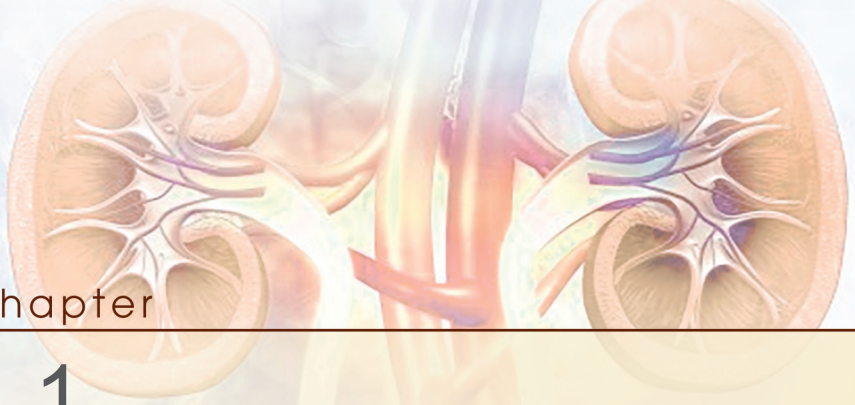


Chapter

1 Understanding Urine Tests

INTRODUCTION

Urinalysis is indispensable part of evaluation of diseases of kidney and renal tract. It may be considered as a fluid biopsy of kidney. It is often part of a routine examination and is frequently performed when people are admitted to hospital and before surgery. Complete urinalysis is done in a laboratory. It usually involves three steps:

1. Assessment of the color, cloudiness and concentration of the urine
2. Examination of the chemical composition of the urine using a test strip
3. Examination of the urine under a microscope to look for bacteria, cells and cast and crystals

Urinalysis is used to find the cause of or monitor urinary tract infections, bleeding in the urinary system in case of kidney or liver disease. It can also be used for diagnosis of diabetic kidney disease, hematological disorder and renal stone disease.

This chapter describes the main aspects of urinalysis, including urine collection, evaluation of physical and chemical features of urine and urine microscopy.

COLLECTION OF URINE SAMPLE

It is a good idea to cleanse the genital area with water, but not soap, before giving a sample. To avoid bacterial contamination, "clean catch" midstream urine is used. One can take a sample of midstream urine by interrupting the flow of urine after a few seconds of starting urination and then collecting this middle portion of the urine (at least 10 mL) in a container. For renal patients, a combined dipstick and urine microscopy on the second morning urine sample is suggested. Sometimes a 24-hour urine collection or spot urine examination is needed for specific indications.

INTERPRETATION OF PHYSICAL CHARACTERISTICS

Color

The color of normal urine ranges from pale to dark yellow and amber. Causes of abnormal urine color are shown in Table 1.1.

Odor

Slightly aromatic, characteristic of freshly voided urine. Urine becomes more ammonia-like upon standing due to bacterial activity.

Table 1.1: Causes of abnormal urine color

Color	*Possible cause*
Pink-red	Hematuria, hemoglobinuria, myoglobinuria
Red-brown	Hematuria, hemoglobinuria, myoglobinuria
Brown-black	Methemoglobin (from hemoglobin or myoglobin), bile pigments
Yellow-orange	Highly concentrated urine, bilirubinuria
Yellow-green	Bilirubin, biliverdin

Turbidity

Normal urine is transparent or clear; becomes cloudy upon standing. Cloudy urine may be evidence of phosphates, urates, mucus, bacteria, epithelial cells, or leukocytes.

pH

Ranges from 4.5–8.0. Average is 6.0, slightly acidic. High protein diets increase acidity. Vegetarian diets increase alkalinity. Bacterial infections also increase alkalinity (Table 1.2).

Specific Gravity

Specific gravity measures the ability of the kidneys to concentrate or dilute urine depending on fluctuating conditions. Normal range 1.005–1.035, average range 1.010–1.025.

Table 1.2: Interpretation of urine pH

Potential causes of aciduria	*Potential causes of alkalinuria*
Meat protein-based diet	Plant protein-based diet
Use of acidifying agents (e.g. NH_4Cl)	Postprandial alkaline tide
Metabolic acidosis	UTI with urease-containing bacteria
Respiratory acidosis	Urine sample exposed to air at room temperature
Paradoxical aciduria with alkalosis	Use of alkalinizing agents (e.g. $NaHCO_3$)
Protein catabolic states	Metabolic alkalosis
Ethylene glycol ingestion	Respiratory alkalosis

CHEMICAL ANALYSIS

For routine chemical analysis of urine, there are several brands of chemical test strips (dipsticks) are commercially available (Fig. 1.1). A dipstick is a paper strip with patches impregnated with chemicals that undergo a color change. Table 1.3 shows abnormal constituents that could be detected by dipstick.

