety
ID	Identification
IQC	Internal Quality Control
SOP	Standard operating procedure
Sens	Susceptibility testing as appropriate
SECs	squamous epithelial cells
MSU	Midstream urine
UTI	Urinary tract infections

1. **Purpose**

   This document describes the procedure for lab investigation of urine samples including microscopy and culture.

2. **Scope**

   This document is applicable for all medical laboratories under MOH and other collaborative governmental and non-governmental health institutions.

3. **Definitions**

   3.1 **leak-proof sterile container**: a container or enclosure that is constructed in such a manner that it will not allow its contents to spill out without being opened and physically discharging the contents

   3.2 **Midstream urine (MSU):** urine collected from clean the urethral meatus after discarding the first voided urine (first 10 - 20 mL) in order to clear the urethra.

   3.3 **Bag or pad urine:** urine collected by clean collection bag placed over the external genitalia and then the urine is transferred into a clean, sterile container. This. method is done for infants and young children only.

   3.4 **Indwelling catheter (Foley catheter) urine:** urine collected by puncturing the catheter tubing aseptically and transfer the urine into a clean, sterile container.

   3.5 **Ileal conduit urine :** urine collected from stomal opening (Urostomy)

   3.6 **In and Out Catheter / Catheter Insertion Urine**: catheter urine Specimen collected by the insertion of a catheter through the urethra into the bladder

   3.7 **Nephrostomy urine**: Urine collected from a nephrostomy tube placed in the renal pelvis.

   3.8 **Suprapubic aspirate:** urine collected by aspiration directly through the bladder using a sterile needle and syringe

   3.9 **Bacteriuria**: Presence of bacteria in the urine and may be cultured from urine .

   3.10 **Cystitis**: Inflammation of the bladder

   3.11 **Dysuria:** Pain or burning on urination, a common complaint on presentation of UTI

   3.12 **Pyelonephritis:** Infection of the kidney and renal pelvis usually presenting acutely with fever, chills, and flank pain.

   3.13 **Pyuria**: is defined as the presence of 10 or more white blood cells per cubic millimeter in a urine specimen, 3 or more white cells per high-power field of unspun urine, a positive result on Gram's staining of an unspun urine specimen, or a urinary dipstick test that is positive for leucocyte esterase.

3.14 **Uncomplicated UTI:** Infection in otherwise healthy who have no structural or functional abnormalities of the urinary tract

3.15 **Complicated UTI:** Infection in a patient with structural and/or functional abnormalities of the urinary tract (e.g., spinal cord injury ,neurogenic bladder, urinary tract obstruction,etc.)

3.16 **Asymptomatic bacteriuria:** Presence of bacteria at ≥105 CFU/ml with no symptoms of infection.

4. Procedure

4.1. Clinical background:

Urine is normally considered a sterile body fluid. However, unless it is collected properly, it may become easily contaminated with normal flora from the urethra, vagina, prostate or perineum.

Urinary tract infections (UTI) are one of the commonly encountered acute infectious diseases. UTI results from the presence and multiplication of microorganisms, in one or more structures of the urinary tract, with associated tissue invasion. This can give rise to a wide variety of clinical syndromes. These include acute and chronic pyelonephritis , cystitis , urethritis , epididymitis and prostatitis . Infection may spread to surrounding tissues (eg perinephric abscess) or to the bloodstream. Clinically, UTIs are categorized as uncomplicated or complicated.

