CHAPTER 4

Chemical Analysis of Urine

Key Terms

ACETEST
ASCORBATE (ASCORBIC ACID)
BILIRUBIN
CHROMOGENS
CLINITEST
GLYCOSURIA
HEMATORIA
HEMOGLOBINURIA
ICTOTEST
KETONES
LEUKOCYTE ESTERASE
MYOGLOBIN
NITRITE
pH
PROTEIN
PROTEIN ERROR OF INDICATORS
PROTEINURIA
REAGENT STRIP
REDUCING SUBSTANCE
RUN-OVER
TAMM–HORSFALL PROTEIN
UROBILINOGEN

Learning Objectives

For Each Chemical Test Performed by Dipstick Methodology
1. Describe the principle and procedure.
2. Compare and contrast reagent strip characteristics among manufacturers.
3. Interpret results.
4. Define expected normal values.
5. Suggest causes for abnormal findings.
6. Identify sources of error.
7. Suggest appropriate confirmatory tests.
8. Correlate results of chemical tests with those of physical examination.
9. Predict findings of microscopic examination.

For the Confirmatory Urine Tests (Acetest, Clinitest, Ictotest)
10. Describe the principle and procedure.
11. Interpret results.
12. Recognize sources of error.
13. Suggest appropriate clinical applications.
The routine urinalysis includes chemical testing for pH, protein, glucose, ketones, occult blood, bilirubin, urobilinogen, nitrate, leukocyte esterase, and strip test method for specific gravity. The urinalysis offered by laboratories depends on the type of dipstick that is used. In addition, most laboratories routinely screen for reducing substances as part of the routine urinalysis for children 2 years old and younger. These procedures are either qualitative (positive or negative) or semiquantitative (e.g., trace through $4^+3$) measurements. Since the introduction of single- and multiple-test reagent strips, test tapes, and tablets, the chemical screening of the urine has become a sensitive and rapid procedure. Completion of urine chemistry using reagent test strips occurs in 2 minutes. Several brands of dipsticks are available worldwide.

### A reagent strip

A reagent strip, also called a dipstick, is a narrow strip of plastic with small pads attached to it. Each pad contains reagents for a different reaction, thus allowing for the simultaneous determination of several tests. The colors generated on each reagent pad vary according to the concentration of the analyte present. Colors generated by each pad are visually compared against a range of colors on brand-specific color charts. Color charts for one brand of reagent strips discussed in this text is included in Appendix C. Figure 4-1 illustrates a typical urine chemistry reagent strip (dipstick).3

The manual method for using a reagent strip to test urine calls for dipping the entire strip into the specimen and withdrawing it in one continuous motion while removing excess urine by dragging across the edge of the specimen container. A critical requirement is that the reactions be read at the prescribed time after dipping and then compared closely with the color chart provided by the manufacturer. To obtain accurate and reliable results with the dipsticks, certain precautions must be taken to help maintain the reactivity of the reagents. The strips must not be exposed to moisture, direct sunlight, heat, or volatile substances; and they should be stored in their original containers. The container should not be kept in the refrigerator nor exposed to temperatures over 30°C. Each vial or bottle contains a desiccant, but the strips should still not be exposed to moisture. Remove only the number of strips needed at the time of testing and then tightly close the container. If the color blocks on the strip do not resemble the negative blocks on the color chart or if the expiration date on the container has past, discard the strips.

Urine should be tested at room temperature. If the urine specimen has been refrigerated, it should be brought to room temperature before testing. The procedure for using the dipstick is as follows:

1. Completely dip the test areas of the strip in fresh, well-mixed, uncentrifuged urine and remove immediately. Care should be taken not to touch the test areas.
2. Remove the excess urine from the stick by touching the edge of the strip to the urine container. Follow the manufacturer’s requirement for maintaining the reagent strip in either a horizontal or vertical position.
3. At the correct times, compare the test areas with the corresponding color charts on the container. The strip should be read in good lighting for accurate color comparison.
4. Record results as prescribed by your laboratory’s protocol.

Several brands of urine chemistry dipsticks are compared in this text. The reagents used for these dipsticks vary according to manufacturer. The reagents for each parameter measured by these manufacturers along with their sensitivities are listed in tables that appear with the discussion of each parameter. Although examples of each parameter’s color reactions are also included, they portray the results obtained by only one manufacturer. Chemical reaction colors vary slightly as do the timing of the reactions. Color charts for one brand of reagent strips discussed in this text is included in Appendix C. Always review and follow the manufacturers’ latest directions, as improvements to the reagent strips may have been made for more recently manufactured lot numbers of strips.

Even with the widespread use of the rapid and convenient screening procedures, it is still necessary to understand the basic principles of the tests as well as the correct technique to be used. This chapter includes a clinical explanation of the chemical constituents most often tested in urine, the principles behind the tests, some causes for abnormal results, and use of confirmatory procedures.

### URINARY pH

One of the functions of the kidney is to help maintain acid–base balance in the body. To maintain a constant pH (hydrogen ion concentration) in the blood (about 7.40), the kidney must vary the pH of the urine to compensate for diet and products of metabolism. This regulation occurs in the distal portion of the nephron with the secretion of both hydrogen and ammonia ions into the filtrate, and the reabsorption of bicarbonate. If sufficient hydrogen ions ($H^+$) are secreted into the tubule, all of the bicarbonate present will be reabsorbed, but if fewer $H^+$ are secreted or if an excess of bicarbonate is present, some of the bicarbonate will be excreted in the urine.4 The continued secretion of $H^+$ after all bicarbonate has been reabsorbed will drop the pH.

**Figure 4-1.** Illustration of Multistix 10 SG. (Modified from Tarrytown, NY: Bayer Corporation Diagnostics Division, 1996.1)
of the filtrate and result in an acidic urine. The secretion of $H^+$ in the tubule is regulated by the amount present in the body. If there is an excess of acid in the body (acidosis), more $H^+$ will be excreted and the urine will be acid. When there is an excess of base in the body (alkalosis), less $H^+$ will be excreted and the urine will be alkaline. The hydrogen ions in the urine are excreted as either free $H^+$, in association with a buffer such as phosphate, or bound to ammonia as ammonium ions. The pH of the urine is determined by the concentration of the free $H^+$.

Because pH is the reciprocal of the hydrogen ion concentration, as the $H^+$ concentration increases, the pH decreases or becomes more acidic. As the $H^-$ concentration decreases, the pH increases or becomes more alkaline. The pH of the urine may range from 4.6 to 8.0 but averages around 6.0, so it is usually slightly acidic. There is no abnormal range as such, since the urine can normally vary from acid to alkaline. For this reason, it is important for the physician to correlate the urine pH with other information to determine whether there is a problem. Metabolic and renal disorders that affect urine pH are discussed in Chapter 5.

### REAGENT TEST STRIPS

All brands of dipsticks discussed in this chapter use the same two indicators, methyl red and bromthymol blue, and measure a range of pH from 5.0 to 8.5. The results may be reported in whole units or interpolated to half units. If a more precise reading is needed, measurement may be made using a pH meter with a glass electrode. Some laboratories report the reaction as “acid,” “neutral,” or “alkaline,” instead of giving numerical values. Figure 4-2 shows the color reactions that correspond to pH values from 5.0 to 8.5.

Most manufacturers recommended that the pH be read immediately as this will prevent misreadings due to the phenomenon of “run-over” effect. This term is used to describe what happens when excess urine is left on the stick after dipping, and so the acid buffer from the reagent in the protein area runs onto the pH area. Run-over can sometimes be recognized by the technologist, because the edge nearest the protein area will usually change first. However, if the strip is not observed constantly after dipping, and so the acid buffer from the reagent in the protein area runs onto the pH area. This type of contamination can cause a false lowering of the pH reading, especially in the case of an alkaline or neutral urine. Run-over can sometimes be recognized by the technologist, because the edge nearest the protein area will usually change first. However, if the strip is not observed constantly after dipping, this occurrence can be overlooked.