Rapid Urine Test

A rapid urine test is the quickest way to test urine. This involves dipping a test strip with small square colored fields on it into the urine sample for a few seconds. After that you have to wait a little for the result to appear. Depending on the concentration of the particular substance you are testing for, the fields on the test strip change color. Then the resulting colors of the fields are compared with a color table. The color table can be found on the urine test package. It shows which colors indicate normal and abnormal values.

Rapid urine tests are usually done as part of routine examinations, e.g. at a family doctor's office, during antenatal visits, when being admitted to the hospital, or before surgery. They are also used in people who have acute symptoms like lower abdominal pain, stomach ache or back pain, frequent painful urination, or blood in their urine. Some people, who have diabetes, use this test to check their sugar levels.

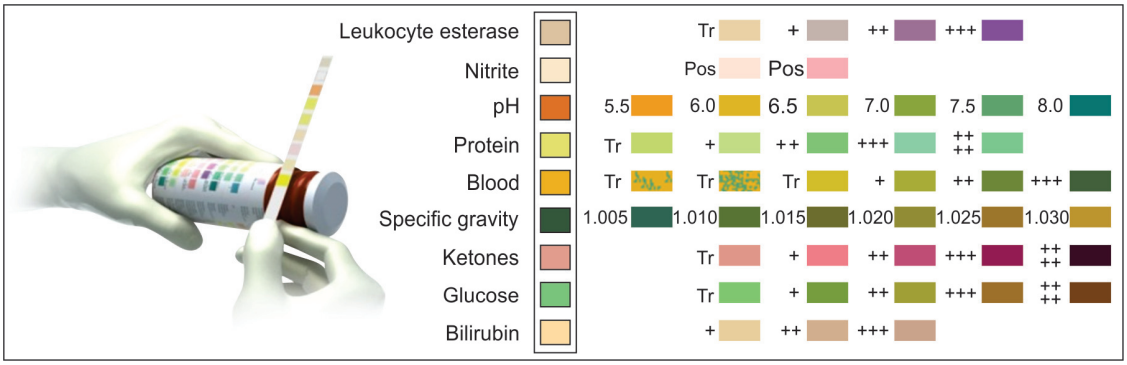


Fig. 1.1: Findings: Leukocyte esterase 3+; Nitrite Pos; pH 7.0; Protein Neg; Blood Neg; Sp Gr 1.015; Ketones 1+, Glucose 1+; Bilirubin Neg

Table 1.3: Abnormal constituents that could be detected by dipstick

Abnormal constituents	*Associated causes*
Protein (albumin)	Albumin is normally too large to pass through glomerulus. Presence of albumin in urine (albuminuria) indicates abnormal permeability of glomerular membrane. *Causes of non-pathological albuminuria:* Pregnancy, increased protein consumption, physical exertion. *Causes of pathological albuminuria:* Chemical poisons, glomerulonephritis bacterial toxins.
Glucose	Glycosuria is the condition of glucose in urine. Normally glucose (filtered) reabsorbed by the renal tubules and returned to the blood (via carrier molecules). The untransported glucose will spill over into the urine, if blood glucose levels exceed renal threshold levels. *Main cause:* Diabetes mellitus
Ketones	Ketone bodies such as beta-hydroxybutyric acid, acetoacetic acid and acetone can appear in urine in small amounts. These intermediate byproducts are associated with the breakdown of fat. *Causes:* Starvation, diarrhea, diabetes mellitus.
Bilirubin	Bilirubin associated by the breakdown of hemoglobin in red blood cells. The globin portion of hemoglobin split off and the heme groups of hemoglobin converted into the pigment bilirubin. Bilirubin is secreted in blood and carried to the liver conjugated with glucuronic acid. Some secreted in blood and some excreted in the bile as bile pigments into the small intestines. *Causes:* Hepatitis, cirrhosis, obstruction of bile duct, liver disorders.
Urobilinogen	Bile pigment derived from breakdown of hemoglobin. The majority of this substance excreted in the stool, but small amounts reabsorbed into the blood from the intestines and then excreted into the urine. *Causes:* Liver diseases, hemolytic anemias.
Hemoglobinuria	Presence of hemoglobin in the urine. *Causes:* Massive burns, blood transfusion reactions, hemolytic anemia, renal disease.
Red blood cells	Hematuria is the presence of intact erythrocytes. Usually pathological. *Causes:* Kidney stones, tumors, glomerulonephritis, physical trauma.
White blood cells	The presence of leukocytes in urine is referred to as pyuria (pus in the urine). *Causes:* Urinary tract infection.
Nitrite	Presence of bacteria. *Causes:* Urinary tract infection.