Colony count is critical in establishing the microbiological significance of bacteriuria, and urine cultures are always reported with an accompanying colony count. Studies conducted in the 1950s found that ≥105 per ml of urine were indicative of a UTI .However, more recent studies suggest that this level of bacteriuria can miss a large group of patients with UTI and support the concept that lower levels (≥102 to ≥104 CFU/ml) should be considered significant in specific patient groups. Other studies have supported the significance of lower colony counts, with counts as low as 102 CFU/ml being associated with infection in symptomatic adult females , infants , and catheterized patients. . Counts as low as 103 cfu/mL of a pure or predominant organism have been shown to be significant in voided urine from men. Clinical guidelines vary on significant colony count threshold in different contexts, ranging anywhere from ≥103 to ≥105 CFU/ml

Most commonly isolated bacteria from patients with acute uncomplicated cystitis are *Escherichia coli, Klebsiella species,* and other *Enterobacteriaceae* and *Staphylococcus saprophyticus*. Hospitalized patients and patients with complicated urinary tract infections are commonly infected with *E. coli, Klebsiella species, Proteus mirabilis, other Enterobacteriaceae, Pseudomonas aeruginosa* and *enterococci. Group B Streptococcus* are markers of colonization in pregnant women. *Corynebacterium urealyticum* are associated with UTI in patient with renal stones.

4.2. Principle:

4.2.1 Except in a few patient groups, interpretations of culture results are made with regard to clinical presentation, the presence or absence of pyuria and squamous epithelial cells (SECs) which indicate contamination.

4.2.2 The number of microorganisms per milliliter recovered on urine culture can aid in the differential diagnosis of UTI. Plastic or wire loops, available commercially, have been calibrated to deliver a known volume of liquid when handled correctly, thus enabling the microbiologist to estimate numbers of organisms in the original specimen based of CFU of growth on culture. The manner of inserting the loop into the urine is critical to the results that are obtained.

- A 0.001 ml calibrated loops are used for urines which are collected using Noninvasive procedures.
- A 0.01 ml calibrated loops are used for urines collected with Invasive procedure.

4.2.3 Delay in processing may give falsely low WBC and RBC due to lysis of cells unless preservatives are used. And it may also result in bacterial overgrowth

4.2.4 Delay in processing may give falsely low crystal count due to precipitation of crystals.

4.2.5 Catheter urine: WBCs may be present in catheter urine as a result of the natural defense mechanism against a foreign body.

4.3. Pre – analytical stage:

4.3.1. Sample:

4.3.1.1 Specimen types: Urine specimens can be divided into categories based on clinical criteria, the possibility of urethral contamination, and the extent of microbiological work-up. Which are**:**

- Non-invasive urine samples:
  - Midstream urine (MSU)
  - Neonatal bagged urine
  - Indwelling catheter (Foley catheter) urine
  - Ileal conduit urine
  - In and out catheter / catheter insertion urine
- Invasive urine sample: Nephrostomy urine
  Bladder/cystoscopy urine
  Suprapubic bladder aspirate

4.3.1.2 Specimen volume: Optimum volume 10-20 mL in plain CE marked leak proof container. In certain cases, a minimum volume of 1mL can be accepted.

4.3.1.3 Specimen transport and storage

- Specimens should ideally be stored and transported in sealed plastic bags.
- Laboratory processing should be done within 2 hours after specimen collection.
- Specimens should be stored at 2-8 c for not more than 24 hrs if delays in processing over two hours are unavoidable, and brought to room temperature before testing.

4.3.1.4 Specimen rejection:

- Do not culture urine specimens delayed longer than 2 h without refrigeration or preservative.
- Do not culture 24-h urine collections.
- Do not culture Foleys catheter tips.
- Do not culture urine from the bag of a catheterized patient.
- Do not culture urine from a container that has leaked.

- Request a repeat specimen or obtain the information when the collection time and method of collection have not been provided.

4.3.2. Material:

Reagents	**Consumables/Supplies**	**Equipment**
MacConkey/ CLED Blood Agar plate (optional)	Microscopic slides and Cover slide Disposable 0.001 ml and 0.01Calibrated loop Incubating Rack Lens paper and Lens cleanser Pipette	Microscope 37˚C incubator Centrifuge

4.3.3. Safety precaution:

4.3.3.1 Any suspected group 3 type of organisms such as: *Mycobaterium, Nisseria. Meningitidis, Brucella, Salmonella paratyphi Salmonella typhi* …etc should be processed in safety cabinet level3.

