Examination of urine

Possible pathogens

_ BACTERIA

Gram positive	***Gram negative***
Staphylococcus	*Escherichia coli*
saprophyticus	*Proteus* species
Haemolytic streptococci	*Pseudomonas aeruginosa*
	Klebsiella strains
	**Salmonella* Typhi
	**Salmonella* Paratyphi
	**Neisseria gonorrhoeae*

*These species are not primarily pathogens of the urinary tract, but may be found in urine. Also *Mycobacterium tuberculosis*, *Leptospira interrogans*, *Chlamydia*, *Mycoplasma* and *Candida*.

Commensals

The bladder and urinary tract are normally sterile. The urethra however may contain a few commensals and also the perineum (wide variety of Gram positive and Gram negative organisms) which can contaminate urine when it is being collected. With female patients, the urine may become contaminated with organisms from the vagina.

COLLECTION AND TRANSPORT OF URINE

Whenever possible, the first urine passed by the patient at the beginning of the day should be sent for examination. This specimen is the most concentrated and therefore the most suitable for culture, microscopy, and biochemical analysis. Midstream urine (MSU) for microbiological examination is collected as follows:

1- Give the patient a sterile, dry, wide-necked, leak proof container and request a 10–20 ml specimen.

***Important*:** Explain to the patient the need to collect the urine with as little contamination as possible.

Female patients: Wash the hands. Cleanse the area around the urethral opening with clean water, dry the area with a sterile gauze pad, and collect the urine.

Male patients: Wash the hands before collecting a specimen (middle of the urine flow). 2- Label the container with the date, the name and number of the patient, and the *time* of collection. *As soon as possible*, deliver the specimen with a request form to the laboratory. When immediate delivery to the laboratory is not possible, refrigerate the urine at 4–6 °C. When a delay in delivery of more than 2 hours is anticipated, add boric acid preservative to the urine.

- Any bacteria in the urine will multiply so that the bacterial count will be unreliable. When the organisms are urease-producing, the ammonia released will increase the pH of the specimen which will result in the destruction of cells and casts. Bacteria will also break down any glucose which may be present.
- When white cells, red cells, and casts are present, these will begin to lyze especially in a concentrated specimen.
- The concentration of protein in the urine will be altered. When bilirubin is present this may be oxidized to biliverdin which will not be detected. Likewise, urobilinogen will not be detected because it will be oxidized to urobilin .

LABORATORY EXAMINATION OF URINE

Day 1

1- Describe the appearance of the specimen

Report:

– Colour of specimen

– Whether it is clear or cloudy (turbid)

Appearance	***Possible Cause***
Cloudy Urine usually has an unpleasant smell and contains WBCs	Bacterial urinary infection
Red and cloudy Due to red cells	-Urinary schistosomiasis – Bacterial infection
Brown and cloudy Due to haemoglobin	– Malaria haemoglobinuria -Other conditions that cause intravascular haemolysis
Yellow-brown, or green-brown	– Acute viral hepatitis – Obstructive jaundiceDue to bilirubin

Yellow-orange Due to urobilin, i.e. oxidized urobilinogen	– Haemolysis – Hepatocellular jaundice
Milky-white Due to chyle	– Bancroftian filariasis

***Note*:** Other changes in the colour of urine can be caused by the ingestion of certain foods, herbs, and drugs especially vitamins.Normal *freshly* passed urine is clear and pale yellow to yellow depending on concentration .

2- Examine the specimens microscopically

Urine is examined microscopically as a wet preparation to detect:

- significant pyuria, i.e. WBCs in excess of 10 cells/_ µl of urine
- red cells
- casts
- yeast cells
- *T. vaginalis* motile trophozoites
- *S. haematobium* eggs
- bacteria (providing the urine is freshly collected)

3-Examination of a Gram stained smear

Prepare and examine a Gram stained smear of the urine when bacteria and, or white cells are seen in the wet preparation.

-Transfer a drop of the urine sediment to a slide and spread it to make a thin smear. Allow to air dry, protected from insects and dust. Heat fix or methanol fix the smear and stain it by the Gram technique .

– Examine the smear first with the 40_ objective to see the distribution of material, and then with the oil immersion objective. Look especially for bacteria associated with urinary infections . especially Gram negative rods. Occasionally Gram positive cocci and streptococci may be seen.

Neisseria gonorrhoeae in urine In male patients with acute urethritis, it is often possible to make a presumptive diagnosis of gonorrhoea by finding Gram negative intracellular diplococci in pus cells passed in urine .

4- Culture the specimen

It is *not* necessary to culture urine which is microscopically and biochemically normal, except when screening for asymptomatic bacteriuria in pregnancy. Culture is required when the urine contains bacteria (as indicated by the Gram smear), cells, casts, protein, nitrite, or has a arkedly alkaline or acid reaction.

- ***Cystine lactose electrolyte-deficient (CLED) agar***

  Cystine lactose electrolyte-deficient (CLED) agar is widely used by laboratories to isolate urinary pathogens because it gives consistent results and allows the growth of both Gram negative and Gram positive pathogens. (The indicator in CLED agar is bromothymol blue and therefore lactose fermenting colonies appear yellow). The medium is electrolyte-deficient to prevent the swarming of *Proteus* species.

Estimating bacterial numbers

It is necessary to estimate the approximate number of bacteria in urine because normal specimens may contain small numbers of contaminating organisms, usually less than 10 000 (10^4) per ml of urine. Urine from a person with an untreated acute urinary infection usually contains 100 000 (10^5) or more bacteria per ml. The approximate number of bacteria per ml of urine, can be estimated by using a calibrated loop or a measured piece of filter paper. Both methods are based on accepting that a single colony represents one organism. For example, if an inoculum of 1\ 500ml produces 20 colonies, the number of organisms represented in1\ 500 ml of urine is 20, or 10 000 in 1 ml (500 × 20).

The calibrated loop method using quarter plates of culture media is recommended because it is inexpensive, simple to perform, and provides individual colonies that are easier to identify and remove for antimicrobial susceptibility testing.

Day 2 and Onwards

Examine and report the cultures *CLED agar culture*

Look especially for colonies that could be:

_ **Appearance of some urinary pathogens on CLED agar**

- *E. coli*: Yellow (lactose-fermenting) opaque colonies often with slightly deeper colored center.
- *Klebsiella species*: Large mucoid yellow or yellow-white colonies.
- *Proteus species*: Translucent blue-grey colonies.

- *P. aeruginosa*: Green colonies with rough periphery (characteristic color).
- *E. faecalis*: Small yellow colonies.
- *S. aureus*: Deep yellow colonies of uniform color.
- *S. saprophyticus* and other coagulase negative staphylococci: Yellow to white colonies.

Reporting bacterial numbers

Count the approximate number of colonies. Estimate the number of bacteria, i.e. colony-forming units (CFU) per ml of urine. Report the bacterial count as:

- Less than 10 000 organisms/ml (104/ml), not significant.
- 10 000–100 000/ml (10^4–10^5/ml), doubtful significance (suggest repeat specimen)
- More than 100 000/ml (105/ml), significant bacteriuria.

Example

If 25 *E. coli* colonies are counted and a1\500 ml loop was used, the approximate number of CFU per ml of urine: 500 × 25 = 12 500

Such a count would be reported as:

10 000–100 000 *E. coli*/ml

***Note*:** Contaminating organisms usually produce a few colonies of mixed growth. Most urinary infections show growth of a single type of organism although mixed infections can occur especially in chronic infections or following catheterization or gynaecological surgery.

Antimicrobial susceptibility testing

Perform susceptibility testing on urines with significant bacteriuria, particularly from patients with a history of recurring UTI. Cultures from patients with a primary uncomplicated UTI may not require a susceptibility test.

Day 1

1	**Describe Appearance**	■ *Describe* – Colour – Whether clear or cloudy	ADDITIONAL INVESTIGATIONS
2	**Examine Microscopically**	■ *Wet preparation* Report: – WBCs (pus cells) – Red cells – Casts – Yeast cells – *T. vaginalis* flagellates – *S. haematobium* eggs – Bacteria (fresh urine only) – Crystals of importance	■ ***Gram smear***: When bacteria or WBCs (pus cells) are seen in wet preparation.
3	**Test Biochemically**	■ ***Tests to help diagnose UTI*** – Protein – Nitrite (Greiss test) – Leukocyte esterase (when microscopy for WBCs not possible)	■ ***Glucose, ketones, bilirubin, urobilinogen***: As indicated

Day 2 and Onwards

5	**Examine and Report Cultures**	■ *CLED culture* Look particularly for: *E. coli* (common cause UTI) *Proteus* species *P. aeruginosa* *Klebsiella* *E. faecalis* *S. aureus* *S. saprophyticus* Report bacterial numbers: • Less than 10^4/ml, not significant • 10^4–10^5/ml, doubtful significance • More than 10^5/ml, significant bacteriuria.	■ ***Antimicrobial susceptibility testing***: As indicated