INSTRUCTIONS: Each question is worth one point. Answer the questions on this paper as appropriate: short answer, fill in the blank, true-false, and multiple choice.

1. List at least two (2) changes that can occur in a urine specimen if it is allowed to sit at room temperature and deteriorate.

   1. 
   2. 

2. As stated in the lab guide, the normal values for urine specific gravity are
   A. 1.000 - 1.015
   B. 1.015 - 1.025
   C. 1.020 - 1.035
   D. 1.035 - 1.050

3. The specific gravity of a liquid is defined as the density of the liquid when compared to the density of _________________ at a specified temperature.

Match the following analytes with the test used to detect them.

4. ___protein
   A. Clinitest
   B. Ictotest

5. ___sugar
   C. 3 % sulfosalicylic acid
   D. Acetest

6. ___acetoacetic acid or acetone

7. ___bilirubin

8. Nitrites are formed in the bladder by the action of certain __________________________ on urinary nitrates.

9. On what reaction is the test for urobilinogen based?
   a. glucose oxidase
   b. Ehrlich
   C. Benedict’s copper reduction
   D. error of indicators

10. Other than intact RBCs, what two substances can be detected by the occult blood pad?
    __________________________ & __________________________
Macroscopic Urinalysis

**Points:** Points are awarded for Pre-test, Skills, including general lab requirements, as well as successful and timely completion of Study Questions.

**Objectives:** According to the standards set by the instructor, the student will be able to perform the following physical and rapid chemical macroscopic examinations within ± 20% accuracy.

1. Color
2. Transparency
3. Specific gravity (by TS meter and dipstick)
4. Urine chemical concentrations of protein, glucose, ketones, bilirubin, blood, nitrite, urobilinogen, leukocyte esterase, and pH using Multistix reagent strip method
5. Protein by sulfosalicylic acid
6. Ketones by Acetest tablet method
7. Bilirubin by Ictotest tablet method
8. Reducing substances by Clinitest tablet method

**Equipment & Supplies:**
1. AO TS Meter
2. Multistix reagent strips
3. Urine controls
4. Color charts for Multistix, Acetest, Ictotest, and Clinitest
5. Pasteur or other transfer pipets @ 5 3/4" size
6. 3% sulfosalicylic acid
7. Acetest tablets & product insert
8. Ictotest tablets, absorbent pads & product insert
9. Clinitest tablets, reaction tubes & product insert
10. Test tubes, racks, marking pens, Kim-wipes, paper towels
11. Distilled water and sodium chloride solution

**References:**
Package inserts – Multistix, Clinitest, Acetest, and Ictotest, Bayer / Ames Company
Strasinger, S. K. *Urinalysis and Body Fluids*, Chapter 4.

**Principle:**
1. Determining the physical properties of urine is done by making simple but important empirical observations. Physical properties can provide clues to subsequent findings and/or dictate the need for additional tests not always performed on each sample. In addition, the physical appearance of a urine sample can often tell a great deal about a patient's condition. A significant change in urinary color or clarity that deviates from accepted normal classifications may indicate the presence of a disease.

2. It is important to work with a freshly collected sample or one that has been properly preserved. A number of changes can occur in an aging sample. They are outlined in the Lecture Guide and in your textbook under preservation. *(HINT: Look
3. The first of the physical properties to be considered is color. The color of urine often varies with its concentration and is most often reported as some shade of yellow (straw, light yellow, yellow, dark yellow, and amber).

4. A urine’s transparency or clarity is best assessed by observing light through a recently mixed sample. Terms used to report transparency include clear, hazy, cloudy, and turbid.

5. The specific gravity of a liquid is defined as the ratio of the density of the substance being measured to the density of water at a specified temperature. Urine specific gravity is a measure of the amount of solutes (electrolytes, urea, etc.) present in the urine sample. Specific gravity indicates how dilute or concentrated is the specimen. The assigned reference value of water is 1.000. Most authors consider 1.015-1.025 to be the normal urine specific gravity range although the kidneys are capable of 1.001-1.030.

6. The kidneys help to regulate the body’s acid-base balance by excreting excess acid or excess alkali. The accepted method for routine measurement of urine pH reaction is by means of pH indicators.

7. Other urine physical properties of volume, odor, and foam are not routinely reported.

8. Multistix or Chemstrips - chemical principles of procedures:
   a. Specific Gravity - check individual manufacture’s package insert.
   b. pH – The indicators methyl red and bromthymol blue provide a broad range of colors covering the entire urinary pH range. Colors range from orange through yellow and green to blue.
   c. Protein – This test is based on the protein-error-of-indicators principle; at a constant buffered pH, the development of any green color is due to the presence of protein. Colors range from yellow for “Negative” through yellow-green, green to blue-green for “Positive” reactions.
   d. Glucose – This test is based on a double enzyme reaction. The enzyme glucose oxidase catalyzes the formation of gluconic acid and hydrogen peroxide from the oxidation of glucose. The second enzyme, peroxidase, catalyzes the reaction of hydrogen peroxide with a potassium iodide chromogen to oxidize the chromogen to colors ranging from green to brown.
EXERCISE 1: Macroscopic Urinalysis

**e. Ketones** – This test is based on the development of a purple color when acetoacetic acid or acetone reacts with nitroprusside.

