Artificial Urine Analysis

Background:

All organisms produce wastes. These waste materials must be removed so that the organism is not poisoned by its own metabolism. In humans, urine is the fluid produced by the kidneys as they remove waste chemicals from the blood. Urine is made up primarily of water, with some salts and organic materials dissolved in it. The concentration of each of these substances varies with a person's health, diet, and degree of activity.

By testing the chemical composition of urine, doctors can learn much about the general health of an individual. Urinary tract infections, kidney malfunction, diabetes, and liver disease are just some of the medical problems that can be diagnosed through urinalysis. Urinalysis involves the physical, chemical, and visual examination of a urine sample. Some substances and their indicators for disease are included below:

Substance	Indications
pH	Acidic – metabolic or respiratory acidosis, methanol poisoning, or metabolic disorders Alkaline – metabolic and respiratory alkalosis and urinary tract infections Normal pH range 5-7
Specific Gravity	<1.015 cardiovascular problems, diabetes insipidus, or renal tubule problems Normal specific gravity 1.015-1.025
Protein	High levels due to excessive exercise, cold exposure, and acute abdominal diseases
Ketones	Presence indicates cold exposure, diabetes mellitus, dietary imbalances, or metabolic abnormalities
Glucose	High levels due to diabetes mellitus, pregnancy, excessive stress, renal tubular damage, and brain damage

In this investigation, you will perform several tests to detect substances in a sample of artificial urine. You will also determine the contents of an artificial urine sample of unknown composition.

Research Problem:

What substances are found in a sample of artificial urine?

Materials:

12 test tubes
glass marking pencil
test tube rack
test tube holder
10 ml graduated cylinder
hot plate
Bunsen burner
400 ml beaker

matches
urine samples
    normal urine
    with glucose
    with ketone
    with protein
    acidic and alkaline pH
    high and low specific gravity
Benedict's solution
Sodium bisulfite reagent
10% acetic acid solution
pH strips or pH meter
hydrometer
unknown urine samples

Safety:

Use Universal Precautions when working with human fluids.
Use caution with hot substances and staining chemicals.
Dispose of urine waste in the designated waste beakers.

Procedures:

Part A Test for Glucose

1. Place two test tubes in a rack. With a glass marking pencil, label one test tube 'G' for glucose. Allow the other test tube to remain unlabeled as the control.
2. Use the 400 ml beaker to prepare a hot water bath.
3. Add 3 ml of Benedict's solution to both test tubes.
4. Add 3 ml of the urine sample with glucose to the test tube labeled 'G'. Add 3 ml of the normal urine sample to the unlabeled test tube. Note the appearance of the substance in each test tube. Record in Data Table 1.
5. Place both test tubes in the hot water bath for 2 minutes.
6. After 2 mins, remove the test tubes from the hot water bath with a test tube holder. Place the test tubes in the test tube rack. Note any color changes in the test tubes. Record in Data Table 1.

Part B Test for Ketone

1. Place two test tubes in a rack. With a glass marking pencil, label one test tube 'K' for ketone. Allow the other test tube to remain unlabeled as the control.
2. Add 5 ml sodium bisulfite reagent to both test tubes.
3. Add 1.5 ml of the urine sample with ketone to the test tube labeled 'K'. Add 1.5 ml of the normal urine sample to the unlabeled test tube. Note the appearance of the substance in each test tube. Record in Data Table 2.
4. Stopper the test tubes and shake vigorously.
5. After shaking, return the test tubes to the test tube rack. Note the formation of a precipitate. Record in Data Table 2.

Part C Test for Protein

1. Place two test tubes in a test tube rack. With a glass marking pencil, label one test tube ‘P’ for Protein. Allow the other test tube to remain unlabeled as the control.
2. Add 5 ml of the urine sample with protein to the test tube labeled ‘P’. Add 5 ml of the normal urine sample to the unlabeled test tube.
3. Using a test tube holder, pass the top surface of each test tube over the flame of a Bunsen burner for 15 to 20 seconds. After heating each test tube, place it in a test tube rack. Note the appearance of each substance. Record your observations in Data Table 3.
4. Add 5 drops of acetic acid to each test tube. Note the appearance of each substance. Record your observations in Data Table 3.

Part D Test for pH

1. Place three test tubes in a test tube rack. With a glass marking pencil, label one test tube ‘A’ for acidic and label another test tube ‘B’ for basic or alkaline. Allow the third test tube to remain unlabeled as the control.
2. Add 3 ml of the acidic urine sample to the test tube labeled ‘A’. Add 3 ml of the basic or alkaline urine sample to the test tube labeled ‘B’. Add 3 ml of the normal urine sample to the unlabeled test tube.
3. Dip a clean pH strip or the probe of a pH meter into the acidic urine sample then place the strip on a paper towel to dry. Repeat this procedure for the basic or alkaline urine sample and for the normal urine sample.
4. Read the strips and record the pH in Data Table 4.

