LABORATORY 2: Macroscopic Urinalysis

Points: Points are awarded for the skills demonstrated in the laboratory, as well as successful and timely completion of study questions.

Objectives: According to the standards set by the instructor, the student will
1. perform the following physical and rapid chemical macroscopic examinations within ± 1 unit difference (± 0.003 on the refractometer)
   - Color & clarity/transparency
   - Specific gravity (by refractometer and dipstick)
   - Urine chemical concentrations of pH, glucose, ketones, leukocyte esterase, nitrite, protein and blood using Bayer Multistix, Chemstrip 10 UA or iChem 10 SG reagent strip method.
   - Protein by sulfosalicylic acid (minimum of 1)
   - Ketones by Acetest tablet method (minimum of 1)
   - Bilirubin by lctotest tablet method (minimum of 1)
   - Reducing substances by Clinitest tablet method (minimum of 1)
2. recognize abnormal urine physical properties and dipstick results.
3. record results accurately, according to the standards set by the instructor.
4. analyze quality control results to determine quality control acceptability.

Equipment and Supplies:
1. refractometer
2. Reagent strips
3. Urine controls
4. Appropriate color charts for Bayer Multistix, Chemstrip 10 UA or iChem 10 SG product.
5. Pasteur or other transfer pipets @ 5 3/4” size
6. 3% sulfosalicylic acid
7. Acetest tablets and product insert
8. lctotest tablets, absorbent pads, and product insert
9. Clinitest tablets, reaction tubes, and product insert
10. Test tubes, racks, marking pens, Kim-wipes, paper towels, distilled water, and saline solution

References:
Package inserts for Bayer Multistix, Chemstrip 10 UA or iChem 10 SG, Clinitest, Acetest, and lctotest reagents.
MLAB 1311 course textbook(s), Lecture and Lab Guides
Wikipedia and other web resources
YouTube videos

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Overview & Related Information:
- Specimen integrity.
  1. *Sample freshness.* The urine sample must be collected in a clean, dry, leakproof container. Urine samples should be handled as biohazardous and appropriate PPE (lab coat and gloves) should be routinely used. Freshly collected samples should be delivered to the laboratory and tested within 2 hours of collection or must be tightly capped and refrigerated or appropriately preserved.
  2. *Sample deterioration.* Unpreserved urine samples kept at room temperature for more than 2 hours will begin to deteriorate. Most of these changes are due to the growth of bacteria. Although urine is normally a sterile body fluid, a few bacteria may be seen due to contamination of the specimen during the voiding process. Without a means of preservation, these bacteria grow quickly utilizing available glucose and converting urea into ammonia which will increase the pH. The
increasing pH causes microscopic structures such as blood cells and urinary casts to deteriorate. Other changes that occur in the unpreserved aging urine include: darkening in the color, decreased clarity, and a decrease / loss of volatile ketones into the atmosphere. In addition bilirubin and urobilinogen levels begin to decrease, especially in samples that are exposed to light (unfortunately, this happens to frequently). Review the listing of changes occurring to unpreserved urine as listed in your textbook.

- Interfering substances.
  1. Substances that mis-color or stain the reaction pad can result in false positive readings.
  2. Vitamin C/ascorbic acid. Patients who take increased levels of vitamin C will excrete ascorbic acid in their urines. The chemical nature of ascorbic acid causes a positive reaction on Clinistest, but suppresses reactions on dipstick for glucose, blood, bilirubin, nitrite, & leukocyte esterase; therefore some dipstick manufacturers include an “ascorbic acid” test square on their dipsticks. A positive reaction on the ascorbic acid square means that the other dipstick reactions for glucose, blood, bilirubin, nitrite, & leukocyte esterase could be falsely decreased. (See ‘Sources of Error’ below for specific examples.)

Physical properties.
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.

1. Color. 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). Normal urine can be found in any of these colors, with the exception of the ‘amber’ color. Urine that is truly an amber color is most often associated with an increased bilirubin level as seen in patients with hepatitis. Review listing of abnormal urine colors provided in lecture guide and textbook - relate the abnormal colors with their probable cause(s).

2. Clarity or transparency. 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. Properly collected freshly voided urine is normally clear or sometimes slightly hazy. Urine that is contaminated is more likely to be hazy. Common sources of contamination include vaginal blood, mucous, or prostatic fluids. Freshly voided urine that is cloudy is strongly associated with bacterial urinary tract infections (UTIs) due to the presence of WBCs. Urine that is turbid contains salt crystals that precipitate out as the specimen cooled.


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

DIPSTICKS
Principles, dipstick manufacturer’s information and limitations of procedures:
The following is a brief overview including the chemical principles and applicable limitations of modern urine chemistry dipstick (Chemstrip) reactions. Refer to the specific manufacturer’s product insert for updates.

