wjpmr, 2018,4(2), 71-84

SJIF Impact Factor: 4.103

WORLD JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.wjpmr.com

Review Article

ISSN 2455-3301

WJPMR

URINALYSIS IN CHEMICAL PATHOLOGY: AN INNOVATIVE REVIEW

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Article Received on 29/11/2017 **Article Revised on 20/12/2017** **Article Accepted on 10/01/2018**

ABSTRACT

A complete urinalysis includes physical, chemical, and microscopic examinations. Midstream clean and sterile collection is required in most of the time, and should be examined within two hours of aseptic collection. Both Kidneys excrete the unwanted substance i.e. metabolic waste including those substances which are present in excessive quantities in the body, through urine. Mostly the concentration of metabolic substances found in the urine is affected by various factors like as dietary intake, body metabolism, endocrine function, physical activity, body position. Complete urinalysis can reveal diseases that have gone unidentified because they do not produce any striking signs and symptoms. The urinalysis can be used as a screening and diagnostic as well as prognostic tool noticed various metabolic and kidney state and disorders. It is evaluated widely and routinely to diagnose any abnormalities that require follow up. In many acute or chronic conditions, such as kidney disease, the urinalysis may be rapidly provides important information about acute renal disorder and metabolic diseases. This review article corrects interpretation and understanding of concepts for performing urinalysis.

KEYWORDS: Urine, Renal disease, metabolic disorder, Urinalysis, Urinary tract etc.

INTRODUCTION

Urinalysis is one of the invaluable tool in the diagnosis of renal states such as calculi, urinary tract infection (UTI), and malignancy. The oldest of Laboratory manual used in traditional medicine is the inspection of urine for diagnostic parameter. The ancients paid concern to the character of urine in renal and metabolic disease. According to the Disease prognosis, a careful examination of all excreta was used as a basis for estimating the course of disease. In advance medical science interpretation of urine examination is possible. Routine urine analysis is mainly performed for the diagnostic and prognostic purpose to detect various intrinsic conditions that may adversely affect the urinary tract or the kidneys, urethra and to reveal metabolic or endocrine abnormalities of the body. This innovative review of analysis is a part of the education of medical students, medical technologists, and other healthcare workers because the analysis of urine chemical constituents, coupled with a careful review of the microscopic elements in urine sediment,can provide physicians with valuable diagnostic information.

MATERIAL AND METHODS OF SAMPLE COLLECTION

A midstream clean-catch technique usually is adequate in men and women after cleansing the external urethral meatus and should be collected with minimum contamination.

- If not possible, bladder catheterization is appropriate for adults-risk of contracting a urinary tract infection is negligible for a single catheterization.
- Suprapubic aspiration is used in infants.

Some points to be noted

- High urine osmolality and low pH favor cellular preservation; hence first voided morning urine is preferred.
- Chemical composition of urine changes with standing and formed elements degrade over time. Hence, urine is best examined when fresh but a brief period of refrigeration is acceptable.
- Bacteria in urine multiply at room temperature; hence bacterial counts from unrefrigerated urine are unreliable.
- The specimen must be properly labeled. For urine routine examination.
- The sample should be examined immediately because on keeping at room temperature, the reaction might change (from acidic to alkaline), casts might disintegrate, crystalline precipitate not originally present may appear and bacterial growth may make the sample turbid.[1]

PROPER URINALYSIS AND INTERPRETAION

Urinalysis is a basic investigation trend in condition characterized by disturb of metabolism. Cultural examination is done for diagnosis of urinary carriers of these infections. In general, the quantity, quality and the content of urine reflects the state of metabolic products of circulatory dynamics, water and electrolyte balance, the structure and functions of the Kidneys and urinary tract as the mechanism of urine formation indulge various processes. Other substances found in urine include pigment, enzymes hormones. RBCs, WBCs, epithelial cells, crystals, mucus and bacteria also may found in urine.[2]

Routine urinalysis includes **physical, chemical, and microscopic** examinations.

Physical or Visual Examination

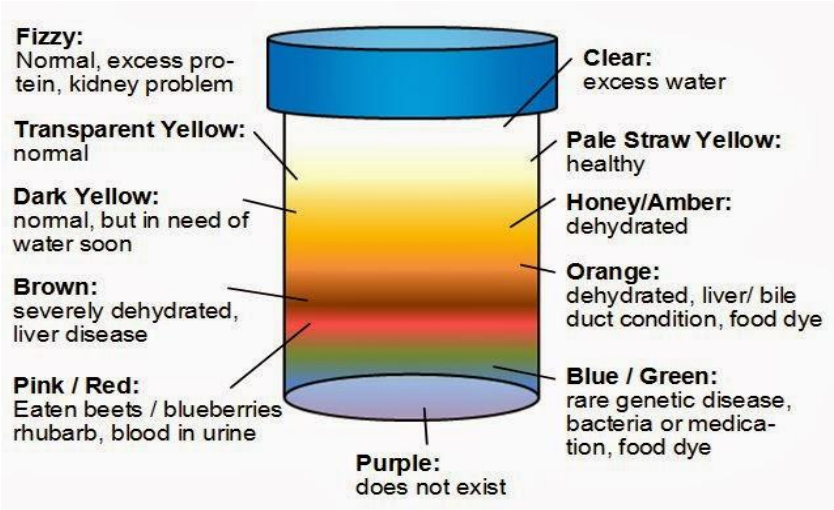


Fig. 1: Various color of Urine.

Physical examination of the urine, observes the urine's color, clarity, concentration, and odor. Normal urine color is pale to straw yellow clear due to urobilin, uroerthrin and urochromes.[3]

Various color of urine can be seen as Foods, medications, metabolic products, and infection can cause abnormal urine color as shown in Table 1. and figure 1.

Urine doesn't usually have a strong smell and normal fresh urine has a slight aromatic odor. On storage urine has an ammoniac odor due to the decomposition of urea. But some foods especially asparagus, which has a smelly sulfur compound can change the odor. So can vitamin B-6 supplements. When you're dehydrated and your urine gets very concentrated, it can smell strongly of ammonia.

