Title: Interpreting Medical Tests: What's in Urine?

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Appropriate Level: Regents, Honors, AP as written, but could be adapted to General.

Abstract: The unique role of the kidneys in regulating the volume and composition of the body's fluid compartments makes urinalysis a powerful diagnostic tool for the assessment of health and disease. Chemical analysis of urine can quantitatively identify the presence of both normally and abnormally occurring substances. Interpretation of urinalysis test results to identify possible disease states requires a basic understanding of normal kidney function. In part 1 of this lab, students will use multifunction test strips to collect data on artificial urine solutions representing samples from 7 case studies, and interpret the results to assess the patient’s problems. The historical method of independently analyzing the many components of urine using wet-chemical methods has been replaced by the development and widespread use of multifunction test strips. In part 2 of this lab, students will prepare a dip stick test using a 2-part enzymatic reaction that produces a color in the presence of glucose.

Special Equipment needed: A hydrometer that fits in a 50 ml graduated cylinder. Multistix® urinalysis test strips, artificial urine solutions, microscope and slides, filter paper, and enzyme solutions for making test strips.

Time Requirement: one 40-minute period to test artificial urine samples.
one 40-minute period to make glucose test strips and analyze data.
Additional Teacher Information

Objectives:

- to understand the significance of standard urinalysis tests.
- to test understanding of kidney function by interpreting urinalysis results.
- to understand the chemical technology used to produce chemical test strips.

Information with Which Students Must Be Familiar:

- the function of the kidney and the filtration/reabsorption mechanism of nephron function.
- the composition of blood and plasma.
- some knowledge of diabetes would be helpful.

Time Required:

In class: Both exercises can be completed in a single class period. Discussion of urinalysis results may require additional time outside of lab.

Before class: Introduction to the function of the kidney. Setup requires making the "urine" solutions and distributing materials. If solutions are prepared from concentrated stocks, this requires less than 1 hour of prep time. If the solutions are made from scratch, using the recipes provided, the time required will be significantly greater (perhaps as much as 3 hours) depending on the equipment available and your solution chemistry skills.

Materials:

Urinalysis

- artificial urine solutions
- multifunction urinalysis test strips
- 50-ml graduated cylinder
- hydrometer
- Pasteur pipettes
- urine sediment chart.

Recipes for making the artificial urine solutions are provided at the end of the teacher’s section. Acetoacetic acid and bilirubin are available through Sigma-Aldrich Chemical Company, St. Louis Mo. All other chemicals are available through general science supply houses or local sources.
Glucose Dip Stick

- **Enzyme solution A:** Make a 50 mM stock solution of pH 7 phosphate buffer. For example, bring 2.0 mmol NaH₂PO₄ and 3.0 mmol Na₂HPO₄ to a final volume of 100ml with (preferably distilled) water. Add 10 mg glucose oxidase and 5 mg horseradish peroxidase to a 1.5 ml Eppendorf tube and add 0.5 ml of the buffer. Mix well. Store on ice.

- **Indicator solution B:** Place 2.4 mg of tetramethylbenzidine (TMB) in a 1.5 ml Eppendorf tube and add 0.5 ml ethyl acetate. Mix well.

- **Sugar solutions:** Try several solutions of different concentration of glucose. For example 10 mg/100ml; 50 mg/100ml; 250 mg/100ml. Use solutions of sucrose and water as negative controls.

- **Filter paper strips:** Cut filter paper into ¼-inch wide strips. Whatman No. 1 filter paper or cone style drip coffee filters may be used.

Reagents for making the dip sticks can be obtained from Sigma-Aldrich Chemical Company, St. Louis, MO.

Safety Tips

The solvent used to prepare the chromogen (TMB) is ethyl acetate. Material Data Safety Sheets for ethyl acetate and TMB will indicate a number of toxic properties. These reagents, in the quantities provided for use in this lab, do not represent a hazard to students if handled properly. If the lab prep personnel are handling bulk containers of the chemicals they should consult the material safety data sheet provided by the manufacturer. Good laboratory practices should always be observed, including no eating or drinking in the lab.

Points to remember

- Ethyl acetate and TMB are no more toxic than many common household products like detergents, bleaches, cleansers, bug sprays, and gasoline, and in this lab are used in very small quantities.

- Like most things in the laboratory, the reagents used in this lab should not be ingested and contact with skin and vapor should be avoided.

- The total quantity of ethyl acetate used in the lab is very small (only 500ul is required.) This really limits potential for this compound to cause problems even in a poorly ventilated classroom.

- When using any chemical, good laboratory technique should be used. Spills should be reported and if contact is made with skin it should be washed off.

- Don't alarm students about risks associated with use of these chemicals. They can be used extremely safely with minimal precautions.
Tips for the Teacher

- The most important aspect of the urinalysis exercise is the interpretation of results. This might best be done as a group, either small student groups or with the whole class.

- The urinalysis lab has been designed to use simulated urine, but it would be easy to have students analyze their own urine after trying out the technique. The advantage of the artificial urines is that they can be designed to reveal aspects of kidney function and dysfunction that (hopefully) would not be represented in samples provided by students.

- The cost of multifunction dip sticks is significant. The school nurse may be a good source of sticks that have reached their expiration date and are no longer suitable for diagnostic use. These sticks deteriorate over time and are very sensitive to light and humidity. In order to prolong the shelf life of sticks it is essential that students use the sticks prudently and that the container be kept closed when not in use. That said, these sticks can remain useful for years past their expiration dates.

- There are 7 different artificial urine samples described in the urinalysis lab. Information about each sample is provided. Depending on time, equipment, or other constraints, groups of students could analyze each sample, or only a subset of samples and then report their findings to the whole class for discussion.

- Patient histories in the lab contain information on the gender and race of the patient. This is done because many conditions have a higher incidence or are exclusive to particular groups. For example, pregnancy can be ruled out of the diagnosis for samples obtained from males, and sickle cell anemia is relatively common in African-Americans but rare in Caucasians.

- A dramatic way to emphasize the magnitude of glomerular filtration and the importance of reabsorption is to compare the quantity of substances filtered by the glomerulus and the quantity of those same substances eliminated in the urine. For example, the kidney filters 7.5 liters of water per hour. In the same time the kidneys excrete about .08 liters of urine. About 65 grams/hour of NaCl filtered, while as little as 2 grams/hour might be excreted. This makes a nice demonstration if a large flask is available.

Answers Questions:

Urinalysis

1. How many of the problems presented in these samples represent a disease of the kidney itself?

   Only the proteinuria is derived from diseased renal tissue.