- Glucose - Although the glucose molecule is small enough to pass through the glomerular capillaries, glucose in the urine (glucosuria/glycosuria) is considered an abnormal finding. Reagent dipsticks utilize the enzyme glucose oxidase, which make the procedure specific for glucose. Sensitivity of the dipstick varies slightly depending upon the manufacturer, but most are sensitive at about 50-100 mg/dL. False positive results may occur in samples contaminated with strong oxidizing cleaning agents, such as peroxide or hypochlorite. Some literature suggests that increased levels of urobilinogen could in certain testing situations falsely increase an already positive glucose reaction. False negative results occur in samples tested when cold (enzyme reactions are slowed) as well as in samples with increased specific gravity and increased (alkaline) pH.
  
  Principle: The detection of glucose is based on the coupled enzymatic reaction of glucose oxidase and peroxidase. The reaction utilizes the enzyme glucose oxidase to catalyze the formation of gluconic acid and hydrogen peroxide from the oxidation of glucose. A second enzyme, peroxidase, catalyzes the
reaction of the hydrogen peroxide with the chromagen tetramethylbenzidine to form a green colored dye. A positive reaction is indicated by a color change from yellow to green.

Correlation with Clinitest: The Clinitest produce has neither the sensitivity nor the specificity of the urine dipstick glucose. The urine dipstick glucose is sensitive down to about 50 mg/dL (depending on specific manufacturer) and is specific for only glucose. The Clinitest product is not specific for glucose, but will detect all reducing substances. The Clinitest can detect urine glucose at levels starting at 250 mg/dL. Clinitest is most commonly used for children less than two years of age to screen for galactosemia.

- **Bilirubin** - The hemoglobin from aged red blood cells is broken down through numerous enzymatically driven steps in the liver resulting in a waste product: bilirubin. By design, conjugated (direct / water soluble) bilirubin is excreted through the bile duct into the small intestine, however very small amounts are able to escape into the circulatory system to be filtered and removed by the kidney. If the liver’s ability to function is compromised by disease such as hepatitis, increased amounts of conjugated bilirubin are found in the urine. Finding increased urine bilirubin is an early marker of obstructive and hepatic jaundice.

  *False positives* can occur if substances staining the pad are misread as a positive reaction. See the textbook for medications that cause a false positive reading. The confirmatory test, Ictotest, is recommended to confirm positive bilirubin readings, especially on samples that are deeply colored.

  *False negative* bilirubin test results are seen in specimens that have been exposed to light. Bilirubin (and the related compound, urobilinogen) are photo-sensitive and degraded by light.

  Principle: The detection of bilirubin is based on the coupling reaction of a diazonium salt with bilirubin in an acid medium. (2,6–dichlorobenzene-diazonium-tetrafluoroborate) The reaction yields a pink to red-violet color proportional to the total bilirubin concentration. NOTE: See General Limitations above; ascorbic acid suppresses reactions which can occur in samples with increased nitrite levels.

- **Ketones** - Ketone bodies (acetone, acetoacetic/diacetic acid, and B-hydroxybutyric acid) are the result of fat catabolism and not normally found in the urine specimen. Ketone bodies can become volatile and evaporate from the sample, therefore to prevent *falsely decreased* results, it is important to keep the container’s lid tight and test fresh samples.

  Principle: Based on the principle of Legal’s test; sodium nitroferricyanide and glycine react with acetoacetate and acetone in an alkaline medium to form a purple-violet dye. A positive result is indicated by a color change from beige to shades of violet. NOTE: *False positive* readings are possible if 1. the sample contains 2-Mercaptoethanesulphonate-sodium (MESNA) or other sulfhydryl- containing compound; or 2. the sample has a strong red to red-orange color (such as caused by phthalein compounds); however, these colors are usually distinguishable from those produced by the ketones. *False negatives* will result if the sample is not properly stored (tight cap and refrigerated), has aged or has been allowed to become heated.

- **Specific Gravity** - The specific gravity of a liquid is defined as the ratio of the density of the substance being measured to the density of pure water- at a specified temperature. Urine specific gravity is a measure of the amount of solutes (electrolytes, urea, etc.) present in the urine sample and indicates the concentration of the specimen. Pure (distilled/deionized) water has an assigned reference value of 1.000. The kidneys are capable of producing urine with a specific gravity as low as 1.001 or as high as 1.030, however most authors consider 1.015-1.025 to be the normal urine specific gravity range.

  Principle: In the presence of cations, protons are released by a complexing agent in the test and produce a color change. The indicator bromthymol blue changes from a blue/blue-green to yellow. NOTE: Lower specific gravity readings can be seen in samples with increased glucose and urea; increased specific gravity readings can be found in samples with increased protein levels as well as samples from patients in ketoacidosis (increased ketones resulting in decreased pH).

