CHAPTER **5**

Chemical Examination of Urine

LEARNING OBJECTIVES

Upon completion of this chapter, the reader will be able to:

1 Describe the proper technique for performing reagent strip testing.
2 List four causes of premature deterioration of reagent strips, and tell how to avoid them.
3 List five quality-control procedures routinely performed with reagent strip testing.
4 Name two reasons for measuring urinary pH, and discuss their clinical applications.
5 Discuss the principle of pH testing by reagent strip.
6 Differentiate between prerenal, renal, and postrenal proteinuria, and give clinical examples of each.
7 Explain the "protein error of indicators," and list any sources of interference that may occur with this method of protein testing.
8 Discuss the sulfosalicylic acid (SSA) test for urine protein, including interpretation and sources of interference.
9 Describe the unique solubility characteristics of Bence Jones protein, and tell how they can be used to perform a screening test for the presence of this protein.
10 Explain why glucose that is normally reabsorbed in the proximal convoluted tubule may appear in the urine, and state the renal threshold levels for glucose.
11 Describe the principle of the glucose oxidase method of reagent strip testing for glucose, and name possible causes of interference with this method.
12 Describe the copper reduction method for detection of urinary reducing substances, and list possible causes of interference.
13 Interpret matching and nonmatching results between the glucose oxidase and the copper reduction tests for glucose.
14 Name the three "ketone bodies" appearing in urine and three causes of ketonuria.
15 Discuss the principle of the sodium nitroprusside reaction, including sensitivity and possible causes of interference.
16 Differentiate between hematuria, hemoglobinuria, and myoglobinuria with regard to the appearance of urine and serum and clinical significance.
17 Describe the chemical principle of the reagent strip method for blood testing, and list possible causes of interference.
18 Discuss methods used to differentiate between hemoglobinuria and myoglobinuria.
19 Outline the steps in the degradation of hemoglobin to bilirubin, urobilinogen, and finally urobilin.
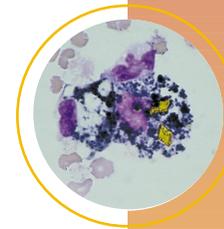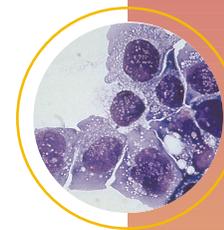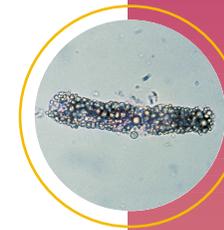
20 Describe the relationship of urinary bilirubin and urobilinogen to the diagnosis of bile duct obstruction, liver disease, and hemolytic disorders.
21 Discuss the principle of the reagent strip test for urinary bilirubin, including possible sources of error.
22 Discuss the advantages and disadvantages of performing an Ictotest for detection of urine bilirubin.
23 State two reasons for increased urine urobilinogen and one reason for a decreased urine urobilinogen.
24 Describe the Watson-Schwartz test used to differentiate among urobilinogen, porphobilinogen, Ehrlich reactive compounds, and the Hoesch screening test for porphobilinogen.
25 Discuss the principle of the nitrite-reagent-strip test for bacteriuria.
26 List five possible causes of a false-negative results in the reagent-strip test for nitrite.
27 State the principle of the reagent strip test for leukocytes.
28 Discuss the advantages and sources of error of the reagent strip test for leukocytes.
29 Explain the principle of the chemical test for specific gravity.
30 Compare reagent strip testing for urine specific gravity with urinometer and refractometer testing.
31 Correlate physical and chemical urinalysis results.

KEY TERMS

bacteriuria	myoglobinuria
bilirubin	orthostatic proteinuria
glycosuria	postrenal proteinuria
hematuria	prerenal proteinuria
hemoglobinuria	protein error of indicators
ketonuria	proteinuria
leukocyturia	renal proteinuria
microalbuminuria	urobilinogen

Reagent Strips

Routine chemical examination of the urine has changed dramatically since the early days of urine testing, owing to the development of the reagent strip method for chemical analysis. Reagent strips currently provide a simple, rapid means for performing medically significant chemical analysis, including pH, protein, glucose, ketones, blood, *bilirubin*, *urobilinogen*, nitrite, leukocytes, and specific gravity. The two major types of reagent strips are manufactured under the tradenames Multistix (Bayer Corporation, Elkhart, IN) and Chemstrip (Roche-Boehringer Mannheim Diagnostics, Indianapolis, IN). These products are available with single- or multiple-testing areas, and the brand and number of tests used are a matter of laboratory preference. Certain variations relating to chemical reactions, sensitivity, specificity, and interfering substances occur among the products and are discussed in the following sections. Reagent strip brands are also specified by instrumentation manufacturers.

Reagent strips consist of chemical-impregnated absorbent pads attached to a plastic strip. A color-producing chemical reaction takes place when the absorbent pad comes in contact with urine. Color reactions are interpreted by comparing the color produced on the pad with a chart supplied by the manufacturer. Several colors or intensities of a color for each substance being tested appear on the chart. By careful comparison of the colors on the chart and the strip, a semiquantitative value of trace, 1+, 2+, 3+, or 4+ can be reported. An estimate of the milligrams per deciliter present is available for appropriate testing areas. Automated reagent strip readers also provide Système International units.

REAGENT STRIP TECHNIQUE

Testing methodology includes dipping the reagent strip completely, but briefly, into a well-mixed specimen; removing excess urine from the strip when withdrawing it from the specimen; waiting the specified length of time for reac-

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PROCEDURE

Reagent Strip Technique

- Dip the reagent strip briefly into a well-mixed uncentrifuged urine specimen at room temperature.
- Remove excess urine by touching the edge of the strip to the container as the strip is withdrawn. Blot the edge of the strip.
- Wait the specified amount of time for the reaction to occur.
- Compare the color reaction of the strip pads to the manufacturer's color chart in good lighting.

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tions to take place; and comparing the colored reactions against the manufacturer's chart using a good light source.

Improper technique can result in errors. Formed elements such as red and white blood cells sink to the bottom of the specimen and will be undetected in an unmixed specimen. Allowing the strip to remain in the urine for an extended period may cause leaching of reagents from the pads. Likewise, excess urine remaining on the strip after its removal from the specimen can produce a runover between chemicals on adjacent pads, producing distortion of the colors. To ensure against runover, blotting the edge of the strip on absorbent paper and holding the strip horizontally while comparing it with the color chart is recommended. The amount of time needed for reactions to take place varies between tests and manufacturers and ranges from an immediate reaction for pH to 120 seconds for leukocytes. For the best semiquantitative results, the manufacturer's stated time should be followed; however, when precise timing cannot be adhered to, manufacturers recommend that reactions be read between 60 and 120 seconds, with the leukocyte reaction read at 120 seconds. A good light source is, of course, essential for accurate interpretation of color reactions. The strip must be held close to the color chart without actually placing it on the chart. Reagent strips and color charts from different manufacturers are not interchangeable. Specimens that have been refrigerated must be allowed to return to room temperature prior to reagent strip testing, as the enzymatic reactions on the strips are temperature dependent.

HANDLING AND STORAGE OF REAGENT STRIPS

In addition to the use of correct testing technique, reagent strips must be protected from deterioration caused by moisture, volatile chemicals, heat, and light. Reagent strips are packaged in opaque containers with a desiccant to protect them from light and moisture. Strips are removed just prior to testing, and the bottle is tightly resealed immediately. Bottles should not be opened in the presence of volatile fumes. Manufacturers recommend that reagent strips be stored at room temperature below 30°C. All bottles are stamped with an expiration date that represents the functional life expectancy of the chemical pads. Reagent strips

must not be used past the expiration date. Care must be taken not to touch the chemical pads when removing the strips.

QUALITY CONTROL OF REAGENT STRIPS

Reagent strips must be checked with both positive and negative controls a minimum of once every 24 hours. Many laboratories perform this check at the beginning of each shift.[12] Testing is also performed when a new bottle of reagent strips is opened, questionable results are obtained, or there is concern about the integrity of the strips. All quality control results must be recorded following laboratory protocol. Several companies manufacture both positive and negative controls, and many methods of preparing and preserving in-house controls have been published.[8] Distilled water is not recommended as a negative control because reagent strip chemical reactions are designed to perform at ionic concentrations similar to urine.[18] All readings of the negative control must be negative, and positive control readings should agree with the published value by ± one color block.[12] Results that do not agree with the published values must be resolved through the testing of additional strips and controls (see Chap. 7).

Summary of Reagent Strip Testing

Care of Reagent Strips

1. Store with desiccant in an opaque, tightly closed container.
2. Store below 30°C; do not freeze.
3. Do not expose to volatile fumes.
4. Do not use past the expiration date.
5. Do not use if chemical pads become discolored.
6. Remove strips immediately prior to use.

Technique

1. Mix specimen well.
2. Dip the strip completely, but briefly, into specimen.
3. Remove excess urine by withdrawing the strip against the rim of the container and by blotting the edge of the strip.
4. Compare reaction colors with the manufacturer's chart under a good light source at the specified time.
5. Perform backup tests when indicated.
6. Be alert for the presence of interfering substances.
7. Understand the principles and significance of the test.
8. Relate chemical findings to each other and to the physical and microscopic urinalysis results.

Quality Control

1. Test open bottles of reagent strips with known positive and negative controls every 24 h.
2. Resolve control results that are out of range by further testing.
3. Test reagents used in backup tests with positive and negative controls.
4. Perform positive and negative controls on new reagents and newly opened bottles of reagent strips.
5. Record all control results and reagent lot numbers.

Demonstration of chemically acceptable reagent strips does not entirely rule out the possibility of inaccurate results. Interfering substances in the urine, technical carelessness, and color blindness also will produce errors. Reagent strip manufacturers have published information concerning the limitations of their chemical reactions, and laboratory personnel should be aware of these conditions. As mentioned in Chapter 4, a primary example of reagent strip interference is the masking of color reactions by the orange pigment present in the urine of persons taking phenazopyridine compounds. If laboratory personnel do not recognize the presence of this pigment or other pigments, they will report many erroneous results.

Nonreagent strip testing procedures using tablets and liquid chemicals are available when questionable results are obtained or highly pigmented specimens are encountered. In the past, many of these procedures were used routinely to confirm positive results. Increased specificity and sensitivity of reagent strips and the use of automated strip readers have reduced the need for routine use of these procedures.[14] The chemical reliability of these procedures also must be checked using positive and negative controls. Specific backup tests are discussed in this chapter under the sections devoted to the chemical parameters for which they are used.

pH

Along with the lungs, the kidneys are the major regulators of the acid-base content in the body. They do this through the secretion of hydrogen in the form of ammonium ions, hydrogen phosphate, and weak organic acids, and by the reabsorption of bicarbonate from the filtrate in the convoluted tubules (see Chap. 2). A healthy individual will usually produce a first morning specimen with a slightly acidic pH of 5.0 to 6.0; a more alkaline pH is found following meals (alkaline tide). The pH of normal random samples can range from 4.5 to 8.0. Consequently, no normal values are assigned to urinary pH, and it must be considered in conjunction with other patient information, such as the acid-base content of the blood, the patient's renal function, the presence of a urinary tract infection, the patient's dietary intake, and the age of the specimen (Table 5–1).

CLINICAL SIGNIFICANCE

The importance of urinary pH is primarily as an aid in determining the existence of systemic acid-base disorders of metabolic or respiratory origin and in the management of urinary conditions that require the urine to be maintained at a specific pH. In respiratory or metabolic acidosis not related to renal function disorders, the urine will be acidic; conversely, if respiratory or metabolic alkalosis is present, the urine will be alkaline. Therefore, a urinary pH that does not conform to this pattern may be used to rule out the suspected condition, or, as discussed in Chapter 2, it may indicate a disorder resulting from the kidneys' inability to secrete or to reabsorb acid or base.

The precipitation of inorganic chemicals dissolved in the urine forms urinary crystals and renal calculi. This precipitation depends on urinary pH and can be controlled by

T A B L E   5 – 1   Causes of Acid and Alkaline Urine

Acid Urine	Alkaline Urine
Emphysema	Hyperventilation
Diabetes mellitus	Vomiting
Starvation	Renal tubular acidosis
Dehydration	Presence of urease-producing bacteria
Diarrhea	Vegetarian diet
Presence of acid-producing bacteria (*Escherichia coli*)	Old specimens
High-protein diet	
Cranberry juice	
Medications (methenamine mandelate [Mandelamine], fosfomycin tromethamine)	

maintaining the urine at a pH that is incompatible with the precipitation of the particular chemicals causing the calculi formation. For example, calcium oxalate, a frequent constituent of renal calculi, precipitates primarily in acidic and not alkaline urine. Therefore, maintaining urine at an alkaline pH will discourage formation of the calculi. Knowledge of urinary pH is important in the identification of crystals observed during microscopic examination of the urine sediment. This will be discussed in detail in Chapter 6.