Recent advances have been made to prevent “run-over.” Multistix has a hydrophobic interpad surface which causes the urine to bead up on it and thereby reduces “run-over.” The design of the Chemstrip is such that a nylon mesh holds the test pads and underlying absorbent papers in place on the plastic strip. The mesh allows for even diffusion of the urine on the test pads, and the underlying paper absorbs excess urine to prevent “run-over.” If pH is the only test needed to be done on a urine specimen, litmus paper or Nitrazine paper can also be used to obtain an approximate reading.

### PROTEIN

The presence of increased amounts of protein in the urine can be an important indicator of renal disease. It may be the first sign of a serious problem and may appear long before other clinical symptoms. There are, however, physiologic conditions such as exercise and fever that can lead to increased protein excretion in the urine in the absence of renal disease. There are also some renal disorders in which proteinuria is absent.

In the normal kidney, only a small amount of low-molecular weight protein is filtered at the glomerulus. The structure of the glomerular membrane prevents the passage of high-molecular weight proteins including albumin (mol wt = 69,000). After filtration, most of the protein is reabsorbed in the tubules with less than 150 mg/24 h (or 20 mg/dL) being excreted. In a child, the normal excretion is less than 100 mg/m²/24 h. The protein that is normally excreted includes a mucoprotein called Tamm–Horsfall protein, which is not contained in the plasma but is secreted by the renal tubules. This protein forms the matrix of most urinary casts (see Chapter 5). Causes for proteinuria are explained in Chapter 5.

### SCREENING TESTS

The screening tests for proteinuria are based either on the “protein error of indicators” principle or on the ability of protein to be precipitated by acid or heat. Sensitivity differs among these tests. The dipsticks are more sensitive to albumin than to other proteins, whereas the heat and acid tests are sensitive to all proteins. In addition, some substances that interfere with the precipitation tests do not interfere with the reaction on the dipstick.

Contamination of the urine with vaginal discharge, semen, heavy mucus, pus, and blood can result in a false-positive reaction with any method that is used. A very dilute urine can give a false-negative reaction because the concentration of protein fluctuates with the urine flow. Therefore, it is important to interpret the protein result by correlating it with the specific gravity. A trace of protein in a dilute urine indicates a greater loss of protein than does a trace amount in a concentrated specimen.

If protein is present in large quantities, the surface tension of the urine will be altered. Agitation of the urine will cause a white foam to develop on the surface of the urine.
Observing foam may be helpful as an indicator of proteinuria. In order to accurately measure the extent of proteinuria and to differentiate the types of protein that are present, positive screening tests may be confirmed by quantitative procedures and/or electrophoretic, immunoelectrophoretic, immunodiffusion, and ultracentrifugation studies.

**REAGENT TEST STRIPS**

This colorimetric method used in dipsticks is based on the concept known as the “protein error of indicators,” a phenomenon which means that the point of color change of some pH indicators is different in the presence of protein from that observed in the absence of protein, because proteins act as hydrogen ion acceptors at a constant pH. Usually, the indicator changes from yellow to blue (or green) between pH 3 and pH 4, but in the presence of protein, this color change will occur between pH 2 and pH 3. Therefore, in the presence of protein an “error” occurs in the behavior of the indicator.8 Indicators used on the various reagent strips vary by manufacturer and are outlined on Table 4-1.

<table>
<thead>
<tr>
<th>BRAND AND SENSITIVITY</th>
<th>INDICATOR</th>
</tr>
</thead>
<tbody>
<tr>
<td>AimStick5 (15 mg/dL)</td>
<td>Tetrabromphenol blue</td>
</tr>
<tr>
<td>Chemistrip5 (6 mg/dL)</td>
<td>3’,3”,5’,5”-Tetrachlorophenol-3,4,5,6-Tetrabromsulfophthalein</td>
</tr>
<tr>
<td>Combi-Screen PLUS10 (15 mg/dL)</td>
<td>Tetrabromphenol blue</td>
</tr>
<tr>
<td>DiaScreen11 (5 mg/dL)</td>
<td>Tetrabromphenol blue</td>
</tr>
<tr>
<td>Dirui H-Series12 (0.15–0.3 g/L)</td>
<td>Tetrabromphenol blue</td>
</tr>
<tr>
<td>Mission13 (18–30 mg/dL)</td>
<td>Tetrabromphenol blue</td>
</tr>
<tr>
<td>Multistix15 (15 mg/dL)</td>
<td>Tetrabromphenol blue</td>
</tr>
<tr>
<td>Self-Stik14 (5–10 mg/dL)</td>
<td>Tetrabromphenol blue</td>
</tr>
<tr>
<td>URiSCAN15 (10 mg/dL albumin)</td>
<td>Tetrabromphenol blue</td>
</tr>
<tr>
<td>Uritest 13G16 (0.1–0.3 g/L albumin)</td>
<td>Tetrabromphenol blue</td>
</tr>
<tr>
<td>Uro-dip 10C17 (not given)</td>
<td>Tetrabromphenol blue</td>
</tr>
<tr>
<td>URS18 (15 mg/dL)</td>
<td>Tetrabromphenol blue</td>
</tr>
</tbody>
</table>

Note: sensitivities are for albumin.

Sensitivities for protein are also listed. Be aware that reagent strips detect primarily albumin and are less sensitive to globulins.

An acid buffer is added to the reagent area to maintain a constant pH of 3, which in the absence of urine protein produces a yellow color. The development of any green to blue color indicates the presence of protein. The intensity of the color is proportional to the amount of protein that is present. The protein area is read at 60 seconds for most brands of dipsticks (follow the manufacturer’s latest directions). The color of the reagent area should be carefully compared with the color chart supplied by the manufacturer. The results are usually reported as negative to 3+ or 4+ and display a range of colors from yellow to blue. Figure 4-3 displays the color chart for positive protein values.

Most brands of dipsticks have differing target areas, so they are not clinically interchangeable.19,20 Refer to each manufacturer’s own color chart for proper reporting of results. Trace readings are only approximate values. Not all urines with those values will necessarily give a trace reaction. Screening tests should be able to discriminate between normal and abnormal concentrations, but it is possible to get a positive reaction with the dipstick in a normal patient because the trace area is too sensitive.20,21 This situation can occur especially if the specimen is very concentrated.

The dipstick procedure is very sensitive to albumin, the protein that is primarily excreted as the result of glomerular damage or disease.22 Other urine proteins such as gamma globulin, glycoprotein, ribonuclease, lysozyme, hemoglobin, Tamm–Horsfall mucoprotein, and Bence-Jones protein are much less readily detected than albumin.5,15 Therefore, a negative urinary dipstick result does not necessarily rule out the presence of these proteins.

**False-Positive Results**

False-positive results may occur in a highly buffered alkaline urine, which may result from alkaline medication or stale urine.5,11 The alkaline pH can overcome the acid buffer in the reagent and the area may change color in the absence of protein. If the dipstick is left in the urine for too long, the buffer will be washed out of the reagent, the pH will increase, and the strip will turn blue or green even if protein is not present.8

Quaternary ammonium compounds that may be used to clean the urine containers will alter the pH and result in a false-positive reaction.5,11 False positives may occur on some
dipsticks during treatment with phenazopyridine and after the infusion of polyvinylpyrrolidone as a plasma expander. Chlorhexidine gluconate, found in skin cleansers, may produce false-positive results. In addition, specimens containing blood may cause a false-positive protein reaction.

False-Negative Results
False-negative results can occur in dilute urines and when proteins other than albumin are present in slightly elevated concentrations.

The various acid precipitation tests that also screen for urinary proteins are not routinely performed in most clinical laboratories. The principles and procedures for these tests are included in Appendix B as reference material.

