Laboratory No. 1  
General Urine Examination (GUE)  
Or Urinalysis

Urinalysis can reveal diseases that have gone unnoticed because they do not produce striking signs or symptoms. Examples include diabetes mellitus, various forms of glomerulonephritis, and chronic urinary tract infections.

**METHODS OF URINE COLLECTION**

1. Random collection taken at any time of day with no precautions regarding contamination. The sample may be dilute, isotonic, or hypertonic and may contain white cells, bacteria, and squamous epithelium as contaminants. In females, the specimen may contain vaginal contaminants such as trichomonads, yeast, and during menses, red cells.

2. Early morning collection of the sample before ingestion of any fluid. This is usually hypertonic and reflects the ability of the kidney to concentrate urine during dehydration which occurs overnight. If all fluid ingestion has been avoided since 6 p.m. the previous day, the specific gravity usually exceeds 1.022 in healthy individuals.

3. Clean-catch, midstream urine specimen collected after cleansing the external urethral meatus. A cotton sponge soaked with benzalkonium hydrochloride is useful and non-irritating for this purpose. A midstream urine is one in which the first half of the bladder urine is discarded and the collection vessel is introduced into the urinary stream to catch the last half. The first half of the stream serves to flush contaminating cells and microbes from the outer urethra prior to collection. This sounds easy, but it isn’t (try it yourself before criticizing the patient).

4. Catherization of the bladder through the urethra for urine collection is carried out only in special circumstances, i.e., in a comatose or confused patient. This procedure risks introducing infection and traumatizing the urethra and bladder, thus producing iatrogenic infection or hematuria.

5. Suprapubic transabdominal needle aspiration of the bladder. When done under ideal conditions, this provides the purest sampling of bladder urine. This is a good method for infants and small children.

**MACROSCOPIC URINALYSIS**

The first part of a urinalysis is direct visual observation. Normal, fresh urine is pale to dark yellow or amber in color and clear. Normal urine volume is 750 to 2000 ml/24hr.

Turbidity or cloudiness may be caused by excessive cellular material or protein in the urine or may develop from crystallization or precipitation of salts upon standing at room temperature or in the refrigerator. Clearing of the specimen after addition of a small amount of acid indicates that precipitation of salts is the probable cause of turbidity.

A red or red-brown (abnormal) color could be from a food dye, eating fresh beets, a drug, or the presence of either hemoglobin or myoglobin. If the sample contained many red blood cells, it would be cloudy as well as red.
Three urine samples are shown. The one at the left shows a red, cloudy appearance. The one in the center is red but clear. The one on the right is yellow, but cloudy.

**PH**

pH estimate by pH meter or by paper strip. The glomerular filtrate of blood plasma is usually acidified by renal tubules and collecting ducts from a pH of 7.4 to about 6 in the final urine. However, depending on the acid-base status, urinary pH may range from as low as 4.5 to as high as 8.0. The change to the acid side of 7.4 is accomplished in the distal convoluted tubule and the collecting duct.

**Specific Gravity (sp.gr.)**

Specific gravity (which is directly proportional to urine osmolality which measures solute concentration) measures urine density, or the ability of the kidney to concentrate or dilute the urine over that of plasma. Dipsticks are available that also measure specific gravity in approximations. Most laboratories measure specific gravity with a refractometer (Urinometer).

Specific gravity between 1.002 and 1.035 on a random sample should be considered normal if kidney function is normal. Since the sp.gr. of the glomerular filtrate in Bowman’s space ranges from 1.007 to 1.010, any measurement below this range indicates hydration and any measurement above it indicates relative dehydration.

If sp.gr. is not > 1.022 after a 12 hour period without food or water, renal concentrating ability is impaired and the patient either has generalized renal impairment or nephrogenic diabetes insipidus. In end-stage renal disease, sp. gr. tends to become 1.007 to 1.010.

Any urine having a specific gravity over 1.035 is either contaminated, contains very high levels of glucose, or the patient may have recently received high density radiopaque dyes intravenously for radiographic studies or low molecular weight dextran solutions. Subtract 0.004 for every 1% glucose to determine non-glucose solute concentration.
• **Chemical analysis**

A. Protein test

1) **HEAT & ACETIC ACID**

Urine is putting in a test tube, heating the upper part of specimen (the lower part not heating for comparison). If the heated part of specimen cloudiness or turbid that may be indicate for protein presence. Add few drops of (10%) acetic acid prove the presence of protein if the cloudiness permanent, and when the cloudiness absent that mean the cloudiness as a result of presence the phosphate or carbonate.

**Results:**

<table>
<thead>
<tr>
<th>-</th>
<th>No cloudiness</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>Definite cloudiness, but no granularity</td>
</tr>
<tr>
<td>++</td>
<td>Granular cloudiness in upper part, dense and transparence</td>
</tr>
<tr>
<td>+++</td>
<td>High dense and opaque cloudiness</td>
</tr>
<tr>
<td>++++</td>
<td>Dense precipitation and often it is solid</td>
</tr>
</tbody>
</table>

2) **SULPHOSALICYLIC ACID TEST:**

In the case of clear and acidic urine specimen add 3 drops of (20%) Sulphosalicylic acid to 1 ml of specimen then heating the specimen. If the cloudiness continue means positive result (presence of protein).