The maintenance of an acidic urine can be of value in the treatment of urinary tract infections caused by urea-splitting organisms because they do not multiply as readily in an acidic medium. These same organisms are also responsible for the highly alkaline pH found in specimens that have been allowed to sit unpreserved for extended periods. Urinary pH is controlled primarily by dietary regulation, although medications also may be used. Persons on high-protein and high-meat diets tend to produce acidic urine, whereas urine from vegetarians is more alkaline, owing to the formation of bicarbonate following digestion of many fruits and vegetables.[13] An exception to the rule is cranberry juice, which produces an acidic urine and has long been used as a home remedy for minor bladder infections. Medications prescribed for urinary tract infections, such as methenamine mandelate (Mandelamine) and fos-

Summary of Clinical Significance of Urine pH

1. Respiratory or metabolic acidosis/ketosis
2. Respiratory or metabolic alkalosis
3. Defects in renal tubular secretion and reabsorption of acids and bases—renal tubular acidosis
4. Renal calculi formation
5. Treatment of urinary tract infections
6. Precipitation/identification of crystals
7. Determination of unsatisfactory specimens
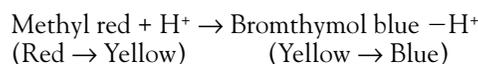
pH Reagent Strip Summary	
Reagents	Methyl red, bromthymol blue
Sensitivity	5–9
Sources of error/interference	No known interfering substances
	Runover from adjacent pads
	Old specimens
Correlations with other tests	Nitrite
	Leukocytes
	Microscopic

fomycin tromethamine, are metabolized to produce an acidic urine.

The pH of freshly excreted urine does not reach 9 in normal or abnormal conditions. A pH of 9 is associated with an improperly preserved specimen and indicates that a fresh specimen should be obtained to ensure the validity of the analysis.

REAGENT STRIP REACTIONS

The Multistix and Chemstrip brands of reagent strips measure urine pH in 0.5- or 1-unit increments between pH 5 and 9. To provide differentiation of pH units throughout this wide range, both manufacturers use a double-indicator system of methyl red and bromthymol blue. Methyl red produces a color change from red to yellow in the pH range 4 to 6, and bromthymol blue turns from yellow to blue in the range of 6 to 9. Therefore, in the pH range 5 to 9 measured by the reagent strips, one will see colors progressing from orange at pH 5 through yellow and green to a final deep blue at pH 9.

$$\text{Methyl red} + H^+ \rightarrow \text{Bromthymol blue} -H^+$$
$$(\text{Red} \rightarrow \text{Yellow}) \qquad (\text{Yellow} \rightarrow \text{Blue})$$

No known substances interfere with urinary pH measurements performed by reagent strips. Care must be taken, however, to prevent runover between the pH testing area and the adjacent, highly acidic protein testing area on Multistix, as this may produce a falsely acidic reading in an alkaline urine.

Protein

Of the routine chemical tests performed on urine, the most indicative of renal disease is the protein determination. The presence of *proteinuria* is often associated with early renal disease, making the urinary protein test an important part of any physical examination. Normal urine contains very little protein: usually, less than 10 mg/dL or 100 mg per 24 hours is excreted. This protein consists primarily of low-molecular-weight serum proteins that have been filtered by the glomerulus and proteins produced in the genitourinary tract. Owing to its low molecular weight, albu-

min is the major serum protein found in normal urine. Even though it is present in high concentrations in the plasma, the normal urinary albumin content is low because the majority of the albumin presented to the glomerulus is not filtered, and much of the filtered albumin is reabsorbed by the tubules. Other proteins include small amounts of serum and tubular microglobulins, Tamm-Horsfall protein produced by the tubules, and proteins from prostatic, seminal, and vaginal secretions.

CLINICAL SIGNIFICANCE

Demonstration of proteinuria in a routine analysis does not always signify renal disease; however, its presence does require additional testing to determine whether the protein represents a normal or a pathologic condition. The causes of proteinuria are varied and can be grouped into three major categories: *prerenal*, *renal*, and *postrenal*, based on the origin of the protein.

PRERENAL PROTEINURIA

As the name implies, prerenal proteinuria is caused by conditions affecting the plasma prior to its reaching the kidney and, therefore, is not indicative of actual renal disease. This condition is frequently transient, caused by increased levels of low-molecular-weight plasma proteins such as hemoglobin, myoglobin, and the **acute phase reactants** associated with infection and inflammation. The increased filtration of these proteins exceeds the normal reabsorptive capacity of the renal tubules resulting in an overflow of the proteins into the urine. Because reagent strips detect primarily albumin, prerenal proteinuria is usually not discovered in a routine urinalysis.

Bence Jones Protein

A primary example of proteinuria due to increased serum protein levels is the excretion of Bence Jones protein by persons with multiple myeloma. In multiple myeloma, a proliferative disorder of the immunoglobulin-producing plasma cells, the serum contains markedly elevated levels of monoclonal immunoglobulin light chains (Bence Jones protein). This low-molecular-weight protein is filtered in quantities exceeding the tubular reabsorption capacity and is excreted in the urine.

When Bence Jones protein is suspected, a screening test that uses the unique solubility characteristics of the protein can be performed. Unlike other proteins, which coagulate and remain coagulated when exposed to heat, Bence Jones protein coagulates at temperatures between 40°C and 60°C and dissolves when the temperature reaches 100°C. Therefore, a specimen that appears turbid between 40°C and 60°C and clear at 100°C can be suspected of containing Bence Jones protein. Interference due to other precipitated proteins can be removed by filtering the specimen at 100°C and observing the specimen for turbidity as it cools to between 40°C and 60°C. Not all persons with multiple myeloma excrete detectable levels of Bence Jones protein. Suspected cases of multiple myeloma must be diagnosed by performing serum electrophoresis.

RENAL PROTEINURIA

Proteinuria associated with true renal disease may be the result of either glomerular or tubular damage. When the glomerular membrane is damaged, selective filtration is impaired, and increased amounts of serum albumin and eventually red and white blood cells pass through the membrane and are excreted in the urine. Conditions that present the glomerular membrane with abnormal substances (e.g., **amyloid material**, toxic substances, and the immune complexes found in lupus erythematosus and streptococcal glomerulonephritis) are the major causes of proteinuria due to glomerular damage.

Increased pressure from the blood entering the glomerulus may override the selective filtration of the glomerulus, causing increased albumin to enter the filtrate. This condition may be reversible, such as occurs during strenuous exercise and dehydration or associated with hypertension. Proteinuria that occurs during the latter months of pregnancy may indicate a pre-eclamptic state and should be considered in conjunction with other clinical symptoms, such as hypertension, to determine if this condition exists.

Increased albumin is also present in disorders affecting tubular reabsorption because the normally filtered albumin can no longer be reabsorbed. Other low-molecular-weight proteins that are usually reabsorbed also will be present. Causes of tubular dysfunction include exposure to toxic substances and heavy metals, severe viral infections, and **Fanconi's syndrome**. The amount of protein that appears in the urine following glomerular damage will range from slightly above normal to 4 g/day, whereas markedly elevated protein levels are seldom seen in tubular disorders.

The discovery of protein, particularly in a random sample, is not always of pathologic significance, because several benign causes of renal proteinuria exist. Benign proteinuria is usually transient and can be produced by conditions such as exposure to cold, strenuous exercise, high fever, and dehydration.

Orthostatic (Postural) Proteinuria

A persistent benign proteinuria occurs frequently in young adults and is termed **orthostatic**, or postural, proteinuria. It occurs following periods spent in a vertical posture and disappears when a horizontal position is assumed. Increased pressure on the renal vein when in the vertical position is believed to account for this condition. Patients suspected of orthostatic proteinuria are requested to empty their bladder before going to bed, collect a specimen immediately upon arising in the morning, and collect a second specimen after remaining in a vertical position for several hours. Both specimens are tested for protein, and, if orthostatic proteinuria is present, a negative reading will be seen on the first morning specimen and a positive result will be found on the second specimen.

Microalbuminuria

The development of diabetic nephropathy leading to reduced glomerular filtration and eventual renal failure is a common occurrence in persons with both type 1 and type 2 diabetes mellitus. Onset of renal complications can first be predicted by detection of **microalbuminuria**, and the progression of renal disease can be prevented through better stabilization of blood glucose levels and controlling of hypertension.[19] The presence of microalbuminuria is also associated with an increased risk of cardiovascular disease.[3]

The term microalbuminuria is used to denote proteinuria that cannot be detected by routinely used reagent strips. Values are reported as the albumin excretion rate (**AER**) in μg/min, mg/24 h, and the albumin:creatinine ratio, depending on the testing methodology in use. Microalbuminuria is considered to be significant when the AER is 20 to 200 μg/min, 30 to 300 mg of albumin are excreted in 24 hours, or the albumin:creatinine ratio is greater than 3.4 mg/mmol.

Determination of AER and 24-hour albumin excretion requires collection of timed specimens. The albumin:creatinine ratio and the semiquantitative Micral-Test (BMC, Indianapolis, IN) can be performed on random specimens. To avoid the presence of orthostatic protein, overnight timed specimens and first morning specimens are recommended for testing. The Clinitek 50 or Clinitek 100 microalbumin reagent strips (Bayer Diagnostics, Elkhart, IN) provide an automated calculation of the albumin:creatinine ratio using semiquantitative results for albumin and creatinine obtained by reactions on the chemical pads (Appendix A). The Micral-Test is a reagent strip test employing an antibody-enzyme conjugate to bind human albumin. The resulting conjugate reacts with substrate to produce a colored reaction that can be compared to a color chart calibrated between 0 to 10 mg/dL. Development of rapid testing methods is increasing the routine testing of patients with diabetes for the presence of microalbuminuria.
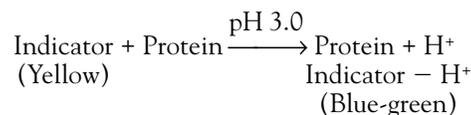
Summary of Clinical Significance of Urine Protein

Prerenal	*Tubular Disorders*
Intravascular hemolysis	Fanconi's syndrome
Muscle injury	Toxic agents/heavy metals
Severe infection and inflammation	Severe viral infections
Multiple myeloma	
Renal	*Postrenal*
Glomerular disorders	Lower urinary tract infections/inflammation
Immune complex disorders	Injury/trauma
Amyloidosis	Menstrual contamination
Toxic agents	Prostatic fluid/spermatozoa
Diabetic nephropathy	Vaginal secretions
Strenuous exercise	
Dehydration	
Hypertension	
Pre-eclampsia	
Orthostatic or postural proteinuria	

POSTRENAL PROTEINURIA

Protein can be added to a urine specimen as it passes through the structures of the lower urinary tract (ureters, bladder, urethra, prostate, and vagina). Bacterial and fungal infections and inflammations produce exudates containing protein from the interstitial fluid. The presence of blood as the result of injury or menstrual contamination contributes protein, as does the presence of prostatic fluid and large amounts of spermatozoa.

REAGENT STRIP REACTIONS

Reagent strip testing for protein uses the principle of the **protein error of indicators** to produce a visible colorimetric reaction. Contrary to the general belief that indicators produce specific colors in response to particular pH levels, certain indicators change color in the presence of protein even though the pH of the medium remains constant. This is because protein (primarily albumin) accepts ions from the indicator. Depending on the manufacturer, the protein area of the strip contains either tetrabromphenol blue or 3′, 3″, 5′, 5″-tetrachlorophenol-3, 4, 5, 6-tetrabromosulfon-phthalein and an acid buffer to maintain the pH at a constant level. At a pH level of 3, both indicators will appear yellow in the absence of protein; however, as the protein concentration increases, the color will progress through various shades of green and finally to blue. Readings are usually reported in terms of negative, trace, 1+, 2+, 3+, and 4+; however, the manufacturers also supply a semiquantitative value in milligrams per deciliter corresponding to each color change. Interpretation of trace readings can be difficult. The specific gravity of the specimen should be considered because a trace protein in a dilute specimen is more significant than in a concentrated specimen.

$$\text{Indicator + Protein} \xrightarrow{\text{pH 3.0}} \text{Protein + H}^+$$
$$\text{(Yellow)} \qquad\qquad \text{Indicator} - \text{H}^+$$
$$\text{(Blue-green)}$$

REACTION INTERFERENCE

The major source of error with reagent strips occurs with highly buffered alkaline urine that overrides the acid buffer system, producing a rise in pH and a color change unrelated to protein concentration. Likewise, a technical error of allowing the reagent pad to remain in contact with the urine for a prolonged period may remove the buffer. False-positive readings will be obtained when the reaction does not take place under acidic conditions. Highly pigmented urine and contamination of the container with quaternary ammonium compounds, detergents, and antiseptics will also cause false-positive readings. When using Multistix, a false-positive trace reading may occur in specimens with a high specific gravity. The fact that reagent strips detect primarily albumin can result in a false-negative reading in the presence of proteins other than albumin.

