Urinalysis Monograph

Urine is produced by the kidney to maintain constant plasma osmotic concentration; to regulate pH, electrolyte and fluid balances and to excrete some 50 grams of waste solids (mostly urea and sodium chloride). Texts on human anatomy and physiology describe in detail the function and mechanism by which the kidney's nephrons accomplish this.

Some normal urine constituents excreted (in g/24 hours):

Urea	25-30
Uric acid	0.6-0.7
Creatinine	1.0-1.2
Hippuric acid	0.7
Ammonia	0.7
Amino acids	3
Sodium	1-5 (NaCl 15.0)
Potassium	2-4
Calcium	0.2-0.3
Magnesium	0.1
Chloride	7
Phosphate	1.7-2.5
Sulfate	1.8-2.5

Routine urinalysis is composed of two examinations:

1) Chemical tests for abnormal chemical constituents
2) Microscopic exam for abnormal insoluble constituents

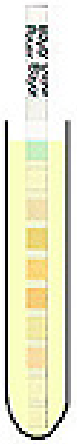
PROCEDURES

The color and appearance of the urine specimen is recorded. Usual colors are colorless, straw, yellow, amber; less commonly pink, red, brown. Usual appearances (opacity) are clear or hazy; less commonly turbid, cloudy and opaque, unless the specimen has remained at room or refrigerated temperatures.

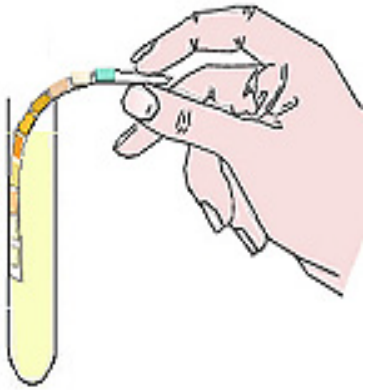
CHEMICAL

The common chemical testing of urine utilizes commercial disposable test strips. Multiparameter *10 or 11* test strips test for Glucose, Bilirubin, Ketone, Specific Gravity, Blood, pH, Protein, Urobilinogen, Nitrite, and Leukocyte Esterase. The result of this testing is regarded as semiquantitative.

A fresh urine specimen is collected in a clean, dry container. A Multiparameter strip is briefly immersed in the urine specimen, covering all reagent areas.



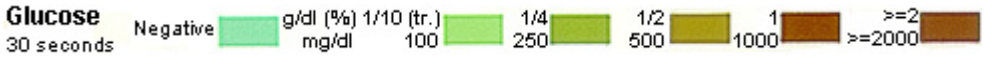
The edge of the Multiparameter strip is run against the rim of the urine container to remove excess urine. The strip is held in a horizontal position.



The reactions are read visually or automatically with an automated reflection photometer. If the strip is evaluated visually, the strip test areas are compared to those on the box color chart at the specified times. The results are recorded, and the strip is discarded.

METHODOLOGIES AND INTERPRETATIONS

Glucose:



This test is based on a double sequential enzyme reaction. One enzyme, glucose oxidase, catalyzes the formation of gluconic acid and hydrogen peroxide from the oxidation of glucose. A second enzyme, peroxidase, catalyzes the reaction of hydrogen peroxide with a potassium iodide chromogen to oxidize the chromogen to colors ranging from green to brown.

In general the presence of glucose indicates that the filtered load of glucose exceeds the maximal tubular reabsorptive capacity for glucose. In diabetes mellitus, urine testing for glucose is often substituted for blood glucose monitoring.

Bilirubin:

This test is based on the coupling of bilirubin with diazotized dichloroanaline in a strongly acid medium. The color ranges through various shades of tan.

Bilirubin in the urine indicates the presence of liver disease or biliary obstruction. Very low amounts of bilirubin can be detected in the urine, even when serum levels are below the clinical detection of jaundice.

Ketone:

This test is based on the development of colors ranging from buff-pink, for a negative reading, to purple when acetoacetic acid reacts with nitroprusside.

Urine testing only detects acetoacetic acid, not the other ketones, acetone or beta-hydroxybuteric acid. In ketoacidosis (insulin deficiency or starvation), it can be present in large amounts in the urine before any elevation in plasma levels.

Specific Gravity: 

This test is based on the apparent pKa change of certain pretreated polyelectrolytes, poly(methyl-vinyl-ether/maleic anhydride), in relation to ionic concentration. In the presence of bromthymol blue, colors range deep blue-green in urine of low ionic concentration through green and yellow-green in urines of increasing ionic concentration.

The specific gravity is a convenient index of urine concentration. It measures density and is only an approximate guide to true concentration. A specific gravity of <1.010 is consistent with a concentrating defect. A specific gravity of >1.025, in the absence of protein, glucose and other large molecular weight substances such as contrast media, usually indicates normal renal concentration and makes chronic renal insufficiency unlikely.

Blood: 

This test is based on the peroxidase-like activity of hemoglobin, which catalyzes the reaction of diisopropylbenzene dihydroperoxide and 3,3',5,5'-tetramethylbenzidine. The resulting color ranges from orange through green; very high levels of blood may cause the color development to continue to blue.

The presence of large numbers of RBCs in the urine sediment establishes the diagnosis of hematuria. If the dipstick is more strongly positive than would be expected from the number of RBCs, then the possibility of hemoglobinuria or myoglobinuria should be considered.

pH: 

The test is based on the double indicator (methyl red/bromthymol blue) principle that gives a broad range of colors covering the entire urinary pH range. Colors range from orange through yellow and green to blue.

The urine pH should be recorded, although it is seldom of diagnostic value. Phosphates will precipitate in an alkaline urine, and uric acid will precipitate in an acidic urine.

