Sample collection

Mid-Stream Clean Catch

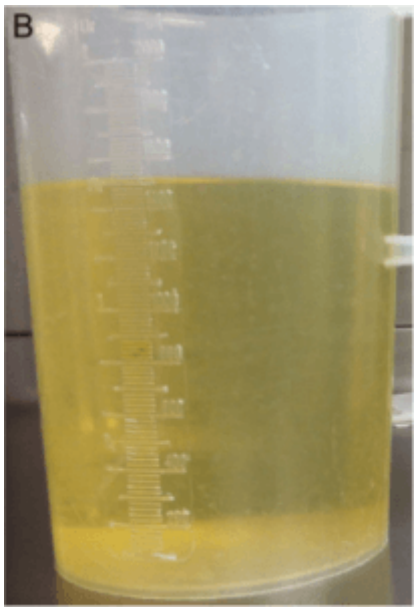
The ideal sample for urinalysis is the **'mid-stream clean catch'** sample obtained from the patient. The sample of urine should be approximately 30–60 ml.

Procedure

Men: Discard the initial 200 ml of urine and obtain the midstream sample.

Women: It is advised to clean the **external genitalia** before urinating with disinfecting wipes to avoid contamination of the sample. Obtain the mid-stream sample after discarding the initial 200 ml. Alternatively, in some patients, the sample can be obtained by inserting a **Foley's catheter** or even inserting a needle in the **suprapubic area** to obtain sterile samples. In case of a **urinary tract infection**, collect the sample before starting the patient on empiric antibiotics. Ideally, the sample should be tested about 30–60 minutes after collecting the sample. If a delay is expected, it is best to keep the sample refrigerated.

24-hour Sample Collection



The other kind of sample collection is the 24-hour sample collection, **used to measure certain parameters** like creatinine, protein, sodium, potassium, nitrogen, calcium, oxalate, urate and the total volume.

Procedure

On day 1, void urine normally in the toilet. Start collecting the urine voided after that in a container provided. Continue collecting the sample throughout the 24-hour period, including the next day's early morning sample. Store the sample in a refrigerator or a cool place during the period of collection.

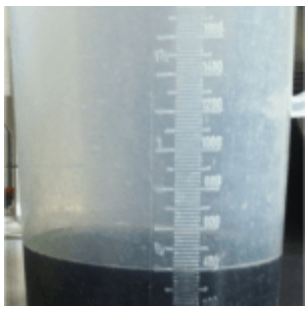
Components of the Analysis of Urine

Analysis of urine includes:

- Macroscopic examination
- Chemical analysis
- Sulfosalicylic acid test
- Microscopic examination

Macroscopic Examination

Macroscopic examination involves **observing the characteristics of the urine sample** that can be observed by the **naked eye**. The macroscopic examination includes color and turbidity.



Color

The normal color of urine is said to be straw yellow in color (spectrum is light/pale to dark/deep amber). Variations in color can be observed with various conditions and medications.

Physiological conditions	**Urine Color**
Food dyes (anthocyanins), beets, blackberries, rhubarb	Red
Carrot, vitamin C	Orange
Asparagus	Green
Fava beans	Brown
Drugs	**Urine Color**
Phenolphthalein	Pink
Rifampicin, phenazopyridine, senna	Orange
Chlorpromazine, thioridazine, Ex-lax	Red
Propofol	Green
Methylene blue, Amitriptyline	Blue
Levodopa, Metronidazole, Nitrofurantoin, Primaquine, Chloroquine, Methocarbamol	Brown
Pathological Conditions	**Urine Color**
Jaundice	Deep yellow
Hematuria	Red
Hemoglobinuria	Red
Myoglobinuria	Brown

Alkaptonuria	Black
PSGN	Cola colored/tea colored
Obstructive jaundice	Dark
UTI	Green

Turbidity

Normal urine should be clear. Increased turbidity or cloudiness of the sample indicates the presence of an underlying pathology with infection, cellular casts, crystals, protein or presence of secretions like semen, prostatic secretion or vaginal discharge.