2. How many different ways can you identify that the filtration/reabsorption mechanism reveals underlying disease processes in the body?

   a. By failing to remove abnormal substances from the urine, e.g., bilirubinuria, ketonuria.

   b. The inability to handle excessive quantities of materials, e.g., glucosuria.

   c. As a target for damage by other disease processes, e.g., glomerulonephritis, oxalate crystals (diet and water intake problems).
Glucose Test Strip

1. Why can't the reagents be applied together?

   *It is necessary to prevent and products formed by the peroxidase reaction for mixing with and reacting with the chromogen (TMB).*

2. What is the purpose of testing the strip in water? In sucrose?

   *These are quality controls. If a reaction occurs in the presence of water then you can not know that the color produced by a positive reaction is due to the glucose, because urine and plasma are aqueous solutions. Similarly, a negative test for sucrose is evidence of specificity. Manufacturers of test strips must test them for false positives against any substance that is likely to be present in urine or plasma.*

3. If we know the chemicals and the reactions involved, how do the drug companies make money on tests like these? What do they provide that makes it worthwhile to buy their product?

   *The test strips are actually quite accurate and it is this quantitative accuracy that is both difficult and expensive to manufacture. In spite of their expense, products like this are desirable because they are accurate and simple to use.*

4. Did your dip sticks turn darker blue in more concentrated glucose solutions?

   *Answers may vary.*

Refer to the equation for the reaction on page 1 to answer the following questions.

5. Which compounds are consumed in this reaction?

   *Glucose and the inactive (brown) form of the TMB. Not shown is a mole of water formed by the breakdown of the peroxide.*

6. What compounds are produced in the reaction?

   *Gluconic acid and the activated (blue) form of the TMB. Peroxide is formed as an intermediate but it too is consumed by the reaction.*

7. What compound determines how much glucose can be detected?

   *The upper limit of detection is determined by the amount of TMB present.*
Notes on the Artificial Urine Samples

Patient A: (urinary tract infection): 25 y/o male complains of feeling he ‘needs to go’ all the time but typical only produces a small volume of urine. The patient experiences a mild degree of discomfort on urination. There is presently no external evidence of urethral discharge. Microscopic examination of urine sediment revealed small to moderate number of round doughnuts-shaped cells and larger round cells with granular appearance and distinct nucleus.

The abnormal findings here are nitrite and occult blood with the dipstick, and the significant presence of red and white blood cells in the sediment. The nitrite is formed by bacteria metabolizing nitrate to nitrite. The white blood cells indicate inflammation. The history is significant because bacteriuria without pain is suggestive of a kidney infection. The presence of even mild discomfort suggests a lower urinary tract (urethra and bladder) infection. The sensation of urgently needing to urinate in the absence of significant volume is also suggestive of lower tract infection. Significant bleeding can sometimes, but not always, accompany urinary tract infection. These infections tend to travel up (ascend) the urinary tract and are common in both males and females. In young sexually active individuals it is important to rule out sexually transmitted diseases. In males it is important to determine if the problem is in the bladder (cystitis) or prostate, as the prostate is more difficult to treat effectively. A culture of the organism would be important as a guide to proper antibiotic therapy.

Patient B: A 28 year old male, overweight. Chief complaints: thirst, frequent urination and lethargy. Urine volume is 3.8 L in 24 hours.

The significant findings here are the glucose (presence due to high blood concentrations and spill over into the urine), the high volume (due to the presence of glucose in the urine holding water), the ketouria (due to abnormal insulin regulation of lipid metabolism), and the high specific gravity (due to the loss of water in urine and subsequent dehydration). The history indicates excess weight which strongly predisposes to adult onset diabetes, the frequent urination and thirst which are the result of the high urine water loss. To confirm this diagnosis a glucose tolerance test would be performed.

Patient C: An 18 y/o healthy female presents for a routine physical examination. Patient has great difficulty producing a very small volume of urine despite not having urinated since early morning. During discussion with physician it is revealed that she has had only 2 cups of coffee and a donut to eat all day.

The only remarkable finding here is the high concentration. Together with the history this would seem to be a normal response to inadequate fluid intake. Drinking coffee early in the morning can produce a diuresis from the caffeine and if the patient then gets busy and fails to replenish water all day before darting off to have a urine test you would expect to see a very concentrated urine. Any scenario where water intake has been severely limited would explain these results. This patient should drink more fluids! Infrequent urination predisposes to urinary tract infection.
Patient D: A healthy 19 y/o male has provided urine for testing following competition in wrestling. The individual normally weighs 190 lb but wrestles at 172 lb.

The abnormal finding here is the presence of ketone bodies in the urine. This individual has voluntarily restricted carbohydrate intake as a means to lose weight in order to wrestle in a lower weight class. As a result the body is using fat to produce energy and the resulting ketones are spilling over into the urine. This is a potentially dangerous situation. Any weight loss program should be supervised by a physician. Wrestlers (and people trying to lose weight in general) do a number of risky things. Spitting to lose weight can result in loss of $K^+$ producing lethargy and muscle weakness. Not the desired result for a wrestler.

Patient E: A 17 y/o female with joint pain and an unusual rash. Microscopic examination of the urine sediment was unremarkable.

The abnormal finding here is the protein in urine. This suggests damage to the filtration barrier allowing protein to filter into the tubular fluid. There are many possible causes of this problem but attack of the glomerular barrier by the immune system is one that occurs in a number of autoimmune diseases. In this case the age and sex of the patient, together with the joint involvement and the rash, are suggestive of Lupus. The absence of evidence of infection as a possible source of proteinuria further supports this finding. The key thing here is to identify glomerular damage as the reason for the finding.

Patient F (bilirubinemia): Elderly female patient presents with abdominal pain following meal. The condition is more severe following a greasy meal.

The abnormal finding here is the bilirubin. There are many possible causes involving liver disease, but a very common one is a gall stone blocking the common bile duct. This prevents bilirubin in bile from being secreted into the intestine for recycling to the liver. As bilirubin accumulates in the liver, it and other bile products infiltrate the blood and are excreted by the kidney. The history of abdominal pain following a meal is suggestive of gall bladder involvement; the fat in the meal would elicit gall bladder contraction to provide bile to the small intestine to aid in digestion of the fat, but the bile duct is blocked.

Patient G: 38 y/o male vegetarian. Complains of mild irritation during urination. Urine volume 1.1 liters/24 hours. Microscopic examination of urine revealed many small pyramid shaped crystals in every high power field.

The abnormal findings in this sample include blood in the urine (indicating some trauma to the urinary system), and the finding of crystals during microscopic examination of the urine sediment. The shape and dietary history of the patient suggest oxaluria. The high pH, low volume, and high specific gravity are all risk factors for the formation of stones. If the patients has a diet high in oxalate containing foods (e.g. spinach and nuts) this 38y/o male is a prime candidate for stone formation. The blood and irritation result from damage to urethra by the jagged crystals. Suggestions might be to drink lots of water (8-12 glasses per day) and eat a low oxalate diet.
Table 1. Expected values of simulated urine samples.

<table>
<thead>
<tr>
<th>sample</th>
<th>condition</th>
<th>volume(^1) (liters)</th>
<th>color</th>
<th>specific gravity(^2)</th>
<th>pH(^3)</th>
<th>glucose (mg/dl)</th>
<th>protein (mg/dl)</th>
<th>ketone (mg/dl)</th>
<th>bilirubin (mg/dl)</th>
<th>nitrite</th>
<th>blood</th>
<th>sediment(^4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>infection</td>
<td>dark</td>
<td>1.010</td>
<td>6.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+++</td>
<td>+</td>
<td>Red and white blood cells</td>
</tr>
<tr>
<td>B</td>
<td>diabetic</td>
<td>3.1 dark</td>
<td>1.025</td>
<td>6.0</td>
<td>500</td>
<td>15</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>dehydrated</td>
<td>.05 very dark</td>
<td>1.030</td>
<td>6.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>ketonuria</td>
<td>pale</td>
<td>1.010</td>
<td>6.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>proteinuria</td>
<td>pale</td>
<td>1.010</td>
<td>6.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>gall stone</td>
<td>dark</td>
<td>1.010</td>
<td>8.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>++</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>kidney stone</td>
<td>.8 dark</td>
<td>1.030</td>
<td>8.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>++</td>
<td></td>
<td></td>
<td>Ca Oxalate, red blood cells</td>
</tr>
</tbody>
</table>

1 This information is provided to students as part of the patient histories.
2 Bayer Corporation manufactures a variety of Multistix® multifunction urine test strips. The 7 function Multistix® does not have a nitrite test. The others are adequate for this exercise. Strips having the SG designation include a specific gravity test. Students should measure the specific gravity using the hydrometer and compare results of the two tests. If they are different, the reasons for this could be discussed.
3 This pH is calculated on the basis of 10mM buffer made using chemicals listed in Table 2 below. The values measured using strip might vary slightly, but should give values to approximate acidic and basic urine. If making mixtures from salts, be aware that these phosphate salts may not dissolve easily and it is important that they do, so be prepared to allow some time for the salts to dissolve completely. Heat and stirring may be helpful but be sure to allow the solution to cool to room temp before testing.
4 A chart of urine sediments are required to interpret this. Where no data is given assume the findings are unremarkable.
Table 2. Recipes for making simulated urine solutions

| sample | color\(^1\) (drops) | NaCl\(^2\) (g) | Na\(_2\)HPO\(_4\)•7H\(_2\)O (g) | NaH\(_2\)PO\(_4\)•H\(_2\)O\(^3\) (g) | glucose\(^4\) (mg) | protein\(^5\) (ml) | ketone\(^6\) (mg) | bilirubin\(^6\) (mg) | NaNO\(_2\) (mg) | Blood\(^7\) (ml) |
|--------|-----------------|-------------|----------------|----------------|----------------|-------------|---------------|----------------|----------------|----------------|----------------|
| A (infect) | 1 | 1.5 | .134 | .0685 | | | | | | | | 2-5 | 0.25 |
| B (diab) | 2 | 2.9 | .0375 | .118 | 500 | | | | | | | | 10 |
| C (conc) | 3 | 3.5 | .088 | | | | | | | | | | | 0.9 |
| D (ket) | 1 | 1.5 | .134 | .0685 | | | | | | | | | | 10 |
| E (lup) | 1 | 1.5 | .134 | .0685 | | | | | | | | | | 1-2 |
| F(bili) | 1 | 1.5 | .252 | .008 | | | | | | | | | | .8 |
| G(stone) | 2-3 | 3.5 | .252 | .008 | | | | | | | | | | 0.50 |

\(^1\) Yellow food coloring produces a nice color for normal urines but doesn’t darken well. Using red color makes urine unrealistically orange. You may try using coffee to darken samples.

\(^2\) Table salt may be substituted for reagent grade NaCl without modification.

\(^3\) These masses using the forms of the chemicals specified (e.g., Na\(_2\)HPO\(_4\)•7H\(_2\)O) provide 10mM buffer.

\(^4\) This must be glucose, other sugars will not work.

\(^5\) Egg white can be used to add protein to this solution. The egg white might make the sample foamy which is realistic.

\(^6\) Ketone must be acetoacetic acid. Bilirubin is available from Sigma Chemical Corp. St. Louis MO as mixed isomers.

\(^7\) Dried blood (12-0-0) from the garden shop can be used to make a solution. Fill a 1.5ml tube about ¼ to ½ with dried blood. Top with water and allow to sit for ½ hour. Use the indicated volume in 100ml for urine solution.

To make artificial urine solutions:
1. Bring the contents of labeled tubes to 100 ml with water. If not using the kit you will need to provide the NaCl, phosphate buffers, glucose for Solution B, and simulated mineral crystals for Solution G.
2. To solution A add the nitrite and a few drops of the blood solution from step 6 below.
3. To solutions B and D, add the aceto-acetic acid.
4. To solution E add 1-2 ml of egg white. Some turbidity is desirable. It might be useful to try this in 100 ml; of .9% NaCl ahead of time to see how your eggs work.
5. If using real bilirubin you will need to combine the bilirubin with 1 ml water in an Eppendorf tube. Add the smallest volume of NaOH required (1 drop) to get bilirubin into solution. Add contents of tube to solution F. Check pH. Solution must remain basic to keep bilirubin from precipitating. Be careful not to make sample unrealistically basic. Mixed isomers of bilirubin are very orange.
6. An hour before making solutions, add 1 ml of water to the dried blood in the labeled tube. Mix well and allow to settle. Add the indicated volume to solution G.
7. Use food coloring to adjust color. This should be tested ahead of time to achieve realistic appearances.
8. Add 1ml water to 0.5 g of blood meal. Allow to stand for half hour with occasional mixing. Let the sediment settle then add ½ml of supernatant to solution G.
Background Information

**Introduction:** Medical tests are often required to assess an individual’s state of health. Although many sophisticated, expensive, highly technological tests have recently become available and popular (e.g., MRI, genetic screening), the most common and useful tests are still the ones that are simple, inexpensive, and non-invasive. Examples include patient history, body temperature, heart rate and blood pressure, and the analysis of blood and urine samples.