- **Blood** - The urine dipstick will detect RBCs, hemoglobin and myoglobin (muscle hemoglobin) all of which are not found in the normal urine specimen. *False positive* results may occur in samples contaminated with strong oxidizing cleaning agents, such as peroxide or hypochlorite. Samples with high amount of bacteria may also show an increased positive reaction due to bacterial peroxidases. Because
the reaction pad is far more sensitive to free hemoglobin, a false negative may occur in samples with well intact RBCs, but which do not contain a measurable amount of free hemoglobin. In addition, a false negative will be recorded if the urine sample was not well mixed just prior to testing because the RBCs would have settled to the bottom of the container. High levels of ascorbic acid decrease the test sensitivity as does increased specific gravity and the preservative formalin.

Principle: This test is based on the ‘peroxidase-like activity of hemoglobin or myoglobin.’ Hemoglobin and myoglobin catalyze the oxidation of the indicator by the organic peroxide contained in the test strip. Intact erythrocytes hemolyze on the test paper; the liberated hemoglobin produces a green dot. Separate color scales are given for hemoglobin and erythrocytes. Scattered or compact green dots on the yellow paper are indicative of intact erythrocytes. A uniform green color indicates free hemoglobin, hemolyzed erythrocytes, or myoglobin. NOTE: See general limitations above; ascorbic acid suppresses reaction. Very high levels of nitrite will cause a delayed reaction.

- **pH** – 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. Dipstick pH range is 4.5 - 9.0 while the kidneys are capable of producing urine in the pH range of 4.5 - 8.0. Most urine will have a pH of @ 6.0 ± 1.0.

  - If a urine sample produces a pH > 8; mishandling of the sample – such as allowing it to remain at room temperature should be first suspected. A properly collected and processed sample with this high pH is strong evidence of a severe UTI, where intra-bladder bacteria have produced ammonia.

  - Principle: This test strip contains the indicators methyl red and bromthymol blue. These give the distinguishable colors over the pH range of 5 - 9. Colors range from orange to yellow to green to blue.

- **Protein** – Of the chemical tests, protein is the most indicative of renal disease. Of the serum proteins, the albumin protein fraction is easily lost through the urine when the kidney integrity is compromised.

  - 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. (That is to say: the pH indicator demonstrates one color in the presence of protein and another in the absence of the protein, even though the pH is held constant.) Colors range from yellow for “Negative” through yellow-green, green to blue-green for “Positive” reactions.

  - **NOTE:** False positive results may be found:
    1. in strongly basic urines (pH 8.5 or higher)
    2. From residues of disinfectants containing quaternary ammonium groups or chlorohexidine are present in the urine container.
    3. following infusions of polyvinylpyrrolidone (blood substitutes)
    4. during therapy with phenazopyridine.

- **Urobilinogen** – Urobilinogen is formed in the intestine by bacterial action on the excreted bilirubin. While most of it continues through the intestinal track, a small amount is absorbed back into the circulatory system where the majority will be again removed by the liver and returned to the intestine. Normally some urobilinogen will escape the liver to be excreted by the kidney. Again, if the liver’s efficiency is reduced, increased amounts of this waste product will be seen in the urine. If, however, the flow of bile into the intestine is reduced (as in obstructive jaundice) urine urobilinogen will be decreased or absent.

  - False positives can occur if substances in the urine stain the pad and are misread as a positive reaction. See the textbook for medications that cause a false positive reading. False negatives are seen in samples that have been exposed to light. It is not possible to detect a true absence of urobilinogen.

  - Principle: Urobilinogen is coupled with 4-methoxybenzene-diazonium-tetra-fluoroborate in an acid medium to form a red azo dye. NOTE: See general limitations above; ascorbic acid suppresses reaction. Test reacts equally well with stercobilinogen, but differentiation is not of diagnostic importance. False positive readings can occur in samples collected with formalin preservative, and in samples with increased nitrite levels. Total absence of urobilinogen cannot be detected.
• **Nitrite** - Nitrite is formed in the bladder by the action of certain bacteria on urinary nitrate. If present, the nitrite reacts with an aromatic amine to give a diazonium salt, ultimately producing a red-violet azo dye. The production of any pink color is considered positive (qualitative results). NOTE: Ascorbic acid suppresses reaction.

• **Leukocytes/leukocyte esterase** - One of three tests on the urine dipstick that is often used to aid in the diagnosis of a urinary tract infection (along with nitrite and pH). Granulocytic leukocytes / neutrophils contain the enzyme ‘esterase’ which catalyzes the hydrolysis of an indoxyl acid ester to indoxyl. The indoxyl formed reacts with diazonium to produce a purple color. NOTE: Ascorbic acid suppresses reaction, drugs, medications, and high concