Urine Analysis

Urine composition is affected mainly by three factors:

1- Nutritional status

2- State of metabolic processes

3- Ability of the kidney to selectively handle the material presented to it.

Collection of Urine

The urine sample should be collected in **a clean, dry container** and should be examined fresh. For cultures sterile containers should be used. With time, RBC, and leucocytes tend to be destroyed due to hypotonicity of urine. Casts too tend to get decomposed. Bacterial contamination of stale urine is frequent and causes alkalinization of the urine due to conversion of urea to ammonia and loss of glucose. This rise in pH accelerates loss of leucocytes and epithelial cells. For ordinary qualitative, tests a random sample is enough. For diabetes mellitus, a 2 hour postprandial sample is desirable; for nephritis, a morning specimen is best as it has higher specific gravity and lower pH desirable for preservation of formed elements.

Reported samples are necessary sometimes, as for orthostatic proteinuria.

Whenever needed, a 24 hour urine should be collected in a large container. Have patient void and discard urine at any particular time, save all urine for the next 24 hour, and then void at the same hour to finish the collection.

Preservation of Specimen

Urinary decomposition occurs quickly in warm temperatures. Hence, fresh specimens should be examined, if not, then it should be refrigerated. As far as possible, the need for preservation should not arise. However, the following preservatives can be used:

1- *Toluol* : best for preservation of chemical constituents. Add 2 ml toluol/100 ml urine.

2- *Thymol* : a small floating lump of thymol can preserve the urine for several days in a bottle. Thymol may, however, cause a flase-positive reaction for protein.

3- *Formalin* : 1 drop / 30 ml urine. Is good for preserving formed elements. It can precipitate proteins and can reduce Benedicts solution.

4- *Boric acid* : 0.3 gm/120 ml of urine. However, yeasts can still grow and uric acid crystals get precipitated.

Gross Examination of Urine

Normal urine is clear and pale yellow(straw) in color.

1- ***Colorless***: Dilution; diabetes mellitus/ insipidus, nervousness, diuretic or alcohol intake.

2- ***Milky*** : Purulent genitourinary tract disease; chyluria.

3- ***Orange***: Urobilinogenuria, fever, excessive sweating, concentrated urine.

4-***Red***: Beet root ingestion, haematuria, haemoglobinuria, phenolphthalein, pyridium, sulfonal.

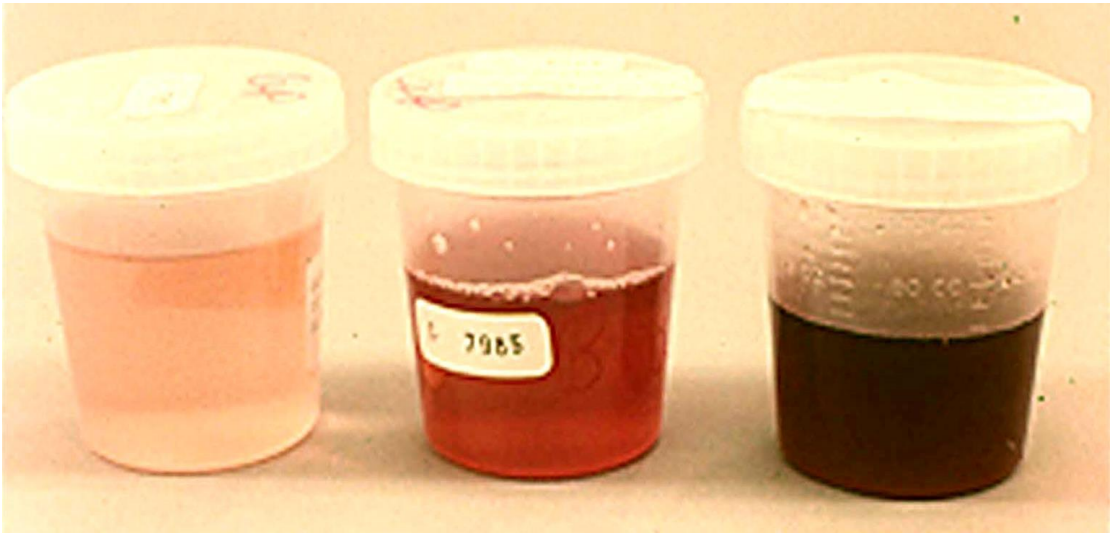
5- ***Greenish***: Jaundice, phenol poisoning.

6- ***Dirty blue or green***: Putrefying urine, in typhus or cholera, methylene blue.