In this exercise, students will perform a simple analysis of simulated urine solutions using commercial multifunction test strips. Students will be provided information about patient history and will conduct a physical and chemical examination of the urine. Chemical tests for the presence of glucose, protein, bilirubin, ketone, blood, and pH will be performed using commercial test strips. Students will test their understanding of normal kidney function by attempting to diagnose and explain the "patients" conditions.

In part 2, the technology for making dipstick-style test strips for chemical analysis will be presented and students will make their own test strips for glucose, using the same chemical principle used in the commercial product.

**Kidney structure and function:**

When studying any organ system, it is important to recognize the difference between the *job* of that organ and the *mechanism* used to accomplish that job. The job of the kidney is to regulate the volume and composition of the body's fluid compartments (in particular the extracellular fluid, ECF). The mechanism used to accomplish this job is a filtration/reabsorption system, where plasma is first non-selectively filtered at the glomerulus, and then substances needed by the body are selective reabsorbed by the tubules, leaving unwanted substances to form urine. The advantages of this system are 1) a very high capacity to get rid of things by monitoring the levels of substances in the plasma on a moment by moment basis and 2) the ability to eliminate a very broad range of substances without requiring special mechanisms for each substance. The filtration/reabsorption mechanism is able to maintain the composition of body fluids within very narrow limits in spite of wide variation in activity and nourishment.

It is important to emphasize to students that the kidneys are relative small organs, representing only 0.5% of the body's mass. Yet, they receive 25% of the blood flow leaving the heart! Even the much larger brain receives less blood flow than the kidneys. This high rate of blood flow supports the high rates of plasma filtration (Figure 1) and is the basis of the kidney's rapid response to changes in ECF volume and composition. The energy required to filter the plasma is provided by the beating heat. The process of glomerular filtration can be likened to a cloth sack filled with wet paste being squeezing so the liquid and other small particles ooze out, while larger substances (in the case of blood, the cells and plasma proteins) are retained. About 20% of the plasma flowing to the glomeruli is filtered to form what is referred to as *glomerular filtrate*.

The functional unit of the kidney is the *nephron* (Figure 2). The renal *filtration locus* is formed by the glomerular capillary and Bowman's capsule, an expansion of the nephron that envelopes the glomerular capillary. It is important to emphasize that the filtration barrier (formed by the wall of the glomerular capillary and the wall of Bowman's capsule) is a very porous and non-selective structure. About the only things in blood that are not filtered by the glomerulus are blood cells and plasma proteins. All the
Figure 1. Maintaining fluid compartment homeostasis in face of highly variable fluid inputs and outputs requires constant monitoring of body fluids. The mechanism evolved to meet this challenge is the high rate of extracellular fluid filtration by the kidney, supported by high rates of renal blood flow, glomerular filtration and selective recovery of essential substances.
The Nephron
"functional unit of the kidney"

**filtration**
- afferent arteriole
- glomerulus
- efferent arteriole
- Bowman's capsule

**reabsorption/secretion**
- H₂O
- NaCl
- amino acids + glucose
- Na⁺, K⁺, H⁺

**Cortex**
- ureter

**Collecting Duct**
- low concentration
- high concentration

**Renal Artery and Vein**
- Medulla

**Figure 2.** Anatomy of the filtration/reabsorption mechanism of renal function. Effect of ADH on collecting duct permeability and the formation of concentrated (ADH<sub>present</sub>) and dilute (ADH<sub>absent</sub>) urine are indicated.
smaller components of blood (water, salt, minerals, sugars, fats, amino acids, hormones, etc.) are freely filtered. This means that, except for protein, the composition of plasma and glomerular filtrate are essentially identical.

The remainder of the nephron, the renal tubule, represents the kidney's reabsorption locus. (Secretion also occurs in the renal tubules, but the emphasis for beginning students should be on the much higher rates of reabsorption.) Tubular reabsorption recovers all of the sugars, amino acids, and other nutrients in the glomerular filtrate. Substances like salt and water that are present in the body in variable quantities are reabsorbed on an as-needed basis but, in general more than 95% of filtered salt and water is reabsorbed. Compare the volume of glomerular filtration (180 l/day) to the volume of urine formed (1-4 l/day). Other materials, specifically metabolic by products and potentially toxic materials are not reabsorbed by the kidney and left to be eliminated in the urine. The result of tubular reabsorption is a dramatic reduction in the volume of tubular fluid and changes in the composition of the tubular fluid as the final urine is formed. It is important to emphasize that some things present in the glomerular filtrate are absent from normal urine (e.g., glucose); other things present in the glomerular filtrate in low concentration become concentrated as water is reabsorbed from the tubule (e.g. urea).

**Five Distinct Functional Regions of the Nephron**

**Bowman's capsule** forms part of the filtration barrier and collects the glomerular filtrate.

The **proximal tubule** reabsorbs the filtered nutrients and more than 90% of the filtered salt and water. The ability of the proximal tubule to completely reabsorb specific substances depends on their concentration in the glomerular filtrate and ultimately in the plasma. The absorption capacity is appropriate for normal concentrations, but if concentrations are too high tubular reabsorption will be incomplete and the substance will "spill over" into the urine and be excreted. This is why glucose appears in the urine of diabetics. The function of the proximal tubule is the most diverse and perhaps complex but it is enough for the beginning student to appreciate its central role in the reabsorption of nutrients.

The **loop of Henle** is required to form an osmotically concentrated urine, and it is unique to birds and mammals. Understanding the mechanism for forming concentrated urine is one of the most conceptually difficult topics in biology. It is important to stress that animals living in air are always losing water to the environment by evaporation. Because water is often in limited supply it is necessary to conserve water where possible and forming a concentrated urine allows organisms living in air to get rid of metabolic waste products with a minimal amount of water loss. Because birds and mammals have high metabolic rates (compared to amphibians and reptiles) this is a much more serious problem for them, hence the fact that only birds and mammals form concentrated urine. The specific function of the loop of Henle is to use metabolic energy to pump salt out of the tubular fluid creating a space inside the kidney that has a very high osmotic concentration. This hyperosmotic compartment will be used by the kidney to make a concentrated urine as needed. Note that the fluid in the tubule is *not* concentrated as it flows out of the loop of Henle (Figure 2). Urine concentration occurs in the collecting duct.
The distal tubule is primarily responsible for regulating the concentration of Na\(^+\), K\(^+\), and H\(^+\) in the blood. Students who have had an introduction to nerve and muscle function will appreciate that the concentrations of Na\(^+\) and K\(^+\) in the ECF are critical to formation of membrane potentials and the propagation of action potentials. As a result, the concentrations of these ions are closely regulated. The hydrogen ion is the most ubiquitous reactant in the body and the pH of ECF is also tightly regulated.

