The Forensic Examiner

Introduction

Mr. Smith was found in his bed deceased. He had no known pre-existing conditions. Perform four tests using his blood and urine to determine the possible cause of death.

Concepts

- Urinalysis
- Blood analysis
- Diseases

Background

The presence and concentrations of chemical species in blood and urine are often used as diagnostic tools for illness and cause of death. Components of blood and urine give insight to the body's current health. Medical professionals commonly use blood and urine samples to diagnose health conditions. These samples are also valuable to toxicologists to determine a possible cause of death.

The composition of blood and urine varies with changes in health. The presence of proteins, glucose, and ketones are often analyzed in urine samples. Healthy individuals exhibit very low concentrations of these chemical compounds. The presence of one or more of these chemical compounds at substantial levels in urine may indicate conditions such as dehydration, kidney damage or diabetes. Both urine and blood are frequently tested for levels of sodium and potassium. Additionally, blood is tested for the presence of ammonium, calcium, chloride and phosphate ions. All of the previously mentioned ions or electrolytes are normally present in urine and/or blood in accepted healthy values. Elevated electrolyte concentrations that vary from the accepted values may be indicative of various diseases or conditions such as diabetes or heart failure. Explanations of the tests that will be done in this laboratory are listed below.

Protein Detection

Proteins can be identified using a simple color test based on the reaction of their polypeptide backbones with copper ions in basic solution. When molecules containing two or more peptide linkages react with copper sulfate in the presence of a strong base, a purple complex is formed. This is called the *biuret test*. The colored product is the result of coordination of peptide nitrogen atoms with copper ions. The amount of product that is formed and thus the intensity of the purple color depend on the nature of the protein and the amount of protein present.

Electrolyte Levels

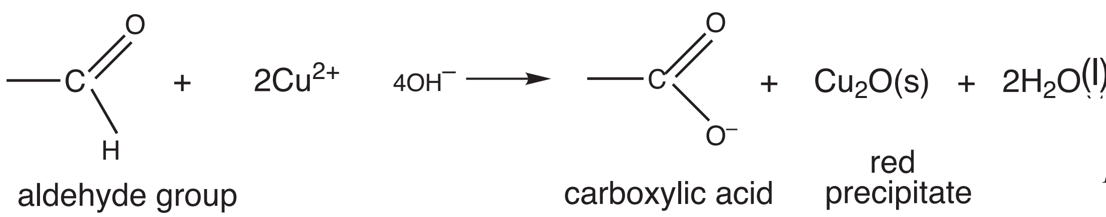
One method of detecting excess or elevated electrolyte levels in urine or blood is by using a flame test. Most metal ions emit a characteristic wavelength or color of light when heated in a flame. Just as a fingerprint is unique to each person, the wavelength of light emitted after excitation of an element is unique to that element. Only a few elements give off a characteristic color of light in the visible region of the spectrum—the region which is visible to the human eye (400–700 nm). For most elements, the characteristic wavelength is detectable only in the ultraviolet or infrared regions of the spectrum. See Table 1 for a list of a few metal ions that emit characteristic visible colors.

Metal	Characteristic Color
Copper	Green
Lithium	Bright red
Potassium	Violet
Sodium	Bright yellow
Strontium	Red/orange

Table 1.

Sugar Detection

Benedict's qualitative solution is used to test for the presence of reducing sugars in urine. A reducing sugar contains an aldehyde or another group which is capable of reducing copper ions in Benedict's solution. In a reaction with copper(II), an aldehyde group in glucose, for example, is oxidized to a carboxylic acid (see Equation 1). All monosaccharides and some disaccharides are reducing sugars. Examples of reducing sugars include glucose, fructose, galactose, and lactose. Notably, sucrose, which is table sugar, is not a reducing sugar.



Equation 1

Initially, the copper(II) ions in the Benedict's solution impart a characteristic blue color to the solution. However, when Benedict's solution is added to a solution containing a reducing sugar, the blue copper(II) ions are reduced to copper(I) ions by the reducing sugar to form red copper(I) oxide, Cu_2O, which precipitates out of solution. Therefore, the formation of a red precipitate indicates a positive test for reducing sugars.

Chloride Detection

Elevated concentrations of chloride ions may be detected using a chemical test with silver ions in solution. Blood contains many dissolved ions from the dissociations of salts such as sodium or potassium chloride.