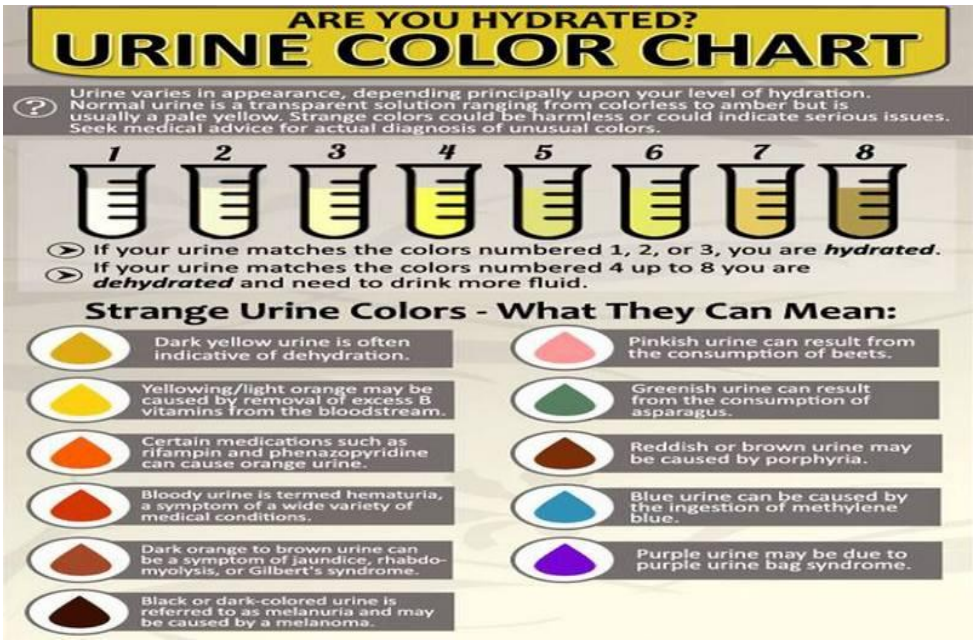


Fig. 2: Urine Color Chart for compare with dehydration state.

Table 1: Abnormal Color and odor of urine.

Common Causes of Abnormal Urine		
Color	**Food and drug causes**	**Pathologic causes**
Turbid or Cloudy	Diet high in purine-rich foods (hyperuricosuria)	Phosphaturia, pyuria, chyluria, lipiduria, hyperoxaluria
Brown	Fava beans Levodopa (Larodopa), metronidazole (Flagyl), nitrofurantoin (Furadantin), some antimalarial agents	Bile pigments, myoglobin
Orange	Phenothiazines, phenazopyridine (Pyridium)	Bile pigments
Red	Beets, blackberries, rhubarb Phenolphthalein, rifampin (Rifadin)	Hematuria, hemoglobinuria, myoglobinuria, porphyria
Brownish- Black	Cascara, levodopa, methyldopa (Aldomet), senna	Bile pigments, melanin, methemoglobin, Phenol Poisoning
Green or Blue	Amitriptyline (Elavil), indigo carmine, IV cimetidine (Tagamet), IV promethazine (Phenergan), methylene blue, triamterene (Dyrenium)	Pseudomonal UTI, biliverdin
Yellow	Carrots Cascara	Concentrated, dehydrated urine
Purple		Purple urine bag syndrome, Blue diaper syndrome. Infection with Escherichia coli, pseudomonas, enterococcus, others
White		Chyle, pus, phosphate crystals
Odor of Urine		
Odor	**Substance or condition**	
Sweet or Fruity	Ketone Bodies	
Maple Syrup	Maple syrup urine disease	
Ammoniacal	Urea splitting bacterial infection	
Musty or mousy	Phenylketoneurea	
Rancid	Hypermethioninemia, tyrosinemia	
Sweet feet	Isovaleric or glutamic acidemia or excess butyric or hexanoic acid	

Specific Gravity

Specific gravity of urine is reveals the presence of solutes represented by particles of varying sizes, from small ions to larger proteins. The normal specific gravity of 24 hours urine is usually between 1.015 and 1.025 compared to distilled water (1.000). Urine osmolality measures the total number of dissolved particles. Urine specific gravity is directly proportional to urine osmolality. If there were no solutes present, the Specific gravity of urine would be 1.000 same as distilled water. 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. Generally the greater the volume, the lower is the specific gravity, except in diabetes mellitus. The substances influencing the specific gravity of urine are urea, sodium chloride, phosphates and abnormally albumin and sugar.

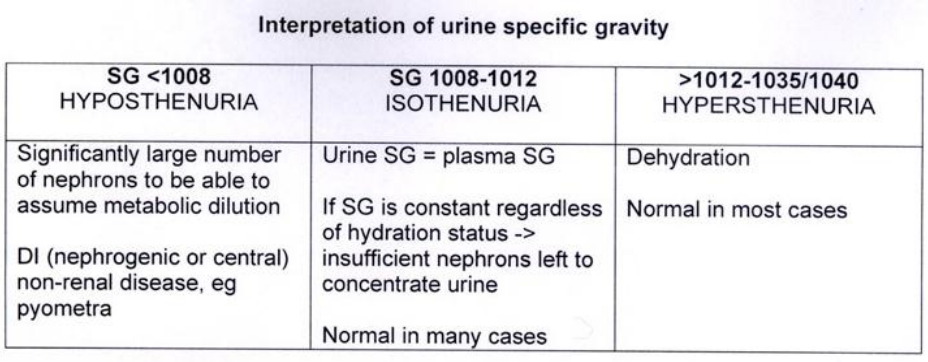


Fig. 2: Interpretation of urine specific Gravity (SG).

Reaction and P^H

Urine normally is slightly acidic in nature, with a pH around 6, although it can range from 4.5 to 8. Urine pH changes, depending on your diet, certain disease processes and the medications. Excreting acid or alkaline urine helps maintain the body's acid-base balance, the balance between acidity and alkalinity. Blue and red litmus papers are put in urine or commercially available

Dipstick to check the reactions of urine. If blue turn red, urine is acidic. If red turns blue, urine is alkaline. Acidic urine: due to high protein intake, e.g. Meat, Ingestion of acidic fruits, Respiratory and metabolic acidosis, UTI by E. coli bacteria Alkaline urine: due to citrus fruits, vegetables, respiratory and metabolic alkalosis. UTI by proteus, pseudomonas. On standing at room temperature, the urine becomes alkaline due to the formation of ammonia. Urinary pH generally reflects the serum pH, except in patients with renal tubular acidosis (RTA). The inability to acidify urine to a pH of less than 5.5 despite an overnight fast and administration of an acid load is the hallmark of RTA. In type I (distal) RTA, the serum is acidic but the urine is alkaline, secondary to an inability to secrete protons into the urine. Alkaline urine in a patient with a UTI suggests the presence of a urea-splitting organism, which may be associated with magnesium-ammonium phosphate crystals and can form calculi. Uric acid calculi are associated with acidic urine.[5]

Fig 3: Physical examination of urine to check reaction and other parameters by dipsticks.

Volume

The normal range for 24-hour urine volume is 100 to 1500 milliliters per day (with a normal fluid intake of about 2 liters per day). Normal value ranges may vary slightly among different laboratories.