Traditionally most laboratories chose to confirm all positive protein results using the sulfosalicyclic acid (**SSA**) precipitation test. Currently this practice is being replaced

Protein Reagent Strip Summary	
Reagents	Multistix: Tetrabromphenol blue
	Chemstrip: 3′, 3″, 5′, 5″ tetrachlorophenol 3, 4, 5, 6-tetrabromosulfophthalein
Sensitivity	Multistix: 15–30 mg/dL albumin
	Chemstrip: 6 mg/dL albumin
Sources of error/ interference	False-positive: Highly buffered alkaline urine
	Pigmented specimens, phenazopyridine
	Quaternary ammonium compounds (detergents)
	Antiseptics, chlorhexidine
	Loss of buffer from prolonged exposure of the reagent strip to the specimen
	High specific gravity
	False-negative: Proteins other than albumin
Correlations with other tests	Blood
	Nitrite
	Leukocytes
	Microscopic

by more selective criteria to determine the need for additional testing. For example, some laboratories perform SSA testing only on highly alkaline urines, and others acidify the specimen and retest using a reagent strip. Also, a laboratory with an automated strip reader can opt not to record trace readings.

SULFOSALICYLIC ACID PRECIPITATION TEST

The SSA test is a cold precipitation test that reacts equally with all forms of protein (Table 5–2). Various concentrations and amounts of SSA can be used to precipitate protein, and methods vary greatly among laboratories. All

T A B L E 5 – 2   **Reporting SSA Turbidity**

Grade	Turbidity	Protein Range (mg/dL)
Negative	No increase in turbidity	<6
Trace	Noticeable turbidity	6–30
1+	Distinct turbidity with no granulation	30–100
2+	Turbidity with granulation with no flocculation	100–200
3+	Turbidity with granulation and flocculation	200–400
4+	Clumps of protein	>400

PROCEDURE ▼

Sulfosalicylic Acid (SSA) Precipitation Test

- Add 3 mL of 3% SSA reagent to 3 mL of centrifuged urine.
- Mix by inversion and observe for cloudiness.
- Grade the degree of turbidity (see Table 5–2).

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precipitation tests must be performed on centrifuged specimens to remove any extraneous contamination.

Any substance precipitated by acid will, of course, produce false turbidity in the SSA test. The most frequently encountered substances are radiographic dyes, tolbutamide metabolites, cephalosporins, penicillins, and sulfonamides.[1] The presence of radiographic material can be suspected when a markedly elevated specific gravity is obtained. In the presence of radiographic dye, the turbidity will also increase on standing due to the precipitation of crystals rather than protein. The patient's history will provide the necessary information on tolbutamide and antibiotic ingestion. In contrast to the reagent strip test, a highly alkaline urine will produce false-negative readings in precipitation tests as the higher pH interferes with precipitation. Use of a more concentrated solution of SSA may overcome the effect of a highly buffered, alkaline urine.

Glucose

Because of its value in the detection and monitoring of diabetes mellitus, the glucose test is the most frequent chemical analysis performed on urine. Owing to the nonspecific symptoms associated with the onset of diabetes, it is estimated that more than half of the cases in the world are undiagnosed. Therefore, blood and urine glucose tests are included in all physical examinations and are often the focus of mass health screening programs. Early diagnosis of diabetes mellitus through blood and urine glucose tests provides a greatly improved prognosis. Using currently available reagent strip methods for both blood and urine glucose testing, patients can monitor themselves at home and can detect regulatory problems prior to the development of serious complications.

CLINICAL SIGNIFICANCE

Under normal circumstances, almost all the glucose filtered by the glomerulus is reabsorbed in the proximal convoluted tubule; therefore, urine contains only minute amounts of glucose. Tubular reabsorption of glucose is by active transport in response to the body's need to maintain an adequate concentration of glucose. Should the blood level of glucose become elevated (**hyperglycemia**), as occurs in diabetes mellitus, the tubular transport of glucose ceases, and glucose appears in the urine. The blood level at which tubular reabsorption stops (renal threshold) for glucose is approxi-

mately 160 to 180 mg/dL. Blood glucose levels will fluctuate, and a normal person may have *glycosuria* following a meal with a high glucose content. Therefore, the most informative glucose results are obtained from specimens collected under controlled conditions. Fasting prior to the collection of samples for screening tests is recommended. For purposes of diabetes monitoring, specimens are usually tested 2 hours after meals. A first morning specimen does not always represent a fasting specimen because glucose from an evening meal may remain in the bladder overnight, and patients should be advised to empty the bladder and collect the second specimen.[5] Urine for glucose testing also may be collected in conjunction with the blood samples drawn during the course of a glucose tolerance test, which is used to confirm the diagnosis of diabetes mellitus or hypoglycemia.

Hyperglycemia that occurs during pregnancy and disappears after delivery is called gestational diabetes. The onset of the hyperglycemia and glycosuria is normally around the sixth month of pregnancy. Hormones secreted by the placenta are believed to block the action of insulin, resulting in hyperglycemia. Detection of gestational diabetes is important to the welfare of the baby, because glucose will cross the placenta whereas insulin does not. Women who have gestational diabetes are prone to developing type 2 diabetes mellitus in later years.

Hyperglycemia of nondiabetic origin is seen in a variety of disorders and also will produce glycosuria. Many of these disorders are associated with hormonal function and include pancreatitis, pancreatic cancer, acromegaly, Cushing's syndrome, hyperthyroidism, and pheochromocytoma. The hormones glucagon, epinephrine, cortisol, thyroxine, and growth hormone, which are increased in these disorders, work in opposition to insulin, thereby producing hyperglycemia and glucosuria. Whereas a primary function of insulin is to convert glucose to glycogen for storage (**glycogenesis**), these opposing hormones cause the breakdown of

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Summary of Clinical Significance of Urine Glucose

Hyperglycemia Associated

Diabetes mellitus
Pancreatitis
Pancreatic cancer
Acromegaly
Cushing's syndrome
Hyperthyroidism
Pheochromocytoma
Central nervous system damage
Stress
Gestational diabetes

Renal Associated
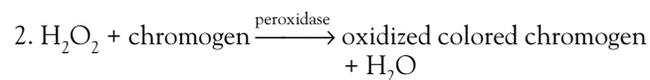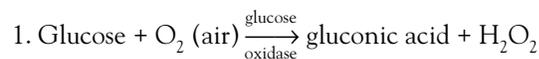
Fanconi's syndrome
Advanced renal disease
Osteomalacia
Pregnancy

glycogen to glucose (**glycogenolysis**), resulting in increased levels of circulating glucose. Epinephrine is also a strong inhibitor of insulin secretion and is increased when the body is subjected to severe stress, which accounts for the glucosuria seen in conjunction with cerebrovascular trauma and myocardial infarction.

Glycosuria will occur in the absence of hyperglycemia when the reabsorption of glucose by the renal tubules is compromised. This is frequently referred to as "renal glycosuria" and is seen in end-stage renal disease, osteomalacia, and Fanconi's syndrome. Glycosuria not associated with gestational diabetes is occasionally seen as a result of a temporary lowering of the renal threshold for glucose during pregnancy.

REAGENT STRIP (GLUCOSE OXIDASE) REACTIONS

Two very different tests have been used by laboratories to measure urinary glucose. The glucose oxidase procedure provides a specific test for glucose, and the copper reduction test is a general test for glucose and other reducing substances. Reagent strips employ the glucose oxidase testing method by impregnating the testing area with a mixture of glucose oxidase, peroxidase, chromogen, and buffer to produce a double sequential enzyme reaction. In the first step, glucose oxidase catalyzes a reaction between glucose and room air to produce gluconic acid and peroxide. In the second step, peroxidase catalyzes the reaction between peroxide and chromogen to form an oxidized colored compound that represents the presence of glucose.

1. $\text{Glucose} + O_2 \text{ (air)} \xrightarrow[\text{oxidase}]{\text{glucose}} \text{gluconic acid} + H_2O_2$

2. $H_2O_2 + \text{chromogen} \xrightarrow{\text{peroxidase}} \text{oxidized colored chromogen} + H_2O$

Reagent strip manufacturers use several different chromogens, including potassium iodide (green to brown) and tetramethylbenzidine (yellow to green). Urine glucose may be reported in terms of negative, trace, 1+, 2+, 3+, and 4+; however, the color charts also provide quantitative measurements ranging from 100 mg/dL to 2 g/dL, or 0.1 percent to 2 percent. The American Diabetes Association recommends quantitative reporting.

REACTION INTERFERENCE

Because the glucose oxidase method is specific for glucose, false-positive reactions will not be obtained from other urinary constituents, including other sugars that may be present. False-positive reactions may occur, however, if containers become contaminated with peroxide or strong oxidizing detergents.

Substances that interfere with the enzymatic reaction or reducing agents, such as ascorbic acid, that prevent oxidation of the chromogen may produce false-negative results. To minimize interference from ascorbic acid, reagent strip
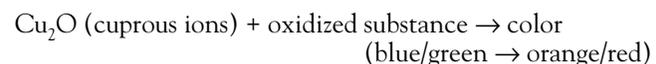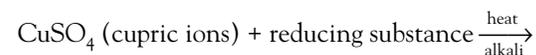
Glucose Reagent Strip Summary	
Reagents	Multistix: Glucose oxidase
	Peroxidase
	Potassium iodide
	Chemstrip: Glucose oxidase
	Peroxidase
	Tetramethylbenzidine
Sensitivity	Multistix: 75–125 mg/dL
	Chemstrip: 40 mg/dL
Interference	False-positive: Contamination by oxidizing agents and detergents
	False-negative: High levels of ascorbic acid
	High levels of ketones
	High specific gravity
	Low temperatures
	Improperly preserved specimens
Correlations with other tests	Ketones

manufacturers are incorporating additional chemicals such as iodate, which oxidizes ascorbic acid into the test pads. Product literature should be carefully reviewed for current information regarding all interfering substances. High levels of ketones also affect glucose oxidase tests at low glucose concentrations; however, because ketones are usually accompanied by marked glycosuria, this seldom presents a problem. High specific gravity and low temperature may decrease the sensitivity of the test. By far the greatest source of false-negative glucose results is the technical error of allowing specimens to remain unpreserved at room temperature for extended periods. False-negative results will be obtained with both the glucose oxidase and the copper reduction methods owing to the rapid glycolysis of glucose.

COPPER REDUCTION TEST

Measurement of glucose by the copper reduction method was one of the earliest chemical tests performed on urine. The test relies on the ability of glucose and other substances to reduce copper sulfate to cuprous oxide in the presence of alkali and heat. A color change progressing from a negative blue ($CuSO_4$) through green, yellow, and orange/red (Cu_2O) occurs when the reaction takes place.

$$CuSO_4 \text{ (cupric ions)} + \text{reducing substance} \xrightarrow[\text{alkali}]{\text{heat}}$$

Cu_2O (cuprous ions) + oxidized substance → color (blue/green → orange/red)

The classic Benedict's solution was developed in 1908 and contained copper sulfate, sodium carbonate, and sodium citrate buffer.[2] Urine was then added to the solution,

heat was applied, and the resulting precipitate was observed for color. A more convenient method that employs Benedict's principle is the Clinitest tablet (Bayer Diagnostics, Elkhart, IN). The tablets contain copper sulfate, sodium carbonate, sodium citrate, and sodium hydroxide. Upon addition of the tablet to water and urine, heat is produced by the hydrolysis of sodium hydroxide and its reaction with sodium citrate, and carbon dioxide is released from the sodium carbonate to prevent room air from interfering with the reduction reaction. Tubes should be placed in a rack and not held in the hand because the reaction heat could cause a burn. At the conclusion of the effervescent reaction, the tube is gently shaken, and the color ranging from blue to orange/red can be compared with the manufacturer's color chart to determine the approximate amount of reducing substance.

Care must be taken to observe the reaction closely as it is taking place, because at high glucose levels, a phenomenon known as "pass through" may occur. When this happens, the color produced passes through the orange/red stage and returns to a blue or blue-green color, and if not observed, a high glucose level may be reported as negative. The manufacturers of Clinitest have suggested a method using two drops instead of five drops of urine to minimize the occurrence of "pass through." A separate color chart must be used to interpret the reaction.