4.3.3.2 All specimens need to be treated as potentially infectious. Standard procedures for handling of biohazard material must be followed at all times. Universal Precautions must be practiced at all stages of these procedures.

4.3.4. Quality control:

4.3.4.1 Check the expiry dates of all media, reagents and stains before use.

4.3.4.2 All media, reagents, kits, and stains MUST be quality controlled before use.

4.3.4.3 Identification tests should be run with appropriate controls.

4.3.4.4 Record the quality control results in the appropriate QC sheet.

4.3.4.5 Inspect the calibrated loops prior to use to ensure that they have a complete loop surface.

4.3.4.6 Change loops that are outdated or appear to be damaged

4.4. Analytical stage:

4.4.1 Clinical acceptability of urine analysis and culture: for more details refer to appendix 2.

4.4.2 Macroscopic examination

Describe the appearance of the urine clarity (clear or cloudy), colour and volume.

4.4.3 Microscopic examination

4.4.3.1 Preparation and examination of a wet preparation centrifuge sample (deposit method)

- NB: Since culturing and urinalysis needs an un-centrifuged urine, this procedure has to be done after urinalysis and culturing the sample.
- Follow the microscopy procedure as described bellow:

1. Place the urine sample container at 1500-2000 rpm for 5 minutes. OR aseptically transfer about 10 ml of well mixed urine to a labelled conical tube
2. Pour the supernatant fluid (by completely inverting the tube) into a second container not the original one.
3. Re-suspend the sediment in the small volume of remaining urine.
4. Place a drop of well mixed, re-suspended sediment onto a clean, glass slide, and cover with a cover slip.

   Note: Do not discard the remaining sediment because this may be needed for further investigation.
5. Examine the preparation microscopically using the X10 and X40 objective with the condenser closed sufficiently to give good contrast
6. First view the sediment under low power (10x).
   - Scan the slide and observe for casts, crystals and other elements that may be present.
   - Switch to high dry power (40x) when necessary to delineate the structures that are seen.
   - Casts have a tendency to move towards the edge of the coverslip; scan the periphery of the coverslip as well.

- Note the type and the average number of casts seen per low power field on the worksheet.
- Note the presence and type of crystals (crystals need only be reported as present unless they are very abundant), refer to annex 8.1.

7. View the sediment under high power (40x)
   - Estimate the number of red blood cells, white blood cells and epithelial cells per high power field by observing 10-15 fields.
   - Note any Trichomonas vaginalis seen and estimate the number per field.
   - Record the results obtained (the presence of: WBC, RBC, epithelial cells, casts, crystals, yeast, sperms, and parasites.

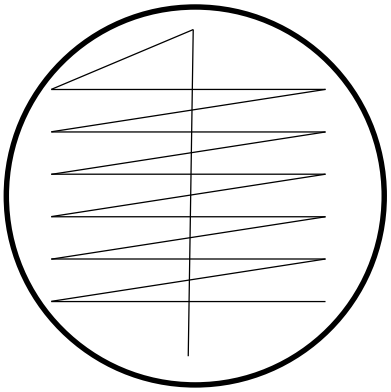
4.4.4 Culturing urine samples.

Plating with calibrated loop instructions:

1. Use the disposable commercially calibrated loop(s) prior to inoculating each specimen.
2. Dip the end of a sterile loop vertically just below the surface of a well-mixed urine specimen (just far enough to cover the loop) and remove it vertically making sure that there is no urine up the loop (as this would mean that a greater volume was cultured).
3. Spread the loopful of urine over the surface in the center of the agar plate by streaking from top to bottom in a vertical line and then Cross-streak again from top to bottom perpendicular to this line in a back-and-forth with the same loop. (figure 1)
4. With the same loop, repeat the process with a blood agar plate and other media if required.
5. Place the streaked plate's batches in an incubating rack.
6. Incubate the plate aerobically at 35-37°C for at 18-24 hours (minimum of 16hr and maximum of 48 hrs .
7. If fungus is requested, inoculate a SAB using the appropriate calibrated loop (s) and incubate in O2, 35oC x 72 hours.
8. After incubation,