**f. Bilirubin** – This test is based on the coupling of bilirubin with diazotized dichloroaniline in a strongly acid medium. The color ranges through various shades of tan.

**g. Blood** – This test is based on the peroxidase-like activity of hemoglobin or myoglobin which catalyzes the reaction of cumene hydroperoxide and orthotolidine. The resulting color ranges from orange through green to dark blue.

**h. Nitrite** – *Nitrite is formed in the bladder by the action of certain bacteria on urinary nitrate. At an acid pH, the nitrite reacts with p-arsanilic acid to form a diazonium compound.

**i. Urobilinogen** – This test is based on the Ehrlich reaction in which paradiemethylaminobenzaldehyde reacts with urobilinogen in a strongly acid medium to produce a brown-orange color. * When there is no change of the test strip color, the result is usually reported as "Normal," not negative.

**j. Leukocyte esterase* – Reaction as follows:

*Nitrite test and leukocyte esterase are not available on all dip stix

URINE BACKUP TESTS

9. **Protein by Sulfosalicylic Acid** – The 3% sulfosalicylic acid precipitates protein in solution, turning the urine specimen milky. The degree of turbidity is graded from trace to 4+.

10. Ketones by **Acetest** reagent tablets – Acetoacetic acid or acetone in urine or blood will form a purple colored complex with nitroprusside in the presence of glycine.

11. **Bilirubin by Ictotest** reagent tablets – The reaction is based on the coupling of a unique solid diazonium salt with bilirubin in acid medium to give the blue or purple reaction product.

12. **Glucose and other Reducing substances by Clinitest** reagent tablets for reducing sugars. This test is based on the classic Benedict's copper reduction reaction. Copper sulfate react with reducing substances in urine, converting cupric sulfate to cuprous oxide. Colors range from blue through green to orange. This test is used to detect sugars other
than glucose (i.e., lactose and galactose).

**Procedure:**

1. Mix specimens well and classify five (5) specimens for color. Note any unusual colors and record.

2. Visually inspect and classify the same five (5) specimens for clarity. Be sure specimen has been well-mixed *before* classifying.

3. Determine the specific gravity of the five (5) specimens using the AO TS meter. Before determining the s.g. of the five (5) urines, check the refractometer's accuracy by measuring the s.g. of a drop of deionized H$_2$O (should be 1.000) and the s.g. of a drop of 5% sodium chloride solution (should be 1.022 ± 0.001). If the s.g. of the deionized H$_2$O and the sodium chloride do not match what they should be, consult the instructor for further directions.

4. Determine the following chemical concentrations on the five specimens using the Multistix reagent strips and the following procedure:
   a. Mix the specimen.
   b. Completely immerse all reagent areas of the strip.
   c. Remove the reagent strip immediately and tap off any excess urine.
   d. Hold the strip in a horizontal position to prevent possible mixing of chemicals and/or soiling of hands with urine.
   e. Compare the test areas on the strip to the corresponding color chart on the bottle at exactly the times specified.*
   1) Specific gravity: As indicated.
   2) pH: Immediately. Report pH to the one-half unit.
   3) Protein: Immediately. Report as negative, trace, or 1+ to 4+.
   4) Glucose: Qualitatively at 10 seconds and semi-quantitatively at 30 seconds.
   5) Ketones: 15 seconds.
   6) Bilirubin: 20 seconds.
   7) Blood: 25 seconds.
   8) Nitrite: 40 seconds.
   9) Urobilinogen: 45 seconds.
   10) Leukocyte esterase: As indicated.

   *NOTE:* If there is a discrepancy between the times stated here and those recommended by the manufacturer, follow the manufacturer's instructions.

5. Perform a sulfosalicylic acid test for protein on each urine showing a positive protein on the dipstick.
   a. Into a small test tube, pour about 1-2 ml (20-40 drops) of either mixed urine or the supernatant from a centrifuged urine.
   b. Addition an equal amount of 3% sulfosalicylic acid solution to the urine
c. Mix well by either “flicking” the urine or placing parafilm on the top of the test tube and inverting 2-3 times.

d. Grade for cloudiness as follows:
   - Negative – no cloudiness
   - Trace – Cloudiness is just perceptible against a black background
   - 1+ – Cloudiness is distinct but not granular
   - 2+ – Cloudiness is distinct and granular
   - 3+ – Cloudiness is heavy with distinct clumping
   - 4+ – Cloud is dense with large clumps that may solidify

6. Perform an Acetest on each specimen showing a positive ketone on the dipstick using the following procedure.
   a. Place an Acetest tablet on a white paper.
   b. Add one (1) drop of urine directly on the tablet.
   c. At 30 seconds, compare the color of the tablet with the color chart provided by the manufacturer.
   d. Report as negative, 1+ (for small amount), 2+ (for moderate amount), or 3+ (for large amount).

