md pg essay on urinalysis

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URINALYSIS : LIQUID BIOPSY OF THE KIDNEY

Abstract :

Urinalysis was the oldest laboratory investigation to be performed on humans and has been in use for several thousand years. However, its clinical value in providing crucial diagnostic information is not known to all. Right from sample collection through transport, storage, preparation and examination of the specimen, there are several loopholes which can lead to reporting with a loss of valuable information. Attempts must be made to squeeze out maximum information from the so called biological waste of the body. Hence, it is essential to know the entire process behind a simple laboratory investigation called The Urinalysis.In the laboratory, the urine can be characterized by physical appearance, chemical composition, and microscopically. Physical examination of the urine includes description of the colour, odor, clarity, volume and specific gravity. Chemical examination of urine includes the identification of protein, blood cells, glucose , pH, bilirubin , urobilinogen, ketone bodies, nitrites and leukocyte esterase. Finally, microscopic examination entails the detection of crystals, cells, casts and micro organisms. Further, there have been recent advances regarding the urine as a metabolome. The following text is an attempt at describing the process of analyzing the urine which if performed accurately can provide pivotal information – precise enough to consider the urine –a liquid

biopsy of the kidney. Being a commonly performed laboratory investigation, it shouldn't be forgotten that it takes great patience, knowledge and application of the same in performing it – and it is firmly believed that trained physicians and nephrologists regain possession of the examination of the urine sediments of the patients.

Introduction :

Laboratory medicine began with the analysis of human urine which was called uroscopy and today is termed urinalysis. As early as 1500 BC, physicians noted that ants were attracted to the urine of certain patients who had complaints of frequent urination, fatigue and thirst. They tagged it the honey'ed urine. Interestingly, Greeks believed that it was the melting down of flesh and limbs into the urine.Apparently, the first test for Diabetes was the urine taste test, where they had people called water tasters to diagnose diabetes. It was only in 1800s when scientists developed tests to detect sugar in urine.

Dipsticks are the most widely used method for urinalysis, and sadly many clinicians are unaware of its limitations. Urine sediment examination is an integral part of urinalysis which should ideally be performed by a trained nephrologist rather than a laboratory personnel who are at times unable to identify important elements and who are not always aware of its clinical correlates.

Sample collection -Peeing in a cup :

The first step in acquiring a reliable lab test would be an ideal sample collection technique – THE CLEAN CATCH sample. Urine, being a sample that can be easily collected from patients is prone to a lot of contamination. It is necessary that the patient be instructed clearly regarding the collection procedure. They must be advised to avoid strenuous physical exercise in the 72 hours preceding collection and to avoid sample collection during menstruation.

The first morning urine is traditionally considered to be the standard specimen for urinalysis as it best correlates with a 24-hour urine sample.Patients should be instructed to wash their hands before sample collection. Men should retract the foreskin and wash or use sterile wipes to clean the external genitalia. Women should separate the labia and clean the area around the urethral meatus with sterile wipes going from front to back. The same procedures are used for children. For infants and very young children, external genitalia are cleaned and dried using wipes and towels, and a sterile urine collection bag is placed over the area using adhesives to adhere to the skin. A mid-stream clean catch sample is preferred as this is least likely to be contaminated.

Urethral catheterization is usually reserved for patients who are unable to void due to urinary obstruction, incontinence, or impaired consciousness. Suprapubic needle aspiration of the bladder is mostly done in infants and is only used in older children or adults if urine cannot be obtained by any other means.Whatever the collection method, it is recommended that a sample be analyzed within 2 to 4 hours of the time of collection to prevent cell lysis and precipitation of solutes.

MACROSCOPIC EXAMINATION

- **Color**

The pigment urochrome imparts the pale yellow colour to normal urine which can be altered in different conditions as given below :

Red, brown, black	Hematuria, hemoglobinuria, myoglobinuria
Milky	Chyluria
Pink	Uric acid crystalluria
Red to black upon standing	Porphyrinuria, alkaptonuria
Brown	Chloroquine, nitrofurantoin
Red	Phenytoin
Yellow-orange	Rifampin

- **Turbidity**

The clear nature of urine may be altered when there is an increased concentration of any particle , especially erythrocytes, leukocytes, bacteria or epithelial cells. Occasionally, the precipitation of phosphate crystals in alkaline urine and urate crystals in acidic urine may give rise to cloudy urine in the absence of any disease. Foamy urine suggests moderate to heavy proteinuria.