- Send the report of negative urines (no bacterial growth, no significant growth, mixed, mixed repeat) for result releasing.
- Cultures with no growth: Discard no growth routine cultures after 18-24 hrs incubation. Except:
    - Suprapubic urine – re-incubate for another 24hrs
    - When yeast or non-specified fungus is requested – re-incubate for another 24 hrs at room temperature.
    - If colonies are too small – re-incubate for another 24hrs
- Send significant urine cultures for further ID and susceptibility testing (Workup cultures according to the criteria in Tables 1, 2 .

Figure 1. Streaking the urine specimen onto agar plate



4.5. Post – analytical stage:

4.5.1. Interpretation / Results / Alerts:

4.5.1.1 Urine microscopy: Identify and quantify urine components as follows:

analyte	Normal ranges	reporting ranges
WBC	0-2/HPF	<1 Rare 1-10 Few 11-25 Moderate >25 Nemours
RBC	0-2/HPF	
Epithelial cells	0-2/HPF	
crystals	none	Report presence
casts	none	Report presence per LPF
bacteria or yeast	none	Report presence
parasites	none	Report presence

4.5.1.2 Urine culture: Criteria for Identification and Workup

- Count the colonies and multiply by the appropriate dilution factor in SI units.

Inoculation loop size	Colony count /m l
0.001 ml (1µL)	1 colony= 1000 CFU/ml $=10^3$ CFU/ml
0.01 ml (10µL)	1 colony= 100 CFU/m l$=10^2$ CFU/ml

- Workup cultures according to the criteria in Tables 1, 2 below.

TABLE 1: Criteria for the identification and susceptibility testing of organisms isolated from non-invasive procedure (MSU, neonatal bagged urine, indwelling catheter, Foley catheter urine, ileal conduit urine using 0.001 ml calibrated loop

No. of Types of Organisms,	**No. of colonies of each type**	**Colony count/ml of uropathogens**	**Work up for uropathogens**	**Report**	**Comment**
No bacterial growth	-	-	-	"Urine culture negative" or" No growth of uropathogens	If Symptomatic with Marked/persistent pyuria Check if Patient on antibiotics, or Consider *Chlamydia*, AFB, Fastidious organism
1	<10	<10,000 CFU/m l	No work-up	No significant growth, please repeat if appropriate	This count might be significant in Symptomatic female ,infant and Prostatitis correlate with clinical symptoms and WBC count .
1	10-100	10,000-100,000 CFU/m l	ID + Sens	Significant growth (10^4 CFU/mL).	
1	>100	≥100,000 CFU/m l	ID + Sens	Significant growth ($\geq 10^5$ CFU/mL))	
2	Both >100	>100,000 CFU/ml	ID + Sens on both	Significant growth ($\geq 10^5$ CFU/mL) for each	

2	Both 10-99, ***or*** one 10-99, and one < 10	Both >10,000 CFU/ml ***or*** one >10,000 CFU/ml and <10,000 CFU/ml	ID+Sens for the 10^4 CFU/mL.	Mixed with predominant Significant growth (10^4 CFU/mL) of	
2	Both <10	<10,000 CFU/ml	No work-up	No significant growth	
≥3	All <100	<100,000 CFU/ml	No work-up	No significant growth(please repeat if appropriate)	
≥3	Any >100	100,000 CFU/ml	No work-up	Mixed significant growth of ≥3 organisms , suggest appropriate recollection, with timely delivery to the laboratory, if clinically indicated	

TABLE 3: Criteria for the identification and susceptibility testing of organisms isolated using invasive procedure, suprapubic bladder aspirates, bladder/cystoscopy urine and nephrostomy urine. **Done using 0.01 mL (10 µL) loop**:

