Teacher's Manual

Urinalysis Using Simulated Urine

W56625

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INTRODUCTION

The detection, followed by the correct diagnosis, of disease is a process that often involves the recognition, investigation, and analysis of a variety of factors. A physician, trained to objectively look for specific abnormalities (symptoms), is generally the first step in detection and diagnosis. A physician will ask probative questions of a patient in order to attempt to narrow down the field of possible causes.   The physician must have good communication skills. As all people communicate somewhat differently, the physician may have to interpret descriptions that vary from patient to patient when being used to describe the same symptom. The physician may have to re-phrase questions or dig deeper to arrive at useable information. After enough dialog with the patient, the physician may then decide to administer one or more specific medical tests based on the verbal analysis.

Medicine, like all sciences, is a constantly advancing field. Increases in technology have led to, and continue to lead to, the development of more and more sophisticated tools, whether they be chemical/biochemical tests (such as DNA genetic screenings, immunochemistry) or pieces of machinery (for example X-rays, MRIs, CAT scans). Technological advance usually leads to increased cost as well, causing some of these options to be quite expensive. For this reason, based on the experience and judgment of the physician, a series of simpler and less expensive tests may first be performed. Using the verbal consultation between the physician and patient, as well as a consideration of the patient's medical history, the physician may first want to know the patient's body temperature, heart rate, or blood pressure for example. Another very effective diagnostic tool is urinalysis.

Urine and Urinalysis

Urine is a liquid containing many waste products that otherwise might be toxic if they were allowed to remain in the body.   The kidneys, part of the excretory system, filter blood circulating throughout the body and remove toxins. The toxins are removed and beneficial materials are returned back to the circulatory system. The kidneys also serve to regulate the amount of substances circulating throughout the body. In other words, a material may be beneficial to the body but in high enough quantities it is able to do harm. The kidneys filter this material and allow necessary, beneficial levels of the substance to be reabsorbed while filtering the rest out with waste.

Urinalysis is the analysis of physical and chemical properties of urine. Urine can be a complex mixture of many components. There is no "set" formula for the composition of urine. It can vary greatly day-to-day or hour-to-hour. Urine appearance and composition can be affected by many factors such as diet, level of physical activity (or inactivity), environmental conditions, or fluid intake. Salts, urea, by-products of metabolism, amino acids and proteins, enzymes, pigments, carbohydrates, or medications are examples of substances that may appear in a normal urine sample and again, the amount of these substances may vary based on circumstances.

When analyzing urinalysis results, a physician is going to consider a variety of factors. The physician may want to know what might not be in the sample that should be there, what might be in the sample that should not be in there, or what may be in the sample but in quantities considered outside of the "normal" range.

Common Factors Examined in Urinalysis

Gross Physical Examination

Gross physical examination is the examination of the collected urine as it appears initially, usually contained in a specimen cup. Some observations of note are:

<u>Color</u> – normally urine varies in color from very pale yellow to a straw/amber color. A pale yellow may simply indicate excessive fluid intake but also may be a sign of diabetes insipidus. A brighter yellow may indicate a high level of vitamins, especially B vitamins. A pink to red tint may be caused by certain foods such as beets but may also be indicative of the presence of blood in the urine. Straw to amber colored urine may be a sign of dehydration resulting from fever or other illness. An olive green to green tint could be caused by certain bacterial infections or the presence of bile pigments. Brown to black tones may be signs of phenol or heavy metal poisoning.

<u>Clarity</u> – regardless of color, urine is usually clear. Cloudiness of the sample may show excessive levels of white blood cells, fats, protein, or other mineral salts. Cloudiness may also indicate bacterial infection.

<u>Odor</u> – like color, odor may also be greatly affected by diet. However, there are certain notable odors that are useful. A foul, ammonia odor may be caused by a bacterial infection. A sweet-smelling, fruity odor may be caused by ketones. Ketones are a by-product of the breakdown of fat and may be signs of diabetes or severe starvation.