**GLUCOSE AND OTHER REDUCING SUBSTANCES**

The presence of significant amounts of glucose in the urine is called **glycosuria** (or glucosuria). The quantity of glucose that appears in the urine is dependent upon the blood glucose level, the rate of glomerular filtration, and the degree of tubular reabsorption. Usually, glucose will not be present in the urine until the blood level exceeds 160–180 mg/dL, which is the normal renal threshold for glucose. When the blood glucose exceeds the renal threshold, the tubules cannot reabsorb all of the filtered glucose, and so glycosuria occurs. Normally, this level is not exceeded even after the ingestion of a large quantity of carbohydrate. A small amount of glucose may be present in the normal urine, but the fasting level in an adult is only about 2–20 mg of glucose per 100 mL of urine.

**SCREENING TESTS**

There are two basic types of tests that are used to screen for or monitor glycosuria. The procedures that use the enzyme glucose oxidase are specific for glucose, while the copper reduction tests will detect any reducing substance. As with all screening procedures, a positive test result should be correlated with other findings. The interpretation of a positive glucose test should be based on the other screening tests, including specific gravity, ketones, and albumin. But more importantly, a correlation must be made with the blood glucose level as well as the case history, family history, and clinical picture. A previously undiagnosed glycosuria should be followed up by such studies as a glucose tolerance test, 2-hour postprandial glucose, and fasting blood sugar. A positive reducing substance other than glucose can best be differentiated by either thin-layer or paper chromatography.

**REAGENT STRIP GLUCOSE OXIDASE TEST**

Reagent strips that are impregnated with the enzyme glucose oxidase detect only glucose. These strips use the following double sequential enzyme reaction:

![Reaction A: Glucose oxidase](image)

![Reaction B: Peroxidase](image)

The chromogen that is used varies among the different reagent strips. Table 4-2 displays the chromogen used by each of the main manufacturers along with their sensitivities.

**Table 4-2 Glucose Chromogens and Sensitivities by Reagent Strip**

<table>
<thead>
<tr>
<th>BRAND AND SENSITIVITY</th>
<th>CHROMOGEN</th>
</tr>
</thead>
<tbody>
<tr>
<td>AimStick9 (50 mg/dL)</td>
<td>Potassium iodide</td>
</tr>
<tr>
<td>Chemistrip5 (40 mg/dL)</td>
<td>Tetramethylbenzine</td>
</tr>
<tr>
<td>Combi-Screen PLUS10 (40 mg/dL)</td>
<td>Glucose oxidase Peroxidase O-tolidine-hydrochloride</td>
</tr>
<tr>
<td>DiaScreen11 (50 mg/dL)</td>
<td>Potassium iodide</td>
</tr>
<tr>
<td>Dirui H-Series12 (2.8–5.5 mmol/L)</td>
<td>Glucose oxidase Peroxidase Potassium iodide</td>
</tr>
<tr>
<td>Mission13 (25–50 mg/dL)</td>
<td>Glucose oxidase Peroxidase O-tolidine</td>
</tr>
<tr>
<td>Multistix2 (75 mg/dL)</td>
<td>Potassium iodide</td>
</tr>
<tr>
<td>Self-Stik14 (50–100 mg/dL)</td>
<td>Glucose oxidase Peroxidase Potassium iodide</td>
</tr>
<tr>
<td>URiSCAN15 (50 mg/dL)</td>
<td>Glucose oxidase Peroxidase Potassium iodide</td>
</tr>
<tr>
<td>Uritest 13G16 (2.2–2.8 mmol/L)</td>
<td>Glucose oxidase Peroxidase 4-Aminoantipyrine</td>
</tr>
<tr>
<td>Uro-dip 10C17 (100–150 mg/dL)</td>
<td>Glucose oxidase Peroxidase Potassium iodide</td>
</tr>
<tr>
<td>URS18 (100 mg/dL)</td>
<td>Glucose oxidase Peroxidase Potassium iodide</td>
</tr>
</tbody>
</table>
Chromogens are subject to change by the manufacturer, so always consult manufacturer package inserts. Glucose results are read at 30 or 60 seconds, depending on the manufacturer. The results are reported as negative to 4+ (negative to 2000 mg/dL). The color changes displayed by these values range from blue to brown. Figure 4-4 displays a glucose color chart.

The results are semiquantitative values, but the color reaction is kinetic and will continue to react after the prescribed time. A reading taken after this time will be falsely elevated. Glucose oxidase methods are more sensitive to solutions of aqueous glucose than to glucose in urine; therefore, they are more sensitive to dilute urine than concentrated urine.8 Urines which have been refrigerated must be first brought to room temperature before accurate testing can be performed, because these methods are enzymatic and are affected by temperature.

False-Positive Results

No known constituent of urine will give a false-positive enzyme test,2,5,11 but if the urine specimen is contaminated with strong oxidizing cleaning agents peroxide or hypochlorite, a false-positive reaction may occur.5

In urines positive for glucose, a falsely elevated glucose may result in the presence of elevated urobilinogen when using automated methods for some brands of reagent strips.11

False-Negative Results

Sensitivity for glucose may be affected by temperature, specific gravity, and pH. Reactivity for glucose can vary with temperature because of the effect temperature can have on enzymatic reactions.9,11 An elevated specific gravity may decrease the sensitivity of glucose oxidase.9,11 Alkaline pH may decrease sensitivity to glucose.11 The combination of high specific gravity and alkaline pH may result in false negatives at low concentrations of glucose.2

High urinary concentrations of ascorbate (ascorbic acid or vitamin C) can inhibit the enzymatic reaction which will result in a reduced or false-negative reading.29,11 The ascorbic acid will be oxidized by the hydrogen peroxide in the second part of the enzyme reaction, and will, therefore, compete with the oxidation of the chromogen, resulting in the inhibition of the color formation.25 The ingestion of a normal amount of vitamin C usually presents no problem, but the recent interest in the self-prescription of large doses of vitamin C (2–15 g/day) to prevent or cure the common cold has created a potential problem. Large concentrations of urinary ascorbic acid can also occur with the parenteral administration of vitamin C or antibiotics that contain ascorbic acid as a stabilizing agent (e.g., tetracycline). If vitamin C interference is suspected, a repeat test should be performed at least 24 hours after the last intake of ascorbic acid.

Moderately high ketone levels (40 mg/dL) may reduce the sensitivity and may cause false negatives with glucose levels of 100 mg/dL.26 However, such a high level of ketones in a patient with diabetes with only a small amount of glucose is unusual.21 For some reagent strips, ketones as high as 250 mg/dL have been shown not to interfere with the glucose test.5

### SCREENING FOR REDUCING SUBSTANCES

In addition to glucose, other sugars that may be found in urine, such as galactose, lactose, fructose, and maltose, are reducing substances. Procedures, which are based on the ability of glucose to reduce copper, will also detect these sugars if they are present. Any other reducing substances which can occasionally be found in the urine such as dextrins, homogentisic acid, and glucuronates will also give positive reduction tests.

**Clinitest** (Benedict’s Test), a copper reduction test, can be used to test for glucose but is usually used to screen for other reducing substances which may be present. This test is based on the fact that in strongly alkaline solutions and in the presence of heat, reducing sugars will reduce cupric ions to cuprous oxide. The reaction produces a color change of blue through green to orange depending upon the amount of reducing substances present in the urine.

A test for reducing substances should be included in the routine urinalysis of all pediatric patients. This will provide for the early detection of those metabolic defects which are characterized by the excretion of reducing sugars such as galactose, which is present in the urine in patients with galactosemia.

### CLINITEST PROCEDURE

Clinitest is a self-heating method for the semiquantitative determination of reducing substances in the urine.27 The tablet contains the following reagents: copper sulfate, citric acid, sodium hydroxide, and sodium carbonate. When placed in a mixture of water and urine, the tablet is rapidly dissolved by the action of sodium carbonate and citric acid which act as an effervescent. The sodium hydroxide provides the alkaline medium necessary for the reaction, and the heat required is provided by the reaction of sodium hydroxide with water and citric acid. The reducing substances in the urine then react with the copper sulfate to reduce the cupric ions to cuprous oxide. Following are the steps involved in the Clinitest procedure, including reporting information.
1. Place five drops of urine into a glass test tube (or use 0.3 mL).
2. Add 10 drops of water (or 0.6 mL) and mix by shaking.
3. Drop one Clinitest tablet into the tube and observe the complete reaction. Do not shake the tube during the reaction or for 15 seconds after the boiling has stopped. Warning: The bottom of the tube will become very hot! Plastic test tubes may expand because of the heat and become difficult to remove from test tube racks.
4. At the end of the 15-second waiting period, shake the tube gently and then compare with the color chart that is provided.

