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BIOL 244L HUMAN ANATOMY AND PHYSIOLOGY LABORATORY
URINARY SYSTEM II : URINE COMPOSITION AND TESTS

Numerous aspects of kidney function can be diagnosed by the chemical and microscopic analysis of urine. Today we will test several analysis techniques for urine. Many procedures that previously required detailed chemical processing are now greatly simplified by use of chemical test strips. Strips of paper or plastic are coated with chemical compounds that when dipped into a sample of urine will develop and display a color reaction that shows the concentration of specific chemical compounds. pH paper has been around for a long time. A "Litmus Test" is a popular expression in political speech, but how many politicians do you think know it comes from an extract of chemical dye from lichens deposited into paper strips that turns red in acid (pH < 7) and blue (pH > 7) in alkaline solutions. Litmus pH tests are pretty crude, but nowadays there are many new sophisticated analyses for other compounds incorporated in a simple test strip dipped into the sample.

We will work with real urine donated by volunteers and two synthetic urines that have an altered chemical composition.

1. We need to collect at least half a cup of urine from one member of the groups at each table. Volunteers can collect it in one of the collection cups provided in the front of class. Label the collection cups with a letter identification: A, B, C,...etc.

2. Synthetic urines are prepared with unusual chemical composition, so they can demonstrate the range of tests better than your own, hopefully normal, urine. Take samples from the front of class of "high" and "low" synthetic urine in small beakers; we don't have much to work with.

Perform the first four tests (test numbers 1-4) on a small sample of the synthetic urines first. Then repeat the test with the urine from the individual at your table. Glassware and droppers are at the laboratory table stations. You can pick up other supplies at the front desk as you need them. **Please leave the stock supplies of test materials at the front desk for other groups to find easily.**

1. Precision Labs ™ multi-purpose urine test strip. These combine several tests in a set of 4 spots on the strip: pH, protein, glucose, and ketones.
Dip the test strip into a urine sample, and be sure all 4 reagent spots are briefly immersed in the sample. Then compare the color of the test spots with the scales on the side of the strip dispenser. Note: after dipping, the ketones and protein reactions will take 1 minute to develop completely.

pH. Record: "High" synthetic urine pH:_____ "Low" synthetic urine pH:_____
Urine sample letter ID (A,B,C etc):_____ pH:_____

Protein. Protein in the urine is a condition called proteinuria.
Record: "High" synthetic urine protein: _____ "Low" synthetic urine protein:_____
Urine sample letter ID (A,B,C etc):_____ protein:_____

Glucose. Glucose in the urine is a condition called glycosuria
Record: "High" synthetic urine glucose _____ "Low" synthetic urine glucose:_____
Urine sample letter ID (A,B,C etc):_____ protein:_____

Ketones. People with diabetes or people fasting may have ketones in their urine (ketonuria)
Record: "High" synthetic urine ketones _____ "Low" synthetic urine ketones:_____
Urine sample letter ID (A,B,C etc):_____ ketones:_____

2. Clinitest Tablets for glucose in urine.

DANGER!: "Clinitest" tablets contain sodium hydroxide (= "LYE" = "caustic soda")!!. Do NOT touch them with bare skin! Use forceps. Rinse your hands with lots of water if you accidentally contact the tablet, or even contact dust from it!

1. With dropper in upright position, place 5 drops of urine in a **wide** test tube (it must be wide to allow addition of the Clinitest tablet).
2. Add 10 drops of water to the urine in the test tube. Swirl to mix.
3. Using forceps with the tablet bottle and the test tube holder for the test tube, drop one Clinitest tablet into the urine-water mixture in the test tube.
CAUTION: The mixture will become boiling hot, so hold the tube upright with the wire clamp holder. **DO NOT SHAKE THE TUBE**!
4. Keep the tube still in the rack for 15 seconds after boiling has stopped, then shake gently to mix the contents. Compare color with the Clinitest chart to estimate the % glucose in the urine.
Record: "High" synthetic urine glucose _____ "Low" synthetic urine glucose:_____
For Your group's urine sample letter ID:_____ glucose:_____
5. Wash and rinse the test tube, then turn it upside down in the rack to drain and dry.

