

Lab 3

Urinalysis: Biochemistry in Medicine

Objectives

1. Assay for glucose in urine.
2. Assay for protein in urine.
3. Perform a dipstick.

Safety Precautions

1. Glucose Color Reagent and the Glucose Standard are irritants. Hydrochloric acid is a corrosive. Use gloves and goggles.
2. Treat urine specimens as though potentially infectious. Do not handle another student's urine collection container or urine. Clean spills immediately and decontaminate all work surfaces after handling. Decontaminate glassware as instructed for each assay.

Materials

Urine collection containers

Markers

Spectrophotometer (340-600 nm)

0.1, 1.0, 5.0 and 10 mL serological pipettes

15 x 125 mixing tubes

cuvettes

0.1 N Hydrochloric acid

Glucose Kit (Sigma 115-A)

Glucose Standard, 100 mg/dl (Sigma 635-100)

Urine control

Micro Protein Determination Kit (Sigma 610-A)

Protein Standard, 50 mg/dl (Sigma 610-50)

0.85% sodium chloride

Microcentrifuge vials (3/group)

Parafilm (1x1 squares)

Multistix Reagent Strips (Fisher cat # AM-2162)

Stopwatch

Bleach

Reagent alcohol

Introduction

Medieval physicians used the odor, color and clarity of urine to aid them in diagnosis and treatment of disease. Urine from diabetic patients smelled sweet and attracted flies. Blood tinted urine pink or red, darkened urine and yellow skin indicated liver disease, and cloudy urine indicated excess cellular material or protein in the blood. Today, physicians have a host of medical tests that they can use to detect and measure biologically important molecules to aid their diagnosis and treatment. However, examination of urine, urinalysis, remains an important screening tool.

Excess glucose in the urine is primarily found when diabetes mellitus is present. Normally, in a 24-hour collection of urine an average of 130 milligrams of glucose are secreted. In diabetics, without uptake of glucose into all the cells of the body, excess glucose remains in the bloodstream. Eventually, the excess glucose is filtered out of the blood in the kidney where it is excreted in urine. Too much glucose in the urine is called glycosuria.

A small amount of protein can be found in urine normally. More than 150 mg/24 hours is considered excessive. Too much protein in the urine is called proteinuria. Proteinuria can be caused by a variety of conditions, from benign to serious. Transient proteinuria can occur with a fever, exposure to cold, emotional stress or severe exercise. However, persistent proteinuria is most commonly associated with kidney diseases such as polycystic kidney disease, and nephrotic syndrome.

Quantitative tests for biologically important molecules in urine are not routinely done. Urine is typically screened using a commercial dipstick. A dipstick has absorbent paper impregnated with specific chemicals. Several different chemical tests can be performed with one dipstick. Dipsticks can screen for the presence of excessive glucose or protein in urine. Other tests that can be performed using a dipstick include detecting the presence of bilirubin, blood, leukocytes, ketones, nitrite, and urobilinogen, and determining pH.

Bilirubin is a breakdown product of hemoglobin, the oxygen-carrying protein in blood. The liver normally metabolizes bilirubin. Its presence in urine suggests liver disease. Urobilinogen is breakdown product of bilirubin. Its presence in urine can also indicate liver disease or hemolytic anemia. (Anemia is a deficiency of oxygen-carrying red blood cells. Hemolytic anemia indicates that the red cells are being formed, but destroyed by some mechanism in the body.) Dipsticks also test for excessive red blood cells in urine. Small numbers of red blood cells can be found in healthy individuals. However, if large numbers are found, it suggests a disease process in the kidneys or along the urinary tract. Leukocytes, white blood cells, appear in large numbers with urinary tract infections.

The presence of ketones, an intermediate breakdown product of fat metabolism, occurs in patients undergoing starvation or who are in diabetic ketosis. Diabetic ketosis is a complication of advanced uncontrolled diabetes caused by the metabolism of fats from the body stores instead of the circulating glucose. The metabolism of fats and glycogen ultimately result in acidosis. Acidosis means that the acid/base balance has been disturbed and the blood has become more acidic than the body tissues. Urine becomes more acidic as the kidneys try to remove the excess acid. The normal pH of urine can vary between 4.6 and 8.0. Diabetic ketosis is dangerous if left untreated. If severe enough to warrant hospitalization, the patient will be given insulin and replacement fluid and electrolytes.

In this laboratory exercise you will quantitatively measure the concentration of glucose in urine using the same spectrophotometric technique you used in the last laboratory exercise. You will also measure the concentration of protein in urine using spectrophotometry. Finally, you will use the common screening tool, a dipstick and compare your quantitative results with the dipstick results.

Procedure

Urine collection and preparation

Because body fluids are potentially infectious, we will treat all urine specimens with utmost care. Gloves and goggles are required for all participants. Each student will handle only his or her urine in all the techniques that follow. As professionals in the health care industry, you will be expected to maintain confidentiality about patients. Begin today; treat your lab partners' results with confidentiality.

Collect some of your urine in a urine collection container. Label the container. The techniques used in specimen collection can have a significant effect on the accuracy of any tests on body fluids. When providing urine specimens, patients are usually instructed to clean the outer genitalia. The patient should begin to urinate, and then introduce the urine collection container. In a clinical setting, additional instructions depend on the type of testing to be completed. For this laboratory exercise, this midstream, clean-catch urine is adequate.

Urine Glucose Assay

Recall that in the previous laboratory exercise you created a standard curve and used it to convert absorbance into concentration. In this laboratory exercise, we will not repeat the entire standard curve. We will determine the slope of a line by establishing the origin point (0,0) that corresponds to absorbance (y_1) and concentration (x_1) of the blank; and a second point that is the absorbance (y_2) and concentration (x_2) of a standard. Measuring a single point on the standard curve line and using it along with the origin establishes a line. From that line, we can calculate the slope and use it to convert absorbance into concentration. This technique is commonly used in clinical laboratories where accurate and precise technique is used. In addition clinical laboratories use a check-and-balance system to verify the accuracy of results. They assay “controls” with patient samples. Clinical **controls** are samples containing known concentrations of an analyte. We will assume that your previous experience with the glucose assay has honed your technique well enough to follow this same protocol.

1. Turn the spectrophotometer on, allowing it to warm at least 5 minutes before taking any absorbance readings.
2. Label mixing tubes, Blank, Standard, Control. Label a tube for each member of your lab group. To each tube, add 1.0 ml of Glucose Assay Reagent
3. Add 20 microliters (0.02 ml) of water to the Blank tube.
4. Add 20 microliters (0.02 ml) of the appropriate sample to each tube. The Control Urine is a simulated urine sample containing known amounts of the analytes we are testing. Each student should handle only his or her urine specimen.
5. Mix by gently swirling the tubes.
6. After 5-10 minutes, add 10 ml of 0.1N hydrochloric acid to each tube, and mix.
7. Transfer the solutions into labeled cuvettes.
8. Turn the wavelength dial to 520 nm.
9. With nothing in the sample chamber, adjust the absorbance to ∞ using the left knob.
10. Pipette or pour the blank solution into a cuvette, until the cuvette is $\frac{3}{4}$ full. Insert the cuvette into the spectrophotometer. Adjust the absorbance to zero using the right knob.
11. Pipette or pour each of the sample solutions into cuvettes, until each cuvette is $\frac{3}{4}$ full. Measure the absorbance. Record the absorbance of the Blank, Standard, Control and your own urine specimen in your assignment. It is important to complete the measurements within 30 minutes after the addition of the hydrochloric acid.
12. Collect all sample waste in a container in which 10 ml of bleach has been added. Rinse sample cuvettes and mixing tubes with 5% fresh bleach, and then rinse several times in deionized water. Place the cuvettes and mixing tubes upside in a rack to dry.
13. To calculate the concentration of glucose in each specimen, we will rearrange and use Beer’s Law.

$$C = \frac{A}{\epsilon L}$$

The slope of the standard curve, ϵL , is calculated:

$$\frac{y_2 - y_1}{x_2 - x_1} = \frac{A_{std} - A_{blank}}{C_{std} - C_{blank}} = \frac{A_{std}}{C_{std}}$$

