Protein in urine…..

Methods :

SSA :

Urine centrifuged , to 3.0 ml of supernatant urine an equal amount of 3.0% SSA added. After 10 min observe the degree of turbidity.

Neg	no turbidity (5 mg/dl or less)
Trace	presence of turbidity (20mg/dl)
1+ 50 mg/dl	distinct turbidity but no discrete granulation
2+ 200mg/dl	turbidity with granulation but no flocculation
3+ 500 mg/dl	**turbidity with granulation and flocculation**
4+ 1.0 gr /dl	clumps of precipitated protein or solid precipitate

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Protein in urine…..

Bence Jones Determination :

By electrophoresis :
(BEST METHOD)

b.j. Proteins precipitate at temperatures between 40° and 60 ° and redissolves near 100 °

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Glucose and other sugar in urine

Various sugars may be found in the urine both pathologic and physiologic , include glucose , fructose , galactose , lactose , maltose , pentose , and sucrose .

Glucose most common.

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Glucose

Glycocuria: the presence of detectable amounts of glucose in urine .

Occurs whenever the glucose level in the blood surpasses the renal tubule capacity for reabsorbtion.

Glucose may appear in the urine at different blood glucose levels and there is not always a concomitant hyperglycemia. Glomerular blood flow , tubular reabsorption rate and urine flow influence its appearance. When hyperglycemia present , glycosuria occurs when blood level > 180-200 mg%.

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Glucose…..

DM: glycosuria accompanied by polyuria and thirst.

Inadequate carbohydrate utilization results in elevated keton level in blood and urine due to increased fat metabolism .Other causes of glycosuria:Pituitary and adrenal disorders : acromegally Cushing's hyperthyroidism

In pregnancy GFR and all filtered glucose may not be reabsorbed. glycosuria without hyperglycemia is usually associated with renal tubular dysfunction.

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Glucose and other sugar in urine

Other sugars :

Small amounts are normally excreted about 50 mg/24h , with intestinal disease such as sever sprue or acute entritis the level may rise to 250 mg or more.

The sugar may be identified by TLC.

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Glucose and other sugar in urine

Methods : strip

Based on a specific glucose oxidase and peroxidase method.

The method is specific for glucose

$$\text{Glucose} + O_2 \xrightarrow{\text{G.Oxidase}} \text{gluconic acid} + H_2O_2$$

$$H_2O_2 + \text{chromogen} \xrightarrow{\text{Peroxidase}} \text{chromogen} + H_2O$$

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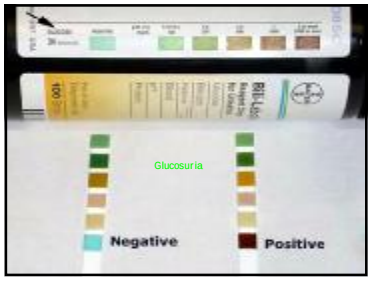

Glucose and other sugar in urine

Copper reduction test :

The glucose oxidase method will not detect increased levels of galactose or other sugars in urine therefore important that a copper reduction method be used especially for young pediatric patients .

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Ketones in urine

Defect in carbohydrate metabolism or absorption or inadequate in the diet ⟶ increasing fatty acids when this increase is large , keton bodies begins to appear in the blood and excreted in the urine .in ketonuria , acetoacetic acid 20% , aceton 2% and butyrate 78% present.

Total keton bodies 17 – 42 mg/dl

Commonly seen in uncontrolled DM

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Ketones in urine

Strip :Based on nitroprusside
(Na nitroferocyanide)

Acetone , acetoacetic and butyrate all present in the urine with ketonuria , methods that indicate the presence of any one is generally satisfactory .

Rothera method detect aceto acetic & acetone

Ferric chloride (Gerhals test detects aceto acetic)

these methods do not measure butyrate , the predominant ketone body

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Blood , Hb , Hemosidrin And myoglobin

Hematuria : the presence of an abnormal number of RBC in urine .

Hemoglobinuria : the presence of free Hb in solution in urine .

Hematuria relatively common , hemoglobinuria uncommon and myoglobinuria rare.

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Blood , Hb , Hemosidrin And myoglobin .....