7- ***Dark brown, brown red, or yellow***: Very concentrated urine. Acute febrile diseases. Bilirubinuria.

8- ***Brown-yellow or brown red (if acidic) or bright red (if alkaline)*:** Due to rhubarb, cascara, aloes.

9- ***Brown, brown black or black***: Haemorrhage in urinary tract if urine is acidic (Acid-haematin); haemoglobinuria; porphyria, methaemoglobinuria; myoglobinuria, melanin, phenol poisoning, homogentisic acid (alkaptonuria). In porphyria, urine turns dark brown on exposure to sunlight or boiling.



Reaction

Average range:4.6-8 , Average pH=6.0

Litmus paper or other pH indicator papers broad range (pH 1-12) or narrow range pH papers can be used. Another simple method is to add 2 drops of **0.4% alcoholic solution of methyl red** to 5 ml of urine. Note the color change – if red= acidic ; orange= neutral ; yellow= alkaline. **Digital electronic pH meters** for better accuracy can be used – here, the electrode is dipped in urine and pH read off directly from the digital display.

Amongst urinary tract infections, *Escherichia coli* produces acidia urine, while *Proteus* (urea splitting) produces alkaline urine. Meat protein diet causes urinary acidification, while consumption of citrus fruits makes the urine alkaline.

Odor

Important in fresh specimens only and is **aromatic** because of volatile fatty acids. Bacterial infection causes **ammoniacal odor**, while ketosis leads to a **fruity odor** in urine.

Specific Gravity

It depends upon the concentration of various solutes in the urine.

1- ***Urinometer:*** Urine should be foamless. Transfer urine (about 70-80 ml) into the urinometer container and let the urinometer float freely without touching the sides or the bottom of the conainer. Read graduations at the lowest level of urinary meniscus. If the urine amount is less, dilute the urine to raise the volume till 70-80 ml, take the reading and multiply the last two digits by the dilution factor.

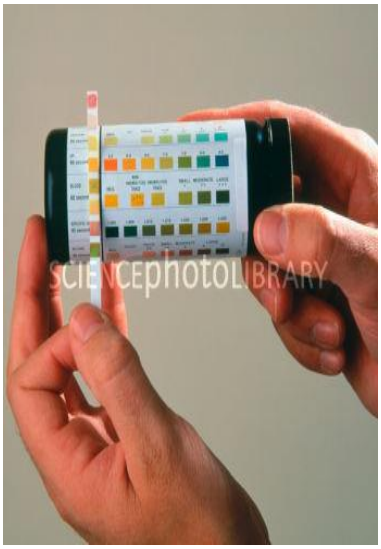
2- ***Refractometer:*** Only small amount of urine is needed. It measures the concentration of solutes (related to refractive index).

3- Can be tested with ***Dipsticks*** also.

4- ***Osmometry:*** Gives the most accurate assessment.

Correction factor for temperature: While using urinometer, add or subtract 0.001 for each 30 C above or below the standardization temperature of the instrument.

Urines of low specific gravity are called **hyposthenuric (< 1.007)** while urines of fixed specific gravity of about **1.010** are known as **isothenuric**.



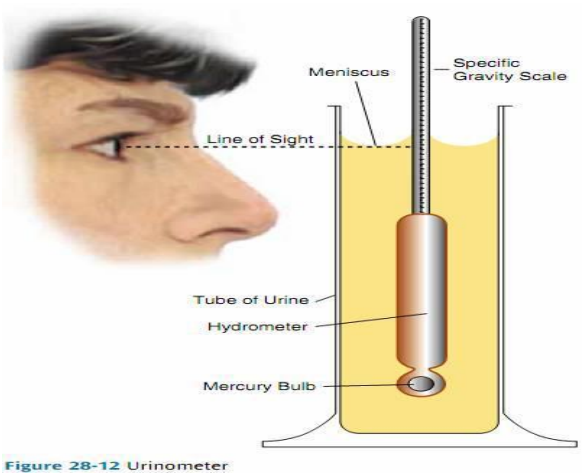
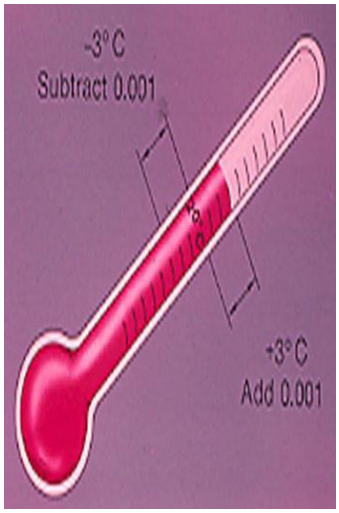
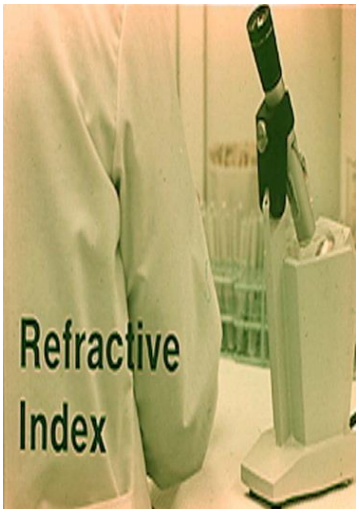


Figure 28-12 Urinometer





Urinary Volume

The average 24 hour urinary output in an adult is around 1200 to 1500 ml and the night urine should not be more than 400 ml.

A volume more than 2000 ml is termed ***polyuria***. ***Oliguria*** implies excretion of urine less than 500 ml and ***anuria*** is complete cessation.

Nocturia is excretion by an adult of urine more than 500 ml with a specific gravity of less than 1.018 at night (characteristic of chronic glomerulonephritis).

Turbidity

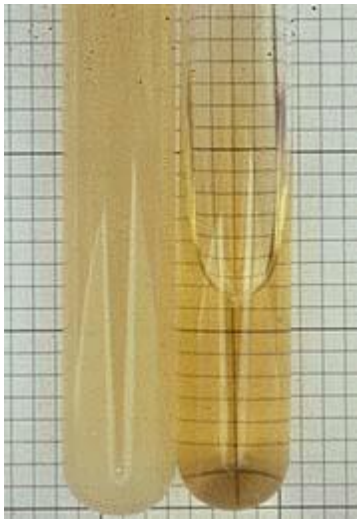
Normal – fresh urine is clear. The appearance of cloudy urine provides a warning of possible abnormality such as the presence of pus, RBCs or bacteria. Sometimes, however, excretion of cloudy urine may

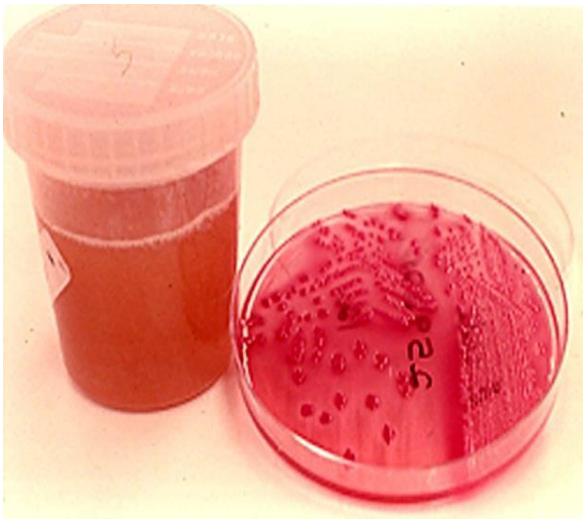
not be abnormal since the change in urine pH may cause precipitation within the bladder of normal urinary constituents. Alkaline urine may appear cloudy because of presence of phosphates, and urine may appear cloudy because of urates.

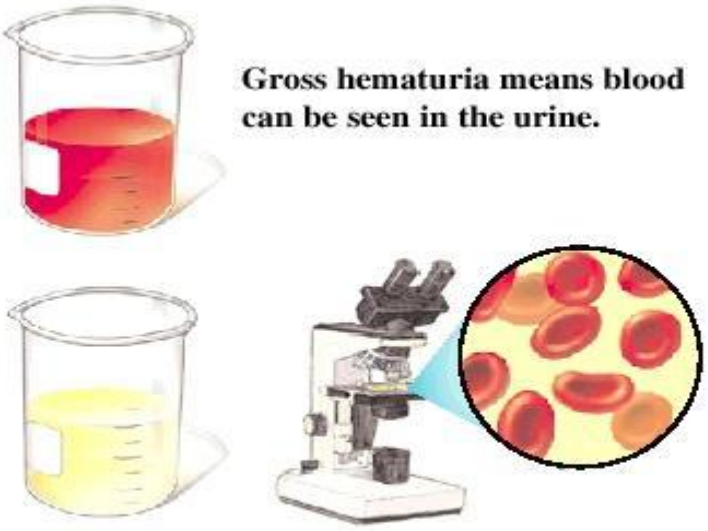
- Pathologic urines are often turbid or cloudy, but so are many normal urines. Cloudy urine may appear from precipitation of crystals due to rapid cooling of the urine.