Many substances are usually found only in certain amounts in urine, so higher or lower levels indicate a deviation from the normal.

Protein

Dipstick screening for protein is done on whole urine, but semiquantitative tests for urine protein should be performed on the supernatant of centrifuged urine since the cells suspended in normal urine can produce a falsely high estimation of protein. Normally, only small plasma proteins filtered at the glomerulus are reabsorbed by the renal tubule. However, a small amount of filtered plasma proteins and protein secreted by the nephron (Tamm-Horsfall protein) can be found in normal urine. Normal total protein excretion does not usually exceed 150 mg/24 hours or 10 mg/100 mL in any single specimen. More than 150 mg/day is defined as proteinuria. Proteinuria >3.5 g/24 hours is severe and known as nephrotic range proteinuria.

24-Hour Urinary Protein

The 24-hour urine collection for protein excretion remains the reference (gold standard) method. It is based on chemical assay, turbidimetric technique, or dye-binding

technique, which quantify total proteins rather than simply albumin.

Protein-Creatinine Ratio on Random Urine Sample

PCR is obtained by the ratio between urine protein excretion (measured by methods in 24-hour protein excretion) and creatinine excretion, expressed as µg/mg or mg/mmol. PCR represents a practical alternative to the 24-hour urine collection because it is easy to obtain and is not influenced by variation in water intake or rate of diuresis.

Some consider that a normal PCR is sufficient to rule out pathologic proteinuria, but an elevated PCR should be confirmed and quantified with a 24-hour collection.

A possible alternative to PCR is the measurement of albumin creatinine ratio (ACR), especially to screen and monitor diabetic patients.

Microalbuminuria is defined as excretion of 30–300 mg of albumin per 24 hours (or 20–200 µg/min or 30–300 µg/mg creatinine) on 2 of 3 urine collections.

The detection of low levels of albumin excretion (microalbuminuria) has been linked to the identification of incipient diabetic kidney disease. This phase calls for aggressive management to prevent or retard overt diabetic nephropathy. The reference ranges for microalbumin are detailed in Table 1.4.

Urine ACR

- Reference range: Less than 3.0 mg/mmol
- Urine ACR 3.0–30.0 mg/mmol: Moderately increased
- Urine ACR >30.0 mg/mmol: Severely increased*

Urine PCR

- Adult reference range: Less than 15 mg/mmol
- Pediatric reference range (<18 years of age): Less than 20 mg/mmol
- In **pregnancy,** a PCR result of more than 30 mg/mmol indicates significant proteinuria.

Localization of Proteinuria

Localization of proteinuria is described in Table 1.5.

MICROSCOPIC ANALYSIS

Crystalluria is frequently observed in urine specimens, stored at room temperature or refrigerated. Such crystals are diagnostically useful when observed in warm, fresh urine by a physician evaluating microhematuria, nephrolithiasis, or toxin ingestion.

In abundance, **calcium oxalate** and/or **hippurate crystals** especially if known to be accompanied by neurological abnormalities, appearance of drunkenness, hypertension, and a high anion gap acidosis may suggest ethylene glycol ingestion. Large numbers of calcium oxalate crystals occur, with acute renal failure following methoxyflurane anesthesia. Urine usually supersaturated in calcium oxalate, often in calcium phosphate, and acid urine often saturated in uric acid. Yet crystalluria is uncommon (in warm, fresh urine) because of the normal presence of crystal inhibitors, the lack of available nidus, and the time factor. When properly observed in fresh urine, crystals may provide a clue to the composition of renal stones even not yet passed, the nidus for such stones, or, as such, have been associated with microhematuria.