Protein: 

This test is based on the protein-error-of-indicators (tetrabromphenol blue) principle. At a constant pH, the development of any green color is due to the presence of protein. Colors range from yellow for negative through yellow-green and green to green-blue for positive reactions.

Heavy proteinuria usually represents an abnormality in the glomerular filtration barrier. The test is more sensitive for albumin than for globulins or hemoglobin.

Urobilinogen: 

This test is based on the modified Ehrlich reaction, in which para-diethylaminobenzaldehyde in conjunction with a color enhancer reacts with urobilinogen in a strongly acid medium to produce a pink-red color.

Urine urobilinogen is increased in any condition that causes an increase in production or retention of bilirubin.

Nitrite: 

This test depends upon the conversion of nitrate (derived from the diet) to nitrite by the action of Gram negative bacteria in the urine. At the acid pH of the reagent area, nitrite in the urine reacts with para-arsanilic acid to form a diazonium compound. This diazonium compound in turn couples with 1,2,3,4-tetrahydrobenzo(h)quinoline-3-ol to produce a pink color.

Bacteriuria caused by some Gram negative bacteria which produce the nitrate reductase enzyme give a positive test.

Leukocytes: 

Granulocytic leukocytes contain esterases that catalize the hydrolysis of the derivatized pyrrole amino acid ester to liberate 3-hydroxy-5-phenyl pyrrole. This pyrrole then reacts with a diazonium salt to produce a purple product.

A positive leukocyte esterase test provides indirect evidence for the presence of bacteriuria.

MICROSCOPIC EXAM

Note that in many laboratories it is a standard practice to exclude the microscopic exam if all chemical testing yields negative or normal results.

In the urinalysis microscopic exam one looks for formed cellular elements, casts, bacteria, yeast, parasites and crystals in centrifuged urine sediment.

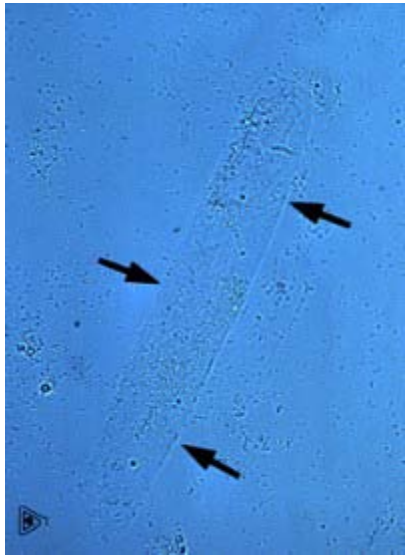
PROCEDURE

Centrifuge 5.0 mL of a mixed, freshly voided or catheterized urine in a conical centrifuge tube for 5 minutes at high speed. Remove 4.5 mL (or 90% of whatever volume was centriguged) of the supernatant fluid, leaving a 10-fold concentration of the urine sediment. Resuspend the sediment by gently mixing with a vortex mixer. Place a drop of stained or unstained suspension in a 1 mm deep chamber; allow the chamber to stand for 2 minutes, so that most elements will settle to the bottom of the chamber. Place the chamber on the microscope stage.

Examine several fields at 100X magnification for casts. Classify and count the number of casts and report as a least-to-most range (eg. 5-10) for each type seen within LPF (Low Power Fields). Switch to 400X magnification and examine for other elements, i.e., WBCs, RBCs, Epithelial cells, yeast, bacteria, Trichomonas vaginalis, Sperm cells, mucous filaments and crystals. Again, classify and report each element by a least-to-most range per HPF (High Power Field). Yeast, bacteria, mucous filaments and crystals are usually graded using the '+' notation (1+ = least significant amount, 4+ = most significant amount). Occasionaly, the fields are packed with cellular elements or casts, so that it is impractical to count their numbers; in this case use the notation 'TNTC' (Too Numerous To Count).

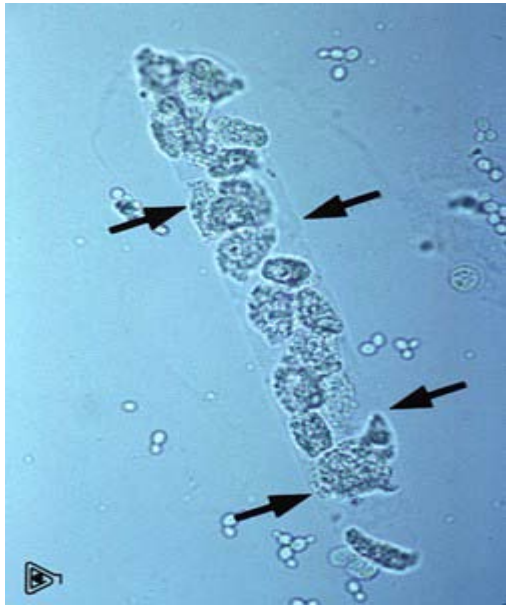
DESCRIPTIONS OF MICROSCOPIC ELEMENTS

Hyaline casts are formed in the absence of cells in the renal tubular lumen. They have a smooth texture and a refractive index very close to that of the surrounding fluid. When present in lower numbers (0-1/LPF) in concentrated urine of otherwise normal patients, hyaline casts are not always indicative of clinically significant disease. Greater numbers of hyaline casts may be seen associated with proteinuria of renal (eg., glomerular disease) or extra-renal (eg., overflow proteinuria as in myeloma) origin.