The sensitivity of Clinitest to glucose is reduced to a minimum of 200 mg/dL. As a nonspecific test for reducing substances, Clinitest is subject to interference from other reducing sugars, including galactose, lactose, fructose, maltose, and pentoses, ascorbic acid, certain drug metabolites, and antibiotics such as the cephalosporins. Therefore, Clinitest does not provide a confirmatory test for glucose.

Clinitest tablets are very hygroscopic and should be stored in their tightly closed packages. A strong blue color in the unused tablets suggests deterioration due to moisture accumulation, as does vigorous tablet fizzing.

PROCEDURE

Clinitest Procedure

- Place five drops of urine into a glass test tube.
- Add 10 drops of distilled water to the urine in the test tube.
- Drop one Clinitest tablet into the test tube and observe the reaction until completion (cessation of boiling).

  CAUTION: The reaction mixture gets very hot. Do not touch the bottom area of the test tube. Use glass test tube only.

- Wait 15 seconds after boiling has stopped and gently shake the contents of the tube.
- Compare the color of the mixture to the Clinitest color chart and record the result (negative, trace, 1+, 2+, 3+, 4+).
- Observe for the possibility of the "pass-through" phenomenon.

Summary of Glucose Oxidase and Clinitest Reactions

Glucose Oxidase	Clinitest	Interpretation
1+ positive	Negative	Small amount of glucose present
4+ positive	Negative	Possible oxidizing agent interference on reagent strip
Negative	Positive	Nonglucose reducing substance present
		Possible interfering substance for reagent strip

COMPARISON OF GLUCOSE OXIDASE AND CLINITEST

Several reasons exist to explain the finding of conflicting results between the two glucose tests. As stated previously, the Clinitest is not as sensitive as the glucose oxidase test, so the finding of a 1+ reagent strip reading and a negative Clinitest should not be surprising. A strongly positive reagent strip and a negative Clinitest, however, should cause concern about the possible contamination by strong oxidizing agents. The most significant discrepancy is the negative reagent strip with a positive Clinitest. Although interfering substances affecting either test may cause this problem, the most frequent cause is the presence of other reducing sugars in the urine. Commonly found reducing sugars include galactose, fructose, pentose, and lactose, of which galactose is the most clinically significant. Galactose in the urine of a newborn represents an "inborn error of metabolism" in which lack of the enzyme galactose-1-phosphate uridyl transferase prevents breakdown of ingested galactose and results in failure to thrive and other complications, including death. All newborns should be screened for galactosuria because early detection followed by dietary restriction will control the condition. Depending on the laboratory population, Clinitest is routinely performed on pediatric specimens from patients up to at least the age of 2 years. The appearance of other reducing sugars is usually of minimal clinical significance, and lactose is frequently found in the urine of nursing mothers. Keep in mind that table sugar is sucrose, a nonreducing sugar, and will not react with Clinitest or glucose oxidase strips.

Ketones

The term ketones represents three intermediate products of fat metabolism, namely, acetone, acetoacetic acid, and beta-hydroxybutyric acid. Normally, measurable amounts of ketones do not appear in the urine, because all the metabolized fat is completely broken down into carbon dioxide and water. However, when the use of available carbohydrate as the major source of energy becomes compromised and body stores of fat must be metabolized to supply energy, ketones will be detected in urine.

Summary of Clinical Significance of Urine Ketones

1. Diabetic acidosis
2. Insulin dosage monitoring
3. Starvation
4. Malabsorption/pancreatic disorders
5. Strenuous exercise
6. Vomiting
7. Inborn errors of amino acid metabolism (see Chap. 9)

PROCEDURE

Acetest Procedure

- Remove the Acetest tablet from the bottle and place on a clean dry piece of white paper.
- Place one drop of urine on top of the tablet.
- Wait 30 seconds.
- Compare the tablet color with the manufacturer-supplied color chart.
- Report as negative, small, moderate, or large.

CLINICAL SIGNIFICANCE

Clinical reasons for increased fat metabolism include the inability to metabolize carbohydrate, as occurs in diabetes mellitus; increased loss of carbohydrate from vomiting; and inadequate intake of carbohydrate associated with starvation and malabsorption.

Testing for urinary ketones is most valuable in the management and monitoring of insulin-dependent (type 1) diabetes mellitus. *Ketonuria* shows a deficiency in insulin, indicating the need to regulate dosage. It is often an early indicator of insufficient insulin dosage in type 1 diabetes and in patients with diabetes experiencing medical problems in addition to their diabetes. Increased accumulation of ketones in the blood leads to electrolyte imbalance, dehydration, and, if not corrected, acidosis and eventual diabetic coma. To aid in the monitoring of diabetes, ketone tests not only are included in all multiple-test strips but also are combined with glucose on strips used primarily for at-home testing by diagnosed patients with diabetes.

The use of multiple-test strips in hospital laboratories will often produce positive ketone tests unrelated to diabetes because the patient's illness is either preventing adequate intake or absorption of carbohydrates or is producing an accelerated loss, as in the case of vomiting. Obesity clinics can use a practical application of ketonuria produced by starvation to determine whether patients on high-protein or fasting diets have been cheating. Frequent strenuous exercise can cause overuse of available carbohydrates and produce ketonuria.

REAGENT STRIP REACTIONS

The three ketone compounds are not present in equal amounts in urine. Both acetone and beta-hydroxybutyric acid are produced from acetoacetic acid, and the proportions of 78 percent beta-hydroxybutyric acid, 20 percent acetoacetic acid, and 2 percent acetone are relatively constant in all specimens.

Reagent strip tests use the sodium nitroprusside (nitroferricyanide) reaction to measure ketones. In this reaction, acetoacetic acid in an alkaline medium will react with sodium nitroprusside to produce a purple color. The test does not measure beta-hydroxybutyric acid and is only slightly sensitive to acetone when glycine is also present; however, inasmuch as these compounds are derived from acetoacetic acid, their presence can be assumed, and it is not necessary to perform individual tests. Results are reported qualitatively as negative, small, moderate, or large, or as negative, 1+, 2+, or 3+.
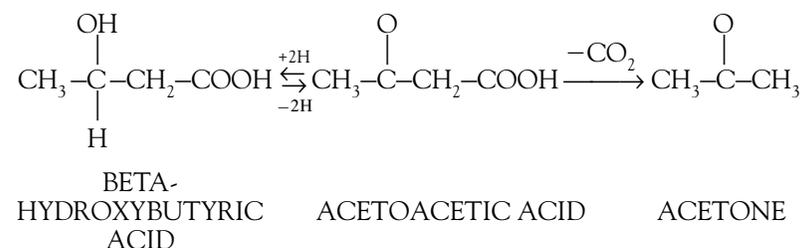
In cases of severe ketosis, it may be necessary to perform tests on serial dilutions to provide more information as to the degree of ketosis.

$$\text{acetoacetate + sodium nitroprusside + (glycine)} \xrightarrow{\text{alkaline}}$$
$$\text{(and acetone)} \qquad\qquad\qquad \text{purple color}$$
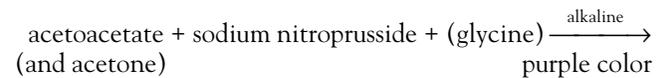
Acetest (Bayer Diagnostics, Elkhart, IN) provides sodium nitroprusside, glycine, disodium phosphate, and lactose in tablet form. The addition of lactose gives better color differentiation; however, the primary advantage of the Acetest tablets is that they can be used for serum and other body fluid testing. Acetest tablets are hygroscopic, and, if the specimen is not completely absorbed within 30 seconds, a new tablet should be used.

REACTION INTERFERENCE

Specimens obtained following diagnostic procedures using the dyes phenolsulfonphthalein and bromsulphalein may produce an interfering red color in the alkaline test medium, as will highly pigmented red urine. Large amounts of levodopa and medications containing sulfhydryl groups, including mercaptoethane sulfonate sodium (MESNA) and captopril, may produce atypical color reactions. Reactions

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$$\underset{\substack{\text{BETA-} \\ \text{HYDROXYBUTYRIC} \\ \text{ACID}}}{CH_3-\overset{\displaystyle OH}{\underset{\displaystyle H}{C}}-CH_2-COOH} \underset{-2H}{\overset{+2H}{\rightleftarrows}} \underset{\text{ACETOACETIC ACID}}{CH_3-\overset{\displaystyle O}{C}-CH_2-COOH} \xrightarrow{-CO_2} \underset{\text{ACETONE}}{CH_3-\overset{\displaystyle O}{C}-CH_3}$$

Ketone Reagent Strip Summary	
Reagents	Sodium nitroprusside
	Glycine (Chemstrip)
Sensitivity	Multistix: 5–10 mg/dL acetoacetic acid
	Chemstrip: 9 mg/dL acetoacetic acid
	70 mg/dL acetone
Interference	False-positive: Phthalein dyes
	Highly pigmented red urine
	Levodopa
	Medications containing free sulfhydryl groups
	False-negative: Improperly preserved specimens
Correlations with other tests	Glucose

with interfering substances frequently fade on standing, whereas color development from acetoacetic acid increases, resulting in false-positive results from improperly timed readings. Falsely decreased values due to the volatilization of acetone and the breakdown of acetoacetic acid by bacteria will be seen in improperly preserved specimens.

Blood

Blood may be present in the urine either in the form of intact red blood cells (*hematuria*) or as the product of red blood cell destruction, hemoglobin (*hemoglobinuria*). As discussed in Chapter 4, blood present in large quantities can be detected visually; hematuria produces a cloudy red urine, and hemoglobinuria appears as a clear red specimen. Because any amount of blood greater than five cells per microliter of urine is considered clinically significant, visual examination cannot be relied on to detect the presence of blood. Microscopic examination of the urinary sediment will show intact red blood cells, but free hemoglobin produced either by hemolytic disorders or lysis of red blood cells will not be detected. Therefore, chemical tests for hemoglobin provide the most accurate means for determining the presence of blood. Once blood has been detected, the microscopic examination can be used to differentiate between hematuria and hemoglobinuria.

CLINICAL SIGNIFICANCE

The finding of a positive reagent strip test result for blood indicates the presence of red blood cells, hemoglobin, or myoglobin. Each of these has a different clinical significance.

HEMATURIA

Hematuria is most closely related to disorders of renal or genitourinary origin in which bleeding is the result of trauma or damage to the organs of these systems. Major causes of hematuria include renal calculi, glomerular dis-

eases, tumors, trauma, pyelonephritis, exposure to toxic chemicals, and anticoagulant therapy. The laboratory is frequently requested to perform a urinalysis when patients presenting with severe back and abdominal pain are suspected of having renal calculi. In such cases, hematuria is usually of a small to moderate degree, but its presence can be essential to the diagnosis. Hematuria of nonpathologic significance is observed following strenuous exercise and during menstruation.

HEMOGLOBINURIA

Hemoglobinuria may result from the lysis of red blood cells produced in the urinary tract, particularly in dilute, alkaline urine. It also may result from intravascular hemolysis and the subsequent filtering of hemoglobin through the glomerulus. Lysis of red blood cells in the urine will usually show a mixture of hemoglobinuria and hematuria, whereas no red blood cells will be seen in cases of intravascular hemolysis. Under normal conditions, the formation of large hemoglobin-haptoglobin complexes in the circulation prevents the glomerular filtration of hemoglobin. When the amount of free hemoglobin present exceeds the haptoglobin content—as occurs in hemolytic anemias, transfusion reactions, severe burns, infections, and strenuous exercise—hemoglobin is available for glomerular filtration. Reabsorption of filtered hemoglobin also will result in the appearance of large yellow-brown granules of denatured **ferritin** called **hemosiderin** in the renal tubular epithelial cells and in the urine sediment.

Summary of Clinical Significance of a Positive Reaction for Blood
Hematuria
1. Renal calculi
2. Glomerulonephritis
3. Pyelonephritis
4. Tumors
5. Trauma
6. Exposure to toxic chemicals
7. Anticoagulants
8. Strenuous exercise
Hemoglobinuria
1. Transfusion reactions
2. Hemolytic anemias
3. Severe burns
4. Infections/malaria
5. Strenuous exercise/red blood cell trauma
Myoglobinuria
1. Muscular trauma/crush syndromes
2. Prolonged coma
3. Convulsions
4. Muscle-wasting diseases
5. Alcoholism/overdose
6. Drug abuse
7. Extensive exertion

MYOGLOBINURIA

Myoglobin, a heme-containing protein found in muscle tissue, not only reacts positively with the reagent strip test for blood but also produces a clear red-brown urine. The presence of myoglobin rather than hemoglobin should be suspected in patients with conditions associated with muscle destruction (**rhabdomyolysis**). Examples of these conditions include trauma, crush syndromes, prolonged coma, convulsions, muscle-wasting diseases, alcoholism, heroin abuse, and extensive exertion. The heme portion of myoglobin is toxic to the renal tubules, and high concentrations can cause acute renal failure. The massive hemoglobinuria seen in hemolytic transfusion reactions also is associated with acute renal failure.
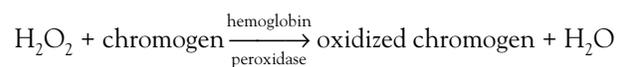
HEMOGLOBINURIA VERSUS MYOGLOBINURIA

Occasionally the laboratory may be requested to differentiate between the presence of hemoglobin and myoglobin in a urine specimen. The diagnosis of *myoglobinuria* usually is based on the patient's history and elevated serum levels of the enzymes creatinine kinase and lactic dehydrogenase. The appearance of the patient's plasma also can aid in the differentiation. The kidneys rapidly clear myoglobin from the plasma, leaving a normal appearing plasma, whereas hemoglobin bound to haptoglobin remains in the plasma and imparts a red color.

The concentration of myoglobin in the urine must be at least 25 mg/dL before the red pigmentation can be visualized. At this concentration, a precipitation test can be used to screen for the presence of myoglobin; 2.8 g of ammonium sulfate are added to 5 mL of centrifuged urine. After mixing and allowing the specimen to sit for 5 minutes, the urine is filtered or centrifuged, and the supernatant is tested for a reaction for blood with a reagent strip. The principle of this screening test is based on the fact that the larger hemoglobin molecules will be precipitated by the ammonium sulfate, and myoglobin will remain in the supernatant. Therefore, when myoglobin is present, the supernatant will retain the red color and give a positive reagent strip test for blood. Conversely, hemoglobin will produce a red precipitate and a supernatant that tests negative for blood. Myoglobin is not stable in acid urine and, if denatured, may precipitate with the ammonium sulfate. Specimens that cannot be tested immediately should be neutralized and frozen.

REAGENT STRIP REACTIONS

Chemical tests for blood use the pseudoperoxidase activity of hemoglobin to catalyze a reaction between hydrogen peroxide and the chromogen tetramethylbenzidine to produce an oxidized chromogen, which has a green-blue color.

$$H_2O_2 + chromogen \xrightarrow[peroxidase]{hemoglobin} oxidized\ chromogen + H_2O$$

Reagent strip manufacturers incorporate peroxide, tetramethylbenzidine, and buffer into the blood testing area.

Two color charts are provided that correspond to the reactions that occur with hemoglobinuria and myoglobinuria and hematuria. In the presence of free hemoglobin/myoglobin, uniform color ranging from a negative yellow through green to a strongly positive green-blue will appear on the pad. In contrast, intact red blood cells are lysed when they come in contact with the pad, and the liberated hemoglobin produces an isolated reaction that results in a speckled pattern on the pad. The degree of hematuria can then be estimated by the intensity of the speckled pattern. Reagent strip tests can detect concentrations as low as five red blood cells per microliter; however, care must be taken when comparing these figures with the actual microscopic values, because the absorbent nature of the pad will attract more than 1 μL of urine. The value of the test lies primarily in its ability to differentiate between hemoglobinuria/myoglobinuria and hematuria, not in the quantitation. The terms trace, small, moderate, and large or trace, 1+, 2+ and 3+ are used for reporting.

REACTION INTERFERENCE

False-positive reactions owing to menstrual contamination may be seen. They also will occur if strong oxidizing detergents are present in the specimen container. Vegetable peroxidase and bacterial enzymes, including an *Escherichia coli* peroxidase, also may cause false-positive reactions. Therefore, sediments containing bacteria should be checked closely for the presence of red blood cells.[21]

Traditionally ascorbic acid has been associated with false-negative reagent strip reactions for blood. Both Mul-

Blood Reagent Strip Summary

Reagents	Multistix: Diisopropylbenzene dehydroperoxide tetramethylbenzidine Chemstrip: 2,5-dimethyl-2,5-dihydroperoxide tetramethylbenzidine
Sensitivity	Multistix: 5–20 RBCs/μL, 0.015–0.062 mg/dL hemoglobin Chemstrip: 5 RBCs/μL, hemoglobin corresponding to 10 RBCs/μL
Interference	False-positive: Strong oxidizing agents Bacterial peroxidases Menstrual contamination False-negative: High specific gravity/crenated cells Formalin Captopril High concentrations of nitrite Ascorbic acid >25 mg/dL Unmixed specimens
Correlations with other tests	Protein Microscopic

tistix and Chemstrip have modified their reagent strips to reduce this interference to very high levels (25 mg/dL) of ascorbic acid. Multistix uses a peroxide that is less subject to reduction by ascorbic acid, and Chemstrip overlays the reagent pad with an iodate-impregnated mesh that oxidizes the ascorbic acid prior to its reaching the reaction pad. False-negative reactions can result when urine with a high specific gravity contains crenated red blood cells that do not lyse when they come in contact with the reagent pad. Decreased reactivity also may be seen when formalin is used as a preservative or when the hypertension medication, captopril, or high concentrations of nitrite (greater than 10 mg/dL) are present. Red blood cells settle to the bottom of the specimen container, and failure to mix the specimen prior to testing will cause a falsely decreased reading.

Bilirubin

The appearance of bilirubin in the urine can provide an early indication of liver disease. It is often detected long before the development of **jaundice**.

PRODUCTION OF BILIRUBIN

Bilirubin, a highly pigmented yellow compound, is a degradation product of hemoglobin. Under normal conditions, the life span of red blood cells is approximately 120 days, at which time they are destroyed in the spleen and liver by the phagocytic cells of the reticuloendothelial system. The liberated hemoglobin is broken down into its component parts: iron, protein, and protoporphyrin. The body reuses the iron and protein, and the cells of the reticuloendothe-

lial system convert the remaining protoporphyrin to bilirubin. The bilirubin is then released into the circulation, where it binds with albumin and is transported to the liver. At this point, the kidneys cannot excrete the circulating bilirubin because not only is it bound to albumin but also it is water insoluble. In the liver, bilirubin is conjugated with glucuronic acid by the action of glucuronyl transferase to form water-soluble bilirubin diglucuronide (conjugated bilirubin). Usually, this conjugated bilirubin will not appear in the urine because it is passed directly from the liver into the bile duct and on to the intestine. In the intestine, intestinal bacteria reduce bilirubin to urobilinogen, which is then oxidized and excreted in the feces in the form of urobilin. In Figure 5–1, bilirubin metabolism is illustrated for reference with this section and the subsequent discussion of urobilinogen.

CLINICAL SIGNIFICANCE

Conjugated bilirubin will appear in the urine when the normal degradation cycle is disrupted by obstruction of the bile duct (e.g., gallstones or cancer) or when the integrity of the liver is damaged, allowing leakage of conjugated bilirubin into the circulation. Hepatitis and cirrhosis are common examples of conditions that produce liver damage resulting in bilirubinuria. Not only does the detection of urinary bilirubin provide an early indication of liver disease, but also its presence or absence can be used in determining the cause of clinical jaundice. As shown in Table 5–3, this determination can be even more significant when bilirubin results are combined with urinary urobilinogen. Jaundice due to increased destruction of red blood cells does not produce bilirubinuria. This is because the serum bilirubin is present in the unconjugated form and the kidneys cannot excrete it.
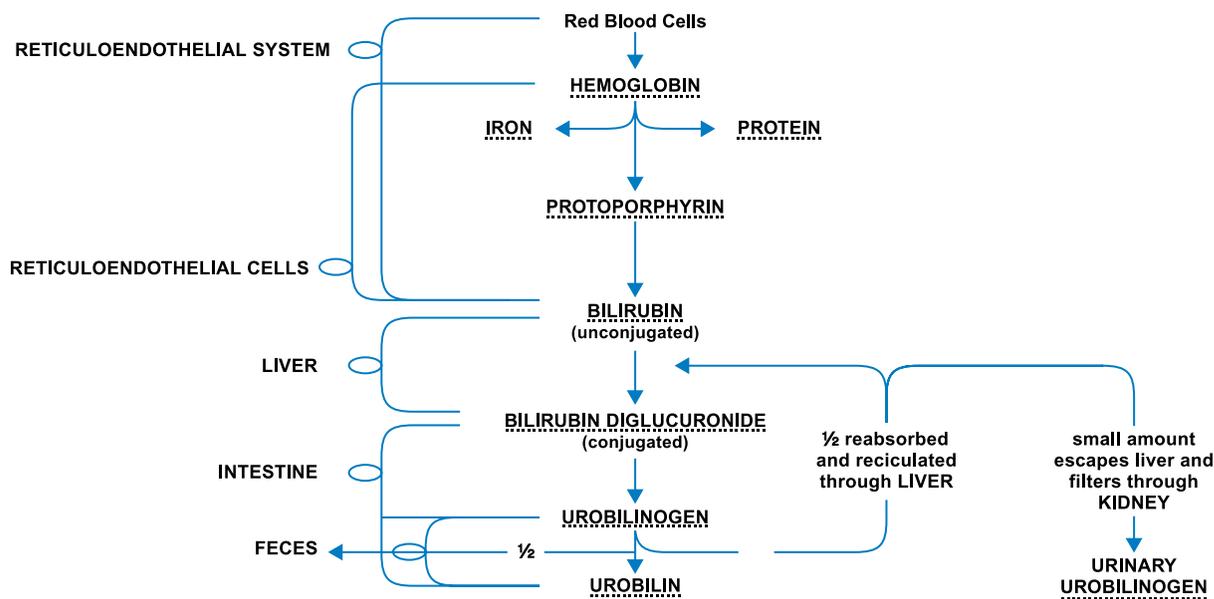
F I G U R E 5 – 1   Hemoglobin degradation.

T A B L E 5 – 3   **Urine Bilirubin and Urobilinogen in Jaundice**

	Urine Bilirubin	Urine Urobilinogen
Bile duct obstruction	+ + +	Normal
Liver damage	+ or −	+ +
Hemolytic disease	Negative	+ + +

REAGENT STRIP (DIAZO) REACTIONS

Routine testing for urinary bilirubin by reagent strip uses the diazo reaction. Bilirubin combines with 2,4-dichloroaniline diazonium salt or 2,6-dichlorobenzene-diazonium-tetrafluoroborate in an acid medium to produce an azodye, with colors ranging from increasing degrees of tan or pink to violet, respectively. Qualitative results are reported as negative, small, moderate, or large, or as negative, 1+, 2+, or 3+. Reagent strip color reactions for bilirubin are more difficult to interpret than other reagent strip reactions and are easily influenced by other pigments present in the urine. Atypical color reactions are frequently noted on visual examination and are measured by automated readers. Further testing should be performed on any questionable results.[15]

$$\text{bilirubin glucuronide} + \text{diazonium salt} \xrightarrow{\text{acid}} \text{azodye}$$
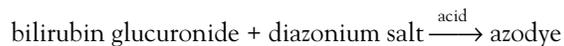
Questionable results can be repeated using the Ictotest (Bayer Diagnostics, Elkhart, IN). The Ictotest is less subject to interference and is sensitive to 0.05 to 0.10 mg/dL of bilirubin, whereas the reagent strips have a lower sensitivity level of 0.40 mg/dL. Ictotest kits consist of testing mats and tablets containing *p*-nitrobenzene-diazonium-*p*-toluenesulfonate, SSA, sodium carbonate, and boric acid. Ten drops of urine are added to the mat, which has special absorbent properties that cause bilirubin to remain on the surface as the urine absorbs into the mat. Following the chemical reaction, a blue-to-purple color will appear on the mat when bilirubin is present. Colors other than blue or purple appearing on the mat are considered to be a negative result. If interference in the Ictotest is suspected, it can usually be removed by adding water directly to the mat after the urine has been added. Interfering substances will be washed into the mat, and only bilirubin will remain on the surface. An Ictotest may be requested

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Ictotest Procedure

- Place 10 drops of urine onto one square of the absorbent test mat.
- Using forceps, remove one Ictotest reagent tablet, recap the bottle promptly, and place the tablet in the center of the moistened area.
- Place one drop of water onto the tablet and wait 5 seconds.
- Place a second drop of water onto the tablet so that the water runs off the tablet onto the mat.
- Observe the color of the mat around the tablet at the end of 60 seconds. The presence of a blue-to-purple color on the mat indicates that bilirubin is present. A slight pink or red color should be ignored. Report as positive or negative.

when early cases of liver disorders, such as hepatitis, are suspected.