<u>Specific gravity</u> – as the concentration of material in the urine increases, the specific gravity increases. Specific gravity is based on the mass of a urine sample as compared to the mass of pure water of an equal volume. One milliliter of water at standard temperature and pressure weighs one gram so water has a specific gravity of 1.000 (1g/1ml). An equal volume of a sample with more material, such as salts, protein, minerals, etc., would have a higher mass and therefore a higher specific gravity. A normal range for urine is 1.005 to 1.030. While specific gravity is useful, certain kidney disorders may

result in the inability to concentrate urine properly so care should be taken to not allow a "normal" specific gravity value to lead to a false security.

Chemical Analysis

Chemical analysis is the determination of "what and how much" is in urine. While gross physical observation may give an indication of this, chemical analysis provides a more accurate determination. Several common factors investigated include:

pH – urine is normally slightly acidic but like many other qualities, many factors can affect the pH of urine at any given moment. Generally the pH ranges from about 4.5 to 7.5. Eating large quantities of meat will reduce urine pH and large amounts of vegetable matter tend to increase the pH of urine. Excessively high pH for urine may indicate a bacterial infection but certain dietary supplements may lead to this condition as well. A consistently low pH may indicate diabetes mellitus.

Protein – little or no protein is typically found in urine as it is filtered out in the kidneys. A little protein may show up due to factors ranging from stress to temporary dehydration but the persistent presence of protein may indicate kidney damage, specifically the glomerulus, a structure in the kidney that normally prevents protein from passing to the urine. Common causes of glomerular damage may be caused by long-term high blood pressure, untreated diabetes, autoimmune diseases, or bacterial infection.

Glucose – also not typically found in urine as it is re-absorbed during processing in the kidneys. Diabetes mellitus may cause glucose levels in the body to reach levels higher than the kidneys are able to filter, resulting in a positive glucose test in urine. Both stress and eating a high carbohydrate meal may cause some glucose to appear temporarily but diabetes results in the persistent presence of glucose. Kidney damage may also cause the presence of glucose as the ability of the kidney to reabsorb glucose may be impaired.

Ketones – as mentioned under odor, ketones impart a sweet smell to urine and are the result of fat metabolism. The need to burn body fat may result from starvation. Diabetics may not be able to regulate levels of insulin in the body which also may lead to ketones in the urine. However, strenuous exercise or pregnancy may also cause the presence of ketones.

Calcium – generally present in small, if any, amounts. Higher levels of calcium may indicate hyperparathyroidism (parathyroid gland overproduces hormones), bone disorders, or kidney disease. Calcium levels may also be affected by diet or large intake of vitamin D. Dehydration may also lead to elevated levels of calcium. The pH of urine may cause calcium to crystallize, which can be observed microscopically.

Blood/hemoglobin – the presence of blood and/or hemoglobin, while possible in the case of extreme physical activity or by taking certain medications, should always be treated as abnormal. Possible causes include liver or kidney damage, infection or damage in the urinary tract, a condition causing excessive red blood cell destruction, and kidney stones.

Bilirubin – this is a normal product of the metabolism of hemoglobin but is not normally present in urine. When red blood cells are broken down, the hemoglobin released is recycled and a byproduct of the process is bilirubin. Bilirubin is formed in the liver and passed to the intestine for excretion. When present in the urine, bilirubin may indicate that the liver is not functioning to capacity. It may also indicate that an excessive amount of red blood cells are being broken down. Possible causes are cirrhosis, gall stones causing bile blockage, types of anemia, or traumatic injury to the liver or gall bladder.

Nitrites – typically a sign of urinary tract infection as nitrite is not found in urine. Nitrates are produced in the body during food metabolism and certain bacteria can reduce nitrates to nitrites in the bladder or urinary tract.