3) **Detecting the protein by strips.**

**Glucose test (Benedict's test)**

In this method the (Cu) ions reduct to the (Cu2O) by the glucose if present. If the glucose concentration 0.1% or less there are no precipitate was seen after cooling specimen.

**Procedure:**

Add 8 drops of urine to 5ml of benedict, heating the tube until boiling and examinant.

**Results**

<table>
<thead>
<tr>
<th>-</th>
<th>BLUE COLOR , Negative result</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>GREEN (&lt;0.5% glucose)</td>
</tr>
<tr>
<td>++</td>
<td>Greenish yellow (0.5-1% glucose)</td>
</tr>
<tr>
<td>+++</td>
<td>Yellow (1-2% glucose)</td>
</tr>
<tr>
<td>++++</td>
<td>Orange to red (over 2% glucose)</td>
</tr>
</tbody>
</table>
B. **Ketone bodies test (Rothera’s test)**

**PROCEDURE:**

1. Saturate 5ml of urine with ammonium sulphate.
2. Add small crystals of sodium nitroprusside and shake.
3. Add ammonia in amount equal to sodium nitroprusside at the side of the test tube.

**RESULTS:** Formation of purple ring indicate (+ve).

C. **Bile pigment test (Harrison test)**

The normal value of bile pigment in urine specimen less than or equal to 0.02 mg%.

**PROCEDURE:**

1. Add 5 ml of 10% Barium chloride to 5 ml of urine in a test tube.
2. Filter the sample by filter paper, and let the filter paper to dry.
3. Add 1-2 drop of Fouchet’s reagent to the dried precipitate.

**RESULTS:** Green color indicate +ve test.

D. **Urobilinogen test (Ehrlich’s test)**

**PROCEDURE:**

1. Add 1 ml of Ehrlich’s reagent to 10 ml of urine.
2. Invert the tube several times and let it stand for 5 minutes.

**RESULTS:** Pink color is normal, while another color is +ve test.
Laboratory No. 2
General Urine Examination (GUE)
Or Urinalysis

**MICROSCOPIC URINALYSIS**

### Methodology

A sample of well-mixed urine (usually 10-15 ml) is centrifuged in a test tube at relatively low speed (about 2-3,000 rpm) for 5-10 minutes until a moderately cohesive button is produced at the bottom of the tube. The supernate is decanted and a volume of 0.2 to 0.5 ml is left inside the tube. The sediment is resuspended in the remaining supernate by flicking the bottom of the tube several times. A drop of resuspended sediment is poured onto a glass slide and cover slipped.

### Examination

The sediment is first examined under low power to identify most crystals, casts, squamous cells, and other large objects. The numbers of casts seen are usually reported as number of each type found per low power field (LPF). Example: 5-10 hyaline casts/L casts/LPF. Since the number of elements found in each field may vary considerably from one field to another, several fields are averaged. Next, examination is carried out at high power to identify crystals, cells, and bacteria. The various types of cells are usually described as the number of each type found per average high power field (HPF). Example: 1-5 WBC/HPF.

### Red Blood Cells

Hematuria is the presence of abnormal numbers of red cells in urine due to: glomerular damage, tumors which erode the urinary tract anywhere along its length, kidney trauma, urinary tract stones, renal infarcts, acute tubular necrosis, upper and lower uri urinary tract infections, nephrotoxins, and physical stress. Red cells may also contaminate the urine from the vagina in menstruating women or from trauma produced by bladder catherization. Theoretically, no red cells should be found, but some find their way into the urine even in very healthy individuals. However, if one or more red cells can be found in every high power field, and if contamination can be ruled out, the specimen is probably abnormal.

RBC's may appear normally shaped, swollen by dilute urine (in fact, only cell ghosts and free hemoglobin may remain), or crenated by concentrated urine. Both swollen, partly hemolyzed
RBC’s and crenated RBC’s are sometimes difficult to distinguish from WBC’s in the urine. In addition, red cell ghosts may simulate yeast. The presence of dysmorphic RBC’s in urine suggests a glomerular disease such as a glomerulonephritis. Dysmorphic RBC’s have odd shapes as a consequence of being distorted via passage through the abnormal glomerular structure.

**White Blood Cells**

Pyuria refers to the presence of abnormal numbers of leukocytes that may appear with infection in either the upper or lower urinary tract or with acute glomerulonephritis. Usually, the WBC’s are granulocytes. White cells from the vagina, especially in the presence of vaginal and cervical infections, or the external urethral meatus in men and women may contaminate the urine.