Chemical Analysis (Dipstick test)

The dipstick is dipped into the urine sample and then let to settle after taking it out. The readings are noted after 2–5 minutes. The chemical agents on the strips change color based on the amount of the parameter in the urine. This includes:

- pH
- Specific gravity
- Glucose
- Ketones
- Nitrites
- Leukocyte esterase
- Bilirubin
- Blood
- Protein

pH

Normal urine is **mildly acidic** (normal range: 4.6–8). pH can be altered by normal compensatory physiological mechanisms of the body, or by disease. Urinary pH can be used to distinguish between the different types of RTA, in assessing the response to treatment of certain medication overdoses or assessing the treatment of rhabdomyolysis.

Parameter (Physiological)	**Change in pH**
Cranberry, protein-rich food	Acidic
Citrus fruits, low carbohydrate diet	Alkaline
Respiratory alkalosis	Acidic
Respiratory acidosis	Alkaline
Parameter (Pathological)	**Change in pH**
RTA (renal tubular acidosis)	Acidic
Uric acid calculi, cystine calculi	Acidic
Calcium oxalate, calcium phosphate, struvite stones	Alkaline
UTI d/t urease splitting organisms	Alkaline

Specific Gravity

The normal specific gravity of urine is 1.005–1.025. It is a measure of the kidney's concentrating ability. It can also give us an idea about the hydration status and the osmolality. For each rise in the specific gravity by 0.001 above 1, the urine osmolality increases by about 30–35 mosmol/kg.

Low Specific Gravity	**High Specific Gravity**
Diabetes insipidus	Dehydration
ATN	Heart failure

Sickle cell nephropathy	Excess Sugar or Protein in urine
Drinking too much fluid	SIADH

Glucose

The normal amount of glucose in urine should be **≤ 130 mg/dl**. When blood glucose levels exceed 180 mg/dl, the proximal tubules lose their ability to absorb any more glucose. The excess glucose in the urine is called **glucosuria** and is detected in the dipstick test.

The most common conditions causing glucosuria are diabetes and **pregnancy**. The other conditions causing glucosuria are: SGLT-1 gene mutations, tubular dysfunction (Fanconi syndrome), Lowe's syndrome (oculocerebrorenal dystrophy), tyrosinemia and Wilson's disease.

A condition called benign glucosuria is generally discovered on routine urinalysis. It is of three types:

- **Type A:** Classic glucosuria, has decreased renal glucose threshold & maximal glucose reabsorption rate
- **Type B:** Has decreased glucose threshold and a normal reabsorptive rate
- **Type O:** Lack of glucose reabsorption

Ketones

Normal urine does not contain ketones at all. The presence of even a small amount of ketones in urine is abnormal. The most common ketones found in urine are **acetone**, **acetoacetic acid** and **beta hydroxybutyrate**. Ketones are seen in:

- Diabetic ketoacidosis
- Uncontrolled diabetes
- Severe vomiting and starvation
- Severe exercise and dehydration
- Pregnancy