Microscopic Examination

Urine may contain various types of sediment of varying composition. While some of the sediment may be visible to the naked eye, a microscopic examination is necessary to reveal enough detail in the sediment to assist in determining its source or cause. Types of sediment are commonly classified as:

Crystals – the occasional appearance of crystals in urine is normal. Diet can lead to mineral salt levels high enough for crystals to appear. Changes in the pH of the urine may also cause the precipitation of mineral salts. If crystal formation is persistent and amount of crystals present seems to increase in amount over time, it can serve as an indication of the formation of kidney stones.

Cells – it is normal for the body to shed dead skin (epithelial) cells. The appearance of these cells in urine is usually not a cause for concern. The presence of very small numbers of white and/or red blood cells is common as well. A high number of epithelial cells may signal degeneration in the kidney. A significant presence of white blood cells is usually a sign of infection as the immune system increases white blood cell production in response. Abnormal red blood cell levels can be caused by a variety of problems such as internal injury, side effects of certain medications, or a variety of blood disorders. Bacterial cells may be present in the case of urinary tract infections.

Casts – as blood is filtered in the kidneys, it is forced through an elaborate series of tube-like structures. In some cases, material may get “stuck” in the certain spots and accumulate. When released, this material is excreted in a distinct tube shape called a cast. Casts may be composed of many materials and the term cast is more in reference to the shape of the structure rather than its composition. Epithelial cells, red blood cells, white blood cells, proteins, pigment molecules, and bacterial cells are some examples. While some casts are normal, others may indicate problems such as kidney disease.

Urinalysis is also used for other applications such as pregnancy testing and drug screening. During pregnancy, a hormone known as human chorionic gonadotropin (HCG) is secreted by embryonic tissues. Some of this hormone eventually makes its way into urine where it can be detected during urinalysis. Traces of ingested drugs, such as prescription medications and illicit drugs, may also end up detectable in urine.

Objectives

- Perform a gross physical examination on simulated patient urine samples.
- Determine if glucose is present in any of the patient urine samples.
- Test for the presence of protein in patient urine samples.
- Use Sulkowitch's reagent to test for calcium in patient urine samples.
- Examine urine samples microscopically for the presence of calcium crystals.

Materials Included in the Kit

4 Simulated urine samples, 250ml each
- Patient X
- Patient Y
- Patient Z
- Control

2 btl. Benedict's reagent, 25ml
2 btl. Biuret solution, 25ml
2 btl. Sulkowitch's reagent, 25ml
1 pkg. pH test strips,100/vial
1 box Microscope slides
1 pkg. Coverslips
60 Graduated plastic cups, 30ml
60 Graduated pipettes, 1ml

Materials Needed but not Supplied

Hot water bath or hot plate and beaker
Compound microscopes (4X/10X/40X)
60 Glass test tubes
Test tube racks
Marking pens (or similar)

Safety

Safety goggles
Gloves
Lab apron

Pre-lab Preparation

Prior to performing the lab, set up a hot water bath or beaker of water on a hot plate. The water temperature should be hot but below boiling (approx. 80-90°C). The hot water bath will be used for the glucose test with the Benedict's reagent.

Instructor's Notes

The urine samples used in this activity are simulated and do not contain any biological material. You may, for the sake of realism and developing good technique, have students treat the samples as if they could potentially be a biohazard risk.

The questions at the end of the lab involving the patient scenarios will most likely require that the students perform research outside of the background information given in this lab. You may also wish to answer the questions in the form of group discussion.

Chemical Disposal

Dispose of all chemicals used in this activity in accordance with local and state regulations.

Procedure

Materials Needed per Group

4	Graduated plastic cups
4	pH test strips
4	Graduated plastic pipettes
4	Glass test tubes
	Test tube rack
	Microscope slides
	Coverslips

Shared Materials

Simulated urine samples:
- Patient X
- Patient Y
- Patient Z
- Control

Benedict's reagent
Biuret solution
Sulkowitch's reagent
Hot water bath
Compound microscopes

Safety

Safety goggles
Gloves
Lab apron

Part 1: Physical Observation/pH

1. Using a marking pen, label each of your four graduated plastic cups as follows: "X," "Y," "Z," and "Control."

2. Using the marking on the cups, fill each cup to 15ml mark with the appropriate urine sample. Be sure to add the correct sample to each labeled cup.

3. Observe each of the samples for color and clarity. Record the color and clarity of each sample in the correct row of the table in the Data Analysis section of the lab.

4. Determine the pH of the sample in the cup labeled “Control.” Dip one end of a pH test strip into the sample for 1-2 seconds. Remove the strip from the sample and shake excess liquid off of the end of the strip. Determine the pH by matching the color of the pH strip to the color chart on the pH strip package. Record the pH of the control sample in the Data Analysis section.

5. Using the same procedure (and a new pH strip for each sample), determine the pH of the samples in the cups labeled “X,” “Y,” and “Z.” Be sure to record your result in the Data Analysis section after each sample.

Part 2: Glucose Test

Benedict’s reagent is a solution that is used to test for the presence of glucose. The reagent is added to a solution and the solution is heated for a brief period. If glucose is present, the solution will form a red precipitate after heating. If glucose is not present, the solution will not form a precipitate. Benedict’s reagent will also react with several (but not all) other sugars such as fructose, lactose, maltose, and galactose but in urinalysis glucose is the sugar of interest from a diagnostic standpoint.

1. Using a marking pen, label each of four test tubes as follows: “X,” “Y,” “Z,” and “Control” and place them in a test tube rack.

2. Using a different graduated plastic pipette for each sample, add 3ml of each urine sample to the appropriately labeled test tube. The 1ml mark on the pipette is the mark closest to the bulb end of the pipette. Keep each pipette with the proper sample as you will use them again in the following sections of the lab.

3. Using a dropper bottle of Benedict’s reagent, add 15 drops of the solution to each of the urine samples in the four test tubes.

4. Using a test tube holder or heat protective gloves, place all four test tubes in a hot water bath. Be sure the test tubes are standing upright and do not allow any of the water from the hot water bath to enter the tubes.

5. Allow the four samples to heat for 10 minutes. After 10 minutes remove the four test tubes from the hot water bath and return them to the test tube rack.

6. Observe each sample for reaction and record the results in the Data Analysis section. Again, if glucose is present in the urine sample, a red precipitate will form. If no glucose is present, there will be no precipitate.

7. After your results are recorded, empty the contents of the four test tubes according to your instructor. Thoroughly wash each of the test tubes before proceeding to the next part of the lab.

Part 3: Protein Test

The biuret test will indicate the presence of protein in a solution. Biuret solution will react with peptide bonds in protein if protein is present. A copper-containing compound in the solution is reduced in the presence of proteins and the solution will form a deep-blue to violet color. If no proteins are present, a deep color will not form.

1. If your marking came off of the test tubes during washing, re-mark each of the four test tubes as follows: “X,” “Y,” “Z,” and “Control” and place them in a test tube rack.
2. Using the correct pipette for each sample, again add 3ml of each urine sample to the appropriately labeled test tube.
3. Using a dropper bottle of biuret reagent, add 15 drops of the solution to each of the urine samples in the four test tubes.
4. Observe each sample for reaction and record the results in the Data Analysis section. Again, if protein is present in the urine sample, a deep blue/violet will form. If no protein is present, the color will appear light blue to green.
5. After your results are recorded, empty the contents of the four test tubes according to your instructor. Thoroughly wash each of the test tubes before proceeding to the next part of the lab.

Part 4: Calcium Test

Sulkowitch’s reagent will react with any calcium to form a white precipitate. After addition of Sulkowitch’s reagent, a sample containing calcium will appear cloudy. If no calcium is present, the sample will maintain its clarity.