- **Odor**

Urine odor usually does not have much clinical significance. A pungent odor may indicate bacterial ammonia production. Sweet or fruity odor suggests ketonuria. Certain rare hereditary metabolic diseases may give rise to strong unusual urine odors as given below :

Odor	**Cause**
Foul , ammoniacal	UTI
Fruity	Ketonuria
Maple syrup	Maple syrup urine disease
Mousy odor	Phenylketonuria
Rancid	Tyrosinemia
Sweaty feet	Isovaleric academia
Cabbage	Methionine malabsorption

DIPSTICK EXAMINATION : A MARVEL OF COLOURS AND INGREDIENTS:

Provides a rapid semiquantitative assessment of urinary characteristics on a series of test pads embedded on a reagent strip.However , being a very convenient test to perform, the prevalence of false positives and false negatives cannot be denied.

- **Relative density :**

Specific gravity (SG) refers to the weight of a volume of urine compared with the weight of the same volume of distilled water and depends on the mass and number of dissolved particles. Relying on the fact that there is generally a linear relationship between urine's ionic strength and its specific gravity, dipstick method is used. The release of hydrogen ions when they are competitively replaced with urinary cations causes a change in the pH-sensitive indicator dye. Specific gravity values measured by dipstick tend to be falsely high if the urine pH is less than 6 and falsely low if the pH is higher than 7.

Osmolality, the gold standard for relative density, is defined as the number of osmoles of solute per kilogram of solvent. It is measured directly with an osmometer. The measurement of osmolality is more reliable than SG by either dipstick or refractometry for the evaluation of pathologic urine.

- **pH :**

The normal range for urine pH is 4.5 to 7.8. Significant deviations from true pH occur with values less than 5.5 or greater than 7.5 with reagent strip methods. Therefore, a pH meter with a glass electrode is mandatory if an accurate measurement is necessary.

- **Hemoglobin :**

Hemoglobin is detected by a dipstick on the basis of the pseudoperoxidase activity of the heme moiety of hemoglobin, which catalyzes the reaction of a peroxide and a chromogen to form a colored product. The presence of hemoglobin is shown as green spots, which results from intact erythrocytes, or as a homogeneous, diffuse green pattern-this can result from marked hematuria because of the high number of erythrocytes that cover the whole pad surface, from lysis of erythrocytes favored by delayed examination, alkaline urine pH, or low SG or from hemoglobinuria secondary to intravascular hemolysis.

False-negative results are mainly caused by (1) ascorbic acid, a strong reducing agent, which can result in low-grade microscopic hematuria being completely missed and (2) high SG.

The most important causes of false-positive results are myoglobinuria, resulting from rhabdomyolysis, and a high concentration of bacteria with pseudoperoxidase activity (Enterobacteriaceae, staphylococci, and streptococci).

- **Glucose :**

With glucose oxidase as catalyst, glucose is first oxidized to gluconic acid and hydrogen per- oxide. Through the catalyzing activity of a peroxidase, hydrogen peroxide then reacts with a reduced colorless chromogen to form a colored product. This test detects concentrations of 0.5 to 20 g/l.

False-negative results with glucose detection occur in the presence of ascorbic acid and bacteria. False-positive findings may be observed in the presence of oxidizing detergents.

- **Protein :**

The presence of protein in a buffer causes a change in pH that is proportional to the concentration of protein itself. Thus, dipstick changes its color from pale green to green and blue according to pH changes induced by the protein. This method is sensitive to albumin (detection limit, 0.020 to 0.025 g/dL [0.20 to 0.250 g/L])whereas it has very low sensitivity to other proteins, such as tubular proteins and light chain immunoglobulins. In addition, dipstick allows only an approximate quantification of urine albumin.

Twenty-Four–Hour Protein Excretion

This approach represents the gold standard method, averages the variation in proteinuria caused by circadian rhythm, and is the most accurate for monitoring proteinuria during treatment. But it's cumbersome procedure and can have pre-analytical errors while collection.

Protein-Creatinine Ratio

This is a recommended alternative to 24-hour urine collection. It is easy to obtain, is not influenced by variation in water intake and rate of diuresis, greatly reduces pre-analytic errors, and the same sample can be used for microscopic investigation.

- **Leukocyte esterase and nitrites**

The leukocyte esterase dipstick test evaluates the presence of leukocytes based on the activity of an indoxyl esterase released from lysed neutrophil granulocytes.False positive results occur when leukocytes are lysed, because of low relative density, alkaline pH, or a delay in sample handling and examination. False-negative results derive from high glucose (≥20 g/l) or high protein (≥5 g/l) concentration or from the presence of antibiotics such as cephalothin and tetracycline , cephalexin, or tobramycin or ascorbic acid.It may take up to 4 hours to convert nitrate to nitrite, so inadequate bladder retention time can also give false-negative results.

- **Ketones**

The ketone dipstick tests for acetoacetate and acetone (but not β-hydroxybutyrate), which are excreted into urine during diabetic acidosis or during fasting, vomiting, or strenuous exercise. It is based on the reaction of the ketones with nitroprusside.

URINE MICROSCOPY : SHOWING THE UNSEEN

Urinalysis was once considered a liquid window through which physicians felt they could view the body's inner workings which is the concept here in microscopy.

- **Preparation :**

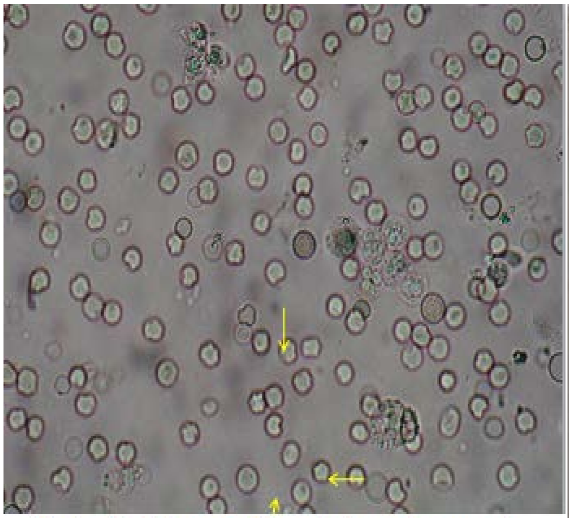
In contrast to the first morning sample which was preferred before, the second catch sample is favored owing to the lysis of urine particles after prolonged standing of urine in the bladder overnight.

Preparation of the urine sediment and examination methods are of the utmost importance. About 10 mL of fresh or properly stored urine is centrifuged in a conical tube at approximately 2,000 revolutions per minute for at least 5 minutes. The supernatant is carefully decanted and the pellet is resuspended in a small amount of urine that remains in the tube by gentle agitation. A pipette is then used to transfer a drop of this resuspended pellet onto the microscope slide. A coverslip is gently placed on top of the urine before transferring the slide to microscope. The sediment is usually examined unstained. Papanicolaou stain may be used to enhance details, and Wright's or Hansel's stain is used in special circumstances to identify eosinophils.

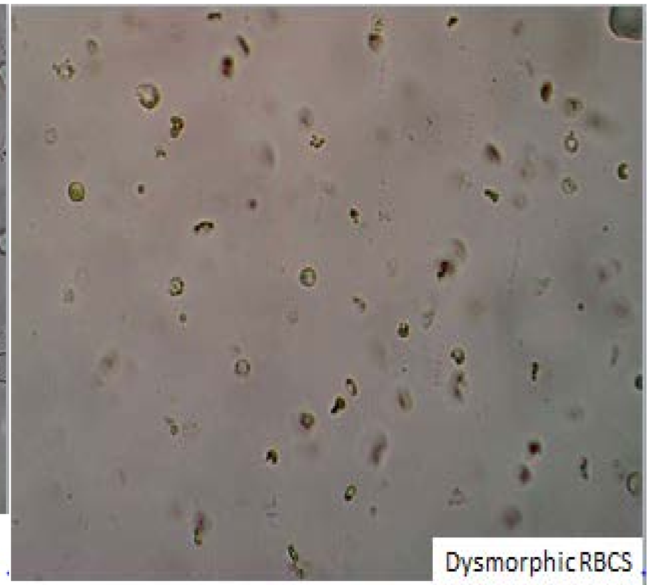
Phase contrast microscopy is recommended because it improves the identification of almost all particles, whereas polarized light is mandatory for the correct identification of some lipids and crystals.At least 20 microscopic fields, in different areas of the sample, should be examined at both low magnification (e.g., ×100 or ×200) and high magnification (e.g., ×400). The use of counting chambers, though recommended by international guidelines is not used in everyday practice. When precise quantification of cells is needed, we count them as total number found over 20 microscopic fields at original magnification.