The test is reported as negative, 1/4 % (or trace), 1/2% (1+), 3/4% (2+), 1% (3+), or 2% (4+). Figure 4-5 displays the colors associated with these amounts of reducing substances.

During the reaction, if the color should rapidly “pass-through” bright orange to a dark brown or greenish-brown, report the result as being greater than 2%. Clinitest is a very accurate procedure if the manufacturer’s directions are carefully followed. Failure to observe the reaction as it takes place will result in a falsely low reading. The “pass-through” phenomenon can occur so rapidly that it can be missed if not observed closely. If measurement beyond 2% is medically desirable, an alternate two-drop method is available. This method involves adding only 2 drops of urine to 10 drops of water, but a special color chart must be used. The two-drop method will allow for quantitation up to 5% but the “pass-through” phenomenon may still occur when very large concentrations of sugar are present.

To determine whether a positive copper reduction test is due to the presence of glucose or another reducing substance, both the glucose oxidase test and the reduction test must be performed and a correlation made of the results. Table 4-3 lists possible results along with the interpretation.

The third possibility of a positive enzyme test but a negative reducing test can occur when only a small amount of glucose is present because the enzyme test can measure as little as 0.1%, but the Clinitest reducing test can detect only 0.25%.

**False-Positive Results**

Nalidixic acid, cephalosporins, probenecid, and the urinary preservatives such as formalin and formaldehyde if present in large quantities may cause false-positive results. High concentrations of ascorbic acid have been considered to give false-positive results, but recent studies question whether this is really a problem. The sensitivity of Clinitest (1/4%) is such that a number of substances which react positively with Benedict’s solution (sensitivity is around 0.05%) will, in most cases, not be present in sufficient quantities to react with Clinitest, for example, salicylates and penicillin.

**False-Negative Results**

If all directions for the procedure are followed closely, no false-negative results will occur. Clinitest is an accurate and reliable test for reducing substances.

**KETONES**

Ketones, or ketone bodies are formed during the catabolism of fatty acids. One of the intermediate products of fatty acid breakdown is acetyl CoA. Acetyl CoA enters the citric acid cycle (Krebs cycle) in the body if fat and carbohydrate degradation are appropriately balanced.

The first step in the Krebs cycle is the reaction of acetyl CoA with oxaloacetate to yield citrate. When carbohydrate is not available or is not being properly utilized, all available oxaloacetate will be used to form glucose, and so there will...
be none available for condensation with acetyl CoA. CoA cannot enter the Krebs cycle; therefore, it is diverted to the formation of ketone bodies.

The ketone bodies are acetoacetic acid (diacetic acid), β-hydroxybutyric acid, and acetone. Acetoacetic acid is the first ketone that is formed from acetyl CoA, and the other ketones are formed from acetoacetic acid as shown in the following reaction:

\[
\begin{align*}
\text{H}_2\text{C-C-CH}_2\text{-COOH} & \rightarrow \text{CH}_3\text{-C-CH}_2\text{-COOH} \\
& \text{Acetoacetic acid} \\
& \text{Acetone}
\end{align*}
\]

\[
\begin{align*}
\text{CO}_2 & \rightarrow \text{O} \\
& \text{β-hydroxybutyric acid}
\end{align*}
\]

β-Hydroxybutyric acid is formed by reversible reduction, and acetone is formed by a slow spontaneous decarboxylation. Acetoacetic acid and β-hydroxybutyric acid are normal fuels of respiration and are important sources of energy. In fact, the heart muscle and the renal cortex prefer to use acetoacetate instead of glucose. But glucose is the major fuel of the brain in well-nourished individuals, even though the brain can adapt to utilize acetoacetate in the absence of glucose. The odor of acetone may be detected in the breath of an individual who has a high level of ketones in the blood because acetone is eliminated via the lungs.

Normally small amounts of ketones are present in the blood, 2–4 mg/dL. The relative proportion of each is approximately 20% acetoacetic acid, 2% acetone, and 78% β-hydroxybutyric acid. There may, however, be considerable proportional variation among individuals.

Acetone is lost into the air if a sample is left standing at room temperature. Therefore, urines should be tested immediately or refrigerated in a closed container until testing.

**REAGENT TEST STRIPS**

Laboratory tests that screen for ketones include reagent test-strip methods and tablet-based tests such as Acetest.

Multistix contains the reagents sodium nitroprusside and an alkaline buffer, which react with diacetic acid in urine to form a maroon color, as in the following reaction:

\[
\begin{align*}
\text{NADH}_2 & \rightarrow \text{NAD}^+ \\
\text{Acetoacetic acid} + \text{Na nitroprusside} + \text{Glycine} & \rightarrow \text{violet–purple color}
\end{align*}
\]

Sodium nitroprusside is used by each manufacturer as listed in Table 4-4. However, sensitivities do vary. Some brands of reagent strips are sensitive only to acetoacetic acid (diacetic acid) whereas others also detect acetone. None of these reagent strips detects β-hydroxybutyric acid.

Multistix and DiaScreen dipsticks do not react with acetone or β-hydroxybutyric acid but will detect as little as 5–10 mg/dL of diacetic acid. In addition to diacetic acid, Chemistrip and AimStick dipsticks detect high levels of acetone but neither detects β-hydroxybutyric acid. Ketone results are read at 40 or 60 seconds, depending on the manufacturer. Color change is from buff-pink to maroon and the reaction is reported as either negative, trace, moderate, or large or negative to 160 mg/dL.

**False-Positive Results**

False-positive results may occur when the urine specimen is highly pigmented or when it contains large amounts of...
levodopa metabolites. Some specimens that have both a high specific gravity and a low pH may give false-positive reactions. Compounds that contain sulfhydryl groups may cause a false-positive or atypical color reaction.

Phenylketones may cause a red–orange coloration. Phthalein compounds used in liver and kidney function tests produce a reddish coloration due to the alkalinity of the test zone. These colors, however, are easily distinguishable from the colors obtained with ketone bodies. Some laboratories chose to confirm positive and questionable results with a tablet test.

**False-Negative Results**

Because of the specificity of Multistix and DiaScreen for diacetic acid, these brands of dipstick will not give a positive ketone result with controls that contain acetone.

**ACETEST TABLETS**

The Acetest tablet contains sodium nitroprusside, glycine, a strong alkaline buffer (disodium phosphate), and lactose. Acetest can be used to test urine, serum, plasma, or whole blood. Diacetic acid and acetone react with sodium nitroprusside and glycine in an alkaline medium to form a purple color. The lactose in the tablet helps enhance the color. Acetest is about 10 times more sensitive to diacetic acid than to acetone. However, Acetest will not react with β-hydroxybutyric acid. In urine it will detect as little as 5–10 mg/dL of diacetic acid and 20–25 mg/dL of acetone.

Procedure for Acetest:

1. Place the tablet on a piece of clean, dry white paper.
2. Put one drop of urine, serum, plasma, or whole blood directly on top of the tablet.
3. For urine, compare the color of the tablet with the color chart at 30 seconds. For serum or plasma, compare the color after 2 minutes. For whole blood, remove the clotted blood from the tablet after 10 minutes and compare the color of the tablet with the chart.

Results are reported as “small, moderate, or large.” For urine, the small color block corresponds to approximately 5–10 mg/dL of diacetic acid, the moderate block is 30–40 mg/dL, and the large block is about 80–100 mg/dL. For serum, plasma, and whole blood, the lowest limit of detection is 10 mg of diacetic acid per 100 mL.

