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Urine Analysis Revisited: A Review.

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ABSTRACT

Analysis of urine represents a basic test in the diagnostic battery for most of the systemic disorders and renal diseases in particular. Significant informations can be obtained by urinalysis as many disease processes invariably display urinary abnormalities. It offers a simple, often quick way of following response to treatment in these diseases, without putting the patient under much distress. For urinalysis to be maximally useful as a diagnostic tool, attention to details is necessary in the methodology of urine collection, timely sample processing and most importantly, a thorough knowledge on the various abnormalities that can be detected during the analysis. This article is a comprehensive review on urinalysis, including the intricacies of sample collection, various normal and abnormal physical and chemical parameters with an emphasis on the importance of a meticulous microscopic examination of urine sample to arrive at a diagnosis.

Key words: Urinalysis, microscopy, proteinuria, specific gravity.

INTRODUCTION

The first medical diagnoses made by humans were based on what ancient physicians could observe with their eyes and ears, which sometimes also included the examination of human specimens. The ancient Greeks attributed all diseases to disorders of bodily fluids called humors, and during the late medieval period, doctors routinely performed uroscopy.[1,2]

Ancient physicians also began the practice of examining patient specimens. The oldest known test on body fluids was done on urine as far back as 400 BC. Urine was poured on the ground and observed to see whether it attracted insects. If it did, patients were diagnosed with boils.[1,3]

Hippocrates advocated a diagnostic protocol that included tasting the patient's urine, listening to the lungs, and observing skin color and other outward appearances.[3]

Hippocrates related the appearance of bubbles on the surface of urine specimens to kidney disease and chronic illness. He also related certain urine sediments and blood and pus in urine to disease. The first description of hematuria, or the presence of blood in urine, by Rufus of Ephesus surfaced at around AD 50 and was attributed to the failure of kidneys to function properly in filtering the blood.[3,4]

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Lay medicine based diagnosis on symptoms, examination, pulse, palpitation, percussion, and inspection of excreta and sometimes semen. Diagnosis by "water casting" (uroscopy) was practiced, and the urine flask became the emblem of medieval medicine.[4,5]

The first book detailing the color, density, quality and sediment found in urine was written around this time, as well. By around AD 1300, uroscopy became so widespread that it was at the point of near universality in European medicine.[5]

Uroscopy was widespread in use and had gained popularity as a method to diagnose "chlorosis," or love-sick young women, and sometimes to test for chastity. Other Frederik Dekkers of Leiden, Netherlands, observed in 1694 that urine that contained protein would form a precipitate when boiled with acetic acid—that urinalysis became more scientific and more valuable. The best qualitative analysis of urine at the time was pioneered by Thomas Willis (1621–1675), an English physician and proponent of chemistry. He was the first to notice the characteristic sweet taste of diabetic urine, which established the principle for the differential diagnosis of diabetes mellitus and diabetes insipidus.[6]

Urinalysis is one of the most ancient and basic tests to evaluate the presence, severity, and course of diseases of the kidney and urinary tract. Therefore, when a patient is first seen by a nephrologist, a complete basic investigation of the urine should always be requested.

In most instances, this is done by means of a dipstick, a widely accepted method for

screeningpurposes because of its quick, simple, and inexpensive use. However, clinicians too often are unaware of the principles and limits of this approach, which allows one to detect and obtain an approximate estimation of concentrations of a number of analytes, including albumin, blood, leukocytes, and bacteria.

Clinicians, and nephrologists among them, also often are unaware of the valuable informations that can be obtained with examination of urinary sediment. Today, this usually is performed in central laboratories, where too many samples are analyzed every day (several hundred in many situations), far from the bedside of the patient, and without the correct methods, equipment, and professional qualification.[7]

To achieve best possible results in the urine examination, the following requirements are mandatory: (A) use of a correct method for patient preparation, urine collection and handling; (B) capability to identify the most important particles of the urinary sediment; (C) knowledge of their clinical meaning; and (D) capability to arrange urinary sediment findings into a clinical context. It must be remembered that all nephrological conditions take advantage of a urinalysis of good quality, and negative urinary findings also help in the correct evaluation of a renal patient.[8]

Collection of Specimens

Results vary greatly depending on the way of urine collection and its handling in the laboratory.

Hence, the patient should be given written, simple, and clear instructions for correct urine collection.[9,10]

Instructions for Urine Collection

- Avoid strenuous physical activity in the 72 hours before the collection to prevent exercise-induced proteinuria and/or hematuria or cylindruria.
- Avoid urinalysis during menstrual cycle because it can contaminate with blood, which can erroneously lead to wrong diagnosis of hematuria.
- In case of mild genital discharge (e.g., leukorrhea), use internal tampons to avoid contamination.
- Wash your hands.
- Urethral meatus should be washed after spreading the vulvar labia (female) or withdrawing the foreskin of the glans (male) and wipe with a towel.
- Collect the urine after discarding the first portion of micturition (midstream technique) to reduce contamination from urethral and/or vaginal cells and secretions.
- Close the container tightly and write the name clearly and in full on the label.

Same procedures can also be used for children. For infants, urine bags are used, although they carry a high chance of contamination. Urine particles can lyse very fast after collection, when urine pH is alkaline and/or specific gravity or osmolality is low. It is recommended that the sample should be analyzed within 2 to 4 hours from collection. Samples can be kept at a temperature of 2°C to 8°C; but this procedure causes precipitation of phosphates or urates, making the examination difficult and inaccurate. Preservatives can alter the appearance of particles. Thus, every effort should be made to examine the samples within 2 to 4 hours from collection. [10,11]

Physical Parameters

a) Volume- Urine 24-hour volume test measures the amount of urine produced in a day. Amount of creatinine, protein, and other chemicals released in the urine during this duration is often tested.

For this test, a person should urinate into a special bag or container each time for a 24-hour period.

- On day 1, urinate into the toilet after getting up in the morning.
- Later, collect all urine in a special container for the next 24 hours.
- On day 2, urinate into the container after getting up in the morning.
- Cap the container. Keep it in refrigerator or a cool place during the collection period.
- Label the container with the name, date, time of collection, and return it as instructed.

For an infant:

Wash the area around the urethra properly. Open a urine collection bag (a plastic bag with an adhesive paper on one end).

- For males, place the entire penis in the bag and attach the adhesive to the skin.
- For females, place the bag over the two folds of skin on the either side of the vagina (labia). Put a diaper on the baby (over the bag).

Check the infant, regularly and change the bag after the infant has urinated. Pour the urine from the bag into the container provided.

An active infant can cause the bag to move. It may take more than one try to collect the sample.

When finished, label the container and collect it as instructed.[12,13]

This test is done if there are signs of damage to the kidney function on blood, urine, or imaging tests.

Urine volume and concentrations of following substances are measures in this test:

- Creatinine
- Sodium
- Potassium
- Urea nitrogen
- Protein

This test can also be done if a person has polyuria (abnormally large volumes of urine, e.g., diabetes insipidus.

The normal range for 24-hour urine volume is 800 to 2000 milliliters per day (with a fluid intake of about 2 liters per day). Oliguria is urine output < 500 mL in 24 h (0.5 mL/kg/h) in an adult. Oliguria in a child is urine output < 1 L in 24 h (1 mL/kg/h). These value ranges may differ slightly in different laboratories.

Disorders which cause a reduced urine volume include dehydration, decreased fluid intake, some types of chronic kidney disease.

Examples of conditions that cause increased urine volume include:

- Diabetes insipidus– renal
- Diabetes insipidus– central
- Diabetes
- High fluid intake
- Few kidney disease
- Use of diuretic medicines.[12-13]

b) Colour- In normal conditions, the color of urine varies from pale to dark yellow and sometimes amber. Abnormal color changes can be due to pathological conditions, drugs, and foods

Main Causes of abnormal color changes in urine

Pathological conditions which causes abnormal colour in urine:

Gross hematuria, hemoglobinuria, myoglobinuria (pink colour, red colour, brown colour, black colour) Jaundice (yellow to brown colour), chyluria (white milky urine), massive uric acid crystalluria (pink colour) porphyrinuria, alkaptonuria (red to black color after standing)

Drugs

Rifampin, Vitamin B2, phenazopyridine, isoniazid(yellow-orange to red colour of urine) phenytoin (red colour), chloroquine, nitrofurantoin (brown colour), triamterene, blue dyes administered by feeding tube. (Green colour) metronidazole, methyldopa, imipenem, cilastatin (darkening of urine upon standing)

Foods

Beetroot (red colour), Senna, rhubarb (yellow to brown colour, red) carrot, blackberries (Pinkish red colour)

c) Turbidity-Normal urine is transparent. Urine may be turbid because of an increased concentration of any particle in urine, like erythrocytes, leukocytes, bacteria, squamous epithelial cells, or crystals. Urinary tract infection and contamination due to genital secretions are most common causes of urine turbidity.

It should be noted that pathological samples may be perfectly clear.

d) Odor- Infection is also the most frequent cause of abnormal pungent odor of urine. It is caused by the production of ammonia by bacteria. Some rare pathological conditions give a specific odor to urine: maple syrup urine disease (maple syrup odor), phenylketonuria (musty or mousy odor), isovaleric acidemia (sweaty feet odor), and hypermethioninemia (rancid butter or fishy odor). Ketones can give a sweet or fruity odor. In severe dehydration urine gets very concentrated, and smell strongly of ammonia.

e) Relative Density- Relative density can be measured by using following methods:

Specific Gravity-It is based on the number and weight of dissolved particles. It is measured by using a urinometer. It is a weighted float marked with a scale from 1.000 to 1.060. The urinometer is simple and quick to use, but now, it is outdated.