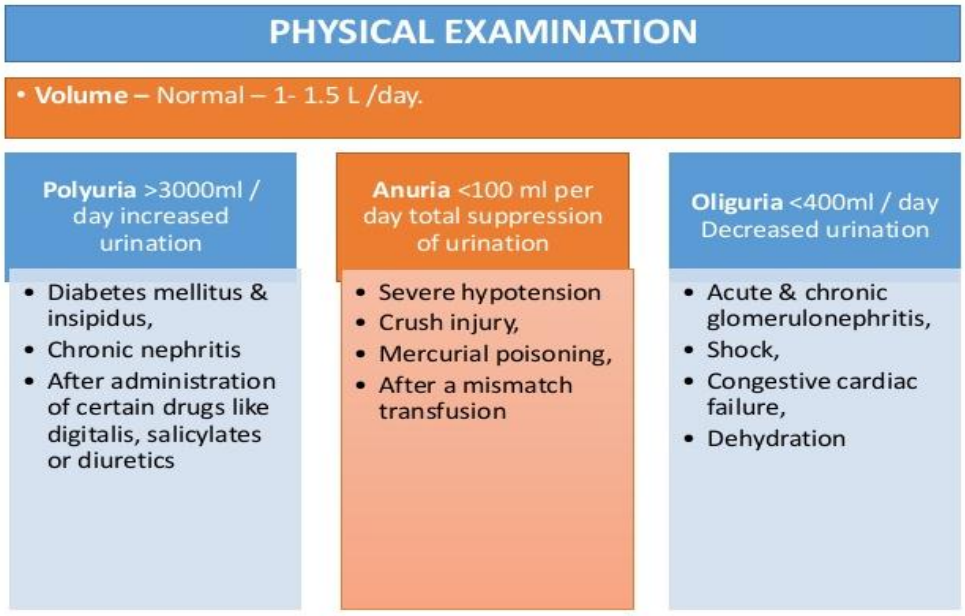


Fig 4: Interpretation of urine volume and associated cause.

CHEMICAL EXAMINATION OF URINE

Proteins in Urine

Urine normally contains only a scant amount of protein which derives both from blood and urinary tract itself. Mainly albumin is filtered from nephrons due to low molecular weight others are reabsorbed by renal tubules. Other protein includes serum or plasma globulin, mucus or mucin, hemoglobin, bence jones protein.

All the methods are based on the principle of precipitation of protein by chemical agents or coagulation by heat. Qualitative tests Methods are applied to detect ie. If urine is alkaline make it slightly acidic by adding 3% glacial acetic acid. Turbid urine should be filtered or centrifuged and supernatant should be used. There are three qualitative methods namely Heat and acetic acid test, Sulphosalicyclic acid test and Purdy's modification.

Qualitative test and semi quantitative test have limitation that they can't detect the exact amount of protein excretion. So quantitative test is done on 24 hr urine Mainly 2 methods are used for this purpose which uses picric acid for precipitation in different proportion methods are: Esbach's method Aufrecht's method.

The most commonly used test are

A. Heat and Acetic Acid Test

Place 5 to 10 ml of clear urine in test tube Boil the upper portion over a flame. If turbidity develops add 1-2 drops of glacial acetic acid. Sometimes turbidity may be due to phosphate or carbonate precipitation. it is so then glacial acetic acid clear up the turbidity. If it is due to protein

then precipitation will be there after the addition of acetic acid Reboil the specimen. If turbidity is present protein is present. If there is no turbidity at upper portion then protein is absent.

Interpretate the turbidity as follows: Negative : No cloudiness, Trace: Barely visible cloudiness.,1+ : definite cloud without granular flocculation,2+ : heavy and granular cloud without granular flocculation,3+ : densed cloud with marked flocculation.,4+ : thick curdy precipitation and coagulation.

B. Sulphosalicyaclic Acid Test

Prepared 3% sulphosalicylic acid by adding Sulphosalicyaclic acid 3.0 gm D/water 100 ml. Place 3-4ml of clean urine in test tube From the side of tube add 2-3 drops of sulphosalicylic acid on top. Let it stand for 5 minutes. Observe the turbidity. No formation of turbidity at upper portion of urine indicates absence of protein Formation of turbidity indicates presence of protein. Turbidity is graded as Trace cloudiness against dark background 1+ dense cloudiness. 2+ cloudiness with granules and definite flocculation. 3+ cloudiness with flocculation. 4+ cloudiness with precipitation. Clinical Significance of Proteinuria mainly due to Glomerular damage and Defect in reabsorption of process of tubules. Always proteinuria in not pathological. So there are mainly 3 types of proteinuria.

a. Accidental Proteinuria

Due to contamination of urine with vaginal seminal discharge after prostatic massage and derivation from diseased condition of genital tract or bladder accidental proteinuria is seen.

b. Functional Proteinuria

Non pathological proteinuria also called physiological albuminuria mainly seen in strenuous exercise, phrexia, exposure to cold, congestive heart failure hypertension atherosclerosis pregnancy dehydration fever if person stand in upright position for longer period. (Postural or orthostatic proteinuria).

c. Renal Proteinuria

Any condition resulting in increased permeability of urinary tract surfaces or in transduction such as glomerulonephritis diabetes nephritis associated with SLE, pyelonephritis, hereditary fructose intolerance, cystitis urinary tract, malignancies, heavy metal poisoning, eclampsia, amyloidosis, sarcoidosis, sickle cell disease, renal transplant rejection, multiple myeloma, degenerative and irritative condition and lower urinary tract.

Bence-Jones protein (BJP)

The Bence-Jones protein (BJP) test measures the level of BJP in urine. Bence-Jones proteins are named for Henry Bence-Jones, a physician and chemist who first isolated them in 1847. These proteins are not present in healthy urine samples and are usually a sign of multiple myeloma. Multiple myeloma is a type of bone marrow cancer that is most common in people who are older than 60 years. Multiple myeloma is a condition where bone marrow makes too much of a type of white blood cell.

Normally, white blood cells make many different types of antibodies. They play an important role in immune system. In multiple myeloma, one white blood cell line grows out of control. It produces only one type of antibody. These cells then crowd out the normal cells and vulnerable to illness. This makes bones more likely to break. Other symptoms include: kidney problems (caused by antibody buildup), anemia, which causes fatigue or weakness, swollen or weak legs, pain in the ribs or back, compressed spinal cord or nerves (due to bone fractures), excessive thirst, dehydration, frequent urination or constipation (from when bones break down and leave excess calcium in the blood), confusion, recurring infections, excessive bleeding, even from slight injuries.

Always keep in concern Orthostatic proteinuria can be differentiated from pathological proteinuria by testing 2 urine samples, one collected immediately after rising and one collected after patient has been in upright position for 3 hour or longer. During heat and acetic acid, If cloudiness is seen it may be due to phosphate or carbonates confirm by adding 3% glacial acetic acid. if it is due to protein cloudiness persist and if it is due to the phosphate the cloudiness disappears and if it is due to carbonate cloudiness disappears with effervescence. If cloudiness is disappeared when nitric acid is added then it is due to mucin and nucleoprotein. If cloudiness appears with the tube is being heated but disappears when boiling point is reached bence jones protein is present. Bence jones protein is low molecular weight protein which is easily filtered through the glomerulus. It has unusual solubility.it precipitates when heated at 40-60 degree centigrade and becomes soluble when boiled and reappears on cooling. it is seen in urine in multiple myeloma where there is malignant proliferative of plasma cells in bone marrow. Heat and acetic acid test detect as little as 2-3 mg/100 ml of protein. Sulfosalicylic acid detects 5 mg/100 ml of protein.