- **Erythrocytes**

In the urine, there are two main types of erythrocytes: isomorphic, with regular shapes and contours, derived from the urinary excretory system; and dysmorphic, with irregular shapes and contours, which are of glomerular origin .



Cluster of WBCs :arrows (larger in size, nucleus present, cytoplasmic granules seen). Rest are all fresh RBCs



In many, glomerular hematuria is diagnosed when there are 40% or more dysmorphic erythrocytes and/or 5% or more acanthocytes and/or one or more red blood cell casts/50 lpf (×160). With this criterion, a good correlation was found between urinary sediment and renal biopsy findings.

- **Leukocytes**

The most common leukocytes found in the urine, neutrophils are usually an indication of infection or contamination. Eosinophils are detectable with Wright's stain or Hansel's stain. The list of diseases that may be associated with eosinophiluria is diverse.The diagnostic value of the presence of other leukocytes, such as lymphocytes and macrophages, is currently limited.

Macrophages are mononucleated or multinucleated cells of variable size and variable appearance.In patients with the nephrotic syndrome, macrophages may be engorged with lipid droplets, appearing as oval fat bodies.

- **Renal Tubular Epithelial Cells**

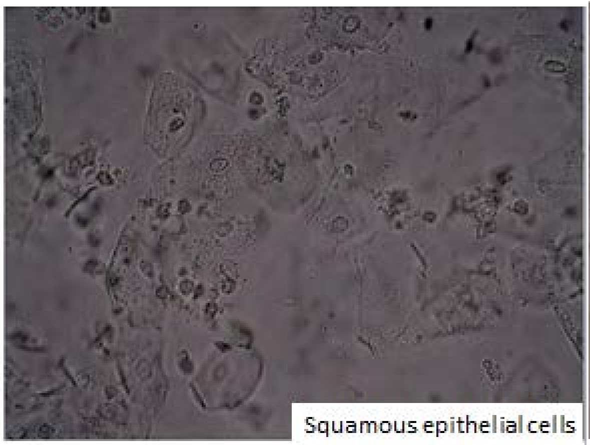
The renal tubular epithelial cells derive from the exfoliation of the tubular epithelium. In the urine, RTECs can differ in size (diameter ~9 to 25 µm) and shape, from roundish to rectangular or columnar, with a central or peripheral large nucleus .

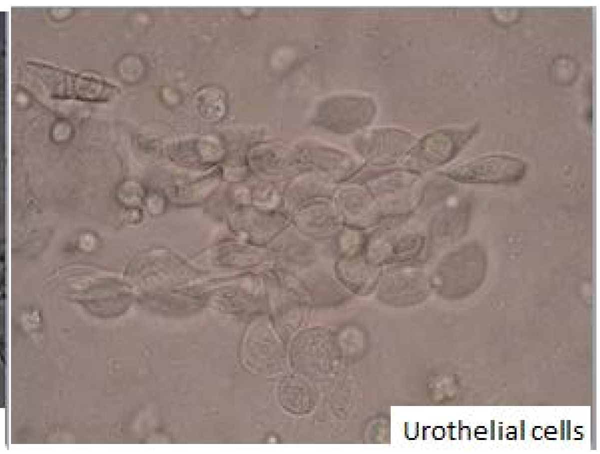
- **Transitional Epithelial Cells**

This multilayered epithelium has small cells in the deep layers and large cells in the superficial layers. When present in large numbers (e.g., ≥1/high- power field [hpf]), cells of the deep epithelial layers suggest severe damage of uroepithelium, as caused by neoplasia, stones, obstruction, or longstanding bladder catheters or ureteral stents.