Part E Test for Specific Gravity

1. Place three test tubes in a test tube rack. With a glass marking pencil, label one test tube ‘H’ for high specific gravity and label another test tube ‘L’ for low specific gravity. Allow the third test tube to remain unlabeled as the control.
2. Add 5 ml of the urine with high specific gravity to the test tube labeled ‘H’. Add 5 ml of the urine with low specific gravity to the test tube labeled ‘L’. Add 5 ml of the normal urine sample to the unlabeled test tube.
3. Fill the hydrometer ¾ with urine from the test tube labeled ‘H’. Rest the hydrometer in water and record the specific gravity reading in Data Table 5. Return the urine to the test tube then rinse the hydrometer thoroughly. Repeat this procedure for the other two urine samples and record the specific gravity readings in Data Table 5.

Part F Test Unknown Urine Samples

Design and perform an experiment to test unknown urine samples for glucose, ketone, protein, acid or alkaline, and specific gravity. Record your results in an appropriate data table.

Data:

Table 1 - Glucose

Substance	Color before heating	Color after heating
Urine with glucose		
Normal urine		

Table 2 - Ketone

Substance	Appearance before shaking	Appearance after shaking
Urine with ketone		
Normal urine		

Table 3 - Protein

Substance	Appearance after heating	Appearance after adding acetic acid
Urine with protein		
Normal urine		

Table 4 - pH

Substance	pH reading (method)
Acidic urine	
Basic or Alkaline urine	
Normal urine	

Table 5 – Specific Gravity

Substance	Specific Gravity
High urine	
Low urine	
Normal urine	

Unknown Urine Samples Table

Discussion:

Bibliography:

Miller and Levine, Biology Laboratory Manual. Prentice-Hall, Inc. 2000

Artificial Urine Analysis Teacher Instructions

Background:

All organisms produce wastes. These waste materials must be removed so that the organism is not poisoned by its own metabolism. In humans, urine is the fluid produced by the kidneys as they remove waste chemicals from the blood. Urine is made up primarily of water, with some salts and organic materials dissolved in it. The concentration of each of these substances varies with a person's health, diet, and degree of activity.

By testing the chemical composition of urine, doctors can learn much about the general health of an individual. Urinary tract infections, kidney malfunction, diabetes, and liver disease are just some of the medical problems that can be diagnosed through urinalysis. Urinalysis involves the physical, chemical, and visual examination of a urine sample. Some substances and their indicators for disease are included below:

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Protein	High levels due to excessive exercise, cold exposure, and acute abdominal diseases
Ketones	Presence indicates cold exposure, diabetes mellitus, dietary imbalances, or metabolic abnormalities
Glucose	High levels due to diabetes mellitus, pregnancy, excessive stress, renal tubular damage, and brain damage

In this investigation, you will perform several tests to detect substances in a sample of artificial urine. You will also determine the contents of an artificial urine sample of unknown composition.

Research Problem:

What substances are found in a sample of artificial urine?

Materials:

12 test tubes
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test tube rack
test tube holder
10 ml graduated cylinder
hot plate
Bunsen burner
400 ml beaker

matches
urine samples
    normal urine
    with glucose
    with ketone
    with protein
    acidic and alkaline pH
    high and low specific gravity
Benedict's solution
Sodium bisulfite reagent
10% acetic acid solution
pH strips or pH meter
hydrometer
unknown urine samples

Sample preparations:

Benedict's solution – 173 g sodium citrate and 100 g sodium carbonate in 700 ml distilled water; heat to dissolve; filter. 17.3 g copper sulfate in 100 ml distilled water; slowly add to first solution stirring constantly; add distilled water to a volume of 1 L.

Sodium bisulfite reagent – 16.6 g sodium bisulfite to 400 ml distilled water (40% solution). Add 100 mL ethanol to 400 mL of 40% sodium bisulfite solution. Decant or filter prior to use.

10 % acetic acid solution – add 10 ml acetic acid to 90 ml distilled water

Normal urine (yellow food color may be added)

36.4 g urea in 1.5 L distilled water; mix until crystals dissolved. 15.0 g sodium chloride, 9.0 g potassium chloride, 9.6 g sodium phosphate to first solution until dissolved. Adjust pH 5-7 range by adding HCl to lower or NaOH to raise. Adjust specific gravity 1.015-1.025 range by adding water to lower. Before use warm to room temp and add 4.0 g creatine and 100 mg albumin.

Abnormal urine (modify from normal urine stock)

Glycosuria – add 300 mg dextrose to 100 mL normal urine
Ketonuria – 0.5 ml acetone to 100 ml normal urine
Proteinuria – 100 mg albumin to 100 ml normal urine
pH – 10 ml HCl to 100 mL normal urine to reduce; 10 ml NaOH to 100 mL normal urine to increase
Specific Gravity – add distilled water to lower; add lead carbonate to raise

Safety:

Use Universal Precautions when working with human fluids.
Use caution with hot substances and staining chemicals.
Dispose of urine waste in the designated waste beakers.