Sugar in Urine

Normally glucose is virtually absent from urine. The renal threshold for glucose ranges from 160-200mg/dl (180mg/dl) depending on the individual. That is blood sugar must rise to its renal threshold before glucose will appear in urine. When glucose is present in urine it is called glysosuria. Other less important sugar that may appear in urine are lactose galactose pentose which may give false positive results for glucose. So specific test must be performed for differentiating glucose from other sugars present in urine.

Glucose is determined by Benedict's test and Fehlings test, Determination of Lactose by Yeast fermentation test, Osazone test and Rubners test. Other tests are

Seliwanoffs Test for Fructose and Bial's Test For Pentoses.

When benedicts qualitative reagent is heated with 8 drops of urine glucose present in urine reduces cupric ions present in reagent to cuprous ions. Alkaline medium is provided to the reaction by sodium carbonate present in reagent. The color changes to green yellow orange or brick red according to concentration of glucose in urine.

Fig 4: Benedict's test (Negative, 1+, 2+, 3+ and 4+ from left to right).

Benedicts qualitative reagents is prepared by adding Sodium citrate 1.73 gm. and Sodium bicarbonate 100 gm. in about 900 ml of distilled water. Boil for 2-3 minutes and add 17.3 gm of cupric sulfate make final volume up to 1 liter The reagent is stable at room temperature.

Pipette 5 ml of benedicts reagent in test tube by using Pasteur pipette add 8 drops of urine heat carefully or place in boiling water bath for 5-10 mins cool under tap water. Interpretate Result as No change in color i.e. blue: Absence of sugar. Pale green with slightly cloudy: Trace Definite cloudy green: 1+ Yellow to orange precipitate with supernatant fluid pale blue: 2+ Orange to red precipitate supernatant fluid pale blue: 3+ Brick red precipitate supernatant decolorized: 4+ Clinically glucose is seen in urine in 2 conditions When blood sugar is elevated and When blood sugar is not elevated but renal tubular absorption-glucose is impaired. Glucose in urine is mainly seen in diabetes mellitus. It is increased in Any cause of increased blood glucose. Rapid intestinal absorption (post gastrectomy dumping normal pregnancy) Endocrine disorders other than diabetes milletus like thyrotoxicosis, gigantism. acromegaly, Cushing syndrome. Major trauma stroke myocardial infarction or circulatory collapse cerebral hemorrhage Burns oral steroid therapy infection pheochromocytoma Glycogen storage disease, obesity, sepsis, carcinoma of pancrease, fanconi's syndrome, cystinosis.

Things to be noted If benedicts show more than 2.5% sugar urine should be diluted. If benedicts test is positive then it is necessary to confirm it by using glucose oxidase uristix Sugar in urine is also detected in gestational diabetes oral glucose tolerance test spot test during post prandial blood glucose. Benedict's reagent gives false positive in certain non-carbohydrate also such as uric acid creatinine salicyaclic acid homogentisic acid and melanogen.

Ketone Bodies

The term ketones refer to 3 intermediate product of fat metabolism, they are acetone acetoacitic acid and beta hydrooxybutyric acid. Ketone is found when there is excessive fat metabolism. excessive fat metabolism occurs in various situation Impaired ability to metabolize carbohydrate Inadequate carbohydrate intake Excessive carbohydrate loss Increased metabolic demand. Various Methods are available to detect ketones, Rothera's test for acetone., Gerhard's test for diacetic acid, Lindeman's test for diacetic acid, Han's method for betahydroxybutyric acid., Tablet test.

Principle based on Nitroprusside used in this test reacts with both acetone and acetoacetic acid in presence of alkali (NH4OH) to produce permanganent calomel red ring at the junction.

Rothera powder mixture is prepared by following mixture of Sodium nitroprusside Ammonium sulphate Liquior ammonia solution. Transfer about 5 ml of urine to a test tube, Saturate with ammonium sulphate, add 1 crystal of sodium nitroprusside, Layer the liquor NH4OH on the side of the tube interpretate by Observing permanganate calomel ring at the junction of two layers.

Ketones are present in urine, Diabetes mellitus, Propanol poisoning, Severe starvation., Severe carbohydrate restriction, Anorexia, Fasting, Fever, Prolonged vomiting, Lactic acidosis, Salicyclate toxicity. Note In diabetes mellitus impaired ability to metabolize carbohydrate takes place. as carbohydrate cannot be used to meet the body energy need, fats are burned which leads to the presence of ketones in the urine. Acetoacetic acid oxidizes rapidly to form acetone therefore test must be carried out in fresh urine specimen. Individuals receiving levadopa paraldehyde pyridium and phathalein compound may produce false positive result when tested for ketonuria. Presence of salicylates give false negative result. When sugar is found in urine, the urine should be tested for ketone.

Occult Blood in Urine

The term occult means hidden. Blood may be present in the urine as either red blood cells or hemoglobin. If enough blood is present the color of sample may be range from pink tinged to red to brownish black. These are tests available for Blood I urine Microscopical Examination to see RBCs, Chemical Examination like Benzidine test, Guaiacum test, Gregersens test, and Ortho-toluidine test. Other is Spectroscopic Test. But mostly done is Benzidine Test.

The test is based on the principle that peroxidase activity of hemoglobin present in urine decomposes hydrogen peroxide and the liberated oxygen oxidized benzidine to form a green- blue colored complex.

Place a pinch of benzidine in a test tube, Add 2- 3 drops of 5% glacial acetic acid. Mix well Add 2 ml of hydrogen peroxide solution. Transfer supernatant to a test tube label as T Add few drops of urine and observe the color.

Clinical significance of Blood in urine is **Hematuria**, hemoglobinuria and Myoglobinuria Presence of more number of red blood cells in urine is called hematuria which is associated with disease of or damage to the genitourinary tract. other disorder commonly used associated with hematuria includes acute infection chronic glomerulonephritis tuberculosis of kidney nephritic syndrome toxic damage to glomerulus malignant hypertension infarction renal calculi trauma to kidney, acute cystitis, calculi, tumors in the ureter or bladder and kidney stones. In other clinical conditions such as bleeding disorder (leukemia, thrombocytopenia, coagulation factor deficiency, sickle disease or traits, scurvy), use of anticoagulant drugs.

Hemoglobinuria: It is the presence of free hemoglobin in urine as a result of intravascular hemolysis. Causes of hemoglobinuria Acute and Chronic Acute, Incompatible blood transfusion, Hemolytic anemia due to drugs and chemicals., Favism., Paroxysmal cold hemoglobunuria., March (exertional) hemoglobunuria., Hemolytic anemia associated with eclampsia, Hemolytic uraemic syndrome., Hemolytic anemia due to burns, Snake and spider bites. Chronic, PNH, Cardiac hemolytic anemia, Cold haemagglutination disease.