Hematuria can occur with disease

Or trauma anywhere in the kidneys or urinary tract , excessive exercise (marathon runners), bleeding originates from the bladder mucosa. A positive test for Hb with normal sediment suggest that a fresh urine sample should be examined for RBC_s since an alkaline PH or SG<1.0010 may cause lyses.

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Blood , Hb , Hemosidrin And myoglobin.....

Hemoglobinuria : any cause of hemolysis has the potential of causing Hemoglobinuria , Hemoglobinuria indicates significant IV hemolysis. Hb binds to plasma haptoglobin and free Hb will pass through glomerulus as Alfa beta dimmer , once this binding capacity is saturated .Hb is reabsorbed by PCT and remaining is excreted. plasma appear pink at level of about 50 mg /dl Hb marked hemolysis plasma levels may reach 1.0 G /dl.

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Blood , Hb , Hemosidrin And myoglobin .....

Hemosidrin:Free Hb is readily filtered by the glomeruli and reabsorbed by PCT cells where it can be categorized into ferritin and hemosidrin. Hemosidrin can be found as yellow-Brown granules that are free or in epithelial cells and occasionally in casts . Hemosidrin also appears in the urine sediment at (hemochromatosis) .urinary iron level (about 0.1 mg /day assay , in pernicious anemia and hereditary spherocytosis are normal but in hemochromatosis increased.

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Blood , Hb , Hemosidrin And myoglobin .....

Myoglobinuria : when there is acute destruction of muscle fibers(rhabdomyolysis) as with trauma , myoglobin is released , rapidly cleared from blood and excreted in the urine as a red – brown pigment. free myoglobin , monomer with MW 17000 is excreted quickly whereas the Hb – haptoglobin complex is more slowly removed .

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Blood , Hb , Hemosidrin And myoglobin .....

The distinction between hematuria , hemoglobinuria , and myoglobinuria may be difficult . In all three cases , the urine can be dark red to brown . Strip for blood is also positive serum often pink with hemoglobinuria but normal with myoglobinuria because this pigment is cleared so rapidly.

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Blood , Hb , Hemosidrin And myoglobin ……

Methods :

Strip : based on the liberation of oxygen from peroxide in the strip by the peroxidase – like activity of heme in free Hb , lysed RBC or MB. Intact RBC are lysed on the strip . The reagent area is impregnated with a buffered mixture of an organic peroxide and the chromogen tetra methyl benzidine .

Blood , Hb , Hemosidrin And myoglobin ……

H_2O_2 + chromogen —Heme / peroxidase activity→ oxidized chromogen + H_2O (color changed)

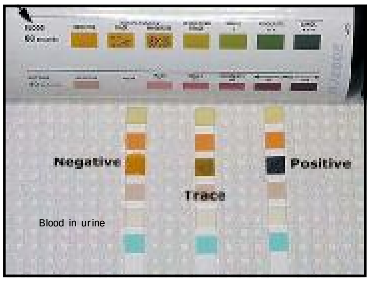
Heme catalyses the oxidation of tetramethylbenzidine to produce a green color.

Strip detect 0.05 to 0.3 mg Hb /dl urine

0.3 mg Hb = 10 lysed RBC / 10^{-6} L

Normal RBC contain 30 Pg of Hb / cell

Capillary elktrophoresis separate Hb from Mb



Blood , Hb , Hemosidrin And myoglobin ……

Detection of hemosidrin in urine :

The Prussian blue reaction is used

Dry and wet methods . Hemosidrin appears as blue granules singly or in groups in renal tubular epithelial cell as amorphous sediment or as blue granules in casts .

Differentiation of hematuria,hemoglobinuria , and myoglobinuria

Condition	Plasma findings	Urine findings
hematuria	Color - normal	Color – normal, smoky ,pink , red , brown Erythrocytes – many Renal – red blood cell casts Protein – marked increase Lower urinart tract – no casts Protein – present or absent
Hemoglobinuria	Color – pink (early) Haptoglobin – low	Color – pink , red , brown Erythrocytes – occasional Pigment casts - occasional Protein – present or absent Hemosiderin – late
myoglobinuria	Color – normal Haptoglobin – normal Creatine kinase – marked increase Aldolase - increased	Color – red , brown Erythrocytes - occasional Dense brown casts - occasional Protein – present or absent