Nitrites

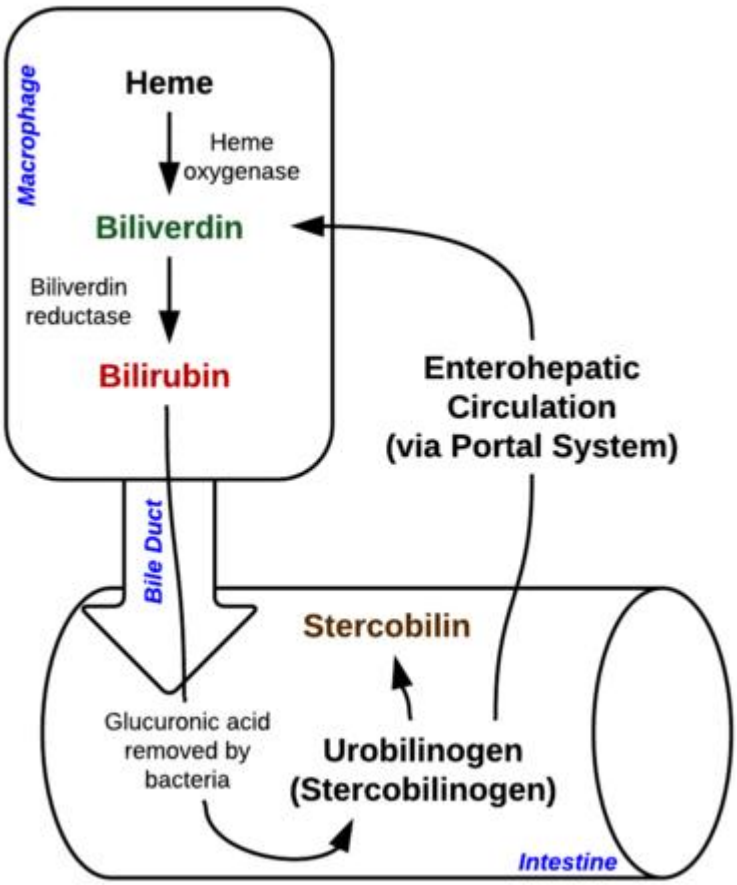
They are not normally seen in urine. Presence of nitrates mostly occurs in the presence of **bacteria** with the inherent capability to convert urinary nitrates into nitrites such as **E. coli, Klebsiella, Proteus, Pseudomonas, Enterobacter and Citrobacter;** hence, the presence of nitrites in urine is highly suggestive of UTI.

However, it does not rule out a urine infection if nitrites are negative because the infection can also be caused by organisms that do not possess the ability to convert urine nitrates; hence, it is a test with high specificity but has low sensitivity.

Leukocyte Esterase

It is normally absent in urine. Leukocyte esterase is an enzyme produced by the lysis of WBCs. In normal sterile urine, the WBCs are very low in number to potentially produce a positive leucocyte esterase test. It is positive only with **pyuria** (increased number of WBCs). Pyuria is caused by infection and analgesic nephropathy (sterile pyuria).

Bilirubin

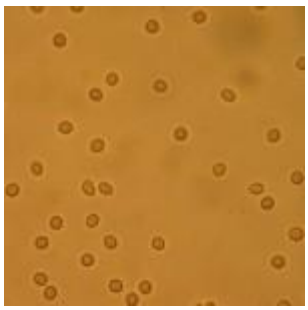


Bilirubin is normally absent in urine. Bilirubin gets converted into urobilinogen in the intestine. Urobilinogen enters the portal circulation again and then is excreted in very small amounts (0.5–1 mg/dL) in the urine. Decreased bilirubin is seen in obstructive biliary disease and severe cholestasis. Excess bilirubin in urine is observed in the following conditions:

Jaundice	**Conditions**
Physiological	Newborn jaundice
Pathological	Biliary tract disease Hepatitis Cirrhosis

	Gallstones Tumors of liver or GB Excessive hemolysis Intestinal bacterial overgrowth

Blood



The test checks for the peroxidase activity of RBCs. Normal amount of RBCs in urine is **≤ 3 RBCs**. If the number of RBCs is > 3, blood is detected in the test. This test is qualitative in nature and does not give any information about the origin of the blood detected.

After centrifugation, if the sediment contains the blood (RBCs), it is **indicative of hematuria**. If the supernatant fluid contains blood, it is due to food dyes, porphyria, hydroxocobalamin and phenazopyridine. Myoglobinuria caused by rhabdomyolysis and hemoglobinuria caused by transfusion-related reactions.

Clostridium and Plasmodium infections do not contain RBCs on microscopic examination, which helps in differentiating them from hematuria, which shows RBC sediments on microscopic examination.