1. If your marking came off of the test tubes during washing, re-mark each of the four test tubes as follows: “X,” “Y,” “Z,” and “Control” and place them in a test tube rack.
2. Using the correct pipette for each sample, again add 3ml of each urine sample to the appropriately labeled test tube.
3. Using a dropper bottle of Sulkowitch’s reagent, add 15 drops of the solution to each of the urine samples in the four test tubes.
4. Observe each sample for reaction and record the results in the Data Analysis section. Again, if calcium is present in the urine sample, a precipitate will form and make the sample appear cloudy. If no calcium is present, the sample will remain clear.

5. If any of your samples displayed a positive reaction for the presence of calcium, place one drop of the sample on a microscope slide. Place a coverslip on the drop.
6. Using a microscope, examine the drop under medium power (10X). After locating a field of view that contains some of the precipitate, switch the microscope to high power (40X) and observe the precipitate in more detail.
7. In the Data Analysis section, draw a representative sample of your microscopic observations. Write a brief description of the material you observe under the microscope.
8. Clean up all materials according to your instructor. Be sure to wash your hands before leaving the lab.

Name:	Instructor:
Date:	Class/Lab Section:

DATA ANALYSIS

	Control	Patient X	Patient Y	Patient Z
Color	yellow	yellow	dark straw, amber	yellow
Clarity	clear	clear	clear	clear
pH	6-7	6-7	8-9	6-7
Glucose (+/-)	(-)	(+)	(-)	(-)
Protein (+/-)	(-)	(-)	(+)	(-)
Calcium (+/-)	(-)	(-)	(-)	(+)

Name:	Instructor:
Date:	Class/Lab Section:

DATA ANALYSIS

Microscopic Observations

Name:	Instructor:
Date:	Class/Lab Section:

DATA ANALYSIS

Questions

1. Patient X is an overweight 36 year old male. He has recently lost a significant amount of weight though he is constantly hungry and thirsty. The patient also complained of feeling tired and run down frequently. Based on the symptoms described and the result of the patient's urine tests, what do you believe is the most probable diagnosis?

The symptoms described are indications of diabetes mellitus. The presence of glucose in the urine as well as the lower pH of the urine supports the likelihood of this as well.

2. Patient Y is a 21 year old female that has been experiencing a fever and nausea. She is a vegetarian but while sick has had trouble keeping both food and liquids down. Her doctor suspects it is a bacterial infection. Are there any symptoms in her urine test results to support this?

Yes, the presence of protein may be indicative of a bacterial infection. The higher pH of her urine may be caused by infection as well, however her vegetarian diet may also be affecting the pH of her urine. The darker color of her urine is a sign of dehydration, which could be a side effect of the infection and her inability to keep liquids down.

Name:	Instructor:
Date:	Class/Lab Section:

DATA ANALYSIS

3. Patient Z is 65 year old female in for a routine checkup. She is not complaining of any symptoms and generally feels fine. Is there anything in the results of her urine test that a doctor may want to investigate further?

Answers will vary based on student research. The presence of calcium could be viewed as suspicious. The doctor may want to seek further information about her diet or if she is taking any nutritional supplements, such as calcium pills. While it may not be an immediate cause for concern, the doctor may want to continue to monitor her calcium levels and check if there any other symptoms of a bone disease such as osteoporosis.

4. What was the purpose of the control urine sample in this investigation?

Since the content of the patient samples was unknown prior to testing, the control sample served as a "negative" for each test, making it easier to read a positive result.

5. Does urinalysis prove the presence of disorder or disease? Explain.

No, urinalysis alone does not necessarily confirm any particular disease or disorder. Even throughout a single day, the contents of urine may vary greatly. Factors such as diet, exertion level, environmental conditions, etc. may all affect the contents of urine at any given moment. Urinalysis best serves as a diagnostic tools that may or may not indicate if further, more reliable (and usually more expensive) medical tests are warranted.