Osmolality

It is gold-standard method. It depends on the number of particles present. It is measured by using an osmometer. High glucose concentrations can significantly increase osmolality (10 g/L of glucose =55.5 mOsmol/L).

f) Refractometry- It is based on measurement of refractive index. It depends on the weight and size of solutes per unit volume. These days, refractometry is used widely because of the simplicity and ease. It also has good correlation with osmolality.[14]

g) Dry Chemistry- It is the method used in dipsticks. In the presence of cations, a complexing agent releases protons. The proton produces a color change in the indicator bromthymol blue. Due to its simplicity, this method is the most widely used. However, underestimation occurs with urine pH greater than 6.5, whereas overestimation occurs with urine protein concentration greater than 7.0 g/L. Further, it is not sensitive to nonionized molecules, e.g.-glucose and urea. The results are poorer than tests of osmolality and refractometry.[15]

Chemical Parameters

a) pH- In routine practice, pH is commonly measured with the help of dipstick method. This is based on an indicator which covers the pH range 5.0 to 8.5 to 9.0. With this method, significant deviations from true pH can be observed for values less than 5.5 and greater than 7.5. Hence a pH meter with a glass electrode is necessary when accurate measurement is required .Apart from its application in clinical practice, measurement of urine pH is required for correct interpretation of urinary microscopy findings. Intake of proteins and acidic fruits (e.g., cranberries) can result in acidic urine, and diets high in citrate can result in alkaline urine.[16-18]

Urinary pH generally indicates the serum pH, except in patients suffering with renal tubular acidosis (RTA). The inability to acidify urine to a pH of less

than 5.5 even after 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. Type II (proximal) RTA is characterized by an inability to reabsorb bicarbonate. This situation initially causes alkaline urine, but as the filtered load of bicarbonate decreases, the urine becomes more acidic. Evaluation of urinary pH is useful in the diagnosis and management of UTIs and renal calculi. Alkaline urine in UTI patient suggests the presence of a urea-splitting organism. It can be associated with magnesium-ammonium phosphate crystals and can form staghorn calculi. Uric acid calculi are seen in acidic urine.[16-18]

Alkaline tide refers to a condition, normally seen after having a meal, where during the production of hydrochloric acid by the parietal cells in the stomach. Parietal cells can secrete high concentrations of hydrochloric acid into the lumen of the stomach. The apical membrane of this cell contains K^+H^+ ATPase, which is required for proton transport into the lumen. Potassium and chloride channels are also present. The basolateral membrane of the parietal cell have transporters that maintain intracellular homeostasis. Large amounts of bicarbonate generated by the carbonic anhydrase enzyme should be removed from the cell in order to prevent alkalinization. Efflux of bicarbonate into the blood after acid secretion can be detected and is known as the alkaline tide. The alkaline tide is neutralised by secretion of H^+ion into the blood during HCO3− secretion in the pancreas.

b) Hemoglobin/ Hematuria-Hemoglobin is commonly detected by means of dipstick or Benzidene test. Dipstick test is based on the pseudoperoxidaseactivity of the heme moiety of hemoglobin.It catalyzes the reaction of a peroxide and a chromogen to form a colored product. The presence of hemoglobin produces either green spots, which are caused by intact erythrocytes, or a homogenous diffuse green pattern. The green pattern may be caused by marked hematuria because of the increased number of erythrocytes that cover the entire pad surface or by lysis of erythrocytes, which can occur on standing or due to alkaline urine pH and/or low relative density. The most significant false-positive results occur for the presence of hemoglobinuria (from intravascular hemolysis), myoglobinuria (from rhabdomyolysis), or high concentration of bacteria with pseudoperoxidase activity (Enterobacteriaciae species, Staphylococci species, and Streptococci species).[19]

False-negative results are mainly obtained due to ascorbic acid, which is a strong reducing agent, the presence of which can cause a low-grade microscopic hematuria which can be completely missed. In such cases, even though dipstick results are negative,microscopy shows hematuria. Detection of hemoglobin by means of dipstick has 95% to 100% sensitivity and 65% to 93%specificity.[20]

According to the American Urological Association, the presence of three or more red blood cells (RBCs) perhigh-powered field (HPF) in two out of three urine samples is considers as hematuria.[21-23]

Glomerular Hematuria. Glomerular hematuria is associated with significant proteinuria, erythrocyte casts, and dysmorphic RBCs. However, 20 percent of patients with biopsy-proven glomerulonephritis present with hematuria only.[24] IgA nephropathy (i.e., Berger's disease) is the most common cause of glomerular hematuria.

Table 1: Common Causes of Hematuria

Glomerular causes	**Renal causes**	**Urologic causes**
Familial causes Fabry's disease Hereditary nephritis (Alport's syndrome) Nail-patella syndrome Thin basement-membrane disease	Arteriovenous malformation Hypercalciuria Hyperuricosuria Loin pain-hematuria syndrome Malignant hypertension	Benign prostatic hyperplasia Cancer (kidney, ureteral, bladder, prostate, and urethral) Cystitis/pyelonephritis Nephrolithiasis
Primary glomerulonephritis	**Medullary sponge kidney**	**Prostatitis**
Focal segmental glomerulonephritis Goodpasture's disease Henoch-Schönleinpurpura IgA nephropathy (Berger's disease) Mesangioproliferative glomerulonephritis Postinfectious glomerulonephritis Rapidly progressive glomerulonephritis	Metabolic causes Papillary necrosis Polycystic kidney disease Renal artery embolism Renal vein thrombosis Sickle cell disease or trait Tubulointerstitial causes	Schistosomahaematobium infection Tuberculosis Other causes Drugs (e.g., NSAIDs, heparin, warfarin [Coumadin], cyclophosphamide [Cytoxan]) Trauma (e.g., contact sports,
Secondary glomerulonephritis	**Vascular cause**	**Trauma,Running,Catherization, Calculi,**
Hemolytic-uremic syndrome Systemic lupus nephritis Thrombotic thrombocytopenic purpura Vasculitis		

Renal (Non-glomerular) Hematuria. Non-glomerular hematuria is secondary to tubulointerstitial, renovascular, or metabolic disorders. Glomerular hematuria, is often associated with significant proteinuria; but there are no associateddysmorphic RBCs or erythrocyte casts. Further evaluation of

patients with glomerular and non-glomerular hematuria should include determination of renal function and 24-hour urinary protein or spot urinary protein-creatinine ratio.

Urologic Hematuria. Urologic causes of hematuria include tumors, calculi, and infections. Urologic hematuria identified amongst other etiologies by the absence of proteinuria, dysmorphic RBCs, and erythrocyte casts. Even significant hematuria will not increase the protein concentration to the 2+ to 3+ range on the dipstick test.[25] Nearly 20 percent of patients with gross hematuria have urinary tract malignancy.A complete investigation with cystoscopy and upper-tract imaging is indicated in patients with maugnancy.[26] In patients with asymptomatic microscopic hematuria (without proteinuria or pyuria), 5 to 22 percent have serious urologic disease, and 0.5 to 5 percent have a genitourinary malignancy.[27-31] Exercise-induced hematuria is more common, benign condition that usually is associated with longdistancerunning. Results of repeat urinalysis after 48 to 72 hours should be negative in patients with exercise induced hematuria Common causes of hematuria are summarized in [Table1].[32]

Proteinuria

Glomerular capillary wall is permeable only to substances that have a molecular weight of less than 20,000 Daltons. Once filtered, low–molecular weight proteins are reabsorbed and metabolized by the proximal tubule cells. Normal urinary proteins include albumin, serum globulins, and proteins secreted by the nephron. Proteinuria is defined as exertion of urinary protein more than 150 mg per day (10 to 20 mg per dL) and is the hallmark of renal disease. Microalbuminuria is defined as the excretion of 30 to 150 mg of protein per day. It is a sign of early renal disease, especially in diabetic patients. Dipstick tests is sensitive to albumin but it may not detect low concentrations of g-globulins and Bence Jones protein. Dipstick tests used for trace amounts of protein yield positive results at concentrations of 5 to 10 mg per dL—lower than the threshold for clinically significant proteinuria.[33] A result of 1+ corresponds to approximately 30 mg of protein per dL and is considered positive; 2+ corresponds to 100 mg per dL, 3+ to 300 mg per dL, and 4+ to 1,000 mg per dL.[34,35]

Orthostatic (postural) proteinuria is a benign condition resulting from prolonged standing. It is confirmed by getting a negative urinalysis result after eight hours of recumbency. Persistent proteinuria is divided into three general categories: glomerular, tubular, and overflow. The glomerular proteinuria, being the most common type, where albumin is the primary urinary protein. Tubular proteinuria results when malfunctioning tubule cells can no longer metabolize or reabsorb normally filtered proteins. In this condition, low–molecular-weight proteins predominate over albumin and rarely exceed 2 g per day. In overflow proteinuria, low–molecular-weight proteins decrease the ability of the tubules to reabsorb the filtered proteins. Evaluation of persistent proteinuria mostly includes determination of 24-hour urinary protein excretion or spot urinary protein-creatinine ratio, microscopic examination of the urinary sediment, urinary protein electrophoresis, and assessment of renal function.[35]