No. of Types of Organisms[1]	**No. of colonies of each type**	**Colony count/ml of uropathogens**	**Work up for uropathogens**	**Report**
1	Any number	Any count	Sens + ID	100 - 1000 cfu/ml
2	One >10 One < 10	≥1000 CFU/m l	ID + Sens for the predominant	Significant growth (indicate colony count per mL ≥1000 cfu/ml
2	Both >10	>1000 CFU/ml	ID + Sens on both	Significant growth (indicate colony count per mL ≥1000 cfu/ml
2	Both < 10	$< 10^3$ CFU/mL	No workup	No significant growth
≥3	All **uropathogens** <10	<1000 CFU/ml	No work-up	Mixed growth-probable contamination
≥3	Any **uropathogen** >10	>1000 CFU/ml	ID + Sens on all	≥1000 cfu/ml

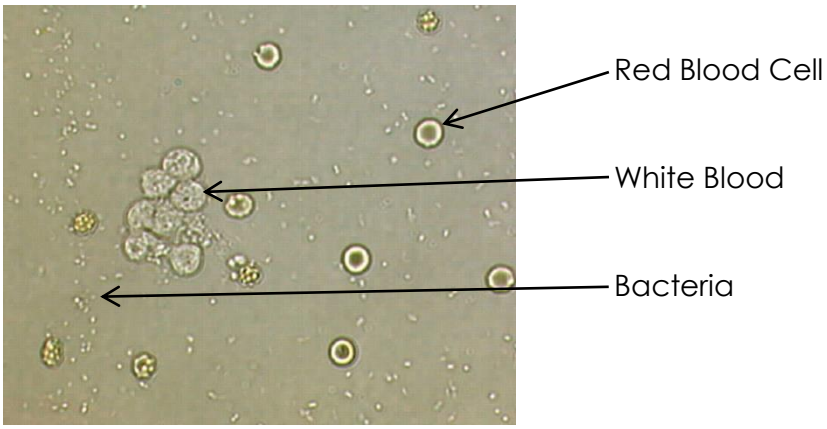
List of Uropathogens and Non-Uropathogens:

Uropathogens	**Non-Uropathogens** (normal skin/urogenital flora)
Enterobacteriaceae *Pseudomonas aeruginosa* Other gram negative bacilli *Enterococcus* species beta-haemolytic streptococci Yeast *Aerococcus uriae** *Corynebacterium urealyticum* *Staphylococcus aureus* *Staphylococcus saprophyticus* (females 12 - 60 yrs only) Other coagulase negative staphylococci*	*Lactobacillus* diphtheroids (not *C. urealyticum*) viridans Streptococci (not *A. uriae*) *Bacillus* species

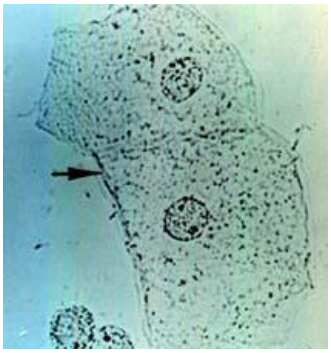
- Consider as **<u>Uropathogens</u>** only when present in amounts >10-fold more than other **<u>non-Uropathogens</u>**
- When counting the types of organisms, do not include <10 colonies of **<u>non-uropathogens</u>**.
- Do not workup or report any number of colonies of **non-uropathogens**