Myoglobinuria: Myoglobin is the haem protein of striated muscle. Myoglobin is very toxic to the renal tubules and in large amounts it is associated with acute renal failure.

Clinical Conditions are, Myocardial infarction Infarction of large skeletal muscle Destruction of muscle with crush injury heat stroke electric shock, Trauma.

Things to be keep in mind while interpretation False positive result is seen in women during menstruation due to contamination of urine with menstrual blood. So this test should be avoided during menstruation cycle. Free Hb is not normally found in the urine. Instead any Hb that could be presented to the glomerulus combines with heptoglobin. The resultant Hb heptoglobin complex is too large to pass through the glomerular membrane. If the amount of free Hb exceeds the amount of heptoglobulin, however the Hb will pass through the glomerulus and ultimately be excreted into the urine. Any disorder associated with hemolysis of red blood cells and resultant release of Hb may lead to the appearance of Hb in urine Hematuria can be differentiated from hemoglobunuria by doing microscopical examination. In hematuria RBC seen in microscopy. In hemoglobunuria, RBC cannot see even though the test for occult blood is positive. This test can be done for stool as occult blood for stool.

Bile in Urine

Bilirubin, bile salt, bile pigment, urobilin, urobilinogen are the constituents of bile.

Determination of Bile Salt by Hay's test Bile salts when present lower the surface tension of urine. When sulphur powder is added to the urine, sulphur particles sink to the bottom of the tube. In the case of normal urine, it will float on the surface. Place about 10 ml of urine in a test tube, Sprinkle a little dry sulphur powder on to the surface of urine. Observe sulphur particles. Other Methods, Foam test, Gmelin's test, Smiths test, Fouchet's test, Ehrlich's aldehyde test Schlesingers test.

Foam test, Shake some urine in a test tube. If the foam on the top is yellow, the bile pigments are present.

Fouchet’s test, When barium chloride is added to urine it combines with sulphate radicals in urine and precipitate of barium phosphate is formed. If bile pigments are present in urine, they will adhere to these large molecules. Ferric chloride present in fouchet reagent then oxidizes yellow bilirubin in presence of trichloroacetic acid to green bilverdin.

Fouchet’s reagent composed of Trichloroacetic acid 25 gm Distilled water 100 ml 10 % ferric chloride solution 10 ml 10ml of urine + 2.5 ml of barium chloride, Filter, Unfold the filter paper and spread it on the dry filter paper. Allow 1 drop of Fauchet’s reagent on the precipitate A green or blue color indicates presence of bilirubin.

Ehrlichs Aldehyde Test for Urobilinogen Take 5 ml of urine in test tube and add half volume i.e. about 2.5 ml of barium chloride. mix well and filter. Take 2.3 ml of filtrate and add 0.5 ml of aldehyde reagent. allow to stand for 3 mins. View the top column of urine against a white background. A pink color denotes the presence of urobilinogen. Repeat the test with 1:10, 1:20, 1:50, 1:100, 1:200 dilution and report a terms of highest dilution giving a positive reaction.

Ehrlich's reagentscomposed of Paradimethylaminobenzaldehyde 2gms. 20% HCl 100 ml.

Schlesingers Test for Urobilin Take 10 ml of urine and 6 drops of tincture of iodine in a test tube. Take 1 gm of powdered zinc acetate and 10 ml 95% alcohol in another test tube. Mix by pouring a into b and vice versa repeatedly until the solid zinc acetate has mostly gone into solution. Filter. Examine the filtrate. A green is due to compound of zinc with urobilin, confirm

spectroscopically absorption band junction of green and blue.

Clinical significance, Determination of bile salts, bile pigments, and urobilinogen is useful in the diagnosis of jaundice. Bilirubin may be found in urine in liver disease and is usually found in clients who have biliary tract obstructions. Conjugated bilirubin appearing in urine generally indicates that there is excess conjugated bilirubin in blood stream. Bilirubinuria is seen when intracanalicular pressure rises secondary to periportal inflammation, fibrosis or hepatocyte swelling. Gallstones in the common bile duct or carcinoma of the head of pancreas are possible sources of extra hepatic biliary obstruction leading to bilirubinuria. Congenital hyperbilirubinemia seen in gilberts disease or crigler najjar disease. When liver cells are damaged, excreation of urobilinogen in the bile decreased, where as its urinary excreation is increased. This may be seen in cirrhosis, hepatitis and congenital heart failure with congestion of the liver. Excessive urobilinogen also may be found in the urine of those with liver disease or hemolytic disorder.

Condition	Urine bilirubin	Urine urobilinogen
Bile duct obstruction	+ + +	Negative
Liver damage	+ or -	+ +
Hemolytic disease	negative	+ + +

The bilirubin in urine is made confirmed by doing confirmatory bilirubin test called as diazo test. The Fouchet's test is also called Harrison spot test. Fresh urine sample should be used for bilirubin determination because exposure of urine to light and room air may give false negative result. large amount of ascorbic acid and nitrates also give false negative result. Acidic urine will result in decreased urinary level of urobilinogen. High levels of nitrates in the urine also may cause false negative results in test for urobilinogen.

Leukocyte Esterase

Leukocyte esterase is an enzyme present in most white blood cells (WBCs). Normally, a few white blood cells (see microscopic examination) are present in urine and this test is negative. WBCs in urine increases significantly, it means that there is inflammation in the urinary tract or kidneys. There is common cause for WBCs in urine (leukocyturia) is a bacterial urinary tract infection (UTI), such as a bladder or kidney infection. Organisms such as Chlamydia and Ureaplasma urealyticum should be considered in patients with pyuria and negative cultures. Other causes of sterile pyuria include balanitis, urethritis, tuberculosis, bladder tumors, viral infections, nephrolithiasis, foreign bodies, exercise, glomerulonephritis, and corticosteroid and cyclophosphamide (Cytoxan) use.