REACTION INTERFERENCE

As discussed previously, false-positive reactions are primarily due to urine pigments. Of particular concern are the yellow-orange urines from persons taking phenazopyridine compounds, because the thick pigment produced may be mistaken for bilirubin on initial examination. The presence of indican and metabolites of the medication Lodine may cause false-positive readings.

The false-negative results caused by the testing of specimens that are not fresh are the most frequent errors associated with bilirubin testing. Bilirubin is an unstable com-

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Summary of Clinical Significance of Urine Bilirubin

1. Hepatitis
2. Cirrhosis
3. Other liver disorders
4. Biliary obstruction (gallstones, carcinoma)

Bilirubin Reagent Strip Summary

Reagents	Multistix: 2,4-dichloroaniline diazonium salt
	Chemstrip: 2,6-dichlorobenzene-diazonium-tetrafluoroborate
Sensitivity	Multistix: 0.4–0.8 mg/dL bilirubin
	Chemstrip: 0.5 mg/dL bilirubin
Interference	False-positive: Highly pigmented urines, phenazopyridine
	Indican, (intestinal disorders)
	Metabolites of Lodine
	False-negative: Specimen exposure to light
	Ascorbic acid >25 mg/dL
	High concentrations of nitrite
Correlations with other tests	Urobilinogen

pound that is rapidly photo-oxidized to biliverdin when exposed to light. Biliverdin does not react with diazo tests. False-negative results also will occur when hydrolysis of bilirubin diglucuronide produces free bilirubin, because this is less reactive in the reagent strip tests.[4] High concentrations of ascorbic acid (greater than 25 mg/dL) and nitrite may lower the sensitivity of the test, because they combine with the diazonium salt and prevent its reaction with bilirubin.

Urobilinogen

Like bilirubin, urobilinogen is a bile pigment that results from the degradation of hemoglobin. As shown in Figure 5–1, it is produced in the intestine from the reduction of bilirubin by the intestinal bacteria. Approximately half of the urobilinogen is reabsorbed from the intestine into the blood, recirculates to the liver, and is excreted back into the intestine through the bile duct. The urobilinogen remaining in the intestine is excreted in the feces, where it is oxidized to urobilin, the pigment responsible for the characteristic brown color of the feces. Urobilinogen appears in the urine because, as it circulates in the blood en route to the liver, it passes through the kidney and is filtered by the glomerulus. Therefore, a small amount of urobilinogen—less than 1 mg/dL or Ehrlich unit—is normally found in the urine.

CLINICAL SIGNIFICANCE

Increased urine urobilinogen (greater than 1 mg/dL) is seen in liver disease and hemolytic disorders. Measurement of urine urobilinogen can be valuable in the detection of early liver disease; however, studies have shown that when urobilinogen tests are routinely performed, 1 percent of the nonhospitalized population and 9 percent of a hospitalized population exhibit elevated results.[6] This is frequently caused by constipation.

Impairment of liver function decreases the ability of the liver to process the urobilinogen recirculated from the intestine. The excess urobilinogen remaining in the blood is filtered by the kidneys and appears in the urine.

The clinical jaundice associated with hemolytic disorders results from the increased amount of circulating unconjugated bilirubin. This unconjugated bilirubin is presented to the liver for conjugation, resulting in a markedly increased amount of conjugated bilirubin entering the intestines. As a result, increased urobilinogen is produced, and increased amounts of urobilinogen are reabsorbed into the blood and circulated through the kidneys where filtration takes place. In addition, the overworked liver does not process the reabsorbed urobilinogen as efficiently, and additional urobilinogen is presented for urinary excretion.

Although it cannot be determined by reagent strip, the absence of urobilinogen in the urine and feces also is diagnostically significant and represents an obstruction of the bile duct that prevents the normal passage of bilirubin into the intestine. See Table 5–3 for an outline of the relationship of urine urobilinogen and bilirubin to the pathologic conditions associated with them.
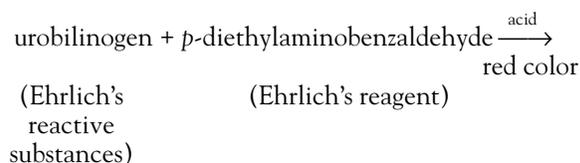

> ### Summary of Clinical Significance of Urine Urobilinogen
>
> 1. Early detection of liver disease
> 2. Liver disorders, hepatitis, cirrhosis, carcinoma
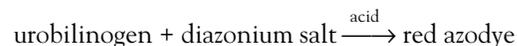> 3. Hemolytic disorders

REAGENT STRIP REACTIONS AND INTERFERENCE

The reagent strip reactions for urobilinogen differ between Multistix and Chemstrip much more significantly than with other reagent strip parameters. Multistix uses Ehrlich's aldehyde reaction, in which urobilinogen reacts with p-diethylaminobenzaldehyde (Ehrlich's reagent) to produce colors ranging from light to dark pink. Results are reported as Ehrlich units (**EU**), which are equal to mg/dL, ranging from normal readings of 0.2 and 1 through abnormal readings of 2, 4, and 8. Chemstrip incorporates an azo-coupling (diazo) reaction using 4-methoxybenzene-diazonium-tetrafluoroborate to react with urobilinogen, producing colors ranging from white to pink. The reaction is much more specific for urobilinogen than the Ehrlich reaction. Results are reported in mg/dL. Both tests will detect urobilinogen present in normal quantities, and color comparisons are provided for the upper limits of normal as well as abnormal concentrations. Reagent strip tests cannot determine the absence of urobilinogen, which is significant in biliary obstruction.

MULTISTIX:

$$\text{urobilinogen} + p\text{-diethylaminobenzaldehyde} \xrightarrow{\text{acid}}$$
$$\text{red color}$$

(Ehrlich's reactive substances)   (Ehrlich's reagent)

CHEMSTRIP:

$$\text{urobilinogen} + \text{diazonium salt} \xrightarrow{\text{acid}} \text{red azodye}$$

REACTION INTERFERENCE

The Ehrlich reaction on Multistix is subject to a variety of interferences, referred to as Ehrlich-reactive compounds, that will produce false-positive reactions. These include porphobilinogen, indican, p-aminosalicylic acid, sulfonamides, methyldopa, procaine, and chlorpromazine compounds. The presence of porphobilinogen is clinically significant; however, the reagent strip test is not considered a reliable method to screen for its presence. Porphobilinogen is discussed later in this section and in Chapter 9.

The sensitivity of the Ehrlich reaction increases with temperature, and testing should be performed at room temperature. Highly pigmented urines will cause atypical readings with both brands of reagent strips.

Urobilinogen Reagent Strip Summary

Reagents	Multistix: *p*-diethylaminobenzaldehyde
	Chemstrip: 4-methoxybenzene-diazonium-tetrafluroborate
Sensitivity	Multistix: 0.2 mg/dL urobilinogen
	Chemstrip: 0.4 mg/dL urobilinogen
Interference	Multistix: False-positive: Porphobilinogen
	Indican
	p-aminosalicylic acid
	Sulfonamides
	Methyldopa
	Procaine
	Chlorpromazine
	Highly pigmented urine
	False-negative: Old specimens
	Preservation in formalin
	Chemstrip: False-positive: Highly pigmented urine
	False-negative: Old specimens
	Preservation in formalin
	High concentrations of nitrate
Correlations with other tests	Bilirubin

False-negative results occur most frequently when specimens are improperly preserved, allowing urobilinogen to be photo-oxidized to urobilin. High concentrations of nitrite will interfere with the azo-coupling reaction on Chemstrip. False-negative readings also are obtained with both strips when formalin is used as a preservative.

EHRLICH'S TUBE TEST

Until development of reagent strip methods, tests for urobilinogen were not performed routinely because the available procedures were time consuming and nonspecific. The tube test described here was used when clinically necessary and serves as the basis for the Watson-Schwartz and Hoesch screening tests for porphobilinogen. The reagent used in both tests is *p*-diethylaminobenzaldehyde (Ehrlich's reagent). Addition of Ehrlich's reagent to urine containing urobilinogen produces a cherry-red color, as do the Ehrlich-reactive compounds. To produce a semiquantitative measurement, the original method of adding one part Ehrlich's reagent to 10 parts urine and observing against a white background for the presence of a red color was modified to test serial dilutions of urine. Positive results in dilutions greater than 1 to 20 were considered significant. To avoid missing the presence of a faint pink color in higher dilutions, tubes should be examined by looking down through the top while holding the bottom against a white background. Sodium acetate can be added to enhance the reaction. Results are reported in Ehrlich units, which are essentially equivalent to 1 mg/mL of urobilinogen. Whereas reagent strip testing for urobilinogen is usually performed on random or first morning specimens, the recommended specimen for quantitative testing is one col-
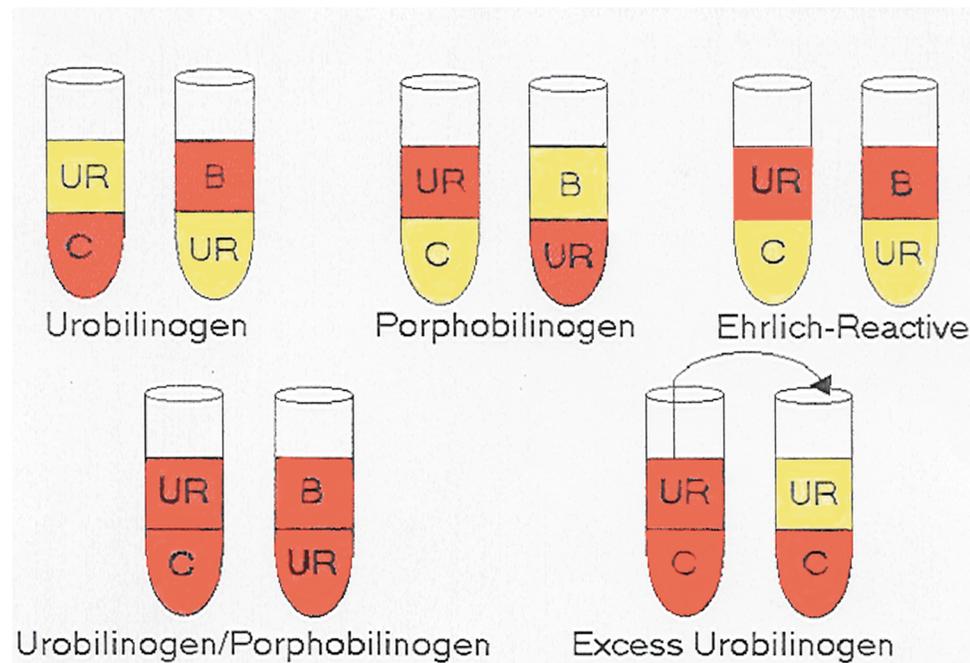

FIGURE 5–2 Typical Ehrlich reactions. (Adapted from Henry, Lauzon, and Schumann,[7] p. 434.)

lected after the noon meal, between 2 PM and 4 PM. This is the time of greatest urobilinogen excretion. As mentioned previously, the Ehrlich reaction is not specific for urobilinogen, and a red color also will be seen in the presence of porphobilinogen and other Ehrlich-reactive compounds. A positive reaction may require additional testing to determine which compound is present.

WATSON-SCHWARTZ DIFFERENTIATION TEST

The classic test for differentiating between urobilinogen and porphobilinogen is the Watson-Schwartz test.[20] After production of the cherry-red color using sodium acetate and Ehrlich's reagent, the specimen is divided into two tubes. The addition of chloroform to one tube will result in the extraction of urobilinogen into the chloroform (bottom) layer, producing a colorless urine (top) layer, and a red chloroform layer on the bottom. Neither porphobilinogen nor other Ehrlich-reactive compounds are soluble in chloroform. Porphobilinogen is also not soluble in butanol; however, urobilinogen and other Ehrlich reactive compounds will be extracted into butanol. Therefore, the addition of butanol to the second tube will produce a red (upper) butanol layer if urobilinogen or Ehrlich reactive compounds are present and a colorless butanol layer if porphobilinogen is present. As shown in Figure 5–2 and Table 5–4, urobilinogen is soluble in both chloroform and butanol, and porphobilinogen is soluble in neither. If both urobilinogen and porphobilinogen are present, both layers will appear red. Before reporting the test as positive for both substances, an additional chloroform extraction should be performed on the red urine layer to ensure that the red color is not due to excess urobilinogen.

HOESCH SCREENING TEST FOR PORPHOBILINOGEN

The Hoesch test is used for rapid screening or monitoring of urinary porphobilinogen. Two drops of urine are added to approximately 2 mL of Hoesch reagent (Ehrlich's reagent dissolved in 6 M HCl), and the top of the solution is immediately observed for the appearance of a red color, which indicates the presence of porphobilinogen. When the tube is shaken, the red color is seen throughout the solution. The test will detect approximately 2 mg/dL of porphobilinogen, and urobilinogen is inhibited by the highly acidic pH. High concentrations of methyldopa and indican and highly pigmented urines may produce false-positive results.