- **Lipids**

Lipids are found in the urine as drops, which are spherical, translucent, yellowish particles of different size that can be isolated or in clusters ; as oval fat bodies,as fatty casts and cholesterol crystals . All these particles contain mainly cholesterol esters and free cholesterol and under polarized light have the appearance of Maltese crosses with symmetric arms . These lipids are typical of glomerular diseases associated with marked proteinuria.

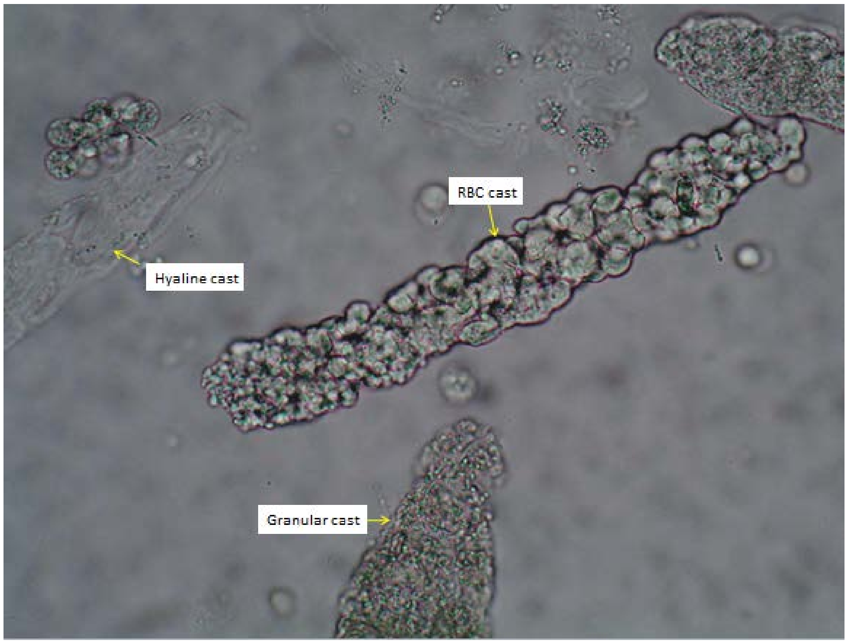

Squamous epithelial cells


Urothelial cells

Cells	Subtype	Clinical association
Erythrocytes	Dysmorphic Isomorphic	Glomerular disease Nonglomerular disease
Leukocytes	Polymorphonuclear Eosinophils Lymphocytes	Infection,contamination,interstitial Nephritis Interstitial nephritis, prostatitis, cholesterol embolism Cellular rejection of kidney graft
Macrophages	Fatty, granular, phagocytic,vacuolar	Marked proteinuria,Glomerulonephritis,IgA Nephropathy
Renal tubular epithelial cells	Ovoidal-colulmnar, depending on the tubular segment they come from	Acute tubular necrosis,interstitial nephritis, Cellular rejection of kidney graft
Uroepithelial	Deep Superficial	Severe urological disease Urinary tract infection, urological disorder
Squamous		Contamination of urine from Genital secretions

- **Casts**

Casts are cylindrical structures that form in the lumen of distal renal tubules and collecting ducts. Their matrix is made of TammHorsfall glycoprotein. Trapping of particles within the cast matrix results in casts with different appearances, each of which may have specific clinical significance as given below:



Cast	Appearance	Clinical association
Hyaline	Colorless , easily missed with bright 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 or coarse granules	Renal disease of any nature
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.	Marked proteinuria Nephrotic syndrome
Erythrocytic	Containing erythrocytes occasionally with a brownish hue	Proliferative/necrotizing GN Glomerular bleeding
Hemoglobin	With a brownish hue, a granular appearance caused by the degradation of erythrocytes	Hemoglobinuria
Leukocytic	Containing leukocytes	Acute interstitial nephritis Acute pyelonephritis
RTEC (epithelial) casts	Containing RTECs	Acute tubular necrosis

		Acute interstitial nephritis GN (proliferative type)
Myoglobin	Similar to hemoglobin casts	Rhabdomyolysis
Bilirubin	Yellow	Bilirubinuria

- **Crystals**

Examination of the urine for crystals is informative in the assessment of patients with stone disease, with some rare inherited metabolic disorder and with suspected drug nephrotoxicity.Crystals can be classified in four categories: common, pathologic, caused by drugs, and other crystals.