7. Perform an Ictotest on each specimen showing a positive bilirubin on the dipstick using the following procedure.
   a. Place a square of the absorbent test mat (provided by manufacturer) onto a white paper.
   b. Place ten (10) drops of urine onto the center of the test mat.
   c. Place one (1) Ictotest tablet on the moistened mat.
   d. Carefully place one (1) drop of distilled/deionized water onto the top of the tablet. *Wait five (5) seconds. Add a second drop of water to the table so that the water runs off the tablet onto the mat.*
   e. The presence of a blue or purple color on the mat indicates a positive test for bilirubin. (Slight pink or red color should be ignored.)
   f. The test is subjectively graded as negative or trace to 4+. See manufacturer's product insert for example of positive results.

8. Perform a Clinitest (5 drop method) on each specimen giving a positive glucose on the dipstick, as well as all children using the following procedure:
   a. Place five (5) drops of urine into a clean glass test tube.
   b. With the same size dropper, add ten (10) drops of deionized water.
   c. Drop one (1) Clinitest tablet into the test tube. Watch while boiling reaction takes place. Do not shake tube during the reaction or for 15 seconds after the boiling has stopped. Remember to observe for the "pass through phenomenon."
EXERCISE 1: Macroscopic Urinalysis

9. Record all results in chart form.

d. At the end of the 15 second waiting period, shake test tube gently to mix contents.

e. Compare the color of the liquid contents to the color chart provided for the five (5) drop method and report the percent (%) of the closest matching color.
## Macroscopic Urinalysis Report Sheet

<table>
<thead>
<tr>
<th>Specimen #</th>
<th>1</th>
<th>2</th>
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<th>4</th>
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<td><strong>Clinitest</strong></td>
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**TS Meter Calibration Check:** Distilled water________ Quality Control solution________________
Study Questions

Unless otherwise noted, each question is worth one point. Using lecture notes, reading assignments and information presented in this lab, answer the following questions.

(2 pts.)
1. According to your lecture and lab notes, what are the two (2) reasons for recording the physical properties of urine?
   1. ____________________________
   2. ____________________________

(5 pts)
2. According to your lecture and lab notes, list ten (10) changes that begin to occur in an un-preserved urine specimen if it is not examined within one (1) hour.

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</table>

3. Define specific gravity of a liquid.

(2 pts)
4. Explain the principle for determining specific gravity by
   a. refractometer

   b. dipstick

5. How should Clinitest tablets be stored?
EXERCISE 1: Macroscopic Urinalysis

6. What is meant by “pass-through”?

7. What is recommended to minimize the occurrence of “pass-through” phenomenon?

(4 pts)
8. Give specific instructions for collecting a 24 hr. urine collection. Start the collection at 8:00am.
   1. ______________________________________
   2. ______________________________________
   3. ______________________________________
   4. ______________________________________
   5. Deliver the specimen to the laboratory as soon as possible.

(3 pts)
9. List at least three (3) precautions that should be taken in handling reagent strips (dipstix).
   1. ______________________________________
   2. ______________________________________
   3. ______________________________________

10. Of the routine chemical tests performed on urine, which is the most indicative of renal disease?

11. What is the major serum protein found in urine?

12. Why should the sulfosalicylic acid precipitation test be performed on centrifuged specimens?

(3 pts)
13. Name three (3) substances that can give a false negative on the dipstix for glucose.
   1. ______________________________________
   2. ______________________________________
   3. ______________________________________

14. What does a positive nitrite test indicate?
EXERCISE 1: Macroscopic Urinalysis

15. What is myoglobin?

16. With what test can myoglobin interfere?

17. Why should a bilirubin test be included in every routine urinalysis?

18. What is the most frequent error associated with bilirubin testing?

19. Why is a small amount of urobinogen normally found in urine?

(2 pts)
20. What two disorders can cause increased urobinogen in urine?
   1. ________________________________
   2. ________________________________

(3 pts)
21. What effect does high levels of ascorbic acid have on the following urinalysis tests?

<table>
<thead>
<tr>
<th>Test</th>
<th>Effect</th>
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<tbody>
<tr>
<td>Clinitest</td>
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<tr>
<td>Glucose</td>
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<td>Blood</td>
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<td>Nitrite</td>
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