Twenty-Four–Hour Protein Excretion

It gives an average variation in proteinuria due to circadian rhythm. It is the most accurate method for monitoring proteinuria during treatment. but it is influenced mainly by the rate of diuresis requires proper instructions for urine collection, and can be impractical in some circumstances (e.g., outpatient setting and elderly patients). Also, during collection, urine may undergo contamination and some pre-analytic errors can occur (e.g., incorrect collection and incorrect calculation of urinary volume).[36]

Table 2: Common Causes of Proteinuria

Transient proteinuria	Secondary glomerular causes	Tubular causes
Congestive heart failure	Alport's syndrome	Aminoaciduria
Dehydration	Amyloidosis	Drugs (e.g., NSAIDs,
Emotional stress	Collagen vascular diseases	antibiotics)
Exercise	(e.g., systemic lupus erythematosus)	Fanconi syndrome
Fever	Diabetes mellitus	Heavy metal ingestion
Orthostatic (postural) proteinuria	Drugs (e.g., NSAIDs, penicillamine	Hypertensive nephrosclerosis
Seizures	[Cuprimine], gold, ACE inhibitors)	Interstitial nephritis
Persistent proteinuria	Fabry's disease	Overflow causes
Primary glomerular causes	Infections (e.g., HIV, syphilis, hepatitis,	Hemoglobinuria
Focal segmental glomerulonephritis	post-streptococcal infection)	Multiple myeloma
IgA nephropathy (i.e., Berger's disease)	Malignancies (e.g., lymphoma, solid	Myoglobinuria
IgM nephropathy	tumors)	
Membranoproliferative glomerulonephritis	Sarcoidosis	
Membranous nephropathy	Sickle cell disease	
Minimal change disease		

Protein-Creatinine Ratio on a Random Urine Sample

This is an alternative to 24-hour urine collection. It is easy to obtain and is not influenced by variation in water intake and rate of diuresis. It significantly reduces the pre-analytic errors, and the same sample can be used for microscopic investigation. Many published literature showed sufficient evidence of a strong association between protein creatinine ratio in

a random urine sample and 24-hour protein excretion. However, it should be noted that a normal protein-creatinine ratio is sufficient to know the presence of pathological proteinuria (which reduces the number of unnecessary 24-hour urine collections), while in the case of a protein-creatinine ratio greater than the cutoff value, a full 24-hour quantification is mandated. Also, correlation between protein-creatinine ratio and 24-hour protein excretion might not be accurate at protein levels greater than 1g/L (_0.1 g/dL), and the protein-creatinine ratio for monitoring proteinuria during treatment is still not reliably proven.[36,37]

Leukocyte Esterase:

Dipstick test detects the presence of leukocytes on the basis of indoxyl esterase activity released from broken neutrophils and macrophages. In urine with alkaline pH and/or low relative density, which favors the lysis of leukocytes, there is mostly a positive dipstick result, but negative microscopy findings. While, high relative density values decrease the sensitivity of this dipstick because of the prevention of leukocyte lysis.

False-negative results is also obtained in the presence of high glucose or protein concentrations, or in the presence of cephalotine and tetracycline (strong interference), cephalexine (moderate interference), or tobramycin (mild interference). False-positive results are very rare (eg, formaldehyde is used as urine preservative). Sensitivity varies from 76% to 94%, and specificity, from 68% to 81%. Dipstick can detect up to is 20 x 106 leukocytes/L.[36,37]

Nitrites

It shows the presence of bacteria that have the ability of reducing nitrates to nitrites with nitrate reductase activity. This is present in most gram-negative uropathogenic bacteria, but is not seen in others, such as Pseudomonas species, Staphylococcus albus, and Enterococcus species. Test positivity also requires a diet rich in nitrates (vegetables), which forms the substrate for nitrite production, and sufficient bladder incubation time. Thus, sensitivity of this test is low, whereas the specificity is greater than 90%.[37]

Bilirubin and Urobilinogen

Urine normally does not contain bilirubin in detectable amounts. Unconjugated bilirubin is water insoluble and cannot pass through the glomerulus; conjugated bilirubin is water soluble. It indicates further evaluation for liver dysfunction and biliary obstruction when detected in the urine. Normal urine contains only small amounts of urobilinogen. Urobilinogen, the end product of conjugated bilirubin after it has passed through the bile ducts and has been metabolized in the intestine. Urobilinogen is reabsorbed into the portal circulation only and a small amount is filtered by the glomerulus. Hemolysis and hepatocellular disease can increase urobilinogen levels, and antibiotic use and bile duct obstruction can decrease urobilinogen levels. [36,37]