Common Crystals

Uric acid crystals have an amber color and a wide spectrum of appearances, including rhomboids and barrels. These crystals are found only in acid urine (pH 5.0 to 5.8) and are polychromatic under polarizing light.

Calcium Oxalate Crystals -two types: bihydrated crystals, which most often have a bipyramidal appearance , and monohydrated crystals, which are ovoid, dumbbell shaped, or biconcave disks.

Brushite (Calcium Phosphate Crystals) and Amorphous Phosphates crystals are pleomorphic, appearing as prisms, star-like particles, or needles of various sizes and shapes .

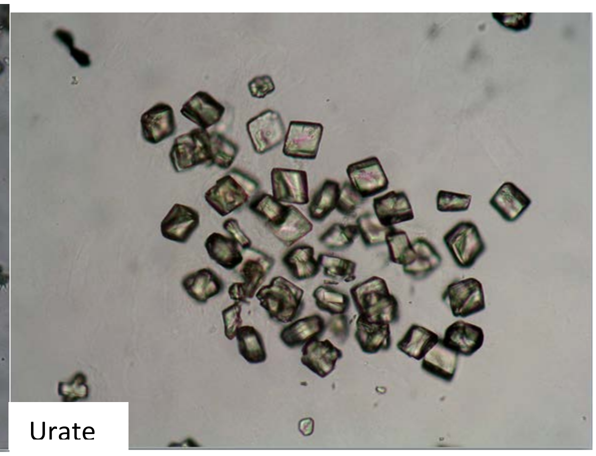
Struvite crystals contain magnesium ammonium phosphate and typically have the appearance of “coffin lids”.

Pathologic Crystals

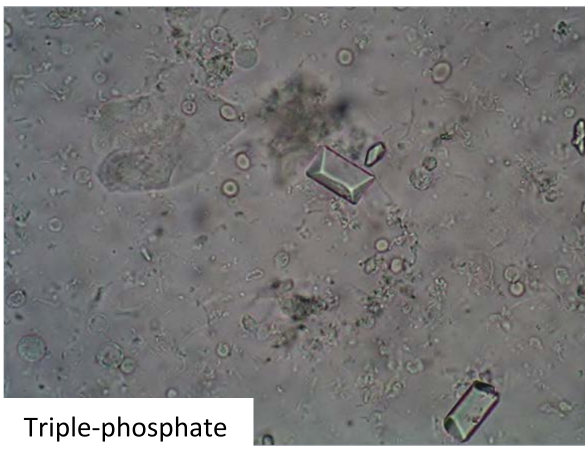
Cholesterol crystals are thin, transparent plates, often clumped together, with sharp edges

Cystine crystals occur in patients with cystinuria and are hexagonal plates with irregular sides that are often heaped on one another . They precipitate in acid urine. Evaluation of their size can be used to predict the recurrence of cystine stones.

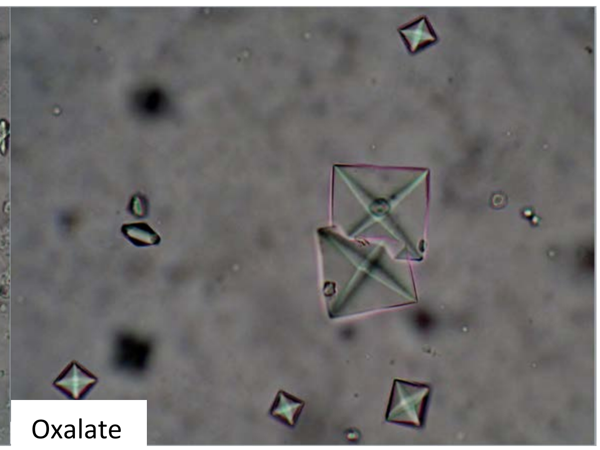
Ammonium-urate



Urate



Triple-phosphate



Oxalate

Crystals Caused by Drugs

Many drugs can cause crystalluria, especially in a setting of drug overdose, dehydration, or hypoalbuminemia in the presence of a specific urinary pH favoring drug crystallization.

