

EXERCISE 13: CHEMICAL EXAMINATION OF URINE

Textbook: Chapter 14

Skill: 15 points

- Objectives:
1. Name 10 routine chemical tests performed on urine and List and describe a condition that will cause an abnormal
 2. Properly use and interpret the reactions of the reagent strip with patient urine samples and control solutions.
 3. Define the following terms: proteinuria, glycosuria, ketonuria, hemoglobinuria, and hematuria.
 4. State the four confirmatory chemical tests on urine and state the substance each confirmatory is used to detect.
 5. Perform chemical testing on 2 urine samples and 2 controls by properly dipping the strips in urine, accurately timing the reaction, interpret the color to determine the results, and record the results using the correct term or units within +/- 1 unit.

Discussion

Tests can be performed on urine samples to detect the presence of certain compounds or chemicals which may be indicative of an underlying disease. These tests are usually considered to be a part of a routine urinalysis. When performed correctly, these tests can provide valuable information to the physicians. The specimen is very easy to obtain. The first morning specimen is the preferred specimen as it is the most concentrated and has less of a chance of giving false negative results.

Methods of Chemical Analysis

Reagent strips are the most widely used technique for detecting constituents present in the urine and are available in a variety of types. A **reagent strip** is a firm plastic strip to which pads containing chemical reactants are attached. Most reagent strips contain reagent areas that test for pH, protein, glucose, ketone, bilirubin and blood. Some strips may also test for urobilinogen, leukocytes, nitrites and specific gravity. The presence or absence of these constituents in the urine provides information on the status of carbohydrate metabolism, kidney and liver function, and acid-base balance of the patient.

Reagent strips are designed to be used only once and discarded. Exact directions for the use of the strips are included in each package and must be followed precisely for accurate results. A color comparison chart is also included, usually on the label of the reagent strip container.

Positive results may need confirmation by a confirmatory test for some constituents.

Quality Control

The performance of the strips should be checked by testing strips with positive and negative urine controls. If the results of the controls do not match the manufacturers published results then patient testing cannot be performed until the cause of the error is determined. Causes of inaccurate results include improper storage and/or handling of the strips, using the strips beyond the expiration date, or contamination of the strips.

Performing the Chemical Test by Reagent Strip

The urine must be collected using appropriate technique and be tested *within one hour of collection*. If tests cannot be performed within this time, the specimen may be refrigerated for *up to 8 hours*. Refrigerated specimens should be allowed to return to room temperature prior to testing.

Chemical testing is performed by dipping a reagent strip into a fresh urine. The color changes on the reagent pads should be visually compared to the color chart after the appropriate time period. This can also be done on an electronic instrument which reads the color and displays it on a lighted panel. Automation eliminates technician error due to differences in timing or interpretation of the colors.

Principles of Chemical Tests

pH. The pH is a measure of the degree of acidity or alkalinity of the urine. A pH below 7 indicates an acid urine; pH above 7 indicates an alkaline urine. Normal, freshly-voided urine may have a pH range of 5.5 - 8.0. The pH of urine may change with diet, medications, kidney disease, and metabolic diseases such as diabetes mellitus. Colors on the pH reagent pad usually range from yellow-orange for acid pH to green-blue when pH is alkaline.

Protein. Protein in the urine is called *proteinuria*. This is an important indicator of renal disease, but can be caused by other conditions as well. At a constant pH, the development of any green color on the protein reagent pad is due to the presence of protein. Colors range from yellow for negative to yellow-green or green for positive.

Glucose. The presence of glucose in urine is called *glycosuria*. This condition indicates that the blood glucose level has exceeded the renal threshold. This condition may occur in diabetes mellitus. The reagent strip is specific for glucose and uses the enzymes glucose oxidase and peroxidase, which react with glucose to form colors ranging from green (low concentration) to brown (high concentration).

Ketone. When the body metabolizes fats incompletely, ketones are excreted in the urine resulting in *ketonuria*. The ketone test is based on the development of colors ranging from light pink to maroon when ketones react with nitroprusside. Ketonuria may be present in diabetes and starvation or fasting. Since ketones will evaporate at room temperature, urine should be tightly covered and refrigerated if not tested promptly.

Bilirubin. Bilirubin is a breakdown product of hemoglobin which produces an extremely yellow color in urine and may be an indication of liver disease, hepatitis or bile duct obstruction. Samples suspected of containing bilirubin should be handled cautiously because of the possibility of hepatitis. These samples should also be protected from light until testing is completed, since direct light will cause decomposition of bilirubin. The test for bilirubin is based on the coupling of bilirubin with a dye to form a color.

Blood. Presence of blood in the urine may indicate infection or trauma of the urinary tract or bleeding in the kidneys. Hemoglobin (*hemoglobinuria*) and red blood cells (*hematuria*) may be detected by the formation of a color due to the enzyme peroxidase (in red cells) reacting with orthotolidine, a chemical which is in the reagent pad. The resulting color ranges from orange through green to dark blue.