**Figure 4-7.** Acetest color chart. Note: This chart is for color demonstration only and should not be used for interpreting reactions for diagnostic testing. (Modified from Siemens [formerly Elkhart, IN: Bayer HealthCare LLC; 2006.)

**HEMATURIA**

**Hematuria** is the presence of blood or intact RBCs in the urine. A urine that is highly alkaline or has a very low specific gravity (<1.007) can cause the red cells to lyse, thus releasing their hemoglobin into the urine. The presence of this type of hemoglobin is still considered to be hematuria as far as the origin is concerned, but it is very difficult to distinguish from true hemoglobinuria. When lysing occurs, the microscopic examination may show the empty red cell membranes which are often referred to as “ghost” cells. In microhematuria there is such a small amount of blood in the urine that the color of the specimen is unaffected and the hematuria can only be detected chemically or microscopically. On the other hand, gross hematuria plays the colors for the Acetest ketone reactions at these various levels.

Those substances which interfere with the dipsticks will also interfere with the Acetest tablet because the same reaction is involved. Other screening tests for ketones that are no longer routinely performed are included in Appendix B.

**OCCULT BLOOD**

The term “occult” means “hidden,” and the methods used to test for blood in the urine are capable of detecting even minute amounts not visualized macroscopically. Another reason for this title is that these procedures actually detect the free hemoglobin from lysed red blood cells (RBCs). Recent improvements in the dipsticks now allow for the detection of intact RBCs by causing them to lyse while on the test pad. Formerly, some intact RBCs could not be detected. In cases in which all of the red cells stayed intact, it was possible to get a negative test for blood even though the microscopic examination revealed the presence of RBCs. The chemical methods used in the routine urinalysis for detecting blood (hematuria) will also detect free hemoglobin (hemoglobinuria) and myoglobin (myoglobinuria). The urine is normally free of all of these substances; therefore, a positive test for occult blood should be followed by determination of the exact cause and origin of this abnormal finding. A correlation must also be made with the microscopic examination, and this may be done by asking the following questions: Are there red cells present? Does the number of red cells agree with the intensity of the chemical test? Are there red cell casts or hemoglobin casts? Are there empty red cell membranes (ghost cells)? Are there numerous squamous epithelial cells present (possible menstrual contamination)? It should be noted that hematuria, hemoglobinuria, and myoglobinuria can occur either individually or together.
alters the color of the urine and is easily visible macroscopically.

HEMOGLOBINURIA

Hemoglobinuria is the presence of free hemoglobin in the urine as a result of intravascular hemolysis. The hemolysis that occurs in the urine while in the urinary tract or after voiding because of a low specific gravity or highly alkaline pH may be considered to be hemoglobinuria, but it does not bear the same significance as true hemoglobinuria. Hemoglobinuria without hematuria occurs as a result of hemoglobinemia and, therefore, has primarily nothing to do with the kidneys even though it may secondarily result in kidney damage.

MYOGLOBINURIA

Myoglobin is the heme protein of striated muscle. It serves as a reserve supply of oxygen and also facilitates the movement of oxygen within muscle. Injury to cardiac or skeletal muscle results in the release of myoglobin into the circulation. Even just subtle injury to the muscle cells can bring about the release of myoglobin. Myoglobin has a molecular weight of approximately 17,000 and so it is easily filtered through the glomerulus and excreted in the urine. Because myoglobin is cleared so rapidly from the circulation, the plasma is left uncolored even though the urine may be red to brown to black, depending on the degree of myoglobinuria. Chapter 5 contains more information on these forms of occult blood.

SCREENING TESTS

Those tests which screen for occult blood will detect hematuria, hemoglobinuria, and myoglobinuria. As previously mentioned, these states can coexist. If the correlation of the microscopic and chemical results does not imply hematuria, then further evaluation and studies may be done to differentiate between hemoglobinuria and myoglobinuria. The definitive diagnostic test for differentiating these two states is electrophoresis. Other methods that can be used are immunodiffusion, hemagglutination inhibition, or immunoelectrophoresis.

Hemoglobinuria and myoglobinuria can be rapidly differentiated by the following screening criteria: red plasma plus red urine equals hemoglobin; clear plasma plus red urine equals myoglobin. Another screening procedure is the ammonium sulfate test described in Appendix B.

Testing for blood by using benzidine has long been the standard procedure for the detection of occult blood. However, benzidine is carcinogenic and the routine use of it has been discouraged. Therefore, benzidine tests procedures are not included in this text.

REAGENT TEST STRIPS

The dipstick procedure is based on the peroxidase-like activity of hemoglobin and myoglobin which catalyzes the oxidation of a chromogen by an organic peroxide as in the following reaction:

\[ \text{Hemoglobin reaction:} \quad \text{H}_2\text{O}_2 + \text{chromogen} \rightarrow \text{oxidized chromogen} + \text{H}_2\text{O} \]

The indicators used by the most common reagent strips are listed in Table 4-5 along with their sensitivities. Most dipsticks are capable of detecting intact erythrocytes as well as free hemoglobin and myoglobin. Intact RBCs in the urine will hemolyze on the test pad. The freed hemoglobin will react with the reagent and will result in green spots on a yellow or orange background. Thus, the presence of intact red cells will give a spotted green reaction, whereas free hemoglobin and myoglobin will give a uniform green or green to dark blue color.

Blood is usually read at 60 seconds, and the color change is from orange to green to dark blue. There are two separate color scales for erythrocytes and hemoglobin. Intact RBCs may display a speckle-pattern reaction in the absence of free hemoglobin. The results are reported as trace or moderate numbers of intact RBCs or trace through 3+ (large) amount of hemoglobin. Figure 4-8 displays a color chart of blood reactions.

False-Positive Results

Most dipsticks will give false-positive results in the presence of certain oxidizing contaminants such as hypochlorites which may be used to clean urine-collection containers. Sodium hypochlorite in the concentration of 100 mg/L of urine gave a 2+ result with both dipsticks, which shows how sensitive the reagents are to oxidizing agents. When the urine is contaminated with a high bacterial content, a false-positive reaction may occur because of bacterial peroxidases.

False positives will result if the urine is contaminated with menstrual blood. False-positive reactions may occur if the urine or test strip is contaminated with povidone-iodine (Betadine).

![Figure 4-8. Blood color chart.](image-url)
False-Negative Results

The test is slightly more sensitive to free hemoglobin and myoglobin than to intact RBCs. If the urine sample is not mixed well before testing, a false-negative result can occur because the red cells tend to settle in the bottom of the container.

Some dipsticks give lower or false-negative readings in the presence of high levels of ascorbic acid.2,11 If necessary, the test should be repeated at least 24 hours after the last dose of vitamin C. Captopril (Capoten) may reduce the reagent pad’s sensitivity.2 Sensitivity is less in urines with high specific gravity, nitrites, or protein.11 In addition, specimens preserved using formalin will yield a false-negative result.5

BILIRUBIN AND UROBILINOGEN

Bilirubin is formed from the breakdown of hemoglobin in the reticuloendothelial system. It is then bound to albumin and transported through the blood to the liver. This free or unconjugated bilirubin is insoluble in water and cannot be filtered through the glomerulus. In the liver, bilirubin is removed by the parenchymal cells and is conjugated with glucuronic acid to form bilirubin diglucuronide. This conjugated bilirubin, which is also called direct bilirubin, is water soluble and is excreted by the liver through the bile duct and into the duodenum.

Normally, very small amounts of conjugated bilirubin regurgitate back from the bile duct and into the blood system.44 Therefore, very small amounts of conjugated bilirubin can be found in the plasma, but not in concentrations higher than 0.2–0.4 mg/dL.45 Because conjugated bilirubin is not bound to protein, it is easily filtered through the glomerulus and excreted in the urine whenever the plasma level is increased. Normally, no detectable amount of bilirubin (sometimes referred to as “bile”) can be found in the urine.