Concentration is then calculated:

$$C = \frac{A_{sample}}{A_{std} / C_{std}}$$

Rearranged, with the concentration of the glucose standard included, this equation is:

$$C = \frac{A_{sample}}{A_{std}} \times 100 \text{ mg / dl}$$

14. Record your results in your assignment. Compare the results you received for the Control sample with the expected values. The expected values of the control will be available from your instructor or TA and will be expressed as a range of acceptable values. If your results do not fall within the expected range, it is likely that your pipetting accuracy and precision needs improvement. Work with more care in the subsequent exercises. In a clinical laboratory, if the control values did not fall within the expected range, the patient values will not be reported without convincing evidence that they are still valid. Typically the test will be repeated until control values fall within the expected range.

Urine Protein Assay

As with the glucose assay, you will need to create a standard curve for protein.

1. First you will make several dilutions of a protein standard solution that has an initial concentration of 50 mg/dl of protein. The procedure will be similar to the method used for the glucose standard curve dilutions, but it will use much smaller volumes.
 - a. Obtain a microcentrifuge vial labeled A from your TA or instructor. This vial contains a protein standard that has a concentration of 50 mg/dl of protein within it.
 - b. Label two empty microcentrifuge vials: B, C.
 - c. Into vials B and C, pipette 100 μ l of deionized water.
 - d. Into vial B, pipette 100 μ l of vial A. Cap the vial, and mix the contents by flicking it with your finger.
 - e. Into vial C, pipette 100 μ l of vial B. Cap and mix
2. Calculate the concentration of protein in each tube using the following equation and record it in your assignment:

$$V_1 C_1 = V_2 C_2$$

Where V_1 = volume of the stock solution

C_1 = the concentration of the stock solution

V_2 = the final diluted volume (volume of stock solution and diluent)

C_2 = the final diluted concentration.

3. Label five cuvettes: Blank, Standard A, Standard B, Standard C, and Control. Label additional cuvettes for each member of your group's urine specimen.
 - a. To each cuvette, add 2.5 ml of Protein Assay Reagent.

- b. To the Blank cuvette, add 0.05 ml (50 μ l) of 0.085% sodium chloride. Parafilm and mix.
 - c. Add 0.05 ml (50 μ l) of the appropriate sample (standard, control, and urine specimens) to each of your cuvettes. Remember to handle only your own urine. Parafilm and mix.
 - d. Wait approximately two minutes.
 - e. Set the spectrophotometer wavelength to 595 nm. With nothing in the sample chamber, adjust the absorbance to ∞ using the left knob.
 - f. Insert the cuvette containing the blank into the spectrophotometer and set the absorbance to zero, using the right knob.
 - g. Measure and record the absorbance of each sample. Measurements should be completed within 30 minutes of the start of the assay.
 - h. Discard the sample waste in a waste container in which 2 ml of bleach has been added. Instead of rinsing the cuvettes with bleach, use ethanol. The blue dye of this assay will eventually stain the glassware. Ethanol will remove the dye and disinfect. Rinse the cuvettes in deionized water and place upside down in racks to dry.
4. Using Microsoft Excel, graph the standard curve. Make a copy for each person in your lab group.
 5. Examine your standard curve. If your pipetting has been accurate and precise, your data points should be very close to the standard curve line, and the line should go through the origin. If the line does not go through the origin, you will have to redraw the line ensuring that it passes through the origin. The quality of your standard curve will directly influence how accurately you can determine the concentration of protein in urine.
 6. Determine the concentration of urine protein in each sample either by calculating it or using the graph. To calculate it, divide its absorbance by the slope of the standard curve.
 7. Record your results in your assignment and compare the results of the Control sample to the expected results.

Screening Urine with a Dipstick

A dipstick can be used to quickly screen urine. Its results are semi-quantitative rather than quantitative like the glucose and protein assays you have just performed. Thus the results are graded with semi-quantitative terms such as trace, or 1+, 2+ rather than quantitative values such as 80 mg/dl.