Table 1.4: American Diabetic Association classification of microalbuminuria

Spot collection	*Timed collection*	*24-hr collection*	*Category*
Less than 30 µg/mg creatinine	Less than 20 µg/min	Less than 30 mg	Normal
30–300 µg/mg creatinine	20–200 µg/min	30–300 mg	Microalbuminuria
More than 300 µg/mg creatinine	More than 200 µg/min	More than 300 mg	Clinical albuminuria

**Including nephrotic syndrome (urine ACR usually >220 mg/mmol*

Table 1.5: Localization of proteinuria

Type of proteinuria	*Examples*	*Diagnosis*
Physiologic or benign proteinuria	Change in exercise level Seizure activity Fever Exposure to extreme temperature Stress	UPC usually <0.5 Compatible history Proteinuria is intermittent, transient Variable UPC
Pathologic proteinuria		
Non-urinary	Congestive heart failure Hemoglobinuria, myoglobinuria Dysproteinemia, dysproteinuria serum Genital tract inflammation or hemorrhage	History/physical examination/echo Urine remains red after centrifugation Electrophoresis Physical examination/imaging/urine sediment
Urinary (non-renal)	Lower urinary tract inflammation (e.g. bacterial cystitis, cystoliths, polyps, neoplasia)	UPC not indicated History/physical examination Compatible urine sediment Lower urinary tract imaging
Urinary (renal)	Renal parenchymal inflammation (e.g. pyelonephritis, renoliths, neoplasia)	Variable UPC Compatible urine sediment Renal imaging
	Tubular proteinuria	UPC usually = 0.5–1 May also see decreased tubular reabsorption of glucose and electrolytes
	Glomerular proteinuria	Persistent UPC ≥1 Inactive urine sediment (with exception of possible hyaline cats)

Leukocyturia may indicate inflammatory disease in the genitourinary tract, including chemical injury, bacterial infection, autoimmune diseases, or inflammatory disease, glomerulonephritis, adjacent to the urinary tract such as appendicitis or diverticulitis.

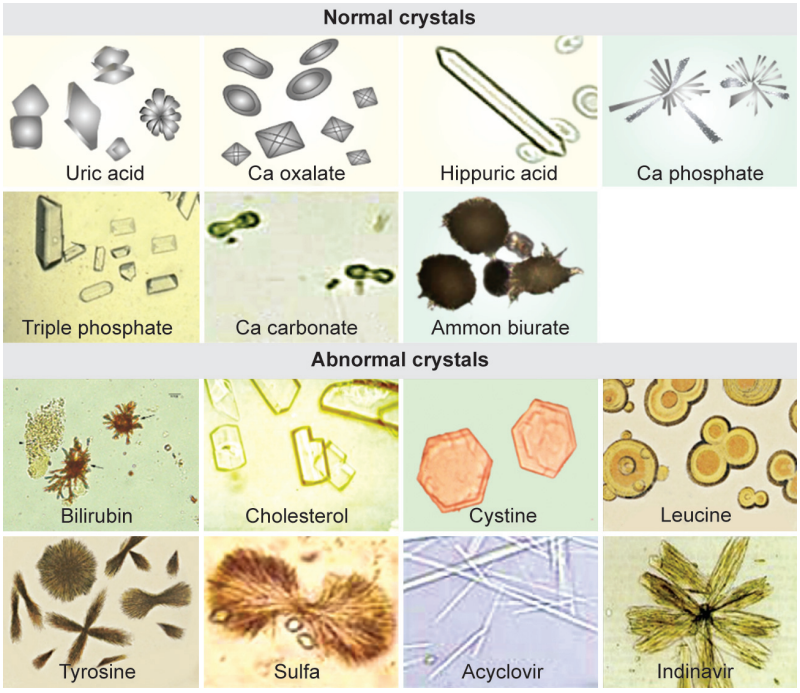
White cell casts indicate the renal origin of leukocytes, most frequently found in acute pyelonephritis. White cell casts also found in glomerulonephritis such as lupus nephritis, and in acute and chronic interstitial nephritis. Leukocyte casts (when nuclei degenerate) resemble renal tubular casts.

Red cell casts indicate renal origin of hematuria and suggest glomerulonephritis, including lupus nephritis. Red cell casts may also be found in subacute sickle cell disease, bacterial endocarditis, vasculitis, renal infarct, Goodpasture syndrome, and in malignant hypertension. Degenerated red cell casts may remain **hemoglobin casts.** Possibly find orange to red casts with myoglobinuria as well.