Appendix 1: Useful images for urine microscopy

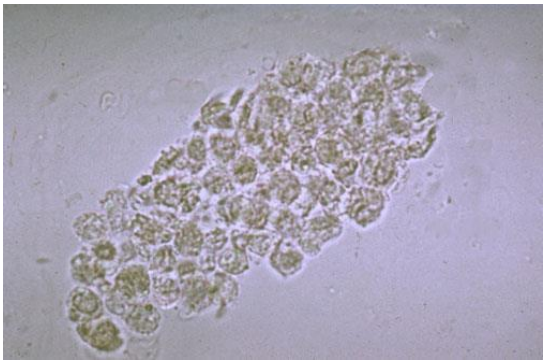
Red cells, White cells, and bacteria



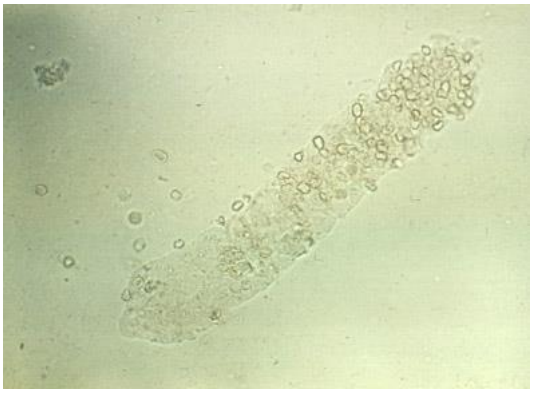
Epithelial cells (indicate that the urine is not a clean catch)



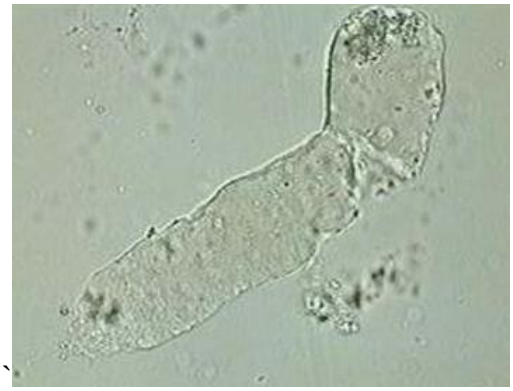
White cell casts (found when there is inflammation of the kidney pelvis or tubules)

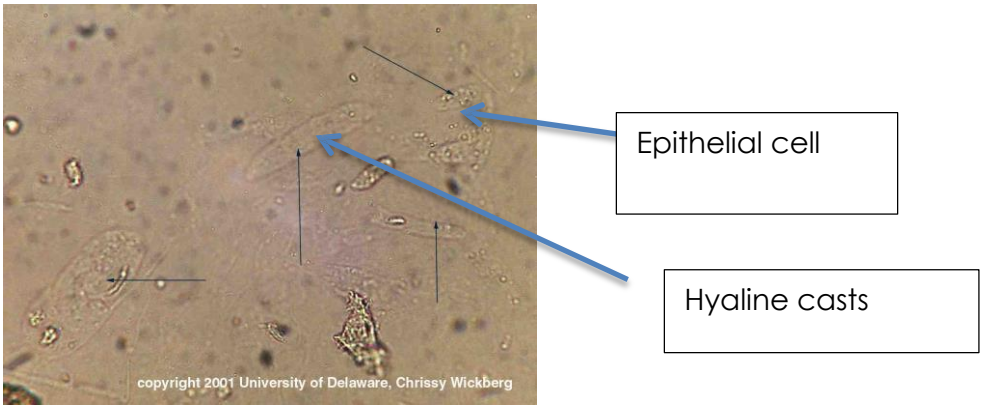


Red cell casts (indicate haemorrhage into the renal tubules or glomerular bleeding; orange red colour)



