

366803

# Simulating Urinalysis Lab Activity

**(student guide)**

Aligned With All Published National Standards

**In this lab, you will be testing simulated urine samples for pH, sugar, and protein and will then observe the samples through a microscope for further analysis. You will be provided with four different case studies and simulated urine for each patient. You will then perform a urinalysis on each sample. The understanding of these exercises will allow you to learn the basics of urinalysis and how it is used to diagnose medical problems.**

## safety precautions

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### general safety:

- Know where all emergency equipment (safety shower, eyewash station, fire extinguisher, fire blanket, first aid kit etc.) is located.
- Remove all dangling jewelry and tie back long hair before you begin.
- Read all instructions and Safety Data Sheets (SDSs) before starting the lab activities, and ask questions about safety and safe laboratory procedures.
- Wear proper protective equipment, such as gloves, safety goggles, and a lab apron.

### at the end of the lab:

- Wash your hands thoroughly with soap and water before leaving the laboratory.
- All laboratory bench tops should be wiped down with a 10% bleach solution or disinfectant to ensure cleanliness.

# vocabulary guide

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**Albumin:** A simple form of protein, soluble in water and coagulable by heat, such as that found in egg white, milk, and blood serum.

**Casts:** Tiny tube-shaped particles made up of white blood cells, red blood cells, or kidney cells.

**Etiology:** The causation of diseases and disorders as a subject of investigation.

**Hemoglobin:** A red protein responsible for transporting oxygen in the blood of vertebrates.

**Intracellular Osmolality:** Determines the distribution of water among the different fluid compartments, particularly between the extracellular and intracellular fluids. Osmolality effects this distribution of water through the generation of osmotic pressure.

**Ketones:** Chemicals produced by the body when fat instead of glucose is burned for energy. Breakdown of fat occurs when not enough insulin is present to channel glucose into body cells.

**Phenylketonuria (PKU):** An inherited disease due to faulty metabolism of phenylalanine, characterized by phenylketones in the urine and usually first noted by signs of mental retardation in infancy.

**Specific Gravity:** The ratio of the density of a substance to the density of a standard, usually water for a liquid or solid, and air for a gas.

**Symptoms:** Subjective evidence of disease or physical disturbance; broadly, something that indicates the presence of bodily disorder.

**Syndromes:** Groups of signs and symptoms occurring in a characteristic pattern.

**Urinalysis:** Analysis of urine by physical, chemical, and microscopical means to test for the presence of disease, drugs, etc.

Recognition of the presence of disease is based to some extent on the existence of objective signs or recognizable abnormalities known as symptoms. Groups of signs and symptoms occurring in a characteristic pattern (referred to as syndromes) are of value in diagnosis and in determining the distribution as well as the cause, or etiology, of diseases.

The process of diagnosing a disease involves several steps. First, the patient will consult with the physician and describe any particular symptoms that he or she has previously noticed. The physician will then examine the patient and note any signs that may be further indicative of the problem. Based on this initial assessment and the patient's medical history, the physician may then make a diagnosis of the disorder. However, in some cases it may be necessary to go a step further and order various laboratory tests, possibly including X-ray examinations. This serves not only to arrive at a correct diagnosis, but also to rule out any other disorders which may share the same signs and/or symptoms.

There are many different types of specimens used in laboratory diagnosis, including blood, feces, sputum, urethral and vaginal secretions, and cerebrospinal fluids. Urine testing is another very important diagnostic tool that involves the physical, chemical, and visual examination of a urine sample. A thorough urinalysis may provide more information about the general condition of the body than any other set of tests. Urinary tract infections, kidney malfunction, diabetes, and liver disease are just some of the medical problems that can be diagnosed through urinalysis.

Urinalysis is often used for the screening of drugs. A urine sample can be tested for drug overdose and toxicity, or for the presence of abused drugs, commonly including amphetamines, barbiturates, cannabinoids, cocaine, methadone, benzodiazepines, methaqualone, and opiates. This test is very useful as a pre-employment drug screen. One limitation, however, is that this test provides only qualitative detection of drugs. Quantitation of drug levels is not recommended because urine levels are time and clearance dependent and are not directly related to toxic symptoms seen clinically.

Another important use of urinalysis is for pregnancy testing. When a woman becomes pregnant, a hormone known as Human Chorionic Gonadotropin begins to be secreted by the embryonic tissues shortly after fertilization. HCG secretion then increases until it reaches a peak in about fifty to sixty days; thereafter, the HCG concentration drops to a much lower level and remains relatively stable throughout the pregnancy. Because HCG is excreted in the urine, urinalysis is used to detect this hormone, thereby indicating the presence of an embryo. Such a pregnancy test may give positive results as early as eight to ten days after fertilization.