Nitrite

CLINICAL SIGNIFICANCE

The reagent strip test for nitrite provides a rapid screening test for the presence of urinary tract infection (**UTI**). The test is designed to detect those cases in which the need for a culture may not be apparent and is not intended to replace the urine culture as the primary test for diagnosing and monitoring bacterial infection. Most UTIs are believed to start in the bladder as a result of external contamination and, if untreated, to progress upward through the ureters to the tubules, renal pelvis, and kidney. The nitrite test is valuable for detecting initial bladder infection (cystitis), because patients are often asymptomatic or have vague symptoms that would not lead the physician to order a urine culture. Pyelonephritis, an inflammatory process of the kidney and adjacent renal pelvis, is a frequent complication of untreated cystitis and can lead to renal tissue damage, impairment of renal function, hypertension, and even septicemia. Therefore, detection of **bacteriuria** through the use of the nitrite screening test and subsequent antibiotic therapy can prevent these serious complications. The nitrite test also can be used to evaluate the success of antibiotic therapy and to periodically screen persons with recurrent infections, patients with diabetes, and pregnant women, all of whom are considered to be at high risk for UTI.[10] As discussed in the following section, many laboratories use the nitrite test in combination with the leukocyte esterase test to determine the necessity of performing urine cultures.

T A B L E 5 – 4   **Watson-Schwartz Test Interpretation**

	Urobilinogen	Other Ehrlich-Reactive Substances	Porphobilinogen
Chloroform Extraction			
Urine (Top Layer)	Colorless	Red	Red
Chloroform (Bottom Layer)	Red	Colorless	Colorless
Butanol Extraction			
Butanol (Top Layer)	Red	Red	Colorless
Urine (Bottom Layer)	Colorless	Colorless	Red

Summary of Clinical Significance of Urine Nitrite

1. Cystitis
2. Pyelonephritis
3. Evaluation of antibiotic therapy
4. Monitoring of patients at high risk for urinary tract infection
5. Screening of urine culture specimens

REAGENT STRIP REACTIONS

The chemical basis of the nitrite test is the ability of certain bacteria to reduce nitrate, a normal constituent of urine, to nitrite, which does nor normally appear in the urine. Nitrite is detected by the Greiss reaction, in which nitrite at an acidic pH reacts with an aromatic amine (para-arsanilic acid or sulfanilamide) to form a diazonium compound that then reacts with tetrahydrobenzoquinolin compounds to produce a pink-colored azodye. To prevent false-positive reactions in externally contaminated specimens, the sensitivity of the test is standardized to correspond with a quantitative bacterial culture criterion of 100,000 organisms per milliliter. Although different shades of pink may be produced, the test does not measure the degree of bacteriuria, and any shade of pink is considered to represent a clinically significant amount of bacteria. Results are reported only as negative or positive.

$$\text{para-arsanilic acid or sulfanilamide} + \underset{\text{(nitrite)}}{NO_2} \xrightarrow{\text{acid}} \text{diazonium salt}$$

$$\text{diazonium salt} + \text{tetrahydrobenzoquinolin} \xrightarrow{\text{acid}} \text{pink azodye}$$
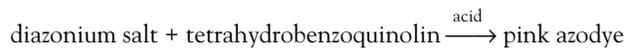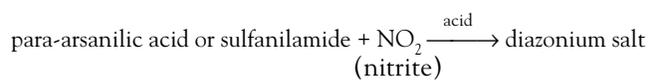
REACTION INTERFERENCE

Several major factors can influence the reliability of the nitrite test, and tests with negative results in the presence of even vaguely suspicious clinical symptoms should always be repeated or followed by a urine culture.

1. Bacteria that lack the enzyme reductase do not possess the ability to reduce nitrate to nitrite. Reductase is found in the gram-negative bacteria (*Enterobacteriaceae*) that most frequently cause UTIs. Non-nitrate-reducing gram-positive bacteria and yeasts, however, cause a significant number of infections, and the nitrite test will not detect the presence of these organisms.
2. Bacteria capable of reducing nitrate must remain in contact with the urinary nitrate long enough to produce nitrite. Therefore, all nitrite tests should be performed on first morning specimens because urine will have been held in the bladder for several hours. The correlation between positive cultures and positive nitrite test results is significantly lower when testing is performed on random samples.[11]

Nitrite Reagent Strip Summary

Reagents	Multistix: *p*-arsanilic acid Tetrahydrobenzo(h)- quinolin-3-ol Chemstrip: Sulfanilamide 3-hydroxy-1,2,3,4- tetrahydro-7,8 benzoquinoline
Sensitivity	Multistix: 0.06–0.1 mg/dL nitrite ion Chemstrip: 0.05 mg/dL nitrite ion
Interference	False-negative: Nonreductase-containing bacteria Insufficient contact time between bacteria and urinary nitrate Lack of urinary nitrate Large quantities of bacteria converting nitrite to nitrogen Presence of antibiotics High concentrations of ascorbic acid High specific gravity False-positive: Improperly preserved specimens Highly pigmented urine
Correlations with other tests	Protein Leukocytes Microscopic

3. The reliability of the test depends on the presence of adequate amounts of nitrate in the urine. This is seldom a problem in patients on a normal diet that contains green vegetables; however, because diet usually is not controlled prior to testing, the possibility of a false-negative result owing to lack of dietary nitrate does exist.
4. Further reduction of nitrite to nitrogen may occur when large numbers of bacteria are present and cause a false-negative reaction.
5. Other causes of false-negative results include inhibition of bacterial metabolism by the presence of antibiotics, large quantities of ascorbic acid interfering with the diazo reaction, and decreased sensitivity in specimens with a high specific gravity.

False-positive results will be obtained if nitrite testing is not performed on fresh samples, because multiplication of contaminant bacteria will soon produce measurable amounts of nitrite. When fresh urine is used, false-positive results will not be obtained, even if a nonsterile container is used. Pink discoloration or spotting on the edges of the reagent pad should not be considered a positive reaction. Highly pigmented urines will produce atypical color reactions. Visual examination of the strip will determine that the characteristic pink color is not present. Automated strip readers will report any color change as positive, and strips should be visually examined when discrepancies are observed.

Leukocyte Esterase

Prior to development of the reagent strip leukocyte esterase (**LE**) test, detection of increased urinary leukocytes required microscopic examination of the urine sediment. This can be subject to variation depending on the method used to prepare the sediment and the technical personnel examining the sediment. Therefore, the chemical test for leukocytes offers a more standardized means for the detection of leukocytes. The test is not designed to measure the concentration of leukocytes, and the manufacturers recommend that quantitation be done by microscopic examination. An additional advantage to the chemical LE test is that it will detect the presence of leukocytes that have been lysed, particularly in dilute alkaline urine, and would not appear in the microscopic examination.

CLINICAL SIGNIFICANCE

Normal values for leukocytes are based on the microscopic sediment examination and vary from 0 to 2 to 0 to 5 per high power field. Women tend to have higher numbers than men as a result of vaginal contamination. Increased urinary leukocytes are indicators of UTI. The LE test detects the presence of esterase in the granulocytic white blood cells (neutrophils, eosinophils, basophils, and monocytes). Neutrophils are the leukocytes most frequently associated with bacterial infections. Esterases also are present in *Trichomonas* and histiocytes. Lymphocytes, erythrocytes, bacteria, and renal tissue cells do not contain esterases. A positive LE test result is most frequently accompanied by the presence of bacteria, which, as discussed previously, may or may not produce a positive nitrite reaction. Infections caused by *Trichomonas*, *Chlamydia*, yeast, and inflammation of renal tissues (i.e., interstitial nephritis) produce *leukocyturia* without bacteriuria.

Screening urine specimens using the LE and nitrite chemical reactions to determine the necessity of performing urine cultures can be a cost-effective measure.[22] The LE test contributes significantly more to the reliability of this practice than does the nitrite test.
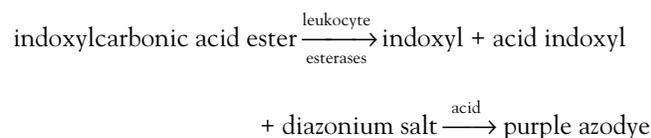
REAGENT STRIP REACTION

The reagent strip reaction uses the action of LE to catalyze the hydrolysis of an acid ester imbedded on the reagent pad to produce an aromatic compound and acid. The aromatic compound then combines with a diazonium salt present on the pad to produce a purple azodye.

Summary of Clinical Significance of Urine Leukocytes
1. Bacterial and nonbacterial urinary tract infection
2. Inflammation of the urinary tract
3. Screening of urine culture specimens

Leukocyte Esterase Reagent Strip Summary	
Reagents	Multistix: Derivatized pyerole amino acid ester
	Diazonium salt
	Chemstrip: Indoxylcarbonic acid ester
	Diazonium salt
Sensitivity	Multistix: 5–15 WBC/hpf
	Chemstrip: 10–25 WBC/hpf
Interference	False-positive: Strong oxidizing agents
	Formalin
	Highly pigmented urine, nitrofurantoin
	False-negative: High concentrations of protein, glucose, oxalic acid, ascorbic acid, gentamicin, cephalosporins, tetracyclines
Correlations with other tests	Protein
	Nitrite
	Microscopic

$$\text{indoxylcarbonic acid ester} \xrightarrow[\text{esterases}]{\text{leukocyte}} \text{indoxyl + acid indoxyl}$$

$$+ \text{ diazonium salt} \xrightarrow{\text{acid}} \text{purple azodye}$$

The LE reaction requires the longest time of all the reagent strip reactions (2 minutes). Reactions are reported as trace, small, moderate, and large or trace, 1+, 2+, and 3+. Trace readings may not be significant and should be repeated on a fresh specimen.

REACTION INTERFERENCE

The presence of strong oxidizing agents or formalin in the collection container will cause false-positive reactions. Highly pigmented urines and the presence of nitrofurantoin will obscure the color reaction.

False-negative results may occur in the presence of high concentrations of protein (greater than 500 mg/dL), glucose (greater than 3 g/dL), oxalic acid, and ascorbic acid. Crenation of leukocytes preventing release of esterases may occur in urines with a high specific gravity.[17] The presence of the antibiotics gentamicin, cephalexin, cephalothin, and tetracycline decreases the sensitivity of the reaction.

Specific Gravity

The addition of a specific gravity testing area to reagent strips has eliminated a time-consuming step in routine urinalysis and has provided a convenient method for routine screening. Replacing osmometry or refractometry for criti-

---

Summary of Clinical Significance of Urine Specific Gravity

1. Monitoring patient hydration and dehydration
2. Loss of renal tubular concentrating ability
3. Diabetes insipidus
4. Determination of unsatisfactory specimens due to low concentration

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Urine Specific Gravity Reagent Strip Summary

Reagents	Multistix: Poly (methyl vinyl ether/maleic anhydride) bromthymol blue
	Chemstrip: Ethyleneglycol-Bis (aminoethylether) bromthymol blue
Sensitivity	1.000–1.030
Interference	False-positive: High concentrations of protein
	False-negative: Highly alkaline urines (>6.5)

---

cal fluid monitoring is not recommended.[16] The clinical significance of the specific gravity test is discussed in Chapter 4.

REAGENT STRIP REACTION

The reagent strip reaction is based on the change in pK_a (dissociation constant) of a polyelectrolyte in an alkaline medium. The polyelectrolyte ionizes releasing hydrogen ions in proportion to the number of ions in the solution. The higher the concentration of urine, the more hydrogen ions are released, thereby lowering the pH. Incorporation of the indicator bromthymol blue on the reagent pad measures the change in pH. As the specific gravity increases, the indicator changes from blue (1.000 [alkaline]), through shades of green, to yellow (1.030 [acid]). Readings can be made in 0.005 intervals by careful comparison with the color chart. The specific gravity reaction is diagramed in Figure 5–3.
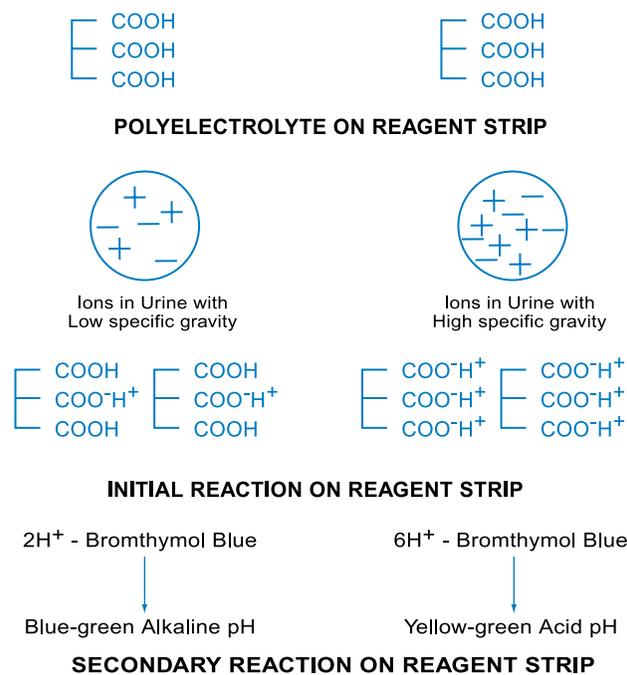
REACTION INTERFERENCE

The reagent strip specific gravity measures only ionic solutes, thereby eliminating the interference by the large organic molecules, such as urea and glucose, and by radiographic contrast media and plasma expanders that are included in physical measurements of specific gravity.[9] This difference must be considered when comparing specific gravity results obtained by a different method. Elevated concentrations of protein will slightly increase the readings as a result of protein anions.