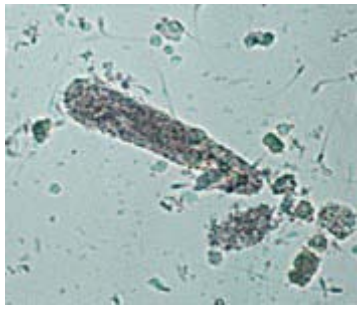


Cellular casts most commonly result when disease processes such as ischemia, infarction or nephrotoxicity cause degeneration and necrosis of tubular epithelial cells. A common scenario is the patient with decreased renal perfusion and oliguria secondary to severe dehydration. Ischemic injury results in degeneration and sloughing of the epithelial cells. The resulting casts often are prominent in urine produced following rehydration with fluid therapy. The restoration of urine flow flushes numerous casts out of the tubules. Leukocytes can also be incorporated into casts in cases of tubulo-interstitial inflammation (eg., pyelonephritis).

Budding yeast cells and mucous filaments are also present in the photomicrograph at right.

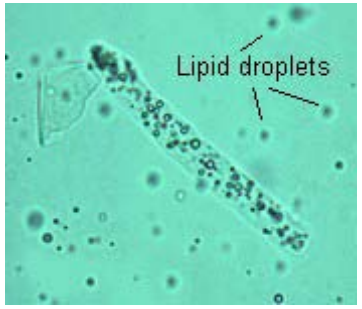


Granular casts have a textured appearance which ranges from fine to coarse. Since they usually form as a stage in the degeneration of cellular casts, the interpretation is similar to that for cellular casts.



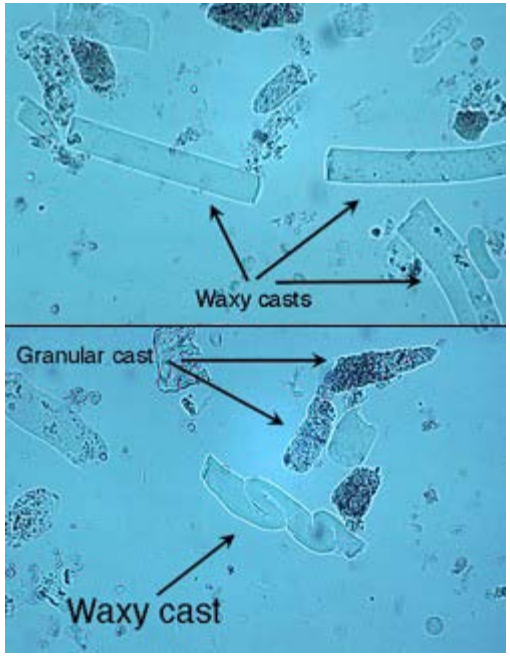
Sperm cells are also present in the photomicrograph at right.

Fatty casts are identified by the presence of refractile lipid droplets. The background matrix of the cast may be hyaline or granular. Often, they are seen in urines in which free lipid droplets are present as well. Interpretation of the significance of fatty casts should be based on the character of the cast matrix, rather than on the lipid content. Pictured is a fatty cast with a hyaline matrix. As an isolated finding, lipiduria is seldom of clinical significance.



Waxy casts have a smooth consistency but are more refractile and therefore easier to see compared to hyaline casts. They commonly have squared off ends, as if brittle and easily broken.

Waxy casts are found especially in chronic renal diseases, and are associated with chronic renal failure; they occur in diabetic nephropathy, malignant hypertension and glomerulonephritis.

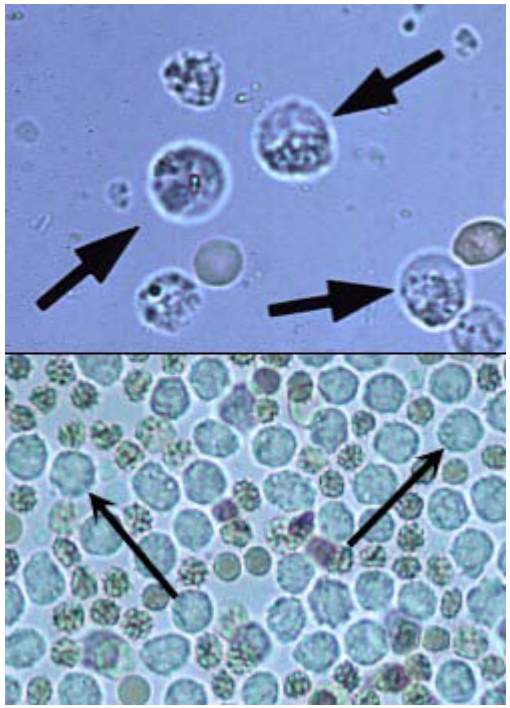


Oval Fat Bodies (OFB) are similar in composition and significance to fatty casts.

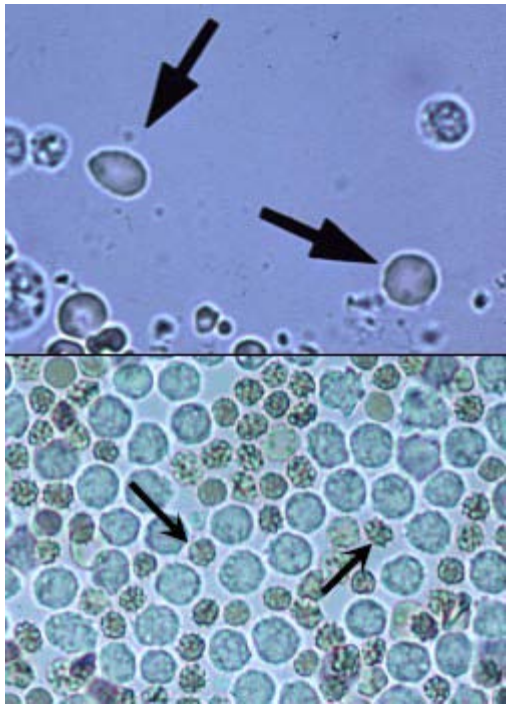
Desmorphic red cells (pictured ar right) are observed in glomerulonephritis. "Dysmorphic" red cells refer to heterogeneous sizes, hypochromia, distorted irregular outlines and frequently small blobs extruding from the cell membrane.