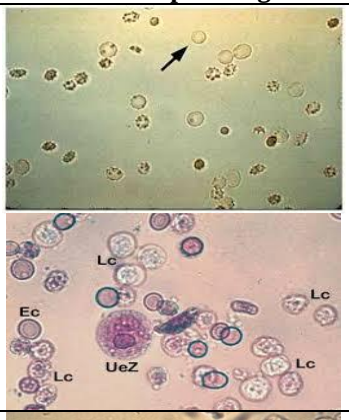
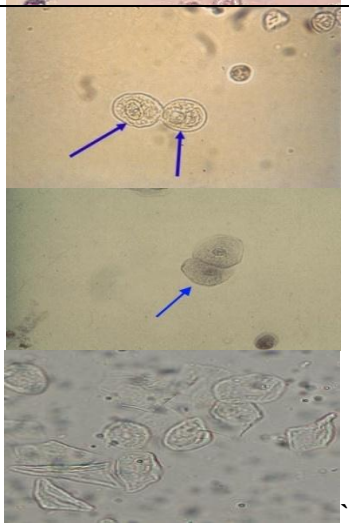
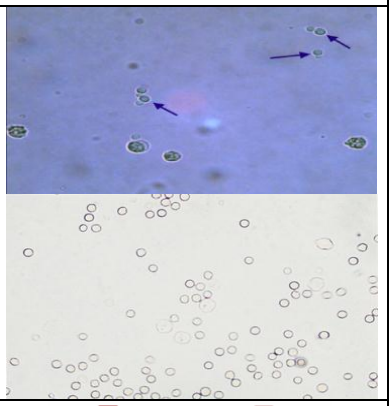
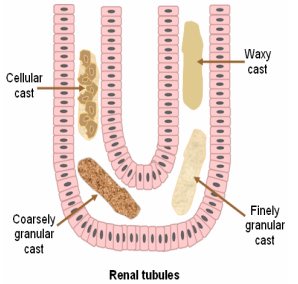
Nitrite

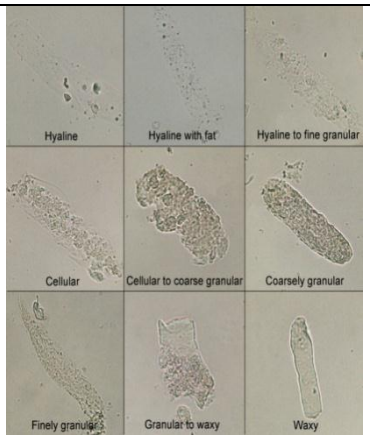
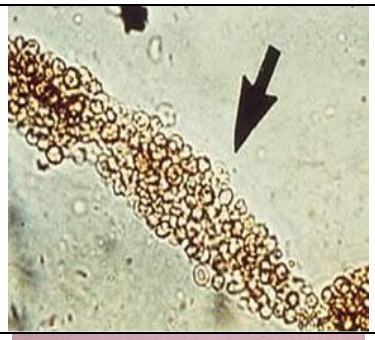
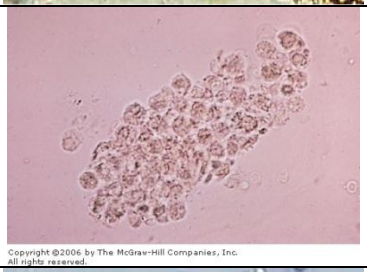
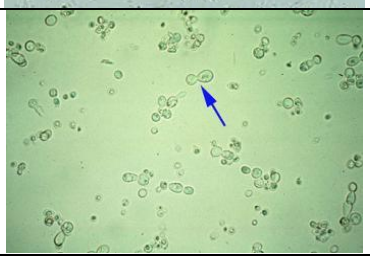
Normally the urinary tract and urine are free of bacteria. When bacteria find, they can cause a urinary tract infection (UTI). A positive nitrite test result can indicate a UTI. Gram negative rods such as E. coli are more likely to give a positive test.

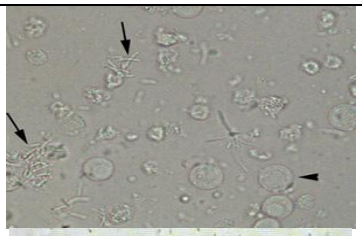
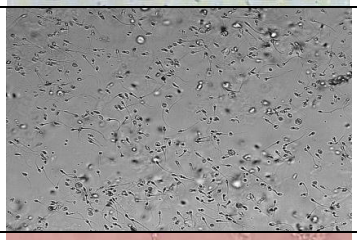
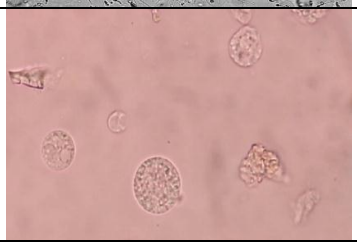
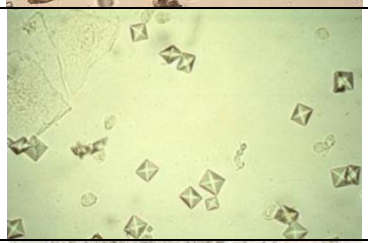
The Microscopic Urinalysis

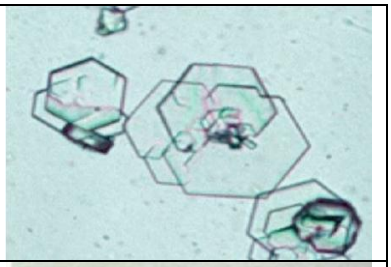
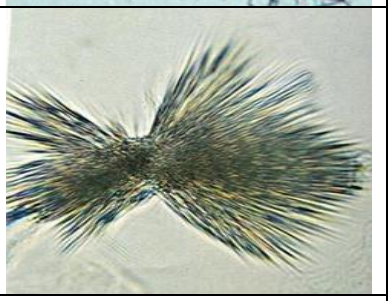
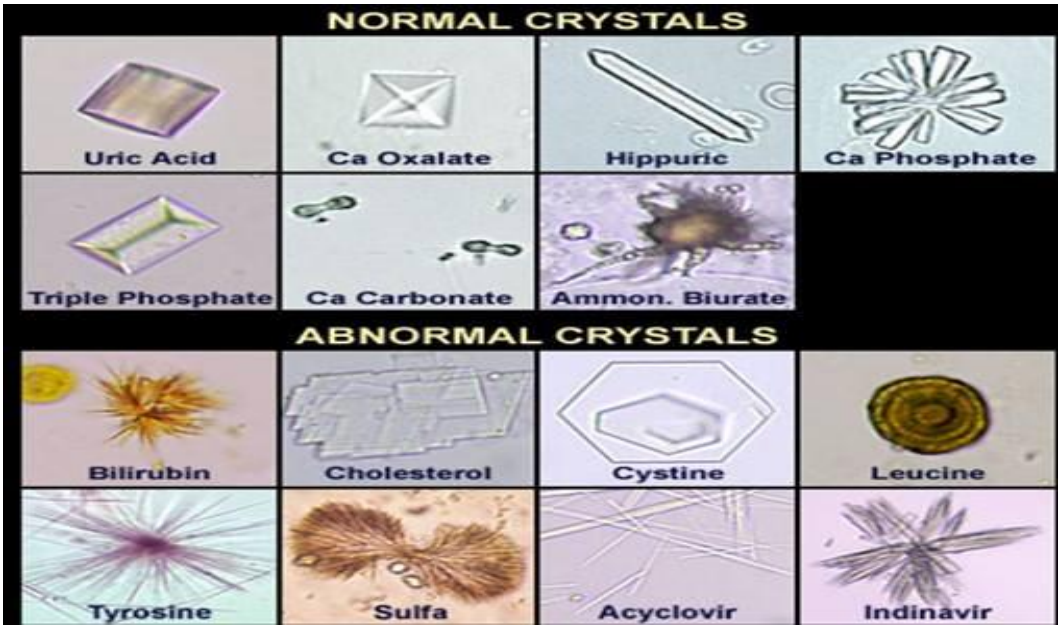
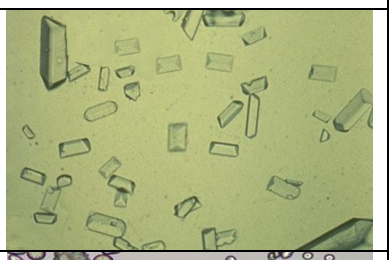
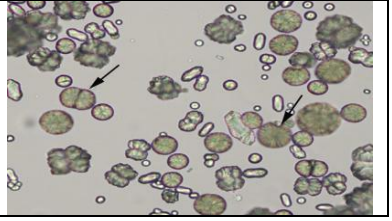
Microscopic examination is an indispensable part of urinalysis; the identification of casts, cells, crystals, and bacteria aids in the diagnosis of a variety of conditions. It will typically be done when there are abnormal findings on the physical or chemical examination. It is performed on urine sediment. To prepare a urine specimen for microscopic analysis; afresh sample of 5 to 10 mL of urine should be centrifuged at 1,500 to 3,000 rpm for five minutes. The supernatant then is discarded and the sediment remixed in the residual liquid. A single drop is transferred to a clean glass slide, and a cover glass is applied smoothly to prevent air bubble. In addition, some entities, if present, are estimated as "few," "moderate," or "many," such as Pus cells (WBCs) epithelial cells, bacteria, and crystals. The numbers of casts seen are usually reported 5-10 hyaline casts/L casts/LPF. Next, examination is carried out at high power to identify crystals, cells, and bacteria. The various types of cells are usually described as 2-4 Pus cells/HPF. Always reported in the standard format i.e. in even number example 0-2, 2-4, 4-6 cells/HPF. Report in difference of 2 up to 10 cells more than that (10 to 20) keep difference of 5 and up to 30 keep difference of 10 i.e. 4-6cell/HPF, 10-15cells/HPF and 20-30cells/HPF. More than 30 cells are reported as Plenty cells/HPF.[5,6,7] Details are tabulated in Table 2.