Protein

The normal amount of protein in urine is **≤ 150 mg/dl**. The Dipstick test measures only albumin and not the other proteins; hence, it is a highly specific test, but with low sensitivity. The Dipstick test becomes positive with levels > 300 mg/ dl; therefore it cannot be used to detect microalbuminuria in diabetic patients.

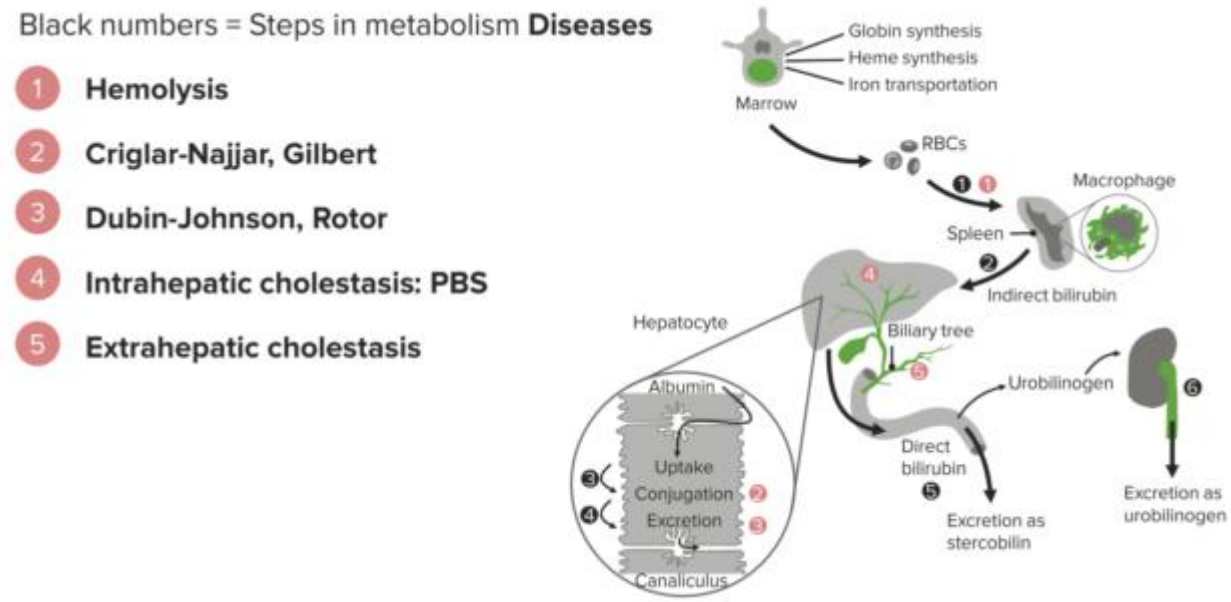
It could give false positive results when tested immediately after giving contrast for testing and also with concentrated urine; hence, caution should be used to prevent such false positives. Approximate inference of dipstick values:

Trace +	**10–30 mg/dl**
1+	> 30 mg/dl
2+	> 100 mg/dl
3+	> 300 mg/dl
4+	> 1000 mg/dl

1+ to 2+ ⇒ equivalent to 24 hr excretion of < 0.5mg
2+ to 3+ ⇒ Glomerular disease more likely
3+ to 4+ ⇒ Nephrotic range, equivalent to 24 hr excretion of > 3g

Types	**Conditions**
Transient proteinuria	Vigorous exercise, fever, heart failure, UTIs
Orthostatic proteinuria	Upright posture (occurs only during the day)
Gross proteinuria	Nephrotic syndrome, nephritic syndrome

Microalbuminuria (not in urinalysis)	Diabetic nephropathy
Bence Jones proteinuria (not in urinalysis)	AL amyloidosis, B cell disorders, Multiple myeloma
Tubular proteinuria	Tubulointerstitial diseases (ATN, Fanconi syndrome, acute interstitial disease)



SSA- Sulfosalicylic Acid Test

This test **detects all proteins** like albumin, globulin and Bence Jones proteins in the urine at any amounts.