The collecting duct is where the final concentration of urine is determined. If there is a shortage of water in the body the kidney will excrete a concentrated urine. It does this by making the walls of the collecting duct permeable to water so that water in the tubular fluid will be absorbed into the compartment made concentrated by the loop of Henle (i.e., the interstitium of the medulla). This is an example of the movement of water down and osmotic gradient. The mechanisms for shunting this water out of the kidney and conserving the concentration gradient are the job of the vasa recta and probably beyond the scope of what high school students can understand, but suffice it to say such mechanisms exist. The conservation of water is called anti-diuresis and it is regulated by the antidiuretic hormone (ADH) which makes the wall of the collecting duct permeable to water.

If there is an excess of water in the body the kidney will excrete a dilute urine. It does this by making the wall of the collecting duct impermeable to water so that as the tubular fluid flows through it no additional water is reabsorbed. Note than the fluid entering the collecting duct is dilute (Figure 2). This condition is called diuresis and is determined by the absence of the hormone ADH.

Two topics of interest are the effects of caffeinated and alcoholic beverages on urine volume and composition. While both beverages are typically more dilute than body fluids, their consumption results in dehydration. Caffeine stimulates water excretion by a number of mechanisms and ethanol inhibits the release of ADH. In each case there is loss of fluid in excess of intake and the result is dehydration. Individuals who consume significant amounts of coffee or alcohol must take care to consume additional water.

In conclusion, the specific sequential action of the different tubular segments determines the final volume and composition of urine. Many disease states can be diagnosed by the absence or presence of substances in the urine. The tests conducted in this lab are typical for routine urinalysis, but it is possible to use urinalysis to test for many specific conditions using the same principle (i.e., there are things that should be in the urine and things that should not be).

Other Uses for Urinalysis

Pregnancy testing: The kidney normally scavenges complex biomolecules such as peptide hormones, which can then be reused or recycled. During pregnancy, however, the placenta produces very high levels of several hormones that spill over into the urine. One in particular, human chorionic gonadotropin (HGC) is diagnostic of pregnancy because its only produced by the placenta, while other hormones that also increase during pregnancy might be increased by factors other than pregnancy. There are currently a number of over-the-counter early pregnancy tests on the market that utilize the presence of HCG in urine to indicate a pregnancy.
**Drug testing:** Because virtually all drugs whether licit or illicit are small enough to be filtered by the kidney and most are substances the kidney has no means to reabsorb, urine can be tested to detect the presence of many drugs. This can be quite sophisticated as in the case of testing athletes for synthetic versions of naturally occurring compounds in the human body (e.g., anabolic steroids) or relatively straight-forward as in the case of detecting the presence of drugs like marijuana. It might be interesting for students to contemplate why urine testing for alcohol is not done. Ethanol is certainly small and filterable and it is present in urine after one has been drinking. But ethanol per se is not illegal, only if the blood concentration is very high. Because substances in the urine can be concentrated passively if the body is trying to save water, it is fairly difficult (although not impossible) to back calculate blood concentrations accurately from urine concentrations. For illegal drugs their presence, not concentration, is the issue and passive concentration actually makes them easier to detect in urine than in blood. There are other, simpler methods for determining blood alcohol levels.

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**Urinalysis**

**Color, volume, and specific gravity** (osmotic concentration) of urine vary tremendously depending on the body’s need for salt and water. Normally, as volume of urine goes down specific gravity goes up and vice versa. The color of urine is due to pigments in the diet or is derived from metabolism. Concentrated urine will have a darker color and stronger smell as chromogens and volatile wastes are concentrated. The artificial urine solutions used in this exercise will not have a detectable odor. For students who take B-vitamin supplements, an interesting observation is to see how quickly their urine turns bright yellow following ingestion of vitamin B. Many people also metabolize a chemical in asparagus to a highly odoriferous and volatile compound that can be smelled in urine literally within minutes of eating asparagus.

**Glucose** is not normally present in the urine because reabsorption in the proximal tubule is complete. Its presence typically indicates that plasma levels are well above normal, as in diabetes. Historically diabetes was diagnosed by tasting the urine for the characteristic sweetness imparted by the sugar present. Students considering careers in medicine will appreciate the development of glucose test strips currently used to make this measurement! Glucose in urine attracts water by osmosis increasing the volume of urine formed. Diabetics urinate frequently and are excessively thirsty as a result. The loss of water dehydrates the body causing a response to conserve water by concentrating urine.

**Ketones** are by-products of the metabolism of fat. Ketosis and ketonuria are characteristic of starvation, various metabolic disorders, and diabetes. For diabetics the production of ketones results from the inability to properly regulate insulin, which in addition to its role in the metabolism of glucose also plays a key role in the metabolism of fats.

**Protein** is not normally present in urine. The glomerulus should not allow filtration of plasma protein. In the early stages of glomerular disease the filtration barrier can become leaky to protein. This generally increases glomerular filtration and can also lead to increased urine volume. Renal disease and damage to the glomerular filtration barrier can result from a bewildering array of conditions. Untreated high blood pressure is probably the most significant and rapidly increasing cause of chronic renal failure. Diabetes and a variety of autoimmune syndromes also result in glomerular damage. Protein in urine might also result from bacterial infection.
Bilirubin and urobilinogen are breakdown products of hemoglobin. Bilirubin is not normally found in urine. Normal levels for urobilinogen are more controversial and not considered in this lab. Red blood cells in the body, live for about 120 days after which time they are broken down and the hemoglobin they contain is recycled in a complex process that results in formation of bilirubin. Bilirubin is formed in the liver and excreted with the bile into the intestine. The presence of bilirubin in the urine indicates blood levels that are too high, either because excessive numbers of red blood cells are being broken down (hemolytic anemia - infrequently observed) or because the liver is failing to properly metabolize the hemoglobin (hepatitis, cirrhosis, or bile blockage due to gall stones). Either situation can result in anemia as valuable blood protein and iron are lost.