White Blood Cells (WBC) in unstained urine sediments typically appear as round, granular cells which are 1.5-2.0 times the diameter of RBCs. WBC in urine are most commly neutrophils. Like erythrocytes, WBC may lyse in very dilute or highly alkaline urine; WBC cytoplasmic granules released into the urine often resemble cocci bacteria.

WBC up to 5/HPF are commonly accepted as normal. Greater numbers (pyuria) generally indicate the presence of an inflammatory process somewhere along the course of the urinary tract (or urogenital tract in voided specimens). Pyuria often is caused by urinary tract infections, and often significant bacteria can be seen on sediment preps, indicating a need for bacterial culture.

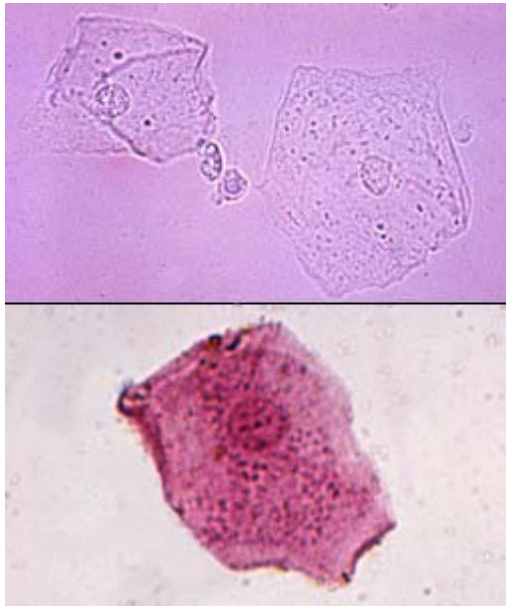


Fresh RBC tend to have a red or yellow color (lower panel). Prolonged exposure results in a pale or colorless appearance as hemoglobin may be lost from the cells (upper panel). In fresh specimens with a Specific Gravity (SG) of 1.010-1.020, RBC may retain the normal disc shape (upper panel). In more concentrated urines (SG>1.025), RBC tend to shrink and appear as small, crenated cells (lower panel). In more dilute specimens, they tend to swell. At a SG<1.008 and/or highly alkaline pH, RBC lysis is likely. Lysed RBC appear as very faint "ghost cells".



Red blood cells up to 5/HPF are commonly accepted as normal. Increased RBC in urine is termed hematuria, which can be due to hemorrhage, inflammation, necrosis, trauma or neoplasia somewhere along the urinary tract (or urogenital tract in voided specimens).

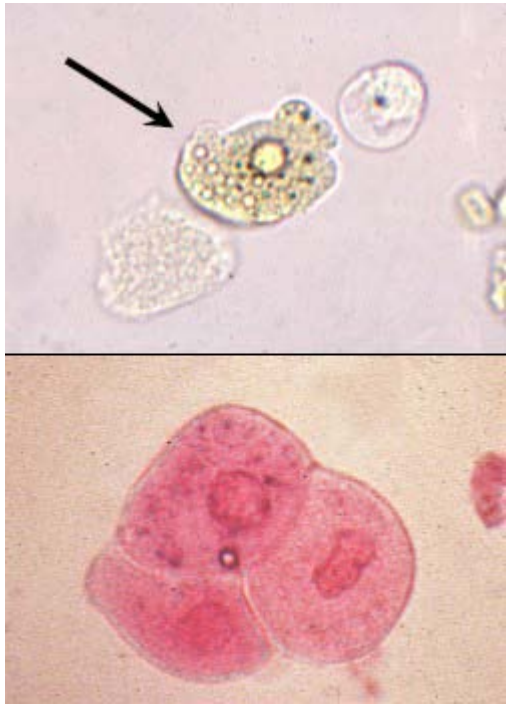
Squamous epithelial cells are the largest cells seen in normal urine specimens. They are thin, flat cells, usually with an angular or irregular outline and a small round nucleus. They may be present as single cells or as variably-sized clusters. Those shown in the upper panel are unstained; that in the lower panel was prepared using Sedi-Stain.



Squamous cells are common in lower numbers in voided specimens and generally represent contamination from the genital tract. Their main significance is as an indicator of such contamination.

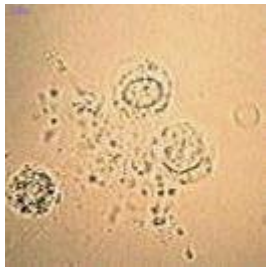
Parabasal squamous epithelial cells are immature squamous epithelial cells. They are commonly seen in urine specimens from postmenopausal women with atrophic vaginitis resulting from decreased estrogen (estradiol) levels.

Transitional epithelial cells originate from the renal pelvis, ureters, bladder and/or urethra. Their size and shape depends on the depth of origin in the epithelial mucosa. Most often they are round or polygonal; less commonly pear-shaped, caudate or spindle-shaped. They are generally somewhat smaller and smoother in outline than squamous cells, but larger than WBC. They may develop refractile, fatty inclusions as they degenerate in older specimens (upper panel).