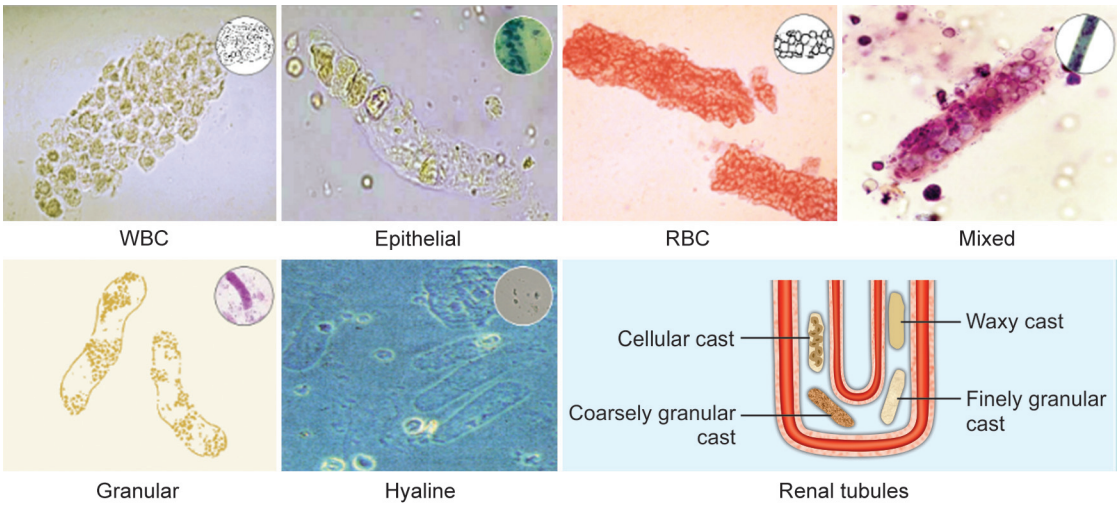
Hyaline casts which occurs in physiologic states (e.g. after exercise) and many types of renal diseases.

Renal tubular (epithelial) casts are most expressive of tubular injury, as in acute tubular necrosis. They also found in other disorders, including heavy metal poisoning, ethylene glycol intoxication, eclampsia and acute allograft rejection.

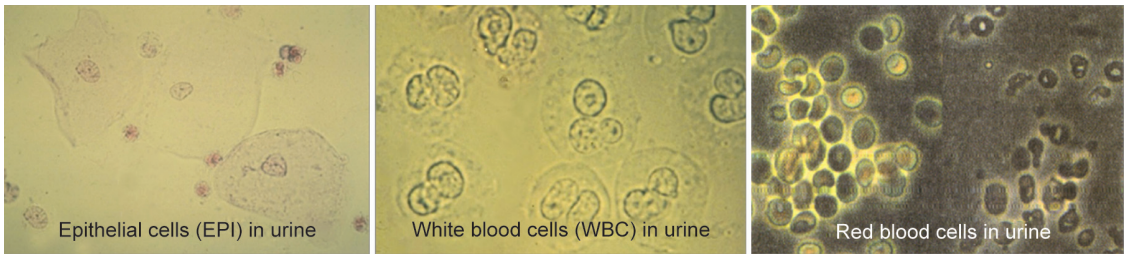
Granular casts: Very finely granulated casts may possibly found after exercise and in a variety of glomerular and tubulointerstitial diseases. Coarse granular casts are abnormal and are present in a wide variety of renal diseases. **"Dirty brown" granular casts** are typical of acute tubular necrosis.