Specimens with a pH of 6.5 or higher will have decreased readings caused by interference with the bromthymol blue indicator (the blue-green readings associated with an alkaline pH correspond to a low specific gravity reading). Therefore, manufacturers recommend adding 0.005 to specific gravity readings when the pH is 6.5 or higher.


```
COOH                              COOH
COOH                              COOH
COOH                              COOH

POLYELECTROLYTE ON REAGENT STRIP

 Ions in Urine with            Ions in Urine with
 Low specific gravity          High specific gravity

COOH      COOH           COO⁻H⁺    COO⁻H⁺
COO⁻H⁺    COO⁻H⁺         COO⁻H⁺    COO⁻H⁺
COOH      COOH           COO⁻H⁺    COO⁻H⁺

INITIAL REACTION ON REAGENT STRIP

2H⁺ - Bromthymol Blue       6H⁺ - Bromthymol Blue

Blue-green Alkaline pH       Yellow-green Acid pH

SECONDARY REACTION ON REAGENT STRIP
```

FIGURE 5–3   Diagram of reagent strip specific gravity reaction.

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(S) TUDY QUESTIONS

1. Describe how each of the following errors in reagent strip technique will affect test results:
   a. Leaving the reagent strip in the urine specimen while recording results of the previous specimen
   b. Failing to remove excess urine while withdrawing the strip from the specimen
   c. Recording all results immediately after withdrawing the strip from the specimen
   d. Using a Chemstrip color chart with a Multistix reagent strip

2. How will failure to allow a refrigerated specimen to warm to room temperature before testing affect reagent strip testing? Why?

3. How are reagent strips protected from deterioration caused by:
   a. moisture
   b. volatile chemicals
   c. light
   d. heat

4. What is the significance of the expiration date stamped on reagent strip containers?

5. List four times when positive and negative controls must be run on reagent strips.

6. Explain the relationship of urine pH to the formation of crystals and renal calculi.

7. What is the significance of a urine pH of 9?

8. Why do reagent strip pH reactions use both methyl red and bromthymol blue indicators?

9. Indicate the source of the following proteinurias by placing a 1 for prerenal, 2 for renal, or 3 for postrenal in front of the condition.
   a. _____ Microalbuminuria
   b. _____ Acute phase reactants
   c. _____ Pre-eclampsia
   d. _____ Vaginal inflammation
   e. _____ Multiple myeloma
   f. _____ Orthostatic proteinuria
   g. _____ Prostatitis

10. Describe the unique solubility characteristics of Bence Jones protein.

11. Differentiate between glomerular and tubular proteinuria.

12. How does detection of microalbuminuria affect patient treatment?

13. Briefly explain the principle of the protein error of indicators. How does a highly alkaline urine affect this?

14. State a pathologic reason that would cause a negative reagent strip protein reaction and a positive SSA test result.

15. Explain why glycosuria occurs in the presence of hyperglycemia.

16. Why do persons with hyperthyroidism exhibit hyperglycemia and glucosuria? Why do persons under extreme stress exhibit hyperglycemia and glucosuria?

17. How can glycosuria occur in the absence of hyperglycemia?

18. Explain the purpose of glucose oxidase and peroxidase in the reagent strip test for glucose.

19. What is the primary cause of a false-negative test result for glucosuria?

20. Does the Clinitest detect oxidizing or reducing substances? How does it do this?

21. How will failure to detect "pass through" affect results?

22. What is the primary reason that laboratories perform the Clinitest?

23. State two reasons for a positive reagent strip test result for glucose and a negative Clinitest result.

24. State the three basic reasons for the presence of ketonuria.

25. What is the primary substance detected by sodium nitroprusside? Why is glycine added to the reaction?

26. How do the reactions between ketones and interfering substances differ on reagent strips?

27. Name three pathologic substances detected by the reagent strip blood reaction. State a reason why each of these appears in the urine.

Substance	Reason
a. _____	_____
b. _____	_____
c. _____	_____

28. Why is hemoglobinuria associated with pink plasma and myoglobinuria with normal-colored plasma?

29. Why may yellow-brown granules appear in the urine sediment?

30. Following precipitation of a clear red urine with ammonium sulfate, what is the significance of a red supernatant?

31. What is the purpose of peroxide on the reagent strip pad for blood?

32. What is the significance of a speckled reaction on the blood pad?

33. Why are high levels of ascorbic acid of concern with both the glucose and blood reactions?

34. List in order the three products formed in the degradation of hemoglobin to urobilin.

35. Name two causes of jaundice that produce bilirubinuria and one cause that does not produce bilirubinuria.

36. State two advantages of the Ictotest over the reagent strip bilirubin test.

37. What is the major cause of false-negative test results for bilirubin?

38. Why is it normal to have 0.1 to 1 mg/dL of urobilinogen in the urine?

39. How does hemolytic anemia affect urine urobilinogen? Why? How does biliary obstruction affect urine urobilinogen? Why?

40. How do the reagent strip reactions for urobilinogen differ between Multistix and Chemstrip? What other pathologic substance does Multistix detect?

41. Describe the reactions obtained with urobilinogen, porphobilinogen, and Ehrlich-reactive compounds in the Watson-Schwartz test.

42. How would you perform a rapid screening test for the presence of porphobilinogen?

43. Describe the diagnostic value of the nitrite test.

44. Why might an automated nitrite reading of positive be changed to negative when the strip is visually examined?

45. State four reasons why a specimen with a large amount of bacteria could have a negative nitrite reaction.

46. Can a nitrite test be performed on a fresh specimen collected in an unsterile container? Why or why not?

47. Why is it possible to have a positive LE reaction and not see any leukocytes in the urine microscopic examination?

48. When is it possible to have a positive LE test in the absence of bacteria?

49. Why do large quantities of ascorbic acid cause false-negative reactions for bilirubin, urobilinogen by Chemstrip, nitrite, and LE?

50. Why does a urine specimen with a low specific gravity produce an alkaline reaction with bromthymol blue in the specific gravity test?

51. How do specific gravity readings differ between reagent strips and refractometers?

52. Explain the need to add 0.005 to the specific gravity readings in urines with a pH of 6.5 or higher.

CASE STUDIES AND CLINICAL SITUATIONS

1. Preadmission laboratory work on an obese patient scheduled for surgery shows a fasting blood glucose level of 230 mg/dL. Results of the routine urinalysis are as follows:

COLOR: Pale yellow	KETONES: Negative
CLARITY: Clear	BLOOD: Negative
SP. GRAVITY: 1.030	BILIRUBIN: Negative
pH: 5.0	UROBILINOGEN: Negative
PROTEIN: 1+	NITRITE: Negative
GLUCOSE: 100 mg/dL	LEUKOCYTES: Negative

  a. Explain the correlation between the patient's blood and urine glucose results.
  b. What is the most probable metabolic disorder associated with this patient?
  c. Considering the patient's condition, what is the significance of the patient's protein result?
  d. What could have been done to delay the onset of proteinuria in this patient?
  e. If the patient in this study had a normal blood glucose level, to what would the urinary glucose be attributed?

2. Results of a urinalysis performed on a patient scheduled for gallbladder surgery are as follows:

COLOR: Amber	KETONES: Negative
CLARITY: Hazy	BLOOD: Negative
SP. GRAVITY: 1.022	BILIRUBIN: Moderate
pH: 6.0	UROBILINOGEN: Normal
PROTEIN: Negative	NITRITE: Negative
GLUCOSE: Negative	LEUKOCYTES: Negative

  a. What would be observed if this specimen were shaken?
  b. What confirmatory test could be performed on this specimen?
  c. Explain the correlation between the patient's scheduled surgery and the normal urobilinogen.
  d. If blood were drawn from this patient, how might the appearance of the serum be described?
  e. What special handling is needed for serum and urine specimens from this patient?

3. Results of a urinalysis on a very anemic and jaundiced patient are as follows:

COLOR: Red	KETONES: Negative
CLARITY: Clear	BLOOD: Large
SP. GRAVITY: 1.020	BILIRUBIN: Negative
pH: 6.0	UROBILINOGEN: 12 EU
PROTEIN: Negative	NITRITE: Negative
GLUCOSE: Negative	LEUKOCYTES: Negative

  a. Would these results be indicative of hematuria or hemoglobinuria?
  b. Correlate the patient's condition with the urobilinogen result.

c. Why is the urine bilirubin result negative in this jaundiced patient?

d. If interference by porphyrins was suspected in this specimen, how could this be resolved? State two methods.

4. A female patient arrives at the outpatient clinic with symptoms of lower back pain and urinary frequency with a burning sensation. She is a firm believer in the curative powers of vitamins. She has tripled her usual dosage of vitamins in an effort to alleviate her symptoms; however, the symptoms have persisted. She is given a sterile container and asked to collect a midstream clean-catch urine specimen. Results of this routine urinalysis are as follows:

COLOR: Dark yellow   KETONES: Negative
CLARITY: Hazy   BLOOD: Trace
SP. GRAVITY: 1.012   BILIRUBIN: Negative
pH: 7.0   UROBILINOGEN: Normal
PROTEIN: Trace   NITRITE: Negative
GLUCOSE: Negative   LEUKOCYTES: 2+

Microscopic

8 TO 12 RBC/HPF   Heavy bacteria
40 TO 50 WBC/HPF   Moderate squamous epithelial cells

a. What discrepancies between the chemical and microscopic test results are present? State a possible reason for each discrepancy.

b. What additional chemical tests could be affected by the patient's vitamin dosage?

c. Discuss the urine color and specific gravity results with regard to correlation, and give a possible cause for any discrepancy.

d. State three additional reasons not previously given for a negative nitrite test in the presence of increased bacteria.

5. Results of a urinalysis collected following practice from a 20-year-old college athlete are as follows:

COLOR: Dark yellow   KETONES: Negative
CLARITY: Hazy   BLOOD: 1+
SP. GRAVITY: 1.029   BILIRUBIN: Negative
pH: 6.5   UROBILINOGEN: 1 EU
PROTEIN: 2+   NITRITE: Negative
GLUCOSE: Negative   LEUKOCYTES: Negative

The physician requests the athlete to collect another specimen in the morning prior to classes and practice.

a. What is the purpose of the second sample?

b. What changes would you expect in the second sample?

c. Is the proteinuria present in the first sample of prerenal, renal, or postrenal origin?

6. A construction worker is pinned under collapsed scaffolding for several hours prior to being taken to the emergency room. His abdomen and upper legs are severely bruised, but no fractures are detected. A specimen for urinalysis obtained by catheterization has the following results:

COLOR: Red-brown   KETONES: Negative
CLARITY: Clear   BLOOD: 4+
SP. GRAVITY: 1.017   BILIRUBIN: Negative
pH: 6.5   UROBILINOGEN: 0.4 EU
PROTEIN: Trace   NITRITE: Negative
GLUCOSE: Negative   LEUKOCYTES: Negative

a. Would hematuria be suspected in this specimen? Why or why not?

b. What is the most probable cause of the positive blood reaction?

c. What is the source of the substance causing the positive blood reaction and the name of the condition?

d. Would this patient be monitored for changes in renal function? Why or why not?

7. Considering the correct procedures for care, technique, and quality control for reagent strips, state a possible cause for each of the following scenarios.

a. The urinalysis supervisor notices that an unusually large number of reagent strips are becoming discolored before the expiration date has been reached.

b. A physician's office is consistently reporting positive nitrite test results with negative LE test results.

c. A student's results for reagent strip blood and LE are consistently lower than those of the laboratory staff.

d. A physician questions the significant number of reports indicating elevated automated reagent strip bilirubin results accompanied by negative Ictotest results.