An ionic salt compound is composed of two parts—*cations* (positively-charged ions) and *anions* (negatively-charged ions). When an ionic salt is dissolved in water, the salt crystal dissociates or separates into its corresponding cations and anions. For example, potassium chloride (KCl) dissociates into potassium cations (K^+) and cloride anions (Cl^-) according to Equation 2.

$$KCl(s) \rightarrow K^+(aq) + Cl^-(aq)$$ *Equation 2*

Similarly, the ionic salt silver nitrate, $AgNO_3$, dissociates into silver cations (Ag^+) and nitrate anions (NO_3^-) according to Equation 3.

$$AgNO_3(s) \rightarrow Ag^+(aq) + NO_3^-(aq)$$ *Equation 3*

When two ionic salts are mixed together in water, two new combinations of cations and anions are possible. In some cases the cation from one salt and the anion from another salt may combine to form an insoluble solid product, called a *precipitate*. For example, if solutions of potassium chloride and silver nitrate are mixed together, a solid precipitate of silver iodide (AgCl) forms as shown by Equation 4.

$$K^+(aq) + Cl^-(aq) + Ag^+(aq) + NO_3^-(aq) \rightarrow AgCl(s) + K^+(aq) + NO_3^-(aq)$$ *Equation 4*

Notice the silver cations (Ag^+) and nitrate anions (NO_3^-) remain dissolved in solution. They do not combine to form a precipitate and thus do not participate in the reaction. They are therefore referred to as *spectator ions*. A net ionic equation is one that includes only the ions participating in the reaction. Thus Equation 4 can be reduced to Equation 5. The reaction shown in Equation 5 is used as the basis of a test to detect excess chloride ions in blood samples.

$$Ag(aq) + Cl^-(aq) \rightarrow AgCl(s)$$ *Equation 5*

Materials

- Benedict's qualitative solution, 5 mL
- Biuret test solution, 2.5 mL
- Silver nitrate, 0.05 M, < 1 mL
- Simulated blood, 6 mL
- Simulated urine, 6 mL
- Water, tap
- Beaker, 200 mL
- Bunsen burner
- Butane safety lighter
- Graduated cylinder, 10-mL
- Hot plate
- Inoculating loop
- Pipets, Beral-type, graduated, 4
- Test tube rack
- Test tubes, 13 × 100 mm, 4

Materials for Pre-Lab Preparation

- Albumin, 2 g
- Graduated cylinder, 250-mL

Dextrose, $C_6H_{12}O_6$, 2 g

Potassium chloride, KCl, 2.5 g

Beaker, 250-mL

Stirring rod

Water, distilled or deionized

Safety Precautions

Biuret test solution is a corrosive liquid and is especially dangerous to eyes. Benedict's qualitative solution is a skin and eye irritant. Silver nitrate solution is moderately toxic by ingestion. It is also irritating to body tissues and will stain skin and clothing. Avoid all body tissue contact. Wear chemical splash goggles, chemical-resistant gloves and a chemical-resistant apron. Wash hands thoroughly with soap and water before leaving the laboratory. Follow all laboratory safety guidelines. Please review current Material Safety Data Sheets for additional safety, handling and disposal information.

Pre-Lab Preparation

Simulated Urine

1. Measure 2 g of albumin and 2 g of dextrose and place in a 250-mL beaker.
2. Measure 200 mL of distilled or deionized water in a graduated cylinder.
3. Add the water to the beaker 10 mL at a time while stirring the albumin and dextrose. Once the solids are dissolved the water may be added in larger increments.
4. Add yellow food coloring for effect.

Simulated Blood

5. Measure 5 g of potassium chloride.
6. Measure 200-mL of distilled or deionized water in a graduated cylinder.
7. Add the water to the graduated cylinder and stir. Add red food coloring for effect.

Silver Nitrate Dilution

8. Prepare 100 mL of 0.05 M silver nitrate measuring 50 mL of 0.1 M silver nitrate and dilute to 100 mL with distilled or deionized water.

Procedure

Part A. Urinalysis

Protein

1. Place 100 mL of tap water in a 200-mL beaker.
2. Heat the beaker of water on a hot plate to prepare a boiling water bath for step 11. Proceed to step 3 while the water heats to a boil.
3. Using a clean graduated cylinder, measure 5 mL of simulated urine.
4. Add the simulated urine to a clean 13 × 100 mm test tube.
5. Using a graduated pipet, measure 2.5 mL of biuret test solution. *Hint:* Use the graduations on the side of the pipet to do this.
6. Add the biuret solution to the test tube containing the 5 mL of simulated urine.
7. After 2–3 minutes, record observations on a separate sheet of paper.