Several factors are examined when analyzing a urine sample. These include appearance of the urine, odor, pH, specific gravity, and microscopic observations.

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## appearance

The color of normal urine can range from pale yellow to amber, depending on the concentration of the pigment urochrome, which is the end product of hemoglobin breakdown. The appearance of the urine may serve as an indication of a pathological condition. For instance, pale yellow urine may indicate diabetes insipidus, granular kidney, or may simply be very dilute due to ingestion of copious amounts of water. A milky color might signify fat globules or pus corpuscles, the latter possibly indicating a urogenital tract infection. Reddish colors may be due to food pigments (such as beets), certain drugs, or blood in the urine. Greenish colors indicate either bile pigment (jaundice) or certain bacterial infections, such as those caused by several species of *Pseudomonas*. Lastly, brown-black urine can indicate phenol or metallic poisonings or hemorrhages due to conditions such as renal injury or malaria.

## odor

The odor of urine can vary greatly according to both diet and pathology. An ammonia smell may result from certain foods, while a fishy smell may indicate cystitis. A fecal smell could be due to an intestinal-urinary tract fistula. Other distinctive smells could be indicative of disorders such as acetonuria, which causes an overripe apple smell, or diabetes, which can cause sweet-smelling urine due to the sugar content.

## pH

The pH of normal urine ranges from 4.5 to 8.0, the acidity or alkalinity can fluctuate depending on the type of food ingested. Pathological conditions can also affect the pH of urine. Fevers and acidosis lower the pH, whereas anemia, vomiting, and ischuria (urine retention) raise the pH.

## specific gravity

Yet another component of urinalysis is the determination of specific gravity. This is a measure of the density of a substance in g/mL as compared to the density of water, which has a specific gravity of 1.00 g/mL. The specific gravity of urine usually ranges between 1.015 and 1.025, although numbers slightly higher or lower may be normal for people with diets either very high or low in fluid content. Specific gravity is generally inversely proportional to urinary volume. A pathological low specific gravity indicates nephritis, whereas a pathological high specific gravity indicates either nephritis or diabetes mellitus.

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## microscopic observations

The microscopic examination of urine is a vital aspect of routine urinalysis. Urine is made up primarily of water, with some salts and organic materials dissolved in it. Inorganic substances normally found in the urine include sulfates, chlorides, phosphates, and ammonia. Casts, cells, crystals, and microorganisms are some of the significant elements found in the urine sediment.

## casts

Casts in the urine are particularly significant because they represent cylindrical molds formed in the renal tubular lumina. They are formed by the precipitation of proteins and agglutination of cells within the renal tubules. Casts are classified into several major types: hyaline, epithelial, granular (coarse and fine), fatty, waxy, red-blood cell, and white-blood cell. Because casts originate within the renal parenchyma, their presence in the urinary sediment often provides important diagnostic clues as to the underlying renal pathology. For example, the presence of red blood-cell casts is always indicative of renal parenchymal disease, especially glomerulonephritis. The formation of casts is favored in a number of pathologic conditions in the nephron. These include: (1) the presence of protein constituents in the tubular urine, (2) increased acidification, and (3) increased osmolar concentration. A reasonable conclusion, then, is that casts will be formed principally within the distal convoluted tubules and the collecting ducts because the urine becomes maximally acidified and concentrated in this segment of the nephron.

## cells

Cells are exfoliated from different parts of the genitourinary tract for various reasons, including normal “wear and tear”, degenerative and inflammatory processes, or secondary process due to infarction or tumor formation. The metabolic activity of the cells found in a urine sample has been impaired to varying degrees, resulting in membrane changes in permeability and selectivity, and causing variations of hydration, intracellular osmolality, density, and microscopic characteristics. Swelling, shrinking, or intracellular structural changes may also occur due to exposure for ill-defined periods of time to wide variations in urine osmolality and pH, toxic substances, excreted drugs and metabolites, and bacterial actions. Cell types include urothelial (transitional), columnar epithelial, prostatic, seminal vesical, decoy, multinucleated giant, squamous, tubular epithelial, oval fat, redblood cell, and white-blood cell. Microscopic evaluation of cells in urinary sediment may help in the diagnosis of neoplastic disease (carcinoma) and some non-neoplastic diseases of the urinary tract.

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## crystals

The variety of crystals and amorphous compounds found in the normal urinary sediment may represent both the end product of tissue metabolism and the excessive consumption of certain foods or drugs. The type of crystal or amorphous compound depends to some extent on the pH and osmolality of the urine. The presence of some crystals are of little or no significance while others constitute a positive diagnostic test. Common crystals may be present normally in acid, neutral, or alkaline urine. However, abnormal types of crystals are almost always associated with only acid or neutral urine.