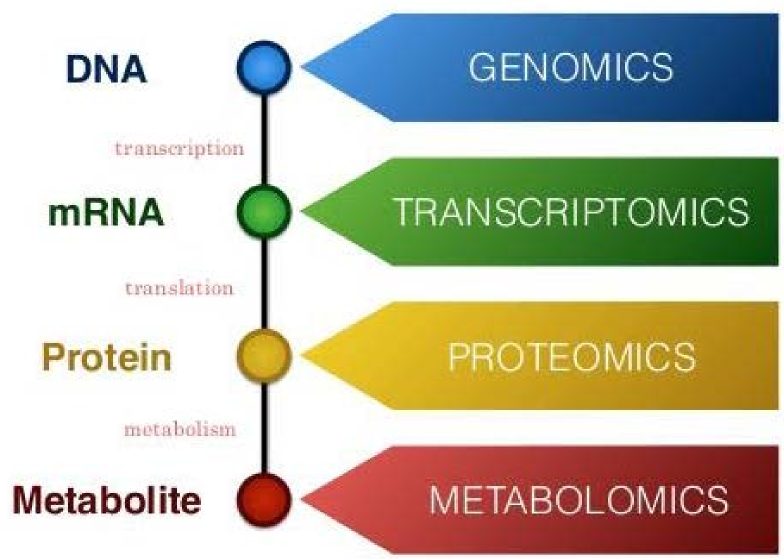
- **Automated urine analyzers**

Automatic analyzers can rapidly analyze a large number of urine samples with high repeatability. However, they do not recognize such particles of nephrological importance as lipids and RTECs and give too many false-negative results for casts (flow cytometry, 15% to 40%; digital imaging system, 60%). That's where a trained nephrologist plays his role.It has been suggested that automatic urine analyzers can be used for screening urine samples. When combined with urine chemistry analysis, these analyzers may provide a rapid and accurate screening in routine urine analysis.

- **The 'Omics' Analysis :**

The term “omics” describes the scientific approach aiming at studying a distinct class of biological molecules in a comprehensive way.Primarily two omics analysis are under studies –(1)Proteome-the entire set of proteins expressed by a cell,tissue or organism at a certain time.They are directly responsible for cell functions and therefore abnormal protein expression is an indication of a cellular pathology.

(2)Metabolomics-the study of all small molecules(exogenous and endogenous with molecular weight<1000Da) in biological fluids and tissue organism. Among the omics technology, metabolomics represent the most distal and the one which is close to the phenotype.



A major goal of omics is to identify unique disease markers so that diagnostic tests with high sensitivity and specificity may be developed. Metabolites in urine, as end products of normal and pathologic cellular processes, are closely linked to phenotypes. Because of this, considerable effort has been made over the last 10 years to study urinary metabolomics as a diagnostic tool.

This non-invasive approach has revealed homeostatic imbalances in biologic systems and has the capability to provide comprehensive information on putative biomarkers for the noninvasive monitoring of disease. It is clear that further research on this forefront technology is clearly warranted to facilitate elucidation of biochemical mechanisms of pathophysiology and early detection thereof.

- **Conclusion :**

Looking at the urine has come a long way from mere visual inspection in antiquity to the current detailed chemical analysis and microscopic examination reported by clinical laboratory. A renaissance of the oldest diagnostic tool of medicine is now under way in the proteomic profiling and detection of biomarkers in the urine – an approach which promises to further extend the merits of the unbroken tradition of looking at the urine. Despite these advances, the history and clinical findings along with a skilled examination of urine is a must before confidently disclosing the fate of the patient ahead. Knowing all these implications, one might as well say –to know your patient, know his urine !

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