Glycosuria

In thedipstick test for glycosuria, glucose is first oxidized to gluconic acid and hydrogen peroxide. Then, hydrogen peroxide reacts with a reduced colorless chromogen to form a colored product in presence of peroxidase (catalyst). This test for glycosuria is sensitive to concentrations of 0.5 to 20 g/L. If more precise quantification of urine glucose is required, enzymatic methods such as a hexokinase must be used. False-negative results occur in the presence of ascorbic acid and bacteria, whereas false-positive results can be observed in the presence of oxidizing detergents and hydrochloric acid. Glucose is filtered by the glomerulus, but it is almost completely reabsorbed in the proximal tubule. Glycosuria occurs when the filtered load of glucose exceeds the ability of the tubule to reabsorb it (i.e., 180 to 200 mg per dL). Causes are diabetes mellitus, Cushing's syndrome, liver and pancreatic disease, and Fanconi's syndrome.[36,37]

Ketonuria

Ketones are the products of fat metabolism and are normally not seen in urine. Dipstick reagents detect acetic acid through a reaction with sodium nitroprusside or nitroferricyanide and glycine. Ketonuria is most commonly associated with uncontrolled diabetes i.e., diabetic ketoacidosis, but it can also occur during pregnancy, starvation, vomiting, strenous exercise and carbohydrate-free diets.[36,37]

Microscopy

Microscopic examination is an absolutely necessary part of urinalysis; the identification of casts, cells, crystals, and bacteria helps in the diagnosis of a variety of conditions. To prepare a urine specimen for microscopic analysis, a fresh sample of 10 to 15 mL of urine should be taken. It is then centrifuged at 1,500 to 3,000 rpm for five minutes. The supernatant then is decanted and the sediment resuspended in the remaining liquid. A single drop is placed on a clean glass slide, and a cover slip is applied and slide is viewed under microscope. Non-centifuged samples can also be used to avoid loss/lysis produced by centrifugation,[38] especially for erythrocyte casts. The use of phase contrast microscopes improve the identification of particles, especially for hyaline cast and erythrocytes with low hemoglobin content (aka. 'ghost cells'). Polarised light filters are mandatory for the correct identification of lipids and crystals with unusual appearances.

While observing under microscope, a minimum 20 microscopic fields, should be observed for correct interpretation of the finding, both pH and specific gravity of the sample should be known. Alkaline pH or low specific gravity favours lysis of erythrocytes and leucocytes. pH helps in crystal identification and albumin level helps in the evaluation for glomerular diseases.

1. Written instructions to patients for method of urine collection.
2. Collection in disposable containers of the second urine of the morning after discarding the first few milliliters of urine (midstream technique)
3. Sample handling and analysis within 2-3 hours from Collection.
4. Centrifugation of a 10-mL aliquot of urine at 400g (2,000 rpm) for 10 minutes.
5. Removal by suction of 9.5 mL of supernatant urine gently but thorough, resuspension with a pipette of the sediment in the remaining 0.5 mL of urine.
6. Transfer by pipette of 50 L of resuspended urine to a Slide.
7. Covering sample with a 24 32-mm cover slip.
8. Examination of all samples by a phase contrast microscope at original magnifications, Use of polarized light to identify doubtful lipids and Crystals.
9. Match of microscopic findings with dipstick for pH, specific gravity, hemoglobin, leukocyte esterase, and albumin.
10. For routine work, cells expressed as lowest to highest number seen/high-power field, casts as number/low power field.

Two groups of cells can be found in urine: cells deriving from the circulation (ie, erythrocytes, leukocytes, and macrophages) and cells deriving from epithelia (ie, renal tubular cells, urethelial cells, and squamous cells.

Table 2: Cells of the Urinary Sediment

Cell	Subtype	Main Clinical Associations
Erythrocytes	Dysmorphic Isomorphic	Glomerular disease Nonglomerular disease
Leukocytes	Polymorphonuclear	Urinary infection and contamination, interstitial nephritis, urological diseases
	Eosinophils	Acute interstitial nephritis, prostatitis, cholesterol embolism, etc
	Lymphocytes	Acute cellular rejection of kidney allograft
Macrophages	Fatty, granular, phagocytic, vacuolar	Marked proteinuria, active glomerulonephritis, immunoglobulin A nephropathy
Renal tubular epithelial cells	Ovoidal to columnar, depending on the tubular segment they come from	Acute tubular necrosis, acute interstitial nephritis, acute cellular rejection of kidney allograft, proliferative glomerulonephritis
Uroepithelial	Deep	Severe urological diseases
	Superficial	Urinary tract infection, urological disorders
Squamous	–	Contamination of urine from genital secretions