Glucose

8. Using a 10-mL graduated cylinder, measure 5 mL of Benedict's qualitative solution.
9. Add the Benedict's qualitative solution to a clean 13 × 100 mm test tube.
10. Using a graduated pipet, add 8 drops of simulated urine to the test tube containing Benedict's qualitative solution.
11. Place the test tube in the boiling water bath prepared in step 2.
12. Allow the test tube to sit in the boiling water for 3–5 minutes and record observations on a separate sheet of paper.
13. Turn off the hot plate and allow the boiling water to cool before discarding.

Part B. Blood Analysis

Sodium and Potassium

14. Using a clean graduated cylinder, measure 5 mL of simulated blood.
15. Add 2–3 mL of simulated blood to a clean 13 × 100 mm test tube. *Note:* The remaining simulated blood will be used in the chloride test.
16. Light a Bunsen burner and obtain an inoculating loop.
17. Dip the inoculating loop in the simulated blood solution and hold the tip of the loop over the flame.
18. Record observations on a separate sheet of paper.

Chloride

19. Using a clean graduated pipet, add 1 mL of simulated blood to a clean 13 × 100 mm test tube.
20. Using a clean graduated pipet, add 3–5 drops of silver nitrate to the test tube containing the simulated blood.
21. Wait 3–5 minutes. Record observations on a separate sheet of paper.
22. After all tests have been completed obtain a Pathology Evidence Chart from the instructor and predict what Mr. Smith may have died from.

Disposal

Please consult your current *Flinn Science Catalog/Reference Manual* for general guidelines and specific procedures, and review all federal, state and local regulations that may apply, before proceeding. Neutralize the simulated urine/biuret mixture by adding dilute hydrochloric acid solution and rinse down the drain with excess water according to Flinn Suggested Disposal Method #10. The simulated blood/silver nitrate mixture may be flushed down the drain with copious amounts of water according to Flinn Suggested Disposal Method #26b. The remaining solution as well as the simulated urine/Benedict's solution mixture may be rinsed of down the drain according to Flinn Suggested Disposal Method #26b.

Connecting to the National Standards

This laboratory activity relates to the following National Science Education Standards (1996):

Unifying Concepts and Processes: Grades K–12
Evidence, models, and explanation
Form and function
Content Standards: Grades 5–8
Content Standard C: Life Science, structure and function in living systems
Content Standard F: Science in Personal and Social Perspectives, personal and community health
Content Standards: Grades 9–12
Content Standard C: Life Science, matter, energy, and organization in living systems
Content Standard F: Science in Personal and Social Perspectives, personal and community health

Tips

- This activity is available from Flinn Scientific as a student laboratory kit, The Coroner's Report (Catalog No. AP7316).
- This is an excellent activity for students to do upon studying renal physiology or diabetes.

Reference

Collins, David. *Investigating Chemistry in the Laboratory;* W. H. Freeman and Company: New York; 2006; pp 49–57.

Materials for *The Forensic Examiner* are available from Flinn Scientific, Inc.

Catalog No.	Description
AP7316	The Coroner's Report—Urine and Blood Analysis Kit
A0258	Albumin, 2 g
B0171	Benedict's Qualitative Solution, 100 mL
B0050	Biuret Test Solution, 100 mL
D0002	Dextrose, 500 g
P0183	Potassium Chloride, 100 g
S0305	Silver Nitrate, 0.1 M, 100 mL

Consult your *Flinn Scientific Catalog/Reference Manual* for current prices.

Pathology Evidence Chart

Condition	Urine					Blood				
	Glucose	Ketones	Potassium	Protein	Sodium	Ammonium	Chloride	Phosphate	Potassium	Sodium
Dehydration		X			X		X		X	X
Diabetes-related	X	X		X	X					X
Heart Failure				X		X				
Heavy Metal Poisoning				X						
Kidney Failure	X		X	X			X	X	X	
Starvation		X			X		X		X	X

X = Positive test results.

Pathology Evidence Chart

Condition	Urine					Blood				
	Glucose	Ketones	Potassium	Protein	Sodium	Ammonium	Chloride	Phosphate	Potassium	Sodium
Dehydration		X			X		X		X	X
Diabetes-related	X	X		X	X					X
Heart Failure				X		X				
Heavy Metal Poisoning				X						
Kidney Failure	X		X	X			X	X	X	
Starvation		X			X		X		X	X

X = Positive test results.