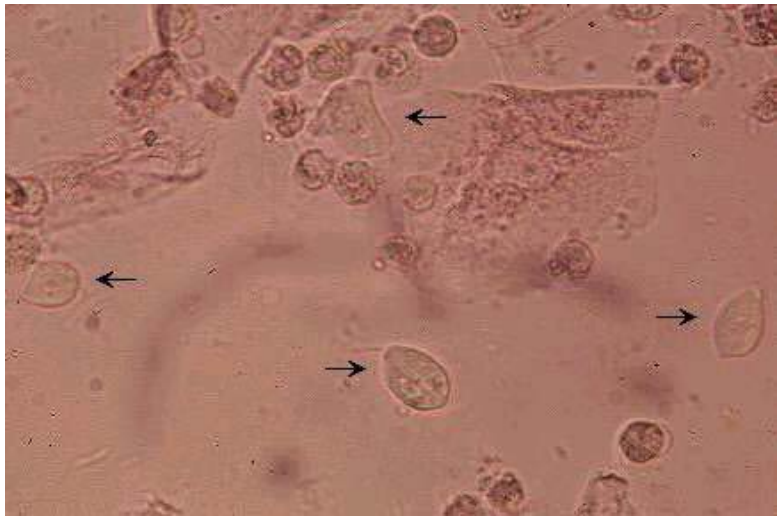


In cleanly-collected normal specimens, transitional cells are few (ca. <2/HPF), but are more frequent in the elderly population. However, the presence of transitional sheets ("brick wall" appearance) is sometimes associated with Transitional Cell Cancer (TCC).

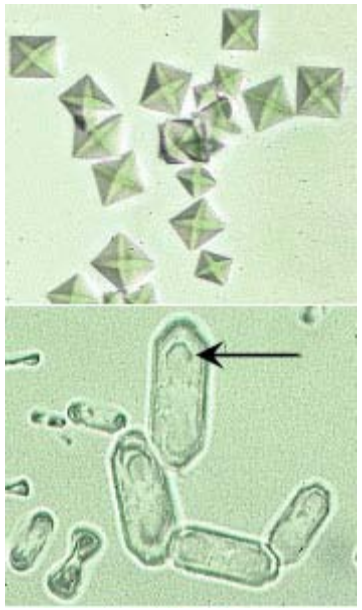
Renal Tubular Cells (RTC) are originally cubic in shape; but once exfoliated, they adopt a rounded shape. These cells are slightly larger than leukocytes (10-14 um) with lightly granular cytoplasm. The nucleus is round, well defined and usually centric. The cytoplasm often shows a perinuclear halo when stained. Note the "glitter" cell (fatty degenerated WBC) in the lower-left corner.



Trichomonas vaginalis is a sexually-transmitted urogenital parasite of men and women. The organism varies in size between 1-2 times the diameter of WBC. The organism is readily identified by its rapid erratic "jerky" movement.

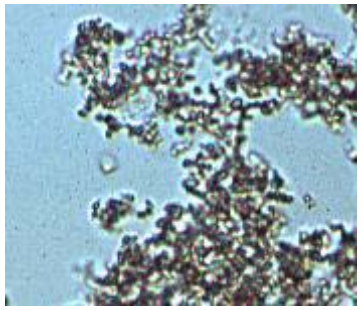


Calcium Oxalate Dihydrate crystals typically are seen as colorless squares whose corners are connected by intersecting lines (resembling an envelope). They can occur in urine of any pH. The crystals vary in size from quite large to very small. Dietary asparagus and ethylene glycol intoxication are notorious for urinary calcium oxalate formation.

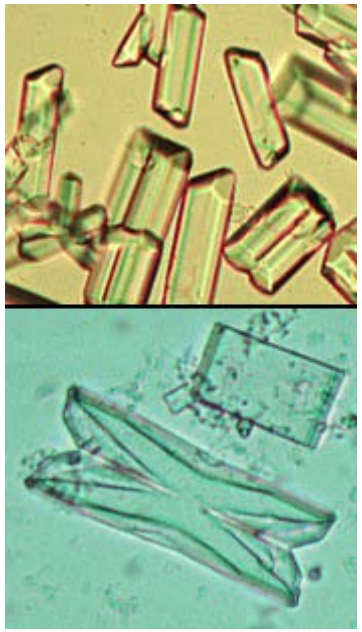


Calcium Oxalate Monohydrate crystals vary in size and may have a spindle, oval, or dumbbell shape. Most commonly, they appear as flat, elongated, six-sided "fence picket" crystals as seen at the right. Sometimes they closely resemble colorless RBCs.

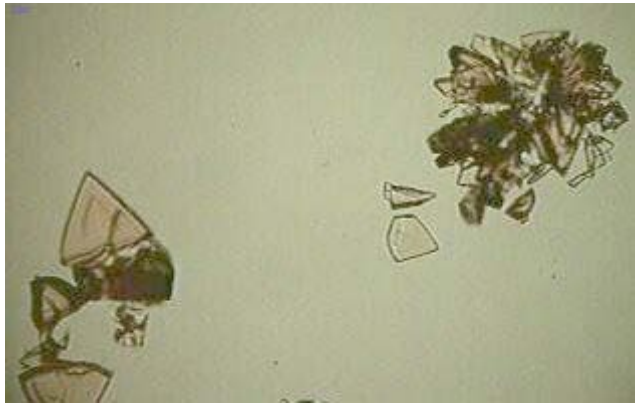
Amorphous crystals appear as aggregates of finely granular material without any defining shape. Amorphous urates of Na, K, Mg or Ca tend to form in acidic urine and may have a yellow or yellow-brown color. Amorphous phosphates are similar in general appearance, but tend to form in alkaline urine and lack color. Generally, no specific clinical interpretation can be made for the presence of amorphous crystals.



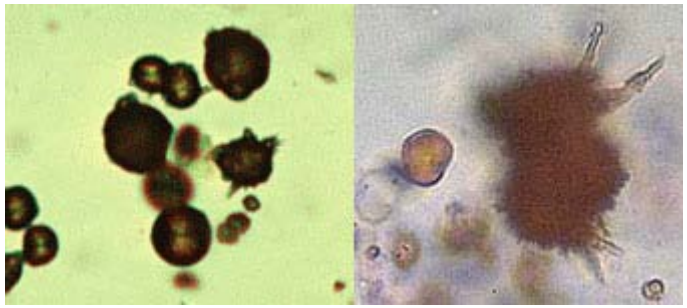
Triple phosphate (Struvite, Magnesium Ammonium Phosphate) crystals usually appear as colorless, prism-like "coffin lids". They are often seen in urine from clinically normal individuals. Although they can be found in urine of any pH, their formation is favored in neutral to alkaline urine. Urinary tract infection with urease producing bacteria (eg. Proteus vulgaris) can promote struvite crystalluria (and urolithiasis) by raising urine pH and increasing free ammonia.



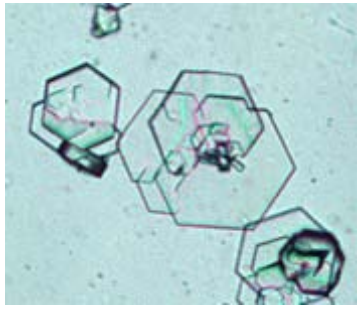
Uric acid crystals may appear as yellow to brown rhombic or hexagonal plates, needles or rosettes. With rare exceptions, the finding of uric acid crystals in urine is of little clinical value.



Ammonium urate (or biurate) crystals generally appear as yellow-brown, radially-striated spheres with irregular "thorn-apple" or "ox-horn" projections. Although they may be seen in acid urine, their formation is favored in neutral to alkaline urine.

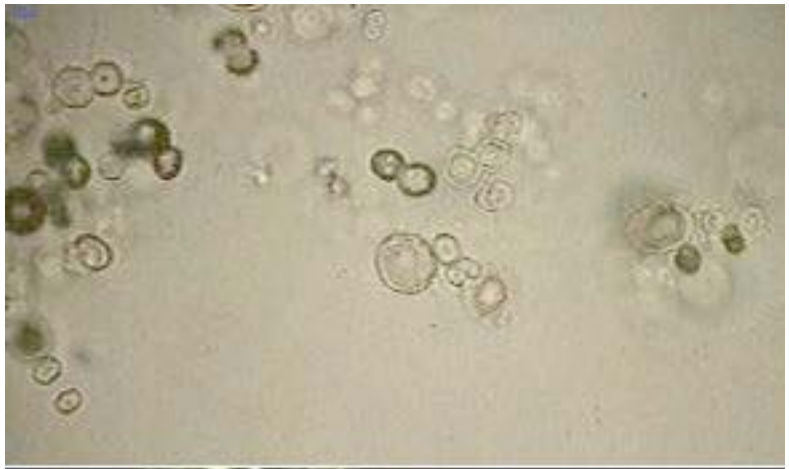


Cystine crystals are seen as flat colorless hexagonal plates. They often aggregate in layers, and their formation is favored in acidic urine.

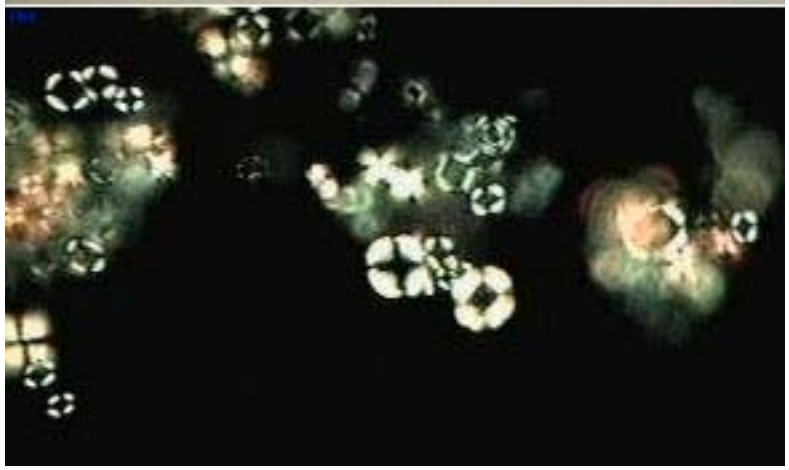


Cystine crystalluria or urolithiasis is an indication of cystinuria, which is an inborn error of metabolism involving defective renal tubular reabsorption of certain amino acids including cystine.

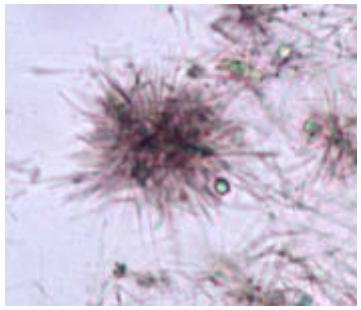
Leucine crystals are seen as yellow spheres with concentric and radial strias. These crystals can sometimes be mistaken for cells, with the center resembling a nucleus.



Under polarized light, leucine crystals transmit a "maltese cross" interference pattern.

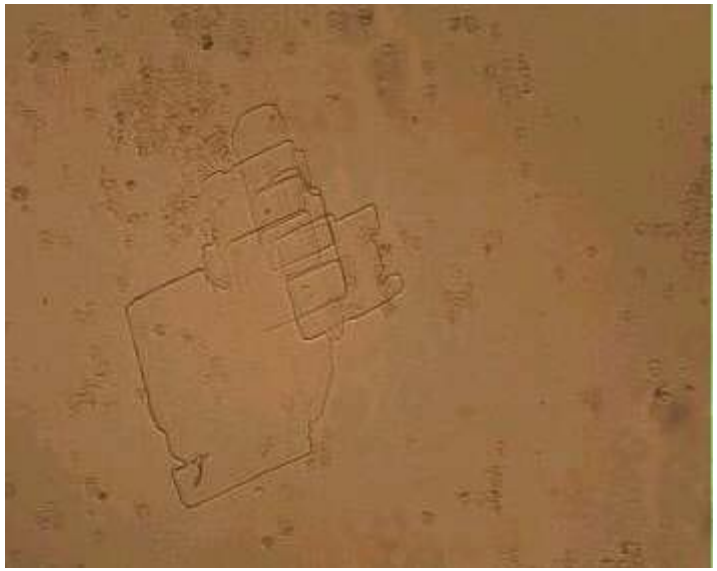


Crystals of the amino acids leucine and tyrosine are very rarely seen in urine sediments. These crystals can be observed in some hereditary diseases like tyrosinosis and "Maple Syrup Disease". More often one finds these crystals concurrently in patients with severe (often terminal) liver disease.