Method

Check turbidity of the solution made by 3 parts of 3% sulfosalicylic acid mixed with one part of urine supernatant.

Interpretation

0	No turbidity (proteinuria – 0 mg/dL)

Trace	Slight turbidity (proteinuria – 20 mg/dL)
1+	Print visible through specimen (proteinuria – 50 mg/dL)
2+	Print invisible (proteinuria – 200 mg/dL)
3+	Flocculation (proteinuria – 500 mg/dL)
4+	Dense precipitate (proteinuria ≥1000 mg/dL)

Microscopic Examination

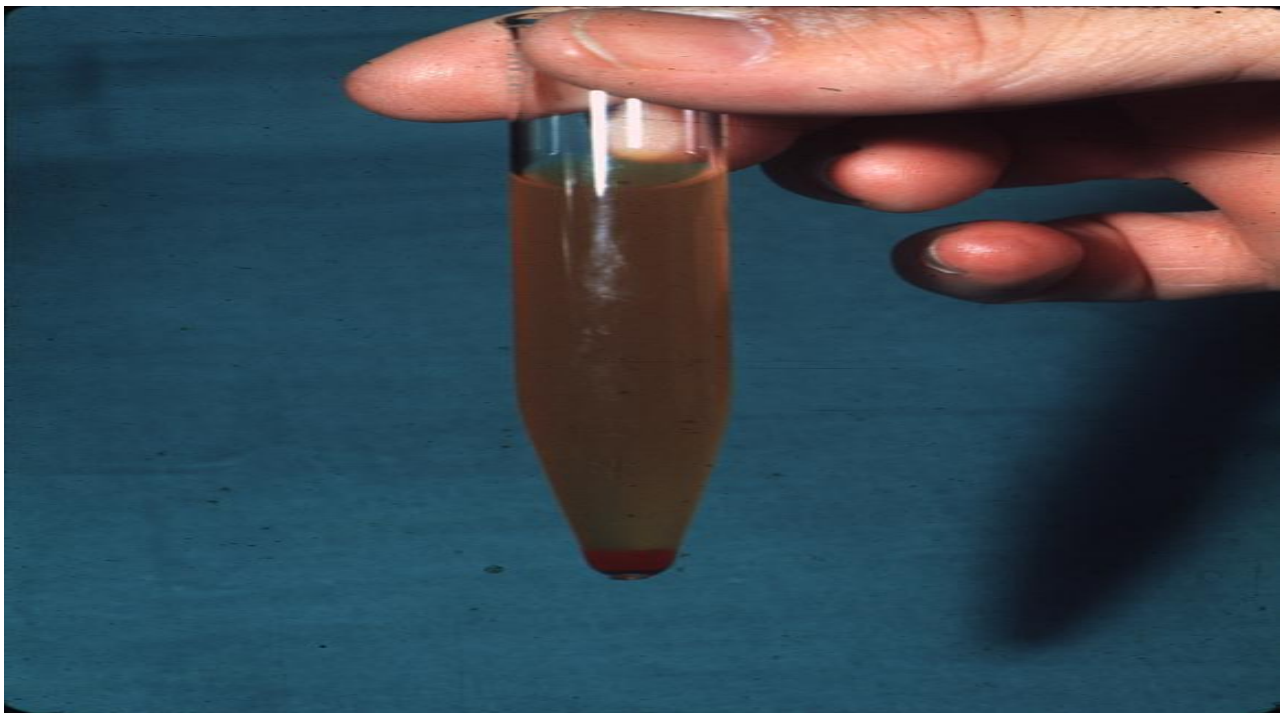
Microscopic examination is done after **centrifuging the urine sample for about 3–5 minutes**. The supernatant fluid is discarded and the underlying sediment is observed under microscope. It is checked for parameters like cells, casts, crystals and living organisms.