Erythrocytes

They are frequently found in the urine of patients with kidney disease. Clinically, they show morphological variations, Isomorphic erythrocytes are similar to erythrocytes found in the blood, suggestive of hematuria of urological origin. Dysmorphic erythrocytes have irregular shape and contours and found in glomerular disease. Hematuria is considered to be of glomerular origin when 40% or more are of dysmorphic and 5% or more erythrocytes are acanthocytes.[39]

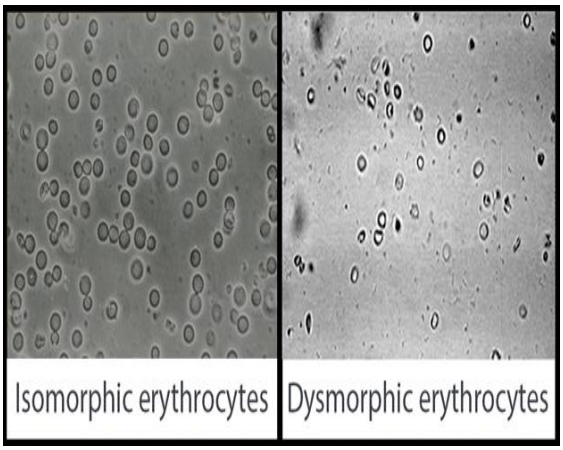

Figure 1: Isomorphic &Dysmorphic Erythrocytes

Leucocytes

Men normally have less than two white blood cells (WBCs) per HPF (High Power Field); Women normally have less than five WBCs per HPF.[39]

Bacteriuria

Clean catch urine sample from female patients are mostly contaminated by the vaginal flora. In these patients, five bacteria per HPF represents roughly 100,000 colony forming units (CFU) per mL, commensurating with asymptomatic bacteriuria and is compatible with a UTI. In symptomatic patients, a colony count as low as 100 CFU per mL suggests UTI, and antibiotics should be Administered. The presence of bacteria in a collected male urine specimen with full precautions is suggestive of infection, and a culture should be performed.

Casts

Casts comprise a coagulum of Tamm- Horsfall mucoprotein and trapped contents of tubule lumen, originate from the distal convoluted tubule or collecting duct during urinary concentration or stasis, high solute concentration, abnormal ionic or

protein constituents or when urinary pH is very low. Their cylindrical shape reflects the tubule in which they were formed and housed when the casts are washed away. The predominant cellular elements determine the type of cast which include: hyaline, erythrocyte, leukocyte, epithelial, granular, waxy, fatty, or broad etc.

Table 3: Classification, Appearance, and Clinical Associations of Casts

Cast	Appearance	Main Clinical Associations
Hyaline	Colorless, easily missed with right field microscopy	Normal subject and renal disease
Hyaline-granular	Variable amounts of granules plunged in the colorless matrix of the cast	Normal subject and renal disease
Granular (finely and coarsely granular)	Fine granules caused by lysosomes containing ultrafiltered proteins	Renal disease of whatever nature
	Coarse granules caused by degenerated RTECs or leukocytes entrapped within the cast	
Waxy	Large, with hard and indented contours and a "melted wax" appearance	Renal insufficiency, either acute or chronic
Fatty	Containing various amounts of lipid droplets, rarely cholesterol crystal	Marked proteinuriaNephrotic syndrome
Erythrocytic	Containing erythrocytes (from few to uncountable, sparse or packed), occasionally with a brownish hue	Proliferative/necrotizing GN Glomerular bleeding (of particular value in patients withisolated microscopic hematuria of unknown origin)
Hemoglobin	With a brownish hue; often with a granular appearance caused by the degradation of erythrocytes	The same as the erythrocytic casts Hemoglobinuria
Leukocytic	Containing leukocytes	Acute interstitial nephritis Acute pyelonephritis
RTEC (epithelial) casts	Containing RTECs	Acute tubular necrosis Acute interstitial nephritis GN (especially of proliferative type)
Myoglobin	Similar to hemoglobin casts	Rhabdomyolysis
Bilirubin	Yellow	All conditions associated with bilirubinuria
Containing microorganisms	Containing bacteria or yeasts	Bacterial or fungal infection of the kidney
Containing crystals	Containing uric acid, calcium oxalate, etc	Crystalluria with/without renal function impairment
Mixed	Waxy-granular, fatty-granular, granular-erythrocytic, etc	See waxy, granular, fatty,erythrocytic casts

Crystals

Table 4: Common Crystals

Crystal	Urine pH	Birefringence	Most Frequent Appearance
Uric acid	≤5.8	+ (polychromatic)	A wide spectrum (most typical: lozenges), amber color common to all appearances
Amorphous urates	≤5.8	+	Irregular granules
Monohydrated calcium oxalate(Whewellite)	5.4-6.7	+	Ovoids, dumb-bell, biconcave disks
Bi-hydrated calcium oxalate (Wedellite)	5.4-6.7	_	Bipyramidal
Calcium phosphate	≥7.0	+	Prisms, star-like particles, or needles of various sizes
		_	Plates
Triple phosphate	≥7.0	+	"Coffin lids
Amorphous phosphates	≥7.0	_	Irregular granules