## albumin, glucose, and ketones

The presence of various substances in the urine that are not normally there can, in some cases, be an indication of a disorder. For example, the presence of albumin (a plasma protein that helps regulate the osmotic concentration of the blood) in a urine sample may indicate a kidney malfunction since kidneys are supposed to filter albumin and glucose out of the waste material and return them to the body. And as such, the presence of glucose in urine may also be a positive indication of a disorder, namely, diabetes mellitus. The hallmark of diabetes is an increase in the concentration of blood sugar (hyperglycemia). When the blood sugar reaches a certain high concentration, it exceeds the renal threshold and the kidneys begin to excrete the excess. At this point, glucose appears in the urine (glycosuria). Another substance that might be found in the urine to indicate diabetes is ketones. These occur when fats are metabolized at abnormally high rates, thus causing ketone bodies to accumulate faster than they can be oxidized. Ketones may also appear in the urine when carbohydrate metabolism is inadequate. So a person may also have ketonuria when he or she suddenly begins a very low carbohydrate diet. Other symptoms of diabetes mellitus include

Urine Type	Crystal Type	Possible Indications
Alkaline	Calcium Phosphate	Calculi (stone) formation
Alkaline	Triphosphate	Calculi formation
		Obstructive uropathy
		Urinary tract infection
		Proteus mirabilis infection
Alkaline	Calcium carbonate	Calculi formation
Acid	Calcium oxalate	Excessive intake of oxalate rich food (e.g. spinach, garlic, tomatoes, oranges)
		Hyperoxaluria
		Ethylene glycol poisoning
		Diabetes mellitus
Acid	Uric acid	Gout
		Leukemia
		High purine metabolism
		Chronic nephritis
Acid	Hippuric acid	No clinical significance
Abnormal (Acid to Neutral)	Leucine	Severe liver disease (leucine & tyrosine may occur together)
	Tyrosine	
	Cystine	Calculi, congenital cystinosis, congenital cystinurea (cystine present)

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the classic triad, that is, excessive urine output (polyuria), dehydration accompanied by great thirst (polydipsia), and increased appetite (polyphagia). The person is also likely to lose weight and the ability to grow or repair damaged tissues is decreased.

## phenylketonuria

Urinalysis is also useful in assisting in the detection of a condition known as phenylketonuria. PKU is a failure of the body to produce the enzyme necessary to oxidize phenylalanine to tyrosine, namely, phenylalanine hydroxylase. PKU is a recessive genetic trait whose incidence is somewhat over 1:11,000 in the United States. PKU causes nerve and brain damage, accompanied by mental retardation if left untreated. However, by reducing or eliminating phenylalanine from the diet, retardation does not occur. Because the phenylketones appear in the urine, infants with PKU often have diapers with distinctive odors; this observation by Swedish mothers ultimately led to the conclusion by scientists that PKU may be detected by checking the urine. However, urinalysis is not used as an initial screening for PKU. Ideally, newborns should be screened via a blood test when they are between 48 to 120 hours of age and have been on a milk (protein) feeding for at least 24 hours. After birth, 2-6 weeks may pass before phenylpyruvic acid is excreted in the urine. After PKU has been diagnosed, urine screening type tests may be used to make sure the disease is being controlled properly. (Look at the nutrition label of carbonated drinks. Some state that they contain phenylketonurics, namely phenylalanine). State laws require PKU testing of infants within 28 days or less; in some states, testing is required prior to hospital discharge regardless of age.

## case studies

**Patient 1** — Jeff Jones is 19 years old. He notices that he has increased urine output (polyuria), increased appetite (polyphagia), and great thirst (polydipsia). He has also experienced unexplained weight loss.

**Patient 2** — Mr. Thompson is 60 years old and has been unusually tired for several weeks. He occasionally feels dizzy and lately he finds it increasingly difficult to sleep at night. He has swollen ankles and feet and his face looks puffy. He experiences a burning pain in his lower back, just below the rib cage. He also notices that his urine is dark in color. He goes to see his physician, who finds that he has elevated blood pressure, and that the kidney region is sensitive to pressure.

**Patient 3** — Ms. Smith is 27 years old and has been experiencing painful and difficult urination (dysuria), frequency of urination and urgency. Her urine has a milky color. She also has fever and malaise, which is evidence of infection. Upon seeking treatment, she is given antibiotic therapy. After a few days on antibiotics, her symptoms disappear.

**Patient 4** — Normal sample (control).



# pre-lab questions

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1. Effectively **communicate** the process of diagnosing a disease.

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2. **Analyze** the importance of a urine test in diagnosing potential medical problems.

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3. Urinalysis can be used to help diagnose medical problems. **Analyze** other uses for this test.

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4. Several factors are examined when analyzing a urine sample. List and **explain** the five factors noted in the background.

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Using the information about the various aspects of urinalysis, analyze the four simulated urine samples and fill in the tables on the results and analysis page. Compare your observations to the chart (on page S7) detailing possible disorders according to pH and crystal type. Based on these results, as well as the case studies, see if you can provide a possible diagnosis of the person's condition.

## physical characteristic observations

1. Label four medicine cups 1-4.
2. Shake each urine sample thoroughly and dispense 10 mL of each sample into the properly labeled medicine cup.
3. For each urine sample provided, observe and record the color, clarity, and smell of the urine. Record your findings in Table 1 in the Results and Analysis section.

## testing pH

1. Dip a pH test strip into the simulated urine from Patient #1.
2. Compare the color of the test strip to the comparator chart within 30 seconds of sampling. Record the pH in Table 1.
3. Repeat steps 1 and 2 for the remaining samples.

## testing for sugar (Benedict's Test)

1. Place 250 mL of water into a 400 mL beaker and place the beaker on a hot plate to create a hot water bath.
2. In to a test tube, add 3 mL of the simulated urine sample from Patient #1 and 3 mL of Benedict's solution.
3. Record the color of the solution, in table 2.
4. Using a test tube holder, place your test tube in the hot water bath and allow it to boil for 2 minutes. Remove the sample from the hot water bath and record any color change, in Table 2.



Be sure to use heat protective gloves when handling hot objects.

5. Repeat steps 2-4 for the remaining samples.
6. A positive reaction will result in a yellow to red color. Examine your data and note whether it was a positive or negative reaction in Table 2.

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## testing for protein (Biuret Test)

1. Remove 3 mL of the simulated urine sample for Patient #1, and place it into a clean test tube. Record its initial color in table 3.
2. Add 1 mL of Biuret solution to the urine and swirl.
3. Record the final color of the solution in Table 3.
4. Repeat steps 1-3 for the remaining samples.
5. A positive reaction will result in an orange-red color, and a negative reaction will result in a green color. Examine your data and note whether it was a positive or negative reaction in Table 3.

## microscopic observations

1. Label four microscope slides 1-4.
2. Gently swirl the urine specimen from Patient #1.
3. Place one drop of simulated urine on a slide and place a coverslip over it.
4. Using a microscope, scan the slide for any cells, red blood cells (visible as small red spheres) or leukocytes (visible as small blue spheres) that may be present.
5. Also look for any crystals that may be visible and record your observations in table 4.
6. Repeat steps 2-5 for the remaining samples.

## further microscopic observations

View the provided microscope slide and identify the crystals.

# results and analysis

**table 1 - physical characteristics**

Sample	Color	Clarity	Smell	pH
Patient #1				
Patient #2				
Patient #3				
Patient #4				

**table 2 - Benedict's test for sugar**

Sample	Color before heating	Color after heating	Result (+ or -)
Patient #1			
Patient #2			
Patient #3			
Patient #4			

**table 3 - Biuret test for protein**

Sample	Initial Color of sample	Final Color after adding biuret	Result (+ or -)
Patient #1			
Patient #2			
Patient #3			
Patient #4			

**table 4 - microscopic observations**

Sample	Red Blood Cells	White Blood Cells	Crystals
Patient #1			
Patient #2			
Patient #3			
Patient #4			

1. **Predict** what disorder patient 1 (Jeff Jones) probably has. Why do you believe this to be so? What type of crystals might be present in his urine?

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2. What diagnosis would you give patient 2 (Mr. Thompson)? **Argue** what type of casts might be found in his urine.

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3. After examining the urine specimen from patient 3 (Ms. Smith) and studying her case history, what disorder does she probably have? What type of crystals do you believe would be found in her urine? To what would you attribute the milky color of her urine?

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4. **Prove** why it is important to perform tests on a control urine sample not containing any chemical substances.

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5. A urine sample from a patient contains albumin, chloride, glucose, and phosphate molecules, while a control urine sample contains only chloride and phosphate molecules. **Explain** what this tells you about one of the functions of the kidneys.

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6. The presence of blood and/or casts in the urine can indicate a serious kidney problem. Why are kidney problems so serious?

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7. Suppose a urine sample revealed abnormal results, such as protein in the urine. If there is a result differing from the norm (e.g., color, pH, substances present), should the physician always make an immediate diagnosis of a disorder? **Argue** why or why not.

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8. Why is it important to develop a case history of the physical symptoms of each patient to be used along with the physical tests performed on the patient's urine specimen?

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9. Urinalysis is an important diagnostic tool for the determination of medical disorders. Urinalysis has many other uses. **Research** one such use and describe it below. Be prepared to share your findings with the class.

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10. Create a poster showing the various structures of the kidney. Label the individual structures and **explain** the function of each in the excretory system. In your poster, include a **diagram** of a nephron and label and explain its features as well.