Nitrite is a product of the bacterial reduction of nitrate and indicates the presence of a bacterial infection. Nitrate is produced in the body through the metabolism of food. Normally there is no nitrite in urine (normal urine is sterile). Infections in the kidney do not normally produce pain during urination. Painful urination is typical of lower urinary tract (urethra and bladder) infections. These infections usually travel up the urinary tract. The amount of nitrite formed depends on many things including: the type of bacteria (whether or not it has reductase), how long the urine is stored in the bladder, the presence of nitrate in the diet. Lower urinary tract infections can travel up into the kidney if left untreated. They may also be sexually transmitted. Antibiotics are used to treat infections. Bacterial culture (identification) is important to guide proper antibiotic therapy.

Blood, often referred to as occult blood (meaning “mysterious origin”) indicates bleeding somewhere in the urinary system and is an abnormal finding.

Sediment and crystals are solids found in urine. Common sediments include dead epithelial cells sloughed from the urinary tract lining, bacteria and white blood cells suggestive of infection, crystals of certain minerals that precipitate out of solution. The most common stone-forming minerals are calcium and oxalate that can lead to the formation of kidney stones that can obstruct tubules, causing significant damage and pain. Twelve percent of males form stones, with those between the ages of 30-50 years at greatest risk for stone disease. Oxalate is common in nuts and dark green leafy vegetables. It is the reason rhubarb leaves are considered toxic. When pets or children drink antifreeze, oxalate is formed from the ethylene glycol in antifreeze by metabolism in the liver. In both cases, kidney failure due to massive tubular damage is the cause of death. To avoid kidney stones, at risk individuals are urged to keep urine volumes high, and oxalate levels and urine pH low. This reduces the concentration and increases solubility of calcium oxalate in tubular fluid.

Use of “Dip-stick” Technology for Chemical Analysis

In the clinical setting it is desirable to be able to obtain quick and highly reproducible analyses - often performed by individuals with limited background and technical expertise. There are a variety of methods used (for example, automated chemical analyzers that can perform a variety of tests on small samples with a high degree of accuracy). These analyzers are expensive, limiting their use to hospital or contract lab settings. The desirability of tests that the consumer can take home and perform themselves for routine monitoring of blood sugar, by diabetics, and recently urine tests for pregnancy has provided a tremendous economic incentive to develop these tests. Most dip stick methods utilize enzyme-based colorimetric reactions. The principles are quite basic and well known. The methods for quality control during production are trade secrets! The difficulty is getting the different reagents to coexist on the paper strip without reacting together during manufacture. The basic technique is to introduce the different
reagents in immiscible phases. In the example described here the paper is impregnated with the enzymes in an aqueous solution, while the chromogenic reagent is added in an organic phase. The point is made that oil and water don't mix!

The reaction used to test for the presence of glucose involves the oxidation of glucose using the enzyme glucose oxidase (G.O.) to form peroxide (H\textsubscript{2}O\textsubscript{2}). The peroxide is activated by another enzyme, peroxidase, forming a reactive oxygen compound that reacts with the indicator substance tetramethyl benzidine (TMB) resulting in the formation of a blue color.

$$G.O. \quad \text{peroxidase}$$

$$\text{glucose} \rightarrow \text{gluconic acid} + \text{H}_2\text{O}_2$$

$$\rightarrow \text{reactive oxygen}$$

$$\text{TMB (brown)} \rightarrow \text{TMB (blue)}$$

By this reaction the quantity of glucose present is estimated by the amount of blue colored TMB that is produced. The enzymes are not consumed in the reaction so all of the glucose absorbed to the dipstick is converted to gluconic acid and H\textsubscript{2}O\textsubscript{2} on a mole for mole basis. Because the TMB is activated by the reactive oxygen (formed when the peroxide is broken down by the peroxidase) on a mole for mole basis, it follows that the amount of blue color is determined by the amount of glucose present. The highest concentration of glucose that can be detected is determined by the amount of TMB present. The amount of glucose that is consumed in the reaction is related to the concentration of glucose in solution by the volume of solution that absorbs to the area of the dipstick containing the chemicals. This can be expressed by the equation: concentration = quantity/volume.
Interpreting Medical Tests: How is Urine Analyzed?

Introduction: Medical tests are often required to assess a patient's state of health. Although many sophisticated, expensive, highly technological tests have recently become available and popular, the most common and useful tests are still ones that are simple, inexpensive, and non-invasive. These include patient history, temperature, heart rate, blood pressure, and analysis of blood and urine. In this experiment you will perform a simple urinalysis using commercial multifunction test strips. After testing several urine samples and using your understanding of normal kidney function, your group will analyze the test results to diagnose the "patient's" conditions. In part 2, you will make dip strips to test for glucose using the same chemical principles used to make the commercial product. This exercise is intended to show how this new, easier technique works, and why it has replaced many of the older chemical tests.

Background for this lab: It is the job of the kidney to regulate the volume and composition of body fluids. Body fluids include the extracellular fluid (ECF, the blood plasma) and the intracellular fluid (ICF, inside body cells). Regulating these fluids requires that the kidney conserves (keeps) or eliminates (discards) materials in just the right amounts to balance intakes and non-renal losses to maintain body fluid homeostasis.

The mechanism of kidney function is a 2-step process. First, the blood is filtered through the glomerulus, forming glomerular filtrate. Second, the filtered fluid is conditioned in the tubule through reabsorption of some chemicals and secretion of others. The final product is urine, a solution that normally contains water, salt, urea and other substances the body needs to eliminate. The kidney responds to body demands and conditions by changing how much of each material is conserved and how much eliminated, so the volume and composition of urine are quite variable, while the volume and composition of the ECF remains constant.

The problem in maintaining constancy in body fluids is that changes caused by eating, drinking, body demands, and activity are very irregular and unpredictable. Even so, the chemical composition of the blood and other body fluids must remain very constant for the body to function normally. The kidneys have the major responsibility for maintaining body fluid homeostasis no matter what the individual eats, drinks, or does.

The solution to the problem is to rapidly turn over the body fluids by glomerular filtration and tubular reabsorption, continually filtering and monitoring the plasma for changes that require correction. The adult kidney filters 170 liters of blood plasma per day. An average adult has a plasma volume of about 3.5 liters, meaning that the kidney completely filters the plasma about 48 times per day, or twice per hour! Obviously, all of the material filtered by the kidney cannot be excreted, so the second step in normal
kidney function is to selectively recover the parts of the filtrate that are necessary and useful to the body. During the process of selective reabsorption, some substances (like glucose) are completely recovered from the urine and other substances (like urea) remain to be eliminated.