- Occasionally, urine turbidity may result from urinary tract infections.

- Abnormal urines may be cloudy on account of presence of RBCs, pus cells or bacteria.







Gross hematuria means blood can be seen in the urine.

Microscopic hematuria means blood can be seen only with a microscope.

Chemical Examination of Urine

Tests of Protein

Normal values – Negative (2-8 mg/dl)

If urine is not clear – filter or centrifuge the specimen. Both bile and protein cause urine to froth.

- Heat and acetic acid test

Take a test tube 2/3rd full with urine, boil upper portion of urine for 2 minutes (lower portion is not heated so that it can be used as a control for comparing). Now turbidity can arise because of phosphates, carbonates, or protein. Add a few drops of 10% acetic acid, persistence or development of turbidity implies proteinuria.

False – positive tests may occur with X-ray contrast media and tolbutamide derivatives.

Sensitivity = 5 – 10 mg%

Interpretation

- No cloudiness.

± Cloudiness barely visible.

+ Define cloudiness, but no granularity and no flocculation.

++ Granular cloudiness, but no flocculation. Seen from above, the cloud is dense but not opaque. Protein content = about 0.1 %.

+++ Dense opaque cloud, clearly flocculated. About 0.2–0.3 % protein.

++++ Very thick precipitation, almost a solid. Protein concentration > 0.5%.

- Sulfosalicylic acid test

Urine should be clear and acid.

To 1 ml of urine, add 3 drops of 20% sulfosalicylic acid. Absence of cloudiness means absence of protein. If the turbidity persists after boiling, it is due to protein. If the cloudiness vanishes on heating and reappears on cooling, it is due to Bence Jones (BJ) protein.

False – positive test may appear if the urine contains tolbutamide derivatives, high concentration of penicillin or X-ray contrast media.

- Paper Strip method

Paper strips impregnated with Bromphenol blue and salicylate buffer are dipped in urine. Presence of protein is indicated by change of color from light yellow to blue. Tolbutamide, X-ray contrast media and preservatives do not react, hence no false-positive tests. However, highly alkaline urine may cause a false-positive test; (sensitivity – 30 mg % or more).

- Quantitative Estimation of Protein in Urine

1- Turbidimetric and chemical procedures: Provide and accurate estimation. Colorimetric readings taken against blanks and calculations done accordingly give the result (example; sulfosalicylic acid turbidity method).

2- Esbach's quantitative method: Acidity the urine if necessary. Cover the bottom of the Esbach tube with pumice, fill urine till the " U" mark and add Esbach's or Tsuchiya's regent till the "R" mark. Stopper the tube and invert it about a dozen times slowly.

Set the tube vertically and read after 30 minutes (if pumice has not been used, read after 24 hours). The tube is graduated to read in per cent or in grams of protein per liter at the top of the sediment. Urine may be diluted for obtaining greater accuracy. After diluting, the Esbach tube reading may be multiplied by the dilution factor. dilutions can be made according to the specific gravity as follows:

1. 1.010 to 1.014 – 1:1 dilution.
2. 1.015 to 1.021 – 1:2 urine : water.
3. 1.022 or more – 1:3 urine : water.
4. If the qualitative test reading is +++, dilute as 1:4 urine : water.

- *Bence Jones Protein Tests*

Seen in multiple myeloma classically.

1- Heat and Sulfsalicylic Acid

As for albumin, the precipitate formed will contain both BJ proteins an albumin. Mix the specimen of urine with the precipitate and divide equally in two test tube. Place both in a water bath and heat to boiling. Remove one from the bath, cool to below 400C and compare the turbidity in the two tubes in good light against a dark background. Cool the other hot tube and heat the cold one and compare again. If the cold tube both the times shows persistently a more densely turbid flocculum of protein, BJ protein is most likely present. If albumin is present also, add 10% acetic acid to a fresh urine sample (pH to be less than 6.0) and bring to boil, keep shaking and break the floc the BJ protein goes into solution. Filter off albumin while it is still hot, BJ proteins will come in the filtrate. Repeat the sulfosalicylic acid test as described above.

2- Toluenesulfonic Acid

Add 1 ml of TSA reagent to 2 ml of urine, let the reagent flow slowly by the side of the test tube. Mix. A precipitate appearing within 5 minutes indicates presence of BJ protein. A negative test excludes.

Sensitivity > 500 mg %.

3- Electrophoresis

Electrophoresis of concentrated urine for proteins would show the dense gammaglobulin band.