The test areas are impregnated with chemicals, therefore handle the strips carefully. Avoid touching the test areas. In a clinical setting, if testing cannot be completed within an hour of collection, the urine sample should be refrigerated to slow the degradation of its constituents by light, heat, and bacteria.

1. Remove one test strip from its storage container for your urine specimen. Cap the container to protect the integrity of the remaining test strips.
2. Completely immerse the test areas of the strip in fresh urine and remove it immediately (to avoid dissolution of the chemicals impregnated on the strip). While removing it, slide the edge of the dipstick along the rim of the urine container to remove excess urine. Hold the dipstick horizontally to prevent the mixing of chemicals between test areas.
3. Compare the color of the test areas to the color chart on the dipstick container at the specified time intervals. Color changes that occur after two minutes are not valid. The test areas are arranged so that you can read tests chronologically.
 - a. Glucose and bilirubin: read at 30 seconds. Glucose is tested in the aqua test area closest to the handle of the dipstick. Bilirubin is the next sequential test area.
 - b. Ketone: read at 40 seconds. It is the third test area from the handle.

- c. Blood, pH, protein, urobilinogen and nitrite: read at 60 seconds. Blood is the 4th, pH the 5th, protein, the 6th, urobilinogen the 7th and nitrite the 8th test areas from the handle.
- d. Leukocytes: read at 2 minutes. It is the last test area on the strip.

References

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Name _____ **Lab Section Day & Time** _____

Lab 3 Assignment

Urine Glucose Assay

1. Record the absorbance of the Standard, Control and your urine specimen in the glucose assay. Determine the glucose concentration of the control and your specimen. Show your calculations.

Sample	Absorbance	Concentration
Standard		100 mg/dl
Control		
Your specimen		

Compare your results with the range of expected values for the control. Your TA or instructor has this information.

Urine Protein Assay

2. Calculate the concentration of protein in Standards B and C. Standard A was not diluted and thus is the same concentration as the stock solution, 50 mg/dl. Show your calculations.
3. Transcribe the concentrations you calculated for each standard solution into the table below. Then record the absorbance of the standards, control and your urine specimen for the urine protein assay.

Solution	Glucose Concentration	Absorbance
Standard A		
Standard B		
Standard C		
Control		
Urine Specimen		

4. Using Microsoft Excel, construct and attach a urine protein assay standard curve. Calculate the quantity of protein present in the control and your urine specimen. Show your calculations.

Unknown Samples	Concentration
Control	
Urine Specimen	

Dipstick Urine Chemistry

5. Record the results of the dipstick on your urine specimen.

Glucose _____

Bilirubin _____

Ketone _____

Blood _____

pH _____

Protein _____

Urobilinogen _____

Nitrite _____

Leukocytes _____

Thought questions

6. A patient in diabetic ketosis has come into the emergency room. What type of results might you expect on the urine dipstick? For all constituents except pH use elevated, decreased or normal for your answer. For pH use acidic, neutral or basic. Justify your answers.

Glucose _____

Bilirubin _____

Ketone _____

Blood _____

pH _____

Protein _____

Urobilinogen _____

Nitrite _____

Leukocytes _____

7. For patient diagnosed with diabetic acidosis, the doctor has ordered an intravenous solution of 1000 ml of electrolytes and fluids to be infused within six hours. IV tubing has a drop chamber in which you can observe the number of drops per minute in the drop chamber. Different sizes of tubing allow different rates of flow. The tubing you are using allows 10 drops/ml. The formula for calculating IV flow rate is:

$$\frac{V_1}{T_1} \times \frac{V_2}{T_2} = \text{drops} / \text{min}$$

Where V_1 is the volume to infuse (the total amount of IV solution to be infused)

T_1 is the time to infuse (in what period of time must this infusion be completed.)

V_2 is drops/ml allowed by the tubing

T_2 is the time in minutes for the infusion. If the infusion will be for more than 1 hour, then this number defaults to 60 because there are 60 minutes in an hour.

In order to infuse 1000 ml in 6 hours, you will need to adjust the flow rate of the IV, how many drops per minute do you need to accomplish this?