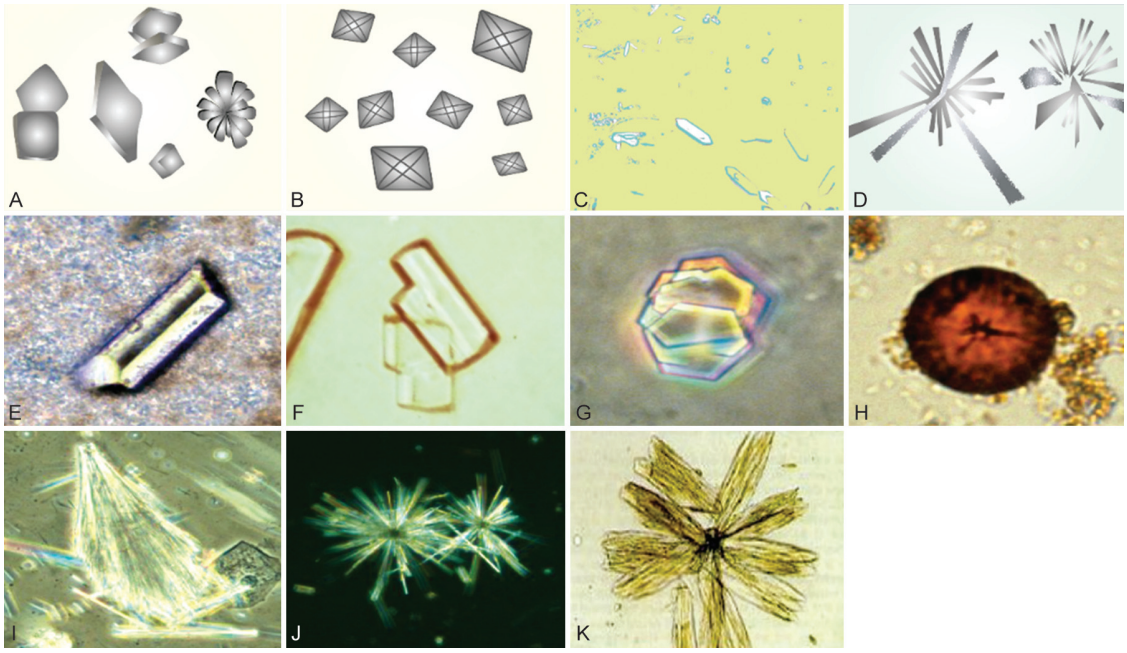
Cast in urine



Cells in urine



Crystals in Urine



A. Uric acid crystals; **B.** Bihydrated calcium oxalate crystals; **C.** Different types of monohydrated calcium oxalate crystals; **D.** Star-like brushite (calcium phosphate) crystal; **E.** Struvite (triple phosphate) crystal, on the background of a massive amount of amorphous phosphate particles; **F.** Cholesterol crystal; **G.** Cystine crystals; **H.** 2, 8-Dihydroxyadenine crystal by bright-field microscopy; *inset*, by polarized light; **I.** Amoxicillin crystal resembling a branch of a broom bush; **J.** Star-like ciprofloxacin crystals and **K.** Large crystal of indinavir.

Waxy casts found especially in chronic renal diseases, and accompanying with chronic renal failure; they occur in malignant hypertension, diabetic nephropathy, and glomerulonephritis, among other conditions. They stand for their waxy or glossy appearance. They often appear brittle and cracked.

Fatty casts found in the nephrotic syndromes generally, other forms of chronic renal diseases, diabetic nephropathy and glomerulonephritis. The fat droplets originate in renal tubular cells when they exceed their capacity to reabsorb protein of glomerular origin. Their inclusions have the features and significance of oval fat bodies.

Broad casts originate from dilated, chronically damaged tubules or the collecting ducts. They can be granular or waxy. **Broad waxy casts** called "renal failure casts."

Phase Contrast Microscopy

Hematuria is a common diagnostic problem that may indicate an underlying glomerulonephritis. The presence of proteinuria and red cell casts helps to distinguish it from other urological diagnosis. The patients with glomerular bleeding but no proteinuria may undergo investigations like cystoscopy and intravenous urography, when renal function testing and biopsy may be more appropriate measures.

In 1979, Birch and Fairley described a method of distinguishing glomerular bleeding from other cause of hematuria by examining the red cells in the urine sediments by using phase contrast microscopy.

If more than 20% of the red blood cells were dysmorphic, a glomerular origin for the site of bleeding was suspected; if less than 20% of the red blood cells were isomorphic, a non-glomerular origin was suspected.

Understanding Urine Sediment

Diagnosis	*Typical sediments*	*Other sediments*
Nephrotic syndrome	Fatty particles	Renal tubular epithelial cells (RTECs) RTEC casts RBC
Nephritic syndrome	RBC RBC casts	WBC RTECs (low number) RTEC casts
Acute tubular necrosis	RTECs RTEC casts Granular casts	Depending on cause: Myoglobin casts in rhabdomyolysis; Uric acid crystals; RBC, etc.
Urinary tract infection	Bacteria Leukocytes	RBC Transitional epithelial cells WBC casts
Urologic diseases	RBC (isomorphic) WBC	Transitional epithelial cells
BK virus infection	Decoy cells	Decoy cell casts (in BK virus nephropathy)

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