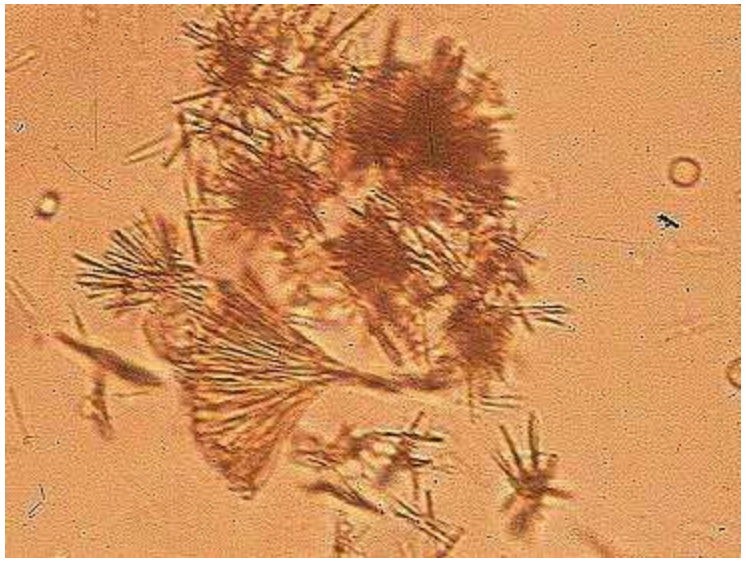


Tyrosine crystals are usually seen as fine brownish needles, either isolated or as rosettes. These are sometimes associated with severe liver disease.

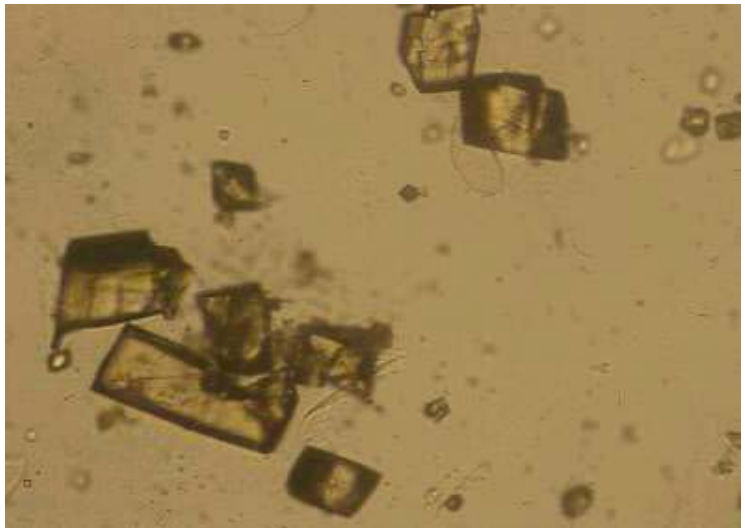
Cholesterol crystalizes as thin rectangular plates with one (sometimes two) of the corners having a square notch. The cause of the presence of crystalized cholesterol is obscure. These crystals are seen in degenerative renal disease and are thought to have an identical clinical meaning as OFB. The presence of cholesterol crystals is usually accompanied by proteinuria, but they are only rarely seen.



Sulfadiazine crystals are a common finding with administration of Trimethoprim-sulfadiazine. They are often seen as "shocks of wheat" or radially-striated spherules.



Sulfonamide crystals are typically yellow in color and often resemble uric acid crystals. However, sulfa crystals are easily distinguished from uric acid by confirmatory tests. Sulfa crystals are readily soluble in acetone and exhibit a positive dextrine/sulfuric acid test ("old yellow newspaper" test).



Many drugs excreted in the urine have the potential to form crystals. Hence, a review of the patient's drug history is useful when an unidentified crystal is found.

REFERENCE RANGES

Normal values or normal ranges may vary considerably, depending on type of specimen collection, the age of the specimen and the method of storage or preservation. The table below represents expected values for a fresh, clean-catch, mid-stream collection.

Test	Reference Range
Color	Straw - Dark yellow
Appearance	Clear - Hazy
Specific Gravity	1.003-1.029
pH	4.5-7.8
Protein	Negative
Glucose	Negative
Ketones	Negative
Bilirubin	Negative
Occult blood	Negative
Leukocyte Esterase	Negative
Nitrite	Negative
Urobilinogen	0.1-1.0 EU/dL
WBCs	0-4/hpf
RBCs	male: 0-3/hpf female: 0-5/hpf

Casts	0-4/lpf
Bacteria	Negative

EU = Ehrlich Units (ca. 1 mg) hpf = High Power Field (400x) lpf = Low Power Field (100X)

OTHER TESTS

PREGNANCY TESTS (Qualitative)