Urobilinogen. Urobilinogen is a degradation product of bilirubin which is formed by intestinal bacteria. Urobilinogen is normally 0.1 to 1.0 Ehrlich units per deciliter of urine. It may be increased in hepatic disease or hemolytic disease. The reagent strip will detect urobilinogen in concentrations as low as 0.1 Ehrlich unit. The reagent pad contains a chemical which reacts with urobilinogen to form a brown-orange color.

Leukocytes. Leukocytes (aka white blood cells) present in large numbers usually indicate a urinary tract infection (UTI). Normal urines should produce no color change.

Nitrites. This test depends upon the conversion of nitrate to nitrite by certain bacterial action in the urine. A positive result indicates a possible UTI and need for a culture.

Specific Gravity. The specific gravity of a solution is the ratio of the weight of a given volume of the solution (urine) to the weight of an equal volume of water. The specific gravity of urine indicates the concentration of dissolved solids such as urea, phosphates, chlorides, proteins present in the urine. Normal specific gravity is 1.005 - 1.030 with most normals falling between 1.010 and 1.025. The higher the number the more concentrated the urine.

Confirmatory Tests Which May be Indicated

Sometimes it may be necessary to measure chemicals in urine other than by the reagent strip methods. The other methods are called confirmatory tests because the most common use is to confirm a positive (or negative) result obtained using the reagent strip. Confirmatory tests are more time consuming and require more reagents and equipment than the reagent strip method. Four most commonly used confirmatory tests are those for protein, reducing sugars, ketone, and bilirubin.

Sulfosalicylic acid which, when added to the urine, will cause precipitation of the protein resulting in turbidity. This is the confirmatory test for a positive protein result.

Clinitest is the most common test performed to detect reducing sugars such as lactose and galactose. These sugars may be present in the urine of infants and indicate a need for immediate investigation. This is the confirmatory test for a positive glucose test.

The **Acetest** is a test for ketones and is available in tablet form. This is the confirmatory test for a positive ketone test.

The **Ictotest** is a specific test for bilirubin and is four times as sensitive as the reagent strip pad. This is the confirmatory for a positive bilirubin test.

Normal Values for Urine Chemical Tests

Substance	Normal Value
pH	5.5 - 8.0
protein	negative to trace
glucose	negative
ketone	negative
bilirubin	negative
blood	negative
urobilinogen	0.1 - 1.0 EU/dl
leukocytes	negative
nitrites	negative
specific gravity	1.010 - 1.025

Precautions

1. Reagent strips should be tested with positive controls on each day of use to be sure that strips are working properly.
2. Failure to observe color changes at the appropriate time intervals may cause inaccurate results.
3. Reagents and reagent strips must be stored properly to retain reactivity.
4. Observe color changes and color charts under good lighting.
5. Proper collection and storage of urine is necessary to insure preservation of components such as bilirubin and ketones.
6. Do not allow the reagent pads of the strip to touch the fingers or other surfaces.

Exercise 13: URINALYSIS - DIPSTICK

Materials:

1. Reagent strips
2. Timer
3. Urine specimens in conical tubes
4. Urine controls
5. Biowipes

Instructions

1. Carefully remove strip from container, taking care not to allow reagent pads to touch hands or other surfaces. Recap bottle finger tight.
2. Briefly (no longer than 1 second) dip test strip into the urine, making sure that all pads are moistened.
3. Draw the edge of the strip along rim of specimen container to remove excess urine.
4. Blot edge of strip on biowipe.
5. At the appropriate time specified on the chart on the bottle compare the color of the pad to the color on the chart.
6. Record results on the report form.
7. Discard the reagent strip.

Name _____

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NOTE: Record the results in the proper format, using “*neg*” for negative, “*pos*” for positive, or appropriate units where indicated.

Dipstick Pad	Positive Control	Negative Control	Name _____ Number _____	Name _____ Number _____
Glucose				
bilirubin				
Ketones				
Specific Gravity				
Blood				
pH				
Protein				
Urobilinogen				
Nitrite				
Leukocytes				

4. Name four *confirmatory* tests performed on urine **and** the constituent of urine detected. (4 points)
- a.
 - b.
 - c.
 - d.
5. State an abnormal condition which would cause the following constituents to be present in the urine (2 points):
- a. bilirubin
 - b. nitrites
 - c. urobilinogen
 - d. leukocytes
6. State the normal values for each of the following (3 points):
- a. pH _____
 - b. protein _____
 - c. glucose _____
 - d. ketone _____
 - e. bilirubin _____
 - f. blood _____
 - g. urobilinogen _____
 - h. leukocytes _____
 - i. nitrites _____
 - j. specific gravity _____

7. State what must be done if the results of the quality control do not fall within the published results provided by the manufacturer? (.5 points)

8. List 2 reasons which may cause inaccurate results which will be detected by the proper performance of the quality control on the reagent strips. (1 point)

9. List three precautions which must be followed in utilizing or testing the urine dipsticks. (1.5 points)