Table 5: Pathological Crystals (Fig 4A and B)

Crystal	Urine pH	Birefringence	Most Frequent Appearance
Cholesterol	5.4-6.7	-	
Cystin	Variable (-to+)		Hexagonal plates with irregular sides, often heaped 1 upon the other
2,8-Dihydroxyadenine	≤5.8	+ (Maltese cross)	Spherical, brownish crystals with radial striations from the center

Table 6: Main Crystals Caused by Drugs

Drug	Crystal Appearance	Clinical Manifestations
Sulfadiazine	Birefringent "shocks" of wheat or "shells" with striations	Isolated crystalluria, hematuria, Acute Renal failure, stones
Acyclovir	Birefringent fine needles	Isolated crystalluria, Acute Renal failure
Indinavir	Birefringent plate-like rectangles, star-like forms, irregular plates	Isolated crystalluria, stones, ARF
Piridoxylate	Asymmetrical hexagons or rectangles with rounded extremities	Crystalluria and stones
Primidone	Birefringent hexagons	Isolated crystalluria, transient hematuria
Felbamate	Needles, cat-tail configuration	Macroscopic hematuria, Acute Renal failure

Amoxicillin	Birefringent needles, shocks of wheat	Isolated crystalluria, hematuria, Acute Renal failure
Ciprofloxacin	Birefringent needles, sheaves, stars, fans, butterflies, etc	Isolated crystalluria, Acute Renal failure
Naftidrofuryl oxalate	Birefringent monohydrate calcium oxalate	Acute Renal failure
Vitamin C	Birefringent monohydrate calcium oxalate	Acute Renal failure
Orlistat	(No better defined) calcium oxalate	Acute Renal failure

Table 7: Main Urinary Sediment Profiles

Clinical Condition	Urine Sediment Hallmark	Associated Urine Sediment Findings
	Marked cylindruria	RTEC casts Microscopic hematuria: absent (ie, minimal change disease) to mild (ie, membranous nephropathy) or moderate (ie, focal segmental glomerulosclerosis)
Nephritic syndrome	Moderate to severe dysmorphic hematuria Erythrocytic/hemoglobin cylindruria	Mild leukocyturia RTECs RTEC casts Waxy casts
Acute tubular necrosis	Necrotic/damaged RTECs Tubular fragments Cylindruria with RTEC casts and muddy brown granular casts	ariable according to cause (eg, high numbers of erythrocytes and erythrocytic casts in proliferative/necrotizing GN, myoglobin casts in rhabdomyolysis, uric acid crystals in acute uric acid nephropathy, calcium oxalate crystals in ethylene glycol intoxication)
Urinary tract infection	Leukocyturia	Superficial transitional cells
	Bacteriuria	Triple phosphate crystals (for infections due to urease-producing bacteria) Leukocyte casts (in renal infection)
Urinary contamination from genital secretions	Leukocyturia Bacteriuria	Massive amounts of squamous epithelial Cells Candida and/or trichomonasvaginalis
Urologic disorders	Isomorphic hematuria	Deep urothelial cells
	Leukocyturia	Superficial urothelial cells

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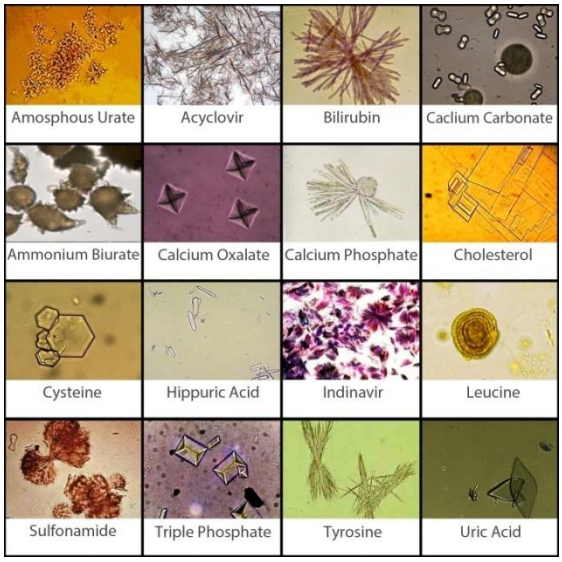

Figure 2: Types of urinary crystals

CONCLUSION

These days, many automated instruments are used in large laboratories to screen large numbers of patients in a short time.We believe this approach is inadequate not only in patients with renal disease but also new patients with complain in the urine. In them, manual microscopy by an experienced examiner represents the gold standard.

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