The composition of normal urine is highly variable because our diet and level of activity are highly variable. However, abnormal urine may contain substances that are never present in normal urine. Sometimes the presence of a substance may indicate a great excess in the body, so that the kidney can't reabsorb it all. If the kidney is diseased, substances that should not be the urine may be present. Depending on what and how much of various substances are present, a physician may be able to determine what's wrong with a patient.

Urinalysis tests for the presence or absence of different substances, so that a physician can analyze the results to find out what's wrong with the patient.

**Prelab Questions:** (answer on a separate piece of paper)

1. Why does the volume and color of urine produced by the kidneys vary so much?
2. Why does urine always contain urea?
3. Why are glucose and amino acids reabsorbed in the nephron?
4. Why can't the kidney just excrete the filtered plasma without adjusting its content?

**Materials:**

- 1 test tube per urine sample analyzed and test tube rack
- 1 Multistix® urinalysis test strip per urine sample analyzed
- artificial urine samples
- 50-ml graduated cylinder
- hydrometer
- Pasteur pipettes
- microscope slides, cover slips, and a microscope
- sediment chart

**Procedure:**

1. According to the instructions provided by your teacher, obtain samples of urine to be tested.

2. For each sample you analyze, record your observations and additional patient history information on the chart provided below.
3. Observe the color of the urine, and check for any cloudiness of precipitate.

4. You will need one test strip for each sample and a color chart to interpret the results. Perform the test by dipping the test strip into the sample until all of the colored patches are completely covered and then quickly removing the strip from the solution and laying it on a paper towel to absorb excess liquid.

5. At the times indicated on the color chart, compare the color of the strips to the colors on the chart. Record the results of the test for each sample.

6. When a description of sediment is provided, check the sediment chart to identify the type of sediment present. If no reference is made to sediment you can conclude that this result is unremarkable.

7. Finally, check the specific gravity (density) of the urine using a hydrometer. Pour 30-ml of sample from the large flask to into a 50-ml graduated cylinder. Gently slip the hydrometer into cylinder. If the hydrometer does not float in the sample you will need to add additional sample to the cylinder until the hydrometer does float. Read the specific gravity of the sample at the top of the meniscus and record the result in the table.
Interpretation of Results:

**Color:** In general, the more intensely colored the urine is, the more concentrated it is. Certain compounds, like Vitamin B, give urine an intensely yellow color. Normal urine should be clear, not cloudy.

**Volume:** Urine volume depends on water intake and can vary considerably. Two liters per day is considered average, although physicians may recommend that individuals at risk for forming kidney stones try to keep urine flow rates higher.

**Specific gravity:** The higher the S.G., the more concentrated the urine. This may reflect either the excretion of large amounts of salt or urea, or the conservation of water by the kidney.

**pH:** Normal urine may range in pH from 5 to 9. Below 7, the urine is acidic; above 7 it's basic. Diet affects pH. A large component of meat in the diet tends to decrease pH, while a large component of plant-based material in the diet tends to increase pH.

**Glucose:** Normally negative. A positive result indicates the presence of more glucose in the blood being filtered than the kidney can reabsorb.

**Protein:** Normally no protein is detected in urine because it's too large to be filtered at the glomerulus.

**Blood:** A positive test indicates the presence of either red blood cells or hemoglobin. Either result is abnormal. The test may be positive even if no cells are seen under the microscope. Blood in urine is usually the result of some trauma to the renal tubule, ureter, bladder, or urethra.

**Ketone:** Normally no ketone is present. Ketone in the blood comes from burning body fat for energy. Its presence in urine may indicate fasting, strenuous exercise, pregnancy, or diabetes.

**Bilirubin/urobilinogin:** Bilirubin is normally negative. Bilirubin and urobilinogen are products formed from the breakdown of red blood cells and recycling of hemoglobin in the body. Their presence in the urine indicates an abnormality in the recycling of red blood cells. Blood cells may be dying too rapidly; there may be a reduction in liver function required to recycle normal numbers of red blood cells.

**Nitrite** is a product of the bacterial reduction of nitrate and indicates the presence of a bacterial infection. Normally there is no nitrite in urine (normal urine is sterile). Painful urination is typical of lower urinary tract (urethra and bladder) infections. These infections usually travel up the urinary tract. Lower urinary tract infections can travel up into the kidney if left untreated. They may also be sexually transmitted. Antibiotics are used to treat infections. Bacterial culture (identification) is important to guide proper antibiotic therapy.

**Sediment:** Common urine sediment includes epithelial cells, blood cells, casts and crystals. White blood cells suggest infection. Small numbers of red cells may be present but significant numbers are not normal. The presence of crystals indicates an increased risk of forming kidney stones. They can result from excess oxalate in the diet (nuts, dark green vegetables) and are made worse by conditions that cause calcium oxalate to precipitate out of solution (high concentration, high pH). Calcium oxalate stones are the most common but other minerals can precipitate out of urine.
**Analysis:** Study the patient histories and test results. Then try to explain the results obtained for the following individuals in terms of how the kidney works.

**Patient A:** 25 y/o male complains of feeling he ‘needs to go’ all the time but typical only produces a small volume of urine (<50ml). The patient experiences a mild degree of discomfort on urination. There is presently no external evidence of urethral discharge. Microscopic examination of urine sediment revealed small to moderate number of round doughnuts-shaped cells and larger round cells with granular appearance and distinct nucleus.

List the abnormal findings.

The amount of nitrite present in a person with a bladder infection is higher if the urine is retained in the bladder for longer periods of time. How can you explain this?

**Patient B:** A 28 y/o male, overweight. Chief complaints: thirst, frequent urination and lethargy. Urine volume 3.8 l/24hours.

List the abnormal findings.

Why is the patient’s urine volume so high?

Why is the patient thirsty?

What is your diagnosis? What other tests might you perform to confirm the diagnosis?
**Patient C:** An 18 y/o healthy female presents for a routine physical examination. Patient has great difficulty producing a very small volume of urine despite not having urinated since early morning. During discussion with physician it is revealed that she has had only 2 cups of coffee and a donut to eat all day.

List the abnormal findings?

Why does the body form concentrated urine?

Where in the kidney does urine concentration occur?

Is this kidney performing properly? What suggestions might you have for this patient?

Why is an extended water fast a bad idea?