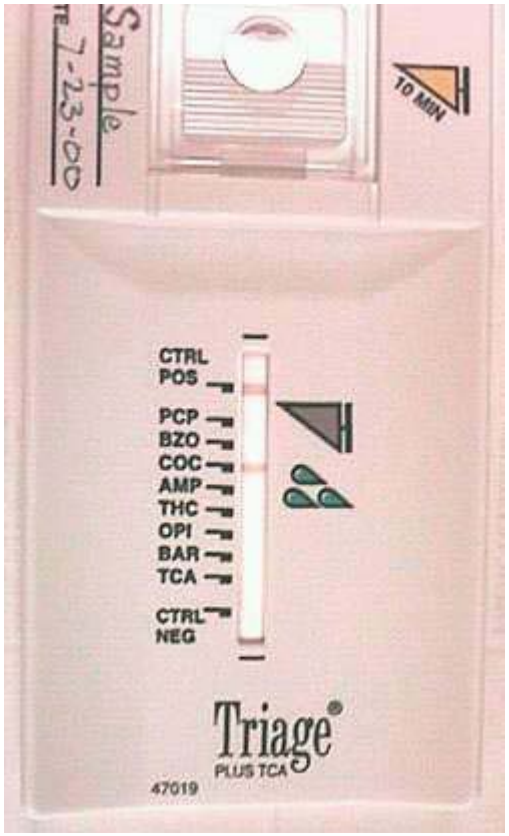
The detection of hCG (human chorionic gonadotropin) in serum and urine is useful in the presumptive diagnosis of pregnancy. This glycoprotein hormone is secreted by the developing placenta after fertilization. The serum hCG hormone level doubles approximately every 2.2 days during the 1st trimester of a normal pregnancy. Detectable levels start at 5 mIU/mL during the 1st week of gestation and rise to 100,000 mIU/mL at 2 to 3 months.

Fisher Scientific Company provides a hCG detection test pack with the proprietary name of *Sure-Vue*. The test utilizes a combination of monoclonal and polyclonal antibody reagents to selectively detect elevated levels of hCG in serum or urine. The test is conducted by the addition of 4 drops of serum or urine into the sample well and observing for the formation of colored lines. The specimen migrates via capillary action along the membrane and reacts with a colored conjugate. A positive specimen reacts with the hCG-specific antibody colored conjugate and forms a colored line in the T(est) window approximately 4-8 minutes after the addition of specimen.

DRUG SCREENING (Qualitative)

Drug abuse in the US continues to be an increasingly significant social and economic problem. Opiates, cocaine, THC, amphetamines, and phencyclidine are recognized as the most frequently abused illicit drugs by the Substance Abuse and Mental Health Services Administration (SAMHSA). Tranquilizers, anti-depressants, barbiturates and opiate compounds are among a group of prescription drugs that also are frequently abused.
These drugs are also associated with drug overdose and accidental or intentional self-poisionings, resulting in increasing admissions to emergency departments. Hence, there is a distinct need for rapid, sensitive and specific testing to detect these Drugs of Abuse (DOA). Immunoassay provides a simple method for drug screening in urine.

Biosite Diagnostics manufactures a convenient test pack with the proprietary label *Triage*, which employs a simultaneous competative binding immunoassay for 8 drugs of abuse and their metabolites.



Triage Plus TCA **provides a rapid qualitative urine screen that analyzes a single urine sample for the following drugs of abuse: Amphetamines/ Methamphetamines, Cocaine, Opiates (heroin), Phencyclidine (PCP) and Tetrahydrocannabinol (marijuana). Prescription drugs tested: Barbiturates, Benzodiazepines, Tricyclic Antidepressants.**

As stated above, the method utilizes a competative binding immunoassay in which a chemically labeled drug (drug conjugate) competes with drug which may be present in the urine for monoclonal antibody binding sites. After a brief (10 minutes) incubation of urine with the drug monoclonal antibodies and competing drugs conjugated to colloidal gold, the reaction mixture is transferred to a detection membrane on which are immobilized zones of monoclonal antibodies. Free drug conjugate that is displaced from antibody binding sites by drug in the urine, binds to a zone on the membrane. The membrane is washed to remove the unbound conjugate. Test results are read visually.

The photo demonstrates a positive test for cocaine. Positive results should be confirmed by quantitative reference methods, such as High Performance Liquid Chromatography or Gas Chromatography with Mass Spectra detection.

Drug Detection Threshold

Threshold detection at the cut-off concentration and clearance times, i.e., the time required to reduce drug concentration to below the cut-off concentration after drug use, vary depending on analytical method used, drug metabolism, patient's condition, fluid intake and method and frequency of drug use. These are general guidelines only. Clinical considerations and professional judgment should be applied to any drug of abuse test result, particularly when preliminary positive results are used.

Threshold Detection and Clearance Times of Several Drugs

Drug	Cut-off Conc.	Approximate Detection Time in Urine using EMIT **
Amphetamines	1000 ng/mL *	2-4 days
Barbiturates	300 ng/mL *	Short-Acting (eg. secobarbital) 1 day.

	300 ng/mL *	Long-Acting (eg. phenobarbital) 2-3 weeks.
Benzodiazepines	300 ng/mL	3-7 days
Cannabinoids	50 ng/mL	3-30 days (half-life = 7 days)
Cocaine	300 ng/mL *	2-4 days
Codeine	----------	2-5 days
Euphorics (LSD, XTC)	----------	? days (Currently, not detectable by EMIT. Detectable by GC/MS, however)
Methadone	----------	3-5 days
Methaqualone	----------	14 days
Opiates	300 ng/mL	2-4 days
Phencyclidine (PCP)	25 ng/mL *	8-14 days
Phenobarbital	300 ng/mL *	10-20 days
Propoxyphene	----------	6 hours to 2 days

* Recommended SAMHSA cut-off concentrations
** EMIT: Syva Corp. acronym for *Enzyme Multiplied Immunoassay Technique*