Normal/Few Hyaline Casts

The presence of up to **0–5 hyaline** casts/hpf* ("casts per high power field") is considered to be normal. Hyaline casts can be seen in normal healthy people. It is composed mostly of **Tamm Horsfall protein**. They are formed only in the **distal convoluted tubule,** or the collecting duct. Low urine pH and high urinary salt concentration promote hyaline cast formation.

RBCs/RBC Casts

The normal amount of urine contains **≤ 2 RBCs/hpf**. The presence of 3 or more RBCs/hpf in 2–3 samples is called hematuria. Red cell casts are diagnostic of glomerulonephritis or vasculitis. Dysmorphic erythrocytes suggest the presence of nephritis.



Transient hematuria is seen in young patients and is considered benign. However, in the elderly, even if hematuria is transient, it needs to be worked up fully to exclude the possibility of cancer.

WBCs/WBC Casts

The normal amount of WBCs seen in urine is **≤ 2–5 WBCs/hpf**. The presence of leucocytes and bacteria in urine is indicative of a renal tract infection. White cell casts are strongly suggestive of pyelonephritis. WBCs and WBC casts are seen in:

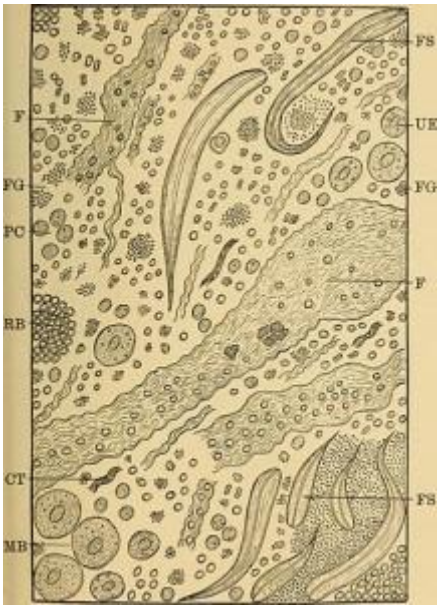
- Interstitial nephritis
- Glomerulonephritis
- Pyelonephritis
- Allograft rejection
- Malignant infiltration of the kidney

Renal Tubular Epithelial Cells/RTE Casts/Pigmented Casts

The normal amount of squamous epithelial cells in urine is **≤ 15–20 squamous epithelial cells/hpf**. If it is > 15–20, it is considered to be an infection. Some of the conditions showing an increased count of cells are:

- Acute tubular necrosis
- Tubulointerstitial nephritis
- Acute cellular allograft rejection
- Myoglobinuria
- Hemoglobinuria

Granular Casts



They are seen in:

- Acute tubular necrosis
- Glomerulonephritis
- Vasculitis
- Tubulointerstitial nephritis

Eosinophiluria

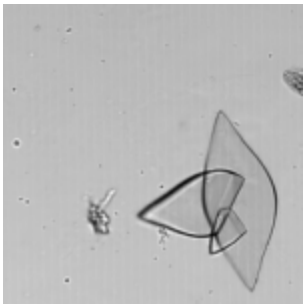
They are observed in the following conditions:

- Allergic interstitial nephritis
- Atheroembolic disease
- Pyelonephritis
- Cystitis
- Glomerulonephritis

Crystalluria

Crystals are seen occasionally in normal urine. It should be noted that calcium oxalate and urate crystals can be found in normal urine that has been left to stand for some time.