In the intestines, bacterial enzymes convert bilirubin, through a group of intermediate compounds, to several related compounds which are collectively referred to as urobilinogen.5 Most of the urobilinogen (a colorless pigment)
and its oxidized variant, urobilin (a brown pigment), are lost in the feces. About 10–15% of the urobilinogen is reabsorbed into the bloodstream, returns to the liver, and is reexcreted into the intestines. A small amount of this urobilinogen is also excreted by the kidneys into the urine, with a normal level of about 1–4 mg/24 h or less than 1.0 Ehrlich unit/2 h. The normal level of total bilirubin in the serum is about 1.0 mg/dL or less. This consists mainly of indirect or unconjugated bilirubin, but there is also a very small amount of direct or conjugated bilirubin present. When the level of total bilirubin exceeds approximately 2.5 mg/dL, the tissues of the body take on the yellow color of bilirubin, and this is called jaundice. If the jaundice is due to an increase in unconjugated bilirubin, no bilirubin will be excreted in the urine because unconjugated bilirubin cannot be filtered at the glomerulus. But if jaundice is due to an increase in the water-soluble conjugated bilirubin, then bilirubin will be present in the urine.

**SCREENING TESTS FOR BILIRUBIN (BILE)**

Bilirubin can be detected in the urine before other clinical symptoms are present or recognizable. The detection of small quantities is very important in the early diagnosis of obstructive and hepatic jaundice. This test is also useful in the differential diagnosis of obstructive (positive) and hemolytic (negative) jaundice.

Bilirubin is light sensitive and so the urine should be protected from the light and examined as quickly as possible. On standing and especially when exposed to light, bilirubin, which is a yellow–brown color, will be oxidized to biliverdin, which is a green color. Many of the procedures used to detect bilirubin will not react with biliverdin, so false-negative results may occur if the urine is not tested when fresh.

Detectable amounts of bilirubin are not normally present in the urine, so the results of some methods are just reported as positive or negative. The procedure of choice when liver disease is suspected is the Ictotest, because of the sensitivity of the test.

**REAGENT TEST STRIPS**

Most dipsticks are based on the coupling reaction of a diazonium salt with bilirubin in an acid medium as show by this reaction:

\[
\text{Bilirubin reaction:} \quad \text{Bilirubin} + \text{diazide} \rightarrow \text{azobilirubin}
\]

Some dipsticks differ, however, in the diazonium salt that is used and the color that develops. The indicators used by the most common manufacturers are listed in Table 4-6 along with their sensitivities.

Bilirubin results are read from 30 to 60 seconds, depending on the manufacturer and display a range of colors from buff through various shades of tan or tannish-purple. These colors correspond to levels of bilirubin from negative to large (3+). Figure 4-9 displays a bilirubin color chart.

<table>
<thead>
<tr>
<th>BRAND AND SENSITIVITY</th>
<th>INDICATOR</th>
</tr>
</thead>
<tbody>
<tr>
<td>AimStick\textsuperscript{9} (0.2 mg/dL)</td>
<td>2,4-Dichlorobenzene amine diazonium salt</td>
</tr>
<tr>
<td>Chemistrip\textsuperscript{4} (0.5 mg/dL)</td>
<td>2,6-Dichlorobenzene-diazonium-tetrafluoroborate</td>
</tr>
<tr>
<td>Combi-Screen PLUS\textsuperscript{10} (0.5–1 mg/dL)</td>
<td>Diazonium salt</td>
</tr>
<tr>
<td>DiaScreen\textsuperscript{11} (0.5 mg/dL)</td>
<td>2,4-Dichlorobenzene diazonium salt</td>
</tr>
<tr>
<td>Dirui H-Series\textsuperscript{12} (8.6–17 nmol/L)</td>
<td>2,4-Dichloroanaline diazonium salt</td>
</tr>
<tr>
<td>Mission\textsuperscript{13} (0.4–1.0 mg/dL)</td>
<td>2,4-Dichloroanaline diazonium salt</td>
</tr>
<tr>
<td>Multistix\textsuperscript{2} (0.4 mg/dL)</td>
<td>2,4-Dichloroanaline diazonium salt</td>
</tr>
<tr>
<td>Self-Stik\textsuperscript{14} (not given)</td>
<td>2,4-Dichloroanaline diazonium Na</td>
</tr>
<tr>
<td>ULRSAN\textsuperscript{15} (0.5 mg/dL)</td>
<td>Sodium nitrite</td>
</tr>
<tr>
<td>Urtest 13G\textsuperscript{16} (8.6–17 nmol/L)</td>
<td>2,4-Dichloroanaline diazonium</td>
</tr>
<tr>
<td>Uro-dip 10C\textsuperscript{17} (not given)</td>
<td>2,4-Dichloroanaline diazonium</td>
</tr>
<tr>
<td>URS\textsuperscript{18} (0.4–0.8 mg/dL)</td>
<td>2,4-Dichloroanaline diazonium salt</td>
</tr>
</tbody>
</table>
False-Positive Results

If the bilirubin pad is observed after the prescribed amount of time, it may develop other colors that may interfere with the reading of bilirubin reactions. Several compounds may produce atypical color reactions on the bilirubin pad. Indican and metabolites of etodolac (Lodine) can produce an interfering color reaction. Patients receiving large doses of chlorpromazine (Thorazine) may have false-positive results. Metabolites of drugs such as phenazopyridine give a red color at an acid pH and cause misinterpretation of results that could lead to false-positive reports. The Ictotest should be used to confirm bilirubin results on urines that generate a positive or atypical color reaction.

False-Negative Results

Large amounts of ascorbic acid decrease the sensitivity of this test. Repeating the test at least 10 hours after the last dose of vitamin C will produce more accurate results. Elevated levels of nitrite will lower the bilirubin result. A false-negative result will be obtained if the bilirubin has been oxidized to biliverdin, as occurs when specimens are exposed to room temperature and light.

ICTOTEST

Ictotest is a tablet test that is based on the same diazo reaction as the dipsticks. However, Ictotest is much more sensitive than the dipsticks, being able to detect as little as 0.05 mg/dL. Because of this sensitivity, Ictotest is the recommended procedure when a test for just bilirubin is ordered. It also serves as a good confirmatory test for a positive dipstick.

The tablet contains 2,6-dichlorobenzene-diazonium-tetrafluoroborate, sulfosalicylic acid, and sodium bicarbonate. The mats that are used in the procedure are made of an asbestos–cellulose mixture. When the urine is placed on the mat, the absorbent qualities of the mat cause the bilirubin to remain on the outer surface. The sulfosalicylic acid provides the acid environment for the reaction. It also acts with the sodium bicarbonate to provide an effervescence which helps partially dissolve the tablet. The diazonium salt then couples with the bilirubin on the mat, giving a blue or purple reaction product.

Procedure

1. Place five drops of urine on one square of the special test mat supplied with Ictotest.

2. Place a tablet in the center of the moistened area.

3. Flow two drops of water onto the tablet so that the water runs off of the tablet and onto the mat.

4. Observe the color of the mat around the tablet at the end of 30 seconds. If a blue or purple color develops, the test is positive. All other colors including pink or red are negative. Figure 4-10 shows the examples of positive and negative Ictotest reactions.

False-Positive Results

Urine from patients receiving large doses of chlorpromazine may give false-positive reactions. If the urine is suspected of containing a large amount of chlorpromazine, the wash-through technique can be used. Prepare duplicate mats with five drops of urine on each. To one mat add 10 drops of water to wash through the drug metabolites. Add a tablet to each mat and perform the Ictotest procedure. If the color is about the same on both mats, bilirubin is present, because it stays adsorbed on the mat surface. If the wash-through mat is either much lighter or if no color is present, then the reaction is probably due to the drug metabolites.