For the following tests, use only the samples of real urine (not the synthetic samples)

3. Specific Gravity: Use the URINOMETER (labeled "urinprober" in German on the box).
This delicate glass instrument is very FRAGILE! Handle it with care!

1. Fill the cylinder about 2/3 to 3/4 full; but not much more, or it will overflow when you add the float.
2. Gently place the scaled urinometer float (with large, weighted end down) in the urine.
3. The urinometer float should float freely in the urine; twirl it to check that it's off the bottom. (Add some more urine, even if it's from someone else, to make the float freely float!)
4. The more dense the urine, the less deep the float will sink.
5. Note where the surface of the urine intersects the scale on the urinometer float.
6. 1.0 is assumed on the scale; the numbers shown are digits (hundredths of a density unit) that follow the 1.0. Therefore, if it reads one line above 20, the reading is 19. The 19 is to be placed to the right of 1.0 (So the density reads 1.019). This means that the specific gravity = 1.019, and that this urine is 1.9% more dense than plain water. Record the density of your group's urine sample letter ID:_____ density:_____.
7. Wash and dry the urinometer float and the cylinder. Put them back into their box.

4. pH: (hydrogen ion concentration) USE THE ELECTRONIC pH METER.

If you are at all uncertain about the care and feeding of the pH meter, ask before using it.

1. Remove the instrument's electrode from the BUFFER container.
2. Dip the tip of the electrode (about 1 cm) into your urine sample, and stir very gently a few turns.
3. Read the pH, and record it for urine sample letter ID:_____ pH:_____
4. Rinse the electrode gently in the "Rinse Bath" container.
5. Place the electrode back into the BUFFER container.
6. How well did this pH reading compare with the pH paper result?

5. Chloride Ions

1. Pour some of the sample of urine into a test tube to a depth of about 1-inch.
2. Add 2 drops of Silver Nitrate solution to the urine in the test tube.
   (**Warning:** Silver nitrate (even though it looks as harmless as water) will stain your skin and clothing dark brown. The stain will not appear for many hours, so you will not know whether you got any on you until it's too late! Therefore, rinse your hands, just in case!!)
3. Look for a change in the urine when the silver nitrate mixes with it.
4. A conspicuous reaction indicates the presence of chloride ions. Most chloride compounds are pretty soluble in water but silver chloride is very insoluble and precipitates.
5. Why are chloride ions likely to be present in urine?
6. Wash and rinse the test tube, then turn it upside down in the rack to drain and dry.

6. Urea

1. Place a sprinkle of urea crystals in a DRY test tube. Urea won't harm your fingers.
2. Smell the tube. Is there any odor?
3. Carefully heat the tube over a burner flame by waving the tube through the flame repeatedly until the crystals are seen to melt and begin to give off fumes .
4. CAUTIOUSLY wave some fumes toward your nose with your hand, and find out if there is any odor now.
5. What is the odor? What is the significance of this observation?
6. When the tube has cooled, add water to dissolve out the urea.

Physiological Considerations.

As we learned in earlier laboratories, the kidney operates with three fundamental processes: filtration, re-absorption, and secretion. The blood filtration step at the glomerlus and Bowman's capsule produces a large volume of filtrate containing pretty much everything in blood except the cells and large proteins greater than 70,000 molecular weight in size. Smaller polypeptides, sugars, amino acids, mineral ions, and large amounts of water are subsequently re-absorbed in the renal tubule. Between re-absorption and secretion, the final urine produced for excretion is adjusted to compensate for scarcity or over-abundance of mineral ions and water in the diet.

If you consume large amounts of flavored soda drinks containing, as they do, lots of citric acid, what would you expect the pH of your urine to be an hour later?

Why might someone have a positive test for glucose in the urine?

Why might someone have a positive test for protein in the urine?

Why might someone have urine with high specific gravity?