If two or more leukocytes per each high power field appear in non-contaminated urine, the specimen is probably abnormal. Leukocytes have lobed nuclei and granular cytoplasm.

**Epithelial Cells**

Renal tubular epithelial cells, usually larger than granulocytes, contain a large round or oval nucleus and normally slough into the urine in small numbers. However, with nephrotic syndrome and in conditions leading to tubular degeneration, the number sloughed is increased.

When lipiduria occurs, these cells contain endogenous fats. When filled with numerous fat droplets, such cells are called oval fat bodies. Oval fat bodies exhibit a ‘Maltese cross’ configuration by polarized light microscopy.
Transitional epithelial cells from the renal pelvis, ureter, or bladder have more regular cell borders, larger nuclei, and smaller overall size than squamous epithelium. Renal tubular epithelial cells are smaller and rounder than transitional epithelium, and their nucleus occupies more of the total cell volume.

Squamous epithelial cells from the skin surface or from the outer urethra can appear in urine. Their significance is that they represent possible contamination of the specimen with skin flora.

Casts

Urinary casts are formed only in the distal convoluted tubule (DCT) or the collecting duct (distal nephron). The proximal convoluted tubule (PCT) and loop of Henle are not locations for cast formation. Hyaline casts are composed primarily of a mucoprotein (Tamm-Horsfall protein) secreted by tubule cells. The Tamm-Horsfall protein secretion (green dots) is illustrated in the diagram below, forming a hyaline cast in the collecting duct:
Even with glomerular injury causing increased glomerular permeability to plasma proteins with resulting proteinuria, most matrix or 'glue' that cements urinary casts together is Tamm-Horsfall mucoprotein, although albumin and some globulins are also incorporated. An example of glomerular inflammation with leakage of RBC's to produce a red blood cell cast is shown in the diagram below:

![Diagram of kidney structure with red blood cell casts]

The factors which favor protein cast formation are low flow rate, high salt concentration, and low pH, all of which favor protein denaturation and precipitation, particularly that of the Tamm-Horsfall protein. Protein casts with long, thin tails formed at the junction of Henle's loop and the distal convoluted tubule are called cylindroids. Hyaline casts can be seen even in healthy patients.

![Hyaline Cast]

Red blood cells may stick together and form red blood cell casts. Such casts are indicative of glomerulonephritis, with leakage of RBC's from glomeruli, or severe tubular damage.

![Red Blood Cell Cast]
White blood cell casts are most typical for acute pyelonephritis, but they may also be present with glomerulonephritis. Their presence indicates inflammation of the kidney, because such casts will not form except in the kidney.

**White Blood Cell Cast**

When cellular casts remain in the nephron for some time before they are flushed into the bladder urine, the cells may degenerate to become a coarsely granular cast, later a finely granular cast, and ultimately, a waxy cast. Granular and waxy casts are believed to derive from renal tubular cell casts. Broad casts are believed to emanate from damaged and dilated tubules and are therefore seen in end-stage chronic renal disease.

**Granular Cast**

**Waxy Cast**

The so-called telescoped urinary sediment is one in which red cells, white cells, oval fat bodies, and all types of casts are found in more or less equal profusion. The conditions which may lead to a telescoped sediment are: 1) lupus nephritis 2) malignant hypertension 3) diabetic glomerulosclerosis, and 4) rapidly progressive glomerulonephritis.

In end-stage kidney disease of any cause, the urinary sediment often becomes very scant because few remaining nephrons produce dilute urine.
BACTERIA

Bacteria are common in urine specimens because of the abundant normal microbial flora of the vagina or external urethral meatus and because of their ability to rapidly multiply in urine standing at room temperature. Therefore, microbial organisms found in all but the most scrupulously collected urines should be interpreted in view of clinical symptoms.

Diagnosis of bacteriuria in a case of suspected urinary tract infection requires culture. A colony count may also be done to see if significant numbers of bacteria are present. Generally, more than 100,000/ml of one organism reflects significant bacteriuria. Multiple organisms reflect contamination. However, the presence of any organism in catheterized or suprapubic tap specimens should be considered significant.

YEAST

Yeast cells may be contaminants or represent a true yeast infection. They are often difficult to distinguish from red cells and amorphous crystals but are distinguished by their tendency to bud. Most often they are Candida, which may colonize bladder, urethra, or vagina.

CRYSTALS

Common crystals seen even in healthy patients include calcium oxalate, triple phosphate crystals and amorphous phosphates.

Very uncommon crystals include: cystine crystals in urine of neonates with congenital cystinuria or severe liver disease, tyrosine crystals with congenital tyrosinosis or marked liver impairment, or leucine crystals in patients with severe liver disease or with maple syrup urine disease.
Miscellaneous

General "crud" or unidentifiable objects may find their way into a specimen, particularly those that patients bring from home. Spermatozoa can sometimes be seen. Rarely, pinworm ova may contaminate the urine. In Egypt, ova from bladder infestations with schistosomiasis may be seen.