FOAM TEST

If the urine is a yellowish-brown or greenish-yellow color and bilirubin is suspected, shake the urine. If a yellow or greenish-yellow foam develops, then bilirubin is most likely present. Bilirubin alters the surface tension of urine and foam will develop after shaking. The yellow color is from the bilirubin pigment. A false-positive foam test occurs when the urine contains phenazopyridine. The foam test must be followed up by another more accurate procedure. It can, however, be a good clue that bilirubin is present, and the technologist should then test out the possibility of bilirubinuria. Other screening tests for bilirubin that are not regularly performed are included in Appendix B.
interpreting urobilinogen results. Patients receiving broad-spectrum antibiotics and other substances which will alter the normal bacterial flora in the intestines will excrete little or no urobilinogen in their urine because urobilinogen cannot be formed in the intestines. In addition, in cases of intestinal obstruction, significant quantities of urobilinogen may be absorbed from the intestine and thus the urine levels will increase.50

Unlike bilirubin, urobilinogen is normally present in the urine but in concentrations of 1 Ehrlich unit or less per 100 mL of urine. Some procedures will detect only amounts in excess of this, but dipsticks are capable of detecting normal amounts. Decreased or absent levels of urobilinogen cannot be detected by any of these screening procedures.

One of the important problems in measuring urobilinogen is its instability. The urobilinogen is converted to urobilin on standing in the presence of oxygen and on exposure to air. For this reason, the test should be performed on a fresh specimen.

Urobilinogen excretion reaches peak levels between 2 and 4 PM. Therefore, when screening for liver damage it is advisable to do a collection during these hours.39,51

### False-Positive Results

Several interfering substances may react with the urobilinogen test pad to produce atypical colors.52 These interfering substances include p-aminosalicylic acid, sulfonamides, and p-aminobenzoic acid.5,9 Reagent strips using p-dimethylaminobenzaldehyde may react with porphobilinogen, although this is not a reliable method for detecting porphobilinogen.2 Urine from patients receiving phenazopyridine may show a false-positive reaction.3

### Table 4-7 Urobilinogen Reagents and Sensitivities by Reagent Strip

<table>
<thead>
<tr>
<th>BRAND AND SENSITIVITY</th>
<th>REAGENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>AimStick9 (0.2 mg/dL)</td>
<td>p-Diethylaminobenzaldehyde</td>
</tr>
<tr>
<td>Chemistrip5 (0.4 mg/dL)</td>
<td>4-Methoxybenzene-diazonium-tetrafluoroborate</td>
</tr>
<tr>
<td>Combi-Screen PLUS10 (not given)</td>
<td>Diazonium salt</td>
</tr>
<tr>
<td>DiaScreen11 (0.4 mg/dL)</td>
<td>4-Methoxybenzene-diazonium-tetrafluoroborate</td>
</tr>
<tr>
<td>Dirui H-Series12 (3.3–0.6 mmol/L)</td>
<td>Fast B blue</td>
</tr>
<tr>
<td>Mission13 (0.2–1.0 mg/dL)</td>
<td>p-Dimethylaminobenzaldehyde</td>
</tr>
<tr>
<td>MultiStix1 (0.2 mg/dL)</td>
<td>p-Dimethylaminobenzaldehyde</td>
</tr>
<tr>
<td>Self-Stik14 (not given)</td>
<td>4-Methoxybenzenediazonium salt</td>
</tr>
<tr>
<td>URISCAN15 (Trace-IEU/dL)</td>
<td>p-Diethylaminobenzaldehyde</td>
</tr>
<tr>
<td>Uritest 13G16 (3.3–16 μmol/L)</td>
<td>Fast blue B salt</td>
</tr>
<tr>
<td>Uro-dip 10C17 (not given)</td>
<td>p-Diethylaminobenzaldehyde</td>
</tr>
<tr>
<td>URS18 (0.2 EU/dL)</td>
<td>p-Dimethylaminobenzaldehyde</td>
</tr>
</tbody>
</table>

### Figure 4-11. Urobilinogen color chart. Note: This chart is for color demonstration only and should not be used for interpreting reagent strip reactions for diagnostic testing. (Modified from Tarrytown, NY: Bayer Corporation Diagnostics Division, 1996.)
False-Negative Results

A true absence of urobilinogen is not detectable. Several substances may decrease the color reaction of this test. Urines containing nitrite or those preserved with formalin may produce false-negative results. False negatives may also occur in improperly stored samples allowing the oxidation of urobilinogen to urobilin.

Other qualitative methods are available for the detection of urobilinogen. The Watson-Schwartz test can be used to differentiate between urobilinogen and porphobilinogen. This and other tests are outlined in Appendix B.

NITRITE

The nitrite test is a rapid, indirect method for the early detection of significant and asymptomatic bacteriuria. Common organisms that can cause urinary tract infections, such as *Escherichia coli*, Enterobacter, Citrobacter, Klebsiella, and Proteus species, produce enzymes that reduce urinary nitrate to nitrite. For this to occur, the urine must have incubated in the bladder for a minimum of 4 hours. Hence, the first morning urine is the specimen of choice.

### REAGENT TEST STRIPS

Reagent strips for the detection of nitrite in the urine commonly use *p*-arsanilic acid and a quinoline compound. Nitrite reacts with *p*-arsanilic acid to form a diazonium compound. This compound then couples with the quinoline compound to produce a pink color as in the following reaction:

\[
\text{Nitrite} + p\text{-arsanilic acid} \rightarrow \text{diazonium compound}
\]

\[
3\text{-Hydroxyl-1,2,3,4 tetrahydro-benzo-(h)-quinoline} + \text{diazonium compound} = \text{pink color}
\]

The reagents used for this reaction vary slightly by manufacturer but have similar sensitivities as seen in Table 4-8.

Nitrite results are read at 30 or 60 seconds, depending on the manufacturer. Any degree of uniform pink color should be interpreted as a positive nitrite test suggesting the

<table>
<thead>
<tr>
<th>BRAND AND SENSITIVITY</th>
<th>REAGENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>AimStick^9^ (0.09 mg/dL)</td>
<td><em>p</em>-Arsanilic acid <em>N</em>-ethylenediamine Tetrahydroquinoline</td>
</tr>
<tr>
<td>Chemistrip^5^ (0.05 mg/dL)</td>
<td>Sulfanilamide 3-Hydroxy-1,2,3,4-tetrahydro-benzo (h) quinoline</td>
</tr>
<tr>
<td>Combi-Screen PLUS^10^ (0.05–0.1 mg/dL)</td>
<td>Tetrahydrobenzoquinoline Sulfanilic acid</td>
</tr>
<tr>
<td>DiaScreen^11^ (0.05 mg/dL)</td>
<td><em>p</em>-Arsanilic acid Hydroxy(3)-1,2,3,4-tetrahydro-benzoquinoline</td>
</tr>
<tr>
<td>Dirui H-Series^12^ (13–22 umol/L)</td>
<td><em>p</em>-Arsanilic acid-N-1-(naphthol)-ethylenediamine Tetrahydroquinoline</td>
</tr>
<tr>
<td>Mission^13^ (0.05–0.1 mg/dL)</td>
<td><em>p</em>-Arsanilic acid-N-1-(naphthol)-ethylenediamine</td>
</tr>
<tr>
<td>Multistix^2^ (0.06 mg/dL)</td>
<td><em>p</em>-Arsanilic acid 1,2,3,4-Tetrahydro-benzo (h) quinoline-3-ol</td>
</tr>
<tr>
<td>Self-Stik^14^ (not given)</td>
<td><em>p</em>-Arsanilic acid N-(1-naphthyl) ethylenediamine 2HCl</td>
</tr>
<tr>
<td>URI SCAN^15^ (0.05 mg/dL nitrite ion)</td>
<td><em>p</em>-Arsanilic acid</td>
</tr>
<tr>
<td>Uritte 13G^16^ (18–26 umol/L)</td>
<td>Sulfanilamide N-(naphthyl) ethylenediammonium dihydrochloride</td>
</tr>
<tr>
<td>Uro-dip 10C^17^ (0.05 mg/dL)</td>
<td>Sulfanilamide N-(naphthyl) ethylenediammonium Dihydrochloride</td>
</tr>
<tr>
<td>URS^18^ (0.075 mg/dL)</td>
<td><em>p</em>-Arsanilic acid</td>
</tr>
</tbody>
</table>
presence of $10^5$ or more organisms per milliliter. The color development is not proportional to the number of bacteria present. Pink spots or pink edges should not be considered a positive result. If the uniform pink color is very light, it may best be seen by placing the strip against a white background. The test is reported as positive or negative. Figure 4-12 displays a nitrite color chart.