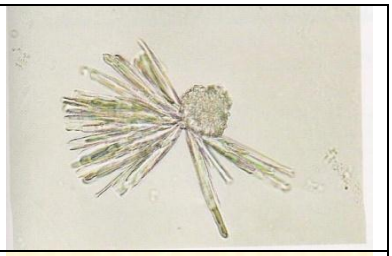
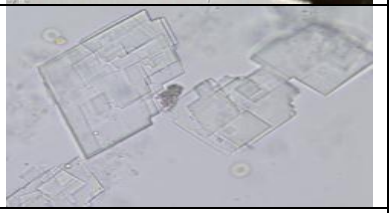
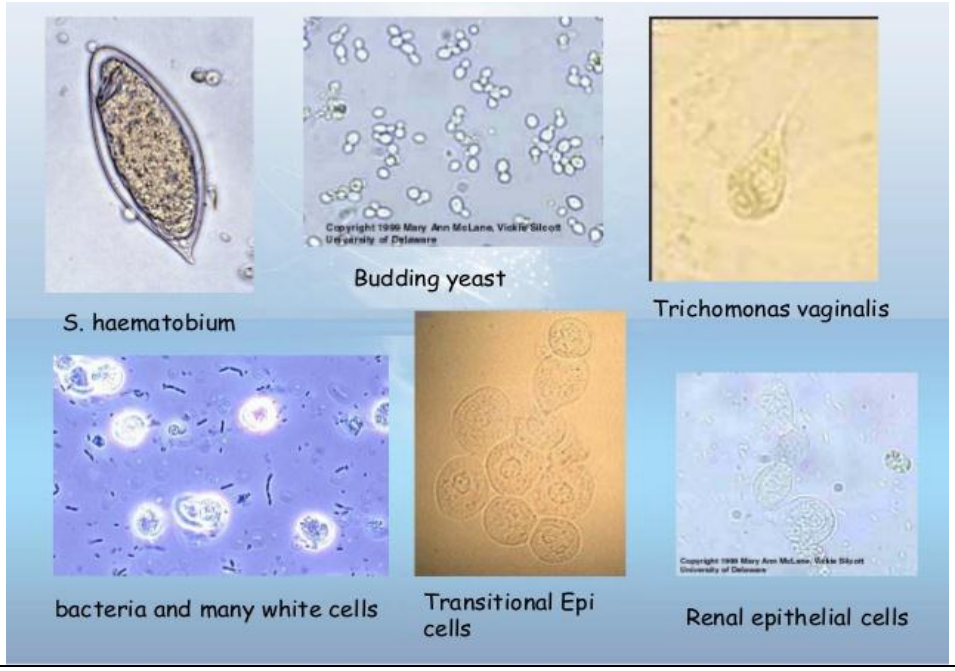
Table 2: Microscopic findings in urine and their description and clinical interpretation.

Variables	Description and Clinical Interpretation	Microscopic Image
Pus Cells(WBCs)	Leukocytes may be seen under low- and high-power magnification. Men normally have fewer than two white blood cells per HPF; women normally have fewer than five WBCs per HPF. The pus cells can enter in urine anywhere from the glomerulus to the urethra. These are mostly neutrophils. Pyuria refers to the presence of abnormal numbers of leukocytes that may appear with infection in urinary tract or with acute glomerulonephritis. When the number is high, it indicates an infection or inflammation somewhere in the urinary tract. WBCs can also be a contaminant, such as those from vaginal and cervical secretions.	
Epithelial Cells	These cells can originate from any site in the urinary tract from the proximal convoluted tubule to the urethra or from vagina. "Three main types of epithelial cells - **Tubular epithelial cells** Slightly larger than leukocytes and large round nucleus. **Transitional epithelial cells** from the renal pelvis, ureter, or bladder have more regular cell borders, larger nuclei, and smaller overall size than squamous epithelium They have pear shaped or round with two nuclei. **Squamous epithelial cells** from the skin surface or from the outer urethra can appear in urine. They are large, flat and irregularly shaped with small central nuclei.	
Erythrocytes (Red Blood Cell)	These seem refrectile particles. 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, nephrotoxins, Inflammation, injury in the bladder or urethra, can cause RBCs to leak out of the blood vessels into the urine, and physical stress. In addition, red cell ghosts may simulate yeast. RBCs can also be a contaminant due to an improper sample collection and blood from hemorrhoids. Normal in menstruation.	
Casts	Urinary casts are formed in the lumen of the tubules of the kidney. Casts can form as the result of precipitation of gelatin of Tammslors fall mucoprotein. Clumping of cells on other material with in protein matrix. Coagulation of material within the lumen.	
a. Granular Casts	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 Significant renal disease, present due to the degeneration of cellular casts.	