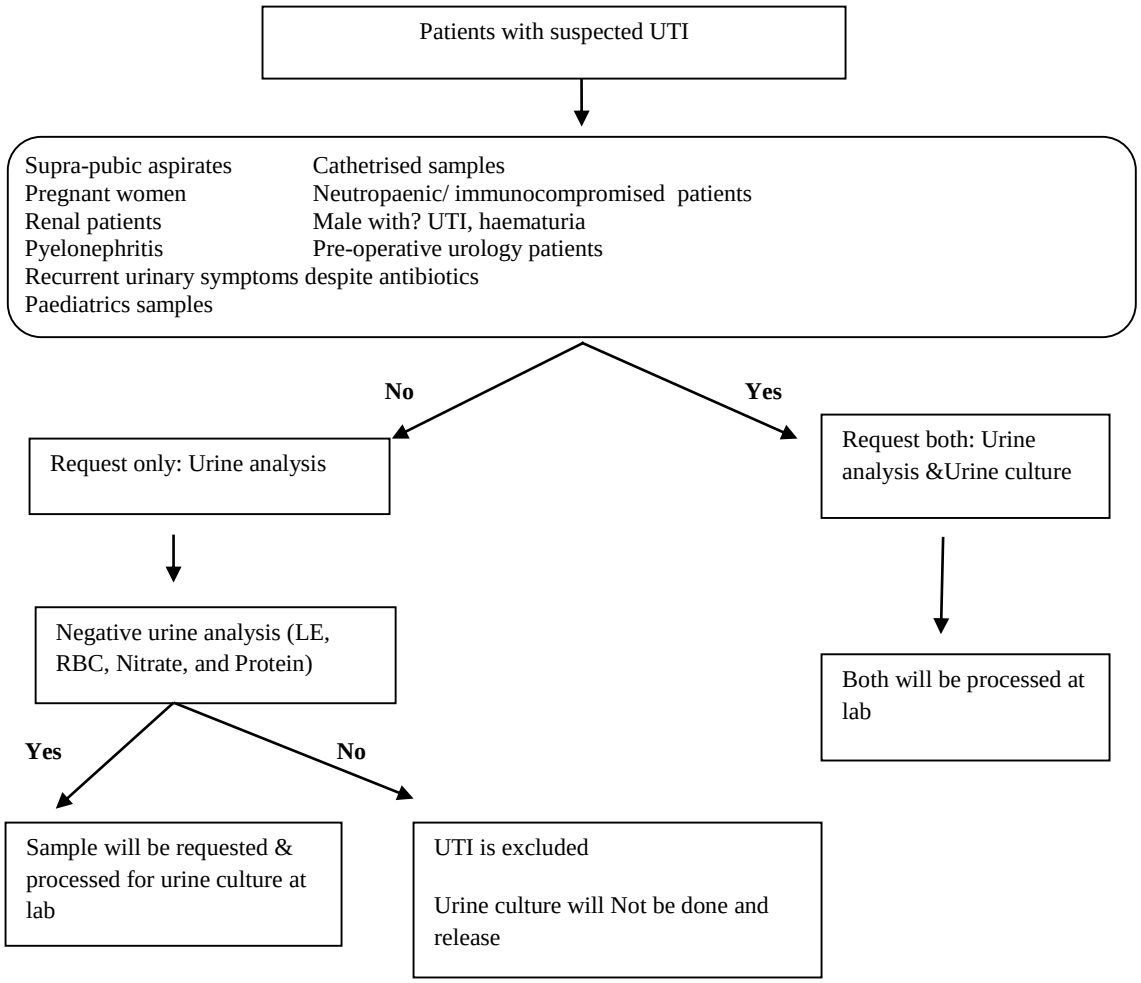
Hyaline casts (associated with damage to the glomerular filter membrane)





Appendix 2: The following flow cart demonstrates the above policy:

Urine analysis screening procedural for referral to culture summary flow chart



5. Responsibilities

5.1. Responsible staff:

5.1.1 To ensure the adherence to critical result communication procedure

5.1.2 To facilitate the alternative channels once needed

5.2. Quality manager /officer

5.2.1 To follow up the implementation of the procedure

5.2.2 To monitor regularly communication of critical results and raise non-conformance with corrective action once needed.

5.3. All lab staff:

5.3.1 To adhere to the procedure.

5.3.2 To document record and release results as recommended

5.3.3 To report test failures or incident

6. Document History and Version Control

Version	Description	Review Date
1	Initial Release	May 2026

7. References

Title of book/ journal/ articles/ Website	Author	Year of publication	Page
Pro68_01_manual_urin_micro_sop(1). .	Caribbean regional microbiology s.o.p. no:crm-sop16		
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. Manual of Clinical Microbiology, 11th ed. ASM Press, Washington, D.C.	Murray P.R., Baron E.J., Pfaller M.A., Yolken R.H..	2015	
Caribbean regional microbiology standard	Strengthening	Prepared by: caribbean regional standard	

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