False-Positive Results

The urine should be tested shortly after being voided, because if the urine is allowed to stand at room temperature for several hours, organisms may grow in the specimen and generate nitrite. Results may be misinterpreted as positive in urines that appear red or contain phenazopyridine and other substances that turn red in acid.

False-Negative Results

The sensitivity of the test is reduced in urine with a high specific gravity or elevated level of ascorbic acid. A negative test should never be interpreted as indicating the absence of bacterial infection. There are several reasons for this:

1. There may be pathogens present in the urine that do not form nitrite.
2. The urine may not have remained in the bladder long enough for the nitrate to be converted to nitrite.
3. There are cases in which the urine does not contain any nitrate, so bacteria may be present but the dipstick will be negative.
4. Under certain circumstances, the bacterial enzymes may have reduced nitrate to nitrite and then converted nitrite to nitrogen, which will give a negative nitrite result.

False-negative nitrite determinations or negative interferences can be the result of abnormally high levels of urobilinogen, the presence of ascorbic acid levels as low as 5 mg/dL, or acidic urine (pH is 6.0 or less). The nitrite test is not meant to take the place of other routine bacteriology studies such as cultures and smears. The dipstick procedure is just used as a screening test which is capable of detecting bacteriuria even when not clinically suspected. If there are clinical symptoms, then regular bacteriology tests should be performed, even if the nitrite test is negative.

Reaction A:
\[ \text{Indoxyl or pyrole} + \text{carbonic acid ester} \rightarrow \text{granulocytic esterase} \rightarrow \text{indoxyl or pyrole} \]

Reaction B:
\[ \text{indoxyl or pyrole} + \text{diazonium salt} = \text{purple} \]

The reagents used for this reaction vary by manufacturer and seem to effect test sensitivities as seen in Table 4-9.

Leukocyte esterase results are read at 2 minutes. A positive reaction produces a lavender to purple color with a reporting range of values from trace to large. Values reflecting cell numbers from negative to 500 may be reported. These results may not correlate with the numbers of neutrophils seen during microscopic examination. Figure 4-13 displays a color chart for leukocyte esterase.

False-Positive Results

Strong oxidizing agents cause a false-positive leukocyte esterase result. This occurs when strong detergents used to clean the collection container remain present. False-positive results may also be obtained on females due to contamination of the urine with vaginal discharge. Some preservatives such as formalin will cause a false-positive result. Nitrofurantoin contributes a color to urine that may cause misinterpretation of this test. False-positive results may be
caused by drugs that contain imipenem, meropenem, and clavulanic acid.5

False-Negative Results

False-negative results may occur with high specific gravity and in urines containing glucose and protein.2,9,11 Significantly high levels of protein or glucose can contribute to increased specific gravity. In such an environment, white blood cells will crenate and be unable to release esterase.

Various drugs and chemicals interfere with this test. Check the packaging insert of the reagent strip manufacturer for specifics concerning interfering substances. Some drugs and chemicals that may cause false-negative results include ascorbic acid, oxalic acid, cephalaxin, cephalothin, gentamicin, and tetracycline.

Some brands of reagent strips are offering additional test parameters including calcium, creatinine, and microalbumin. In addition, some brands of reagent strips include a test pad for ascorbic acid. Excess ascorbic acid can interfere with the chemical reactions for bilirubin, blood, and glucose and may result in false low or negative results in these parameters. Detecting the presence of ascorbic acid may be helpful in correlating negative results with other findings.

### Summary

The chemical examination of urine includes measuring urinary pH and strip test method for specific gravity. In addition, chemical analysis of urine involves screening for abnormal levels of protein, glucose, ketones, occult blood, bilirubin, urobilinogen, nitrite, and leukocyte esterase. Technological advances have provided for the development of reagent test strips (dipsticks) that allow for the rapid, simultaneous determination of these substances. Abnormal urine chemistry results not only aid in the assessment of renal disorders but can also disclose many systemic disorders.

Several manufacturers have developed reagent test strips that will provide results in 2 minutes. Instructions for each brand of reagent strips must be reviewed and adhered to carefully to avoid reporting of misinterpreted results.

### STUDY QUESTIONS

1. Specimens for urine chemistry analysis must be well mixed to ensure an accurate reading of:
   a. pH and specific gravity
   b. blood and leukocytes
   c. glucose and ketones
   d. bilirubin and urobilinogen

2. Timing of reagent strip readings is especially critical for:
   a. diazo compound formation
   b. dye-binding reactions
   c. enzymatic reactions
   d. protein error of indicators

3. Testing specimens that contain high levels of ascorbate may effect the reading of all of these EXCEPT:
   a. bilirubin
   b. glucose
   c. nitrite
   d. urobilinogen
4. A high specific gravity will affect all of the following reactions EXCEPT:
   a. glucose
   b. leukocytes
   c. nitrite
   d. protein

5. Which of the following tests does not have a negative reading on reagent strip color charts?
   a. blood
   b. glucose
   c. ketone
   d. urobilinogen

6. Purple colors are observed in the positive reactions for:
   a. blood and glucose
   b. ketone and leukocytes
   c. bilirubin and urobilinogen
   d. protein and nitrite

7. The ketone most detectable by all reagent strips is:
   a. acetoacetic acid
   b. acetone
   c. β-hydroxybutyric acid
   d. phenylketone

8. A false-positive protein may be produced by:
   a. albumin
   b. alkaline pH
   c. ascorbic acid
   d. run-over effect

9. Positive bilirubin reactions should be confirmed by:
   a. Acetest
   b. Clinistix
   c. Foam Test
   d. Ictotest

10. The principle of “protein error of indicators” is based on:
    a. protein changing the pH of the specimen.
    b. protein changing the pK of the specimen.
    c. protein accepting hydrogen from the indicator.
    d. protein giving up hydrogen to the indicator.

Match the reagents listed below to the test in which they are used.
   a. bilirubin
   b. blood
   c. glucose
   d. ketone
   e. leukocytes
   f. nitrite
   g. pH
   h. protein
   i. urobilinogen

11. ________ arsanilic acid
12. ________ bromthymol blue
13. ________ cumene hydroperoxide
14. ________ dichloroanaline
15. ________ dimethyaminobenzaldehyde
16. ________ indoxylcarboxonic acid ester
17. ________ methyl red
18. ________ potassium iodide
19. ________ sodium nitroprusside
20. ________ tetrabromphenol blue
21. ________ tetrachlorphenol-tetrabromsulfophthalein
22. ________ tetrahydroquinoline
23. ________ tetramethylbenzine

CASE STUDIES

Case 4-1 When performing routine urinalysis quality control you observe when you remove the dipsticks from the bottle that the urobilinogen pad is a brown color. What is your course of action?

Case 4-2 When performing routine urinalysis you observe a pink color on the bilirubin pad. How should you proceed?

Case 4-3 When performing a routine urinalysis you observe a 1+ leukocyte esterase. No cells are seen upon microscopic examination. What can account for these results?

Case 4-4 A urine test on a 1-month-old baby shows a positive copper reduction test with a negative oxidase test. How should these results be reported and what is their significance?

Case 4-5 A physician questions the results of a urinalysis which was reported to show a negative nitrite, yet contained 2+ bacteria. Suggest a course of action and explanation why these findings are consistent.

REFERENCES

Chapter 4—Chemical Analysis of Urine

34. Acetest® [Package insert]. Elkhart, IN: Siemens (formerly Elkhart, IN: Bayer HealthCare LLC); 2006.