b. Hyaline Casts	These are cylindrical particles sometimes found in urine that are formed from coagulated protein secreted by kidney cells. They are formed in the long, thin, hollow tubes of the kidneys known as tubules and usually take the shape of the tubule, Colorless, homogenous, transparent and with round ends. Under the microscope, they often look like the shape of a "hot dog" and in healthy people they appear nearly clear. This type of cast is called a "hyaline" cast. Increased in the mildest kind of renal disease.	
c. Waxy Casts	Have very high refractive index, yellow grey or colorless and have a homogenous appearance found in degeneration of granular casts.	
d. Fatty casts	Formed by incorporated free fat droplets or oral fat bodies. These are seen when there is fatty degeneration of the tubular epithelium, nephritic syndrome and toxic renal poisoning.	
e. Red cell casts	When a disease process is present in the kidney, other things such as RBCs can become trapped in the protein as the cast is formed. Red blood cells may stick together and form red blood cell casts. Contain few RBCs in protein matrix and always pathogenic. Found in acute glomerulonephritis, sub-acute endocarditis and also in severe pyelonephritis and in renal infraction. Glomerular inflammation with leakage of RBC's to produce a red blood cell cast	
f. White cell casts	Appear in polymorphonuclear neutrophils. The cells may be few or many, tightly packed together. Present in renal infection and inflammation. 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.	
g. Epithelial cell casts	Arranged haphazardly and vary in size and shape and rarely seen in urine. Presence of casts indicates tubular degeneration and necrosis. Also present in severe chronic renal disease.	
Mucus threads	Long, thin, waxy threads of ribbon like structures. Found in condition of inflammation or irritation of urinary tract	
Yeast cells	Smooth colourless and usually ovoid cells, vary in size and have doubly retractile walls. 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. Found in urinary tract infection, Diabetes Mellitus and present as a result of vaginal contamination.	

Bacteria	Normally urine is free of bacteria. Less frequently, bacteria from a blood infection (septicaemia) may move into the urinary tract. This also results in a UTI 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. Present in large number with pus cells indicate Urinary Tract Infections. 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.	 
Spermatozoa	Sperms are present in urine. They are present in urine of male after epileptic convulsions, nocturnal emissions and in diseases of genital organ. After coitus they may be present in urine of both sexes. Spermatozoa can sometimes be seen. Rarely, pinworm ova may contaminate the urine.	
Oval fat bodies and fat droplets	When lipiduria occurs, these cells contain endogenous free fat droplets in urine i.e.lipiduria (excretion of lipids in urine). Present in nephrotic syndrome, D.M. lipoid nephrosis and in fat embolism.	
Crystals	Urine contains many dissolved substances (solutes) – waste chemicals that the body needs to eliminate. Found in urine, in calculus formation, metabolic disorders and in medication. These solutes can form crystals, solid forms of a particular substance, in the urine if: The urine pH is increasingly acidic or basic; The concentration of dissolved substances is increased; and The urine temperature promotes their formation. Crystals are identified by their shape, color, and by the urine pH. Abnormal crystals may indicate an abnormal metabolic process.	
Crystals found in ACIDIC URINE		
a. Uric acid	In diamond rhomic or roselt form stained with urinary pigments as yellow or red brown. These are found in gout, chronic nephritis, and acute febrile conditions.	
b. Calcium Oxalate	Colourless and octahedral shaped and dumb-bell shaped also frequently found in acid and neutral urine. These can be present after ingestion of tomatoes, spinach, garlic, and Vit. C Increased of crystals in D.M. Liver disease and chronic liver disease and suggest the possibility of oxalate calculi.	
c. Amorphous urates	There are urate salts of sodium, potassium, magnesium and calcium. Appearance is yellow-red granular. They have no clinical significance.	

d. Cystine crystals	Colourless, retractile, hexagonal plates with equal or unequal sides. They can form calculi. Cystine crystals in urine of neonates with congenital cystinuria or severe liver disease, tyrosine crystals with congenital tyrosinosis or marked liver impairment.	
e. Tyrosine	These appear in the form of fine, vefractile needles occurring in clusters or shaves. Found in liver disease and tyrosinosis.	
f. Leucien	These are oily, highly retractile spheroids with radial and concentric striations, havind yellow or brown colour found in severe hepatitis and acute yellow atrophy Leucine crystals in patients with severe liver disease or with maple syrup urine disease. 	
Crystals found in ALKALINE URINE		
a. Triple phosphate	Colourless prisms with 3 or 6 sides and frequently with oblique end. They can form calculi, found in chronic cystitis, chronic pyelitis, enlarged prostate.	
b. Calcium Carbonate	Small, colorless and in the form of spherical, dumbbell shape or as granular type.	

c. Calcium phosphate	Long, thin, colourless and prism like with one pointed end, arranged as rosettes. These appear as irregular, granular plates. They may also form calculi.	
d. Ammonium biurates	Yellow brown spherical bodies with or without long, irregular spicules. Presence of ammonium bio rates is abnormal if found in fresh urine.	
Cholesterol	These are large, flat and transparent plates with notched corners. Found in nephritis, chyluria, excessive tissue breakdown.	
Parasite, Microorganisms (Bacteria, Trichomonas, Yeast)	In health, the urinary tract is sterile; there will be no microorganisms seen in the urine sediment. Microorganisms are usually reported as "none," "few," "moderate," or "many" present per high power field (HPF). In women (and rarely in men), yeast can be present in urine. They are most often present in women who have a vaginal yeast infection, because of contamination with vaginal secretions during collection. Trichomonads are parasites that may be found in the urine of women or men (rarely). As with yeast, the trichomonads are actually infecting the vaginal canal and their presence in urine is due to contamination. 1.Trichomonas Vaginalis Trophozoites 2.Enterobius Vermicularis 3.Schistosoma Hematobium Ovum. 	

CONCLUSION

In summary, a properly collected clean-catch, midstream fresh voided urine after cleansing of the urethral meatus is adequate for complete urinalysis. A period of dehydration may precede urine collection if testing of renal concentration is desired, but any specific gravity > 1.022 measured in a randomly collected specimen denotes adequate renal concentration so long as there are no abnormal solutes in the urine.

The sample should transported to Laboratory within a hour and should analyzed within two hours of collection. Changes which occur with time after collection include: 1) decreased clarity due to crystallization of solutes, 2) rising pH, 3) loss of ketone bodies, 4) loss of bilirubin, 5) dissolution of cells and casts, and 6) overgrowth of contaminating microorganisms. Urinalysis also called as routine and microscopy examination of urine, and one of the most common tools of clinical diagnosis. The target parameters that can be measured or quantified in urinalysis include naked eye (gross) examination for color and smell plus analysis for many substances and cells, as well as other properties, such as specific gravity. 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.[8]

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