**Patient D:** A healthy 19 y/o male has provided urine for testing following competition in wrestling. The individual normally weighs 185lb but wrestles at 172lb.

List the abnormal findings.

Where do ketones come from?
**Patient E:** A 17 y/o female with joint pain, and an unusual rash. Microscopic examination of the urine sediment was unremarkable.

List the abnormal findings.

How does the kidney normally handle proteins?

Why are proteins present in this sample?

**Patient F:** Elderly female patient presents with abdominal pain following meal. The condition is more severe following a greasy meal.

List the abnormal findings.

Is bilirubin normally found in blood?

In the urine?
Patient G: 38 y/o male vegetarian. Complains of mild irritation during urination. Urine volume 1.1 liters/24 hours. Microscopic examination of urine revealed many small pyramid shaped crystals in every high power field.

List the abnormal findings.

What type of sediment is present in this sample?

How does diet affect urine?

Have you ever tried drinking 12 large glasses of water in a day? Why would drinking water be helpful to this individual?

Post Lab Questions:

1. How many of the problems presented in these samples represent a disease of the kidney itself?

2. How many different ways can you identify that the filtration/reabsorption mechanism reveals underlying disease processes in the body?
Urinalysis data table: Enter data and observations from patient histories and urine sample observations and tests.

<table>
<thead>
<tr>
<th>patient</th>
<th>volume</th>
<th>color</th>
<th>specific gravity</th>
<th>pH</th>
<th>glucose</th>
<th>protein</th>
<th>ketone</th>
<th>bilirubin</th>
<th>nitrite</th>
<th>blood</th>
<th>sediment</th>
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Making a glucose test strip

Introduction:

Drug companies have invested a lot of money in developing simple, 1-step tests to replace the traditional chemical methods used to analyze urine and other body fluids. The new techniques improve the reliability of medical testing, and don't require as much skill to perform the tests correctly. The Multistix® tests used in the first part of this lab are a good example of this new technology.

Most dip stick methods utilize enzyme based colorimetric reactions. The challenge in performing these reactions in a single step is getting the different reagents to coexist on the paper strip without reacting together during manufacture. The basic technique is to introduce the different reagents in solvents that won't mix. In the test described here the paper is impregnated with the enzymes in a water solution and the indicator in an organic solution.

The reaction uses the enzyme glucose oxidase (G.O.) to form peroxide (H₂O₂) during the oxidation of glucose to gluconic acid. The peroxide formed is activated by peroxidase forming a highly reactive oxygen compound that combines with the indicator substance tetramethyl benzidine (TMB), resulting in the formation of a blue color.

\[
\begin{align*}
\text{G.O.} & \quad \text{peroxidase} \\
\text{glucose} & \quad \rightarrow \quad \text{gluconic acid} + \text{H}_2\text{O}_2 \\
& \quad \rightarrow \quad \text{reactive oxygen} \\
& \quad \downarrow \\
\text{TMB (brown)} & \quad \rightarrow \quad \text{TMB (blue)}
\end{align*}
\]

You will try to reproduce this technique by making a test strip with the necessary reagents, and testing its accuracy.

Materials:

<table>
<thead>
<tr>
<th>• Filter paper strips</th>
<th>• Microliter pipette</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Enzyme solution A (containing glucose oxidase and peroxidase)</td>
<td>• Indicator solution B (containing tetramethyl benzidine)</td>
</tr>
<tr>
<td>• Glucose solution(s)</td>
<td></td>
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</tbody>
</table>
**Procedure:**

To manufacture a dip stick:

1. Use a pipette to carefully add 10 microliters of **Solution A** to the end of a test strip and let the strip dry thoroughly. A hair dryer or gentle blowing can be used to speed the drying process. Do not expose the chemicals to high heat.

2. Using a different pipette, place 10 microliters of **Solution B** directly over the dried spot. Let this second spot dry.

To test your product:

3. Dip the test strip into various test solutions.

4. Observe any color change. Devise a system using +’s and –’s to record the intensity of the reaction you observe in the table below. For example, +++ = strong, + = weak, - = no reaction.

5. Make additional strips and test them in solutions of water only, sucrose, and glucose solutions of different concentration.

6. If time and materials permit, try varying the amount of solutions A and B used to make the strips. Record your observations in the table below.

**Observations:**

<table>
<thead>
<tr>
<th>Test Solution</th>
<th>Concentration</th>
<th>Reaction (+/-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
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<tr>
<td>Sucrose</td>
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<tr>
<td>Glucose</td>
<td>concentration</td>
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</tr>
<tr>
<td>Glucose</td>
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</tr>
</tbody>
</table>
Questions:

1. Why can't the reagents be applied together?

2. What is the purpose of testing the strip in water? In sucrose?

3. If we know the chemicals and the reactions involved, how do the drug companies make money on tests like these? What do they provide that makes it worthwhile to buy their product?

4. Did your dip sticks turn darker blue in more concentrated glucose solutions?

Refer to the equation for the reaction on page 1 to answer the following questions.

5. Which compounds are consumed in this reaction?

6. What compounds are produced in the reaction?

7. What compound determines how much glucose can be detected?
Some Common Substances Found in Urine

**1. Crystals of calcium oxalate** appear in alkaline urine after ingestion of certain foods, especially green leafy vegetables, nuts, and chocolate. Ca oxalate crystals are common and are a risk factor for the formation of kidney stones.

**2. Triple phosphate (or struvite) crystals** are found in alkaline urine in the presence of ammonia. Ammonia is uncommon in normal urine and results mainly from bacterial breakdown of urea. Struvite crystals in a fresh specimen can indicate a urinary tract infection.

**3. Cysteine crystals** are quite rare. Urine acid crystals (4) are less common than calcium crystals. The main risk factors for uric acid crystals are dietary, especially excess intake of purines in the form of meat, fish and fowl.

**4. Urine acid crystals** are less common than calcium crystals. The main risk factors for uric acid crystals are dietary, especially excess intake of purines in the form of meat, fish and fowl.

**5. Casts** form when various cells become embedded in a protein gel. Casts are normal in urine sediment. Large numbers of casts and specific kinds of casts are associated with certain diseases.

**6. In theory, red blood cells should never be present in urine, but are found as a common external contaminate. Abnormal numbers of red cells indicate injury to renal tissue.**

**7. White blood cells generally indicate inflammation somewhere along the urinary tract and are commonly associated with bacterial infection.**

**8. Epithelial cells** like the squamous cells shown here are common in urine sediment and of no special significance.