Procedures:

Part A Test for Glucose

1. Place two test tubes in a rack. With a glass marking pencil, label one test tube 'G' for glucose. Allow the other test tube to remain unlabeled as the control.
2. Use the 400 ml beaker to prepare a hot water bath.
3. Add 3 ml of Benedict's solution to both test tubes.
4. Add 3 ml of the urine sample with glucose to the test tube labeled 'G'. Add 3 ml of the normal urine sample to the unlabeled test tube. Note the appearance of the substance in each test tube. Record in Data Table 1.
5. Place both test tubes in the hot water bath for 2 minutes.
6. After 2 mins, remove the test tubes from the hot water bath with a test tube holder. Place the test tubes in the test tube rack. Note any color changes in the test tubes. Record in Data Table 1.

Part B Test for Ketone

1. Place two test tubes in a rack. With a glass marking pencil, label one test tube 'K' for ketone. Allow the other test tube to remain unlabeled as the control.
2. Add 5 ml sodium bisulfite reagent to both test tubes.
3. Add 1.5 ml of the urine sample with ketone to the test tube labeled 'K'. Add 1.5 ml of the normal urine sample to the unlabeled test tube. Note the appearance of the substance in each test tube. Record in Data Table 2.
4. Stopper the test tubes and shake vigorously.
5. After shaking, return the test tubes to the test tube rack. Note the formation of a precipitate. Record in Data Table 2.

Part C Test for Protein

1. Place two test tubes in a test tube rack. With a glass marking pencil, label one test tube 'P' for Protein. Allow the other test tube to remain unlabeled as the control.
2. Add 5 ml of the urine sample with protein to the test tube labeled 'P'. Add 5 ml of the normal urine sample to the unlabeled test tube.
3. Using a test tube holder, pass the top surface of each test tube over the flame of a Bunsen burner for 15 to 20 seconds. After heating each test tube, place it in a test tube rack. Note the appearance of each substance. Record your observations in Data Table 3.
4. Add 5 drops of acetic acid to each test tube. Note the appearance of each substance. Record your observations in Data Table 3.

Part D Test for pH

1. Place three test tubes in a test tube rack. With a glass marking pencil, label one test tube 'A' for acidic and label another test tube 'B' for basic or alkaline. Allow the third test tube to remain unlabeled as the control.
2. Add 3 ml of the acidic urine sample to the test tube labeled 'A'. Add 3 ml of the basic or alkaline urine sample to the test tube labeled 'B'. Add 3 ml of the normal urine sample to the unlabeled test tube.

3. Dip a clean pH strip or the probe of a pH meter into the acidic urine sample then place the strip on a paper towel to dry. Repeat this procedure for the basic or alkaline urine sample and for the normal urine sample.
4. Read the strips and record the pH in Data Table 4.

Part E Test for Specific Gravity

1. Place three test tubes in a test tube rack. With a glass marking pencil, label one test tube 'H' for high specific gravity and label another test tube 'L' for low specific gravity. Allow the third test tube to remain unlabeled as the control.
2. Add 5 ml of the urine with high specific gravity to the test tube labeled 'H'. Add 5 ml of the urine with low specific gravity to the test tube labeled 'L'. Add 5 ml of the normal urine sample to the unlabeled test tube.
3. Fill the hydrometer ¾ with urine from the test tube labeled 'H'. Rest the hydrometer in water and record the specific gravity reading in Data Table 5. Return the urine to the test tube then rinse the hydrometer thoroughly. Repeat this procedure for the other two urine samples and record the specific gravity readings in Data Table 5.

Part F Test Unknown Urine Samples

Design and perform an experiment to test unknown urine samples for glucose, ketone, protein, acid or alkaline, and specific gravity. Record your results in an appropriate data table.

Data:

Table 1 - Glucose

Substance	Color before heating	Color after heating
Urine with glucose	Blue	Orange-red
Normal urine	Blue	Blue

Table 2 - Ketone

Substance	Appearance before shaking	Appearance after shaking
Urine with ketone	Clear	Precipitate
Normal urine	Clear	Clear

Table 3 - Protein

Substance	Appearance after heating	Appearance after adding acetic acid
Urine with protein	White haze at top	White haze remains
Normal urine	Clear	Clear

Table 4 - pH

Substance	pH reading (method)
Acidic urine	<5
Basic or Alkaline urine	>7
Normal urine	5-7

Table 5 – Specific Gravity

Substance	Specific Gravity
High urine	>1.025
Low urine	<1.015
Normal urine	1.015-1.025

Unknown Urine Samples Table

Test	Unknown #1	Unknown #2	Unknown #3
Glucose			
Ketone			
Protein			
pH			
Specific Gravity			

Discussion:

What was the purpose of testing the known samples and normal urine prior to testing the unknown samples? What are the limitations of this type of testing? What substances would you expect to find in your own urine? Prediction of disease using urinalysis must be confirmed by another test. When would you notify the patient?

Extensions may include research into dialysis for kidney failure and methods of waste removal in organisms other than humans.

Bibliography:

Miller and Levine, Biology Laboratory Manual. Prentice-Hall, Inc. 2000

Shmaefsky, Brian R. Artificial Urine for Laboratory Testing. National Association of Biology Teachers. 2004