Acute Uric Acid Nephropathy

- 

  Seen in tumor lysis syndrome, hyperuricosuria and gout

- Radiolucent
- Rhomboid or rosette shaped crystals

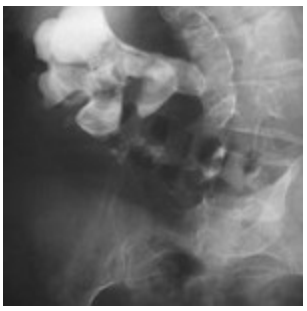
Calcium Oxalate Crystals

- Envelope or dumbbell-shaped crystals
- Seen in ethylene glycol intoxication
- Radio-opaque
- Conditions causing hypercalciuria

Cystine Crystals

- Hexagonal crystals
- Seen in cystinuria
- Radiopaque
- Nitroprusside test positive

Struvite Stones

- 

Composed of magnesium ammonium phosphate, also called triple phosphate

- Shaped like a staghorn and radio-opaque
- Caused by urease positive organisms
- Coffin lid shaped crystals

Bacteria

Normal urine is sterile and does not contain bacteria. The presence of bacteria indicates an infection or contamination. The bacteria can be cultured in an appropriate medium to find out the exact species. Empiric antibiotics should be started while waiting for culture results.

Overview

General Examination	
Color	Dark yellow: Concentrated urine, bilirubinuria, ↑ UBG, vitamins Red or pink: Hematuria, hemoglobinuria, myoglobinuria, drugs (e.g., phenazopyridine, a urinary aesthetic), porphyria Smoky-colored urine: Acid pH urine converts Hb to hematin; common finding in the nephritic type of glomerulonephritis Black urine after exposure to light: Alkaptonuria (AR disease with deficiency of homogentisate oxidase) with an increase in homogentisic acid in the urine; turns black when exposed to light
Clarity	Cloudy urine with alkaline pH: Normal finding most often due

	to phosphates Cloudy urine with acid pH: Normal finding most often due to uric acid Other: Bacteria, WBCs, Hb, myoglobin also decrease clarity
Specific gravity	Evaluates urine concentration and dilution Specific gravity > 1.023 (UOsm 900 mOsm/kg) indicates urine concentration and excludes intrinsic renal disease Hypotonic urine has a specific gravity < 1.015 (~ UOsm 220 mOsm/kg) UOsm is the best indicator of urine concentration/dilution Fixed specific gravity (1.008—1.010): correlates with UOsm; lack of concentration and dilution (e.g., chronic renal failure)
Chemical Dipstick	
pH	Determined by diet and acid-base status of the patient; pure vegan usually has alkaline pH (citrate converted into bicarbonate); meat eater usually has acid pH (organic acids in meat) Alkaline pH + smell of ammonia: Urease-producing pathogen (e.g., Proteus)
Protein	Detects albumin (not globulins) SSA: Detects albumin and globulins (e.g. BJ protein) Albuminuria: Reagent strip and SSA have the same results BJ protein: SSA greater than reagent strip result; always confirm BJ protein with urine immunoelectrophoresis

Glucose	Specific for glucose; will not detect fructose or other sugars Detect glucose in urine as los as 30 mg/dL ↑ Serum glucose + glucosuria: Diabetes mellitus Normal serum glucose + glucosuria: Normal pregnancy (normally have a low renal threshold for glucose), benign glucosaria (low renal threshold for glucose) Microalbuminuria dipsticks: More sensitive than standard dipstick; sensitive to 1.5—8 mg/dL; microalbuminuria is the first sign of diabetic nephropathy
Ketones	Detects acetone, acetoacetic acid (not β-OHB); nitroprusside in the test system only reacts with AcAc and acetone, not β-OHB
Bilirubin	Detects conjugated (water-soluble) bilirubin Bilirubinuria: Viral hepatitis, obstructive jaundice
Urobilinogen	Normal to have trace amounts (normal urine color is due to urobilin) Absent urine UBG, ↑ urine bilirubin: Obstructive jaundice ↑ Urine UBG, absent urine bilirubin: Extravascular hemolytic anemia (e.g., hereditary spherocytosis) ↑ Urine UBG, ↑ urine bilirubin: Hepatitis