Bilirubin in urine

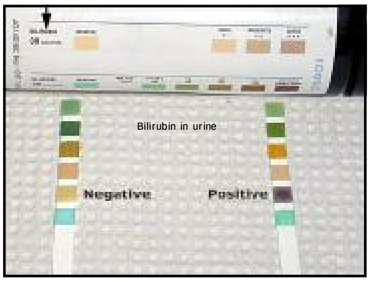
Bilirubin is a breakdown product of Hb that is formed in the RES of spleen , liver and bone marrow . It is carried in the blood linked to Alb , this unconjugate bili or indirect bili is water insoluble and therefore unable to pass through the glomerular barrier. Unconjugate bili is transported to the liver where it is conjugated with glucoronid acid . This conjugate bili (Direct) is water soluble and able to pass thought the glomeruleus into urine. Bilirubin will appear in the urine in Dobbin – Johnson and Rotor type but not present with Gilberts or Crigler – Najjar syndrome .

Bilirubin in urine …..

Bilirubinuria is associated with yellow – Brown to greenish brown urine that may have a yellow foam , elevated serum bili (conj.) jaundice and pale – colored feces (acholic stool) urinary bili (+) , urobilinogen (-) is indicative of intra or extrahepatic biliary obstruction.

Bilirubin in urine …..

Strip : the test is based on the coupling of bili with a diazonium salt in acid medium.



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Urobilinogen

Conjugated bili from the liver eventually reaches the duodenum , complexed with ch , bile salts and phospholipids with the bile. The conj bili is not absorbed from small intestine but instead passes on into the colon , where resident bacteria hydrolyze the conjugate the free bili is then reduced to urobilinogen . Up to 50 % of the urobilinogen is reabsorbed into the portal circulation and re – excreted , unconjugated into the bile .the vast majority of remaining uroblinogen is excreted in feces as colored urobilins or stercobilin . A small amount is excreted in urine .Normal output of urobilinogen in the urine is 0.5 to 2.5 mg or unit /24h. These substances are colorless and labile , the oxidation products of urobilinogen impart yellow – orange color to normal urine .

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Urobilinogen

Methods :

test is based on either the Ehrlich aldehyde reaction or the formation of a red azo dye from a diazonium compound .

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Porphyrins…..

Urine specimen for urobilinogen or porphobilinogen must be fresh. If the testing will be delayed , the ph should be adjusted to near neutral and the specimen stored in a refrigerator , where it is stable for about one week.urine may be darken if the patient has porphyria ,especially if left at room temperature.

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porphyrins

The porphyrias are a group of diseases resulting from defects in the synthesis of heme .These are inherited enzyme deficiencies in which the enzyme substrate is usually excreted in excess in urine and / or feces.During the acute porphyric attack , high level of porphobilinogen are excreted, but between attacks levels of porphobilinogen may be increased or normal .the pattern of excretion of the various porphyrins vary with the different diseases , and together with the clinical finding helps establish the diagnosis.

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Porphyrins…..

Watson- Schwartz test :

The Ehrlich's aldehyde reaction and Watson- Schwartz tests are based on solubility differences between urobilinogen urobilinogen and porphobilinogen. Urobilinogen can be extracted by chloroform and / or butanol,whereas porphobilinogen will be remain in an aqueous phase.

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Indirect test for UTI

1- Nitrite : reduction of nitrate to nitrite , > 10^5 organism , nitrite positive Ex: E.coli

2 – leukocyte esterase :

Leukocyte esterase activity can be indicative of remnants of cells (PMN) that are not visible .

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Examination of urine sediment

With microscopy , one can detect those cellular and non cellular elements of urine that do not give distinct chemical reaction . Microscopy can also serve as a confirmatory test in some circumstances e.g. RBC , WBC and bacteria. cellular elements are from two sources …….

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Examination of urine sediment

…… 1- desquamated / spontaneously exfoliated epithelial lining cells of the kidney and lower urinary tract , and
2- cells of hematogenous origin (leukocytes and erythrocytes) .

Cellular and non cellular cast may be seen , these are formed in the renal tubules and collecting ducts.

Organisms (bacteria , fungi , viral inclusion cells , parasites) and neoplastic cells represents elements that are typically foreign to urine .

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Examination of urine sediment

Methods :

It is recommended that examination take place when the sample is fresh , particularly if no preservative has been added . Cells and casts begin to lyses within two hours of collection . Midstream collection is recommended for female to reduce contamination from vaginal elements .

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Examination of urine sediment

1 - Bright field microscopy

Staining by a 2% solution of methylene blue and toluidine blue

2- Phase – contrast microscopy (casts)

3 – polarized microscopy

crystals

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Microscopic

Cells (WBc , RBc , Epithelial)•

Crystals•

Organisms and artifacts•

Trichomonas vaginalis •

Sperm•

Bacteria •

Fiber •

Starch •

Yeast •

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Examination of urine sediment
Microscopic components , cells

Erythrocytes :

Under high power RBSs appear as pale biconcave disks usually about 7µm in diameter. If specimen is *not* ***fresh*** **, *may appear as faint colorless circles*** *or (shadow cells)* . In dilute urine , the cells will swell and rapidly lyses , releasing Hb and leaving only empty cell membranes referred to as (ghost cells) . …….

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Erythrocytes :

May be confused with oil droplets or yeast cells . Oil droplets exhibit a greater variation and highly refractile and yeast show budding . Acid acetic added , RBC lysed 0-2 cell/ hpf in normal urine > 3.0 abnormal RBC

1- renal disease
2- lower UT disease
3- extra renal disease
4- toxic reaction
5- physiologic causes

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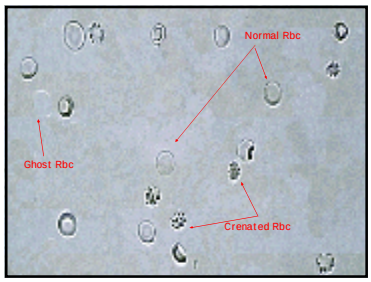

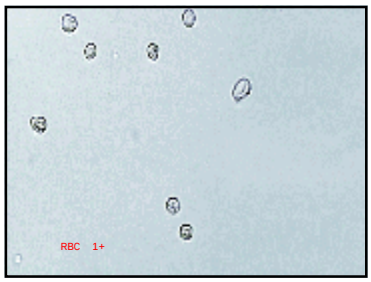

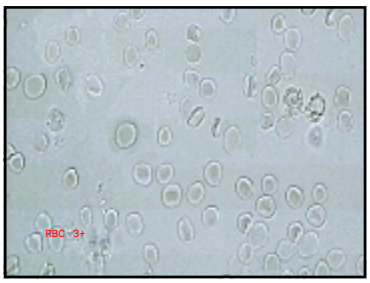

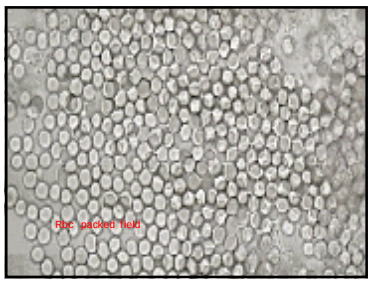

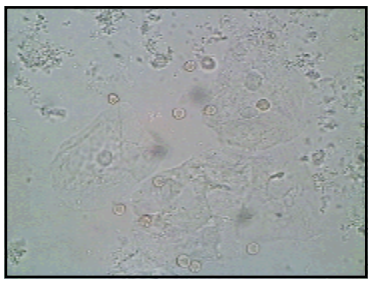

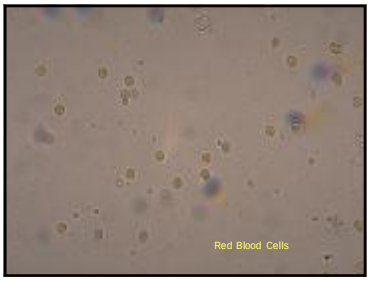

Leukocytes

Neutrophils :

The predominant type of leukocyte in urine , appear as granular spheres about 12µm with multilobated nuclei.

In dilute urine PMN swell and cytoplasmic granuls exhibit Brownian movement , known as (glitter cells)

Leukocyte are rapidly lysed in hypotonic or alkaline urine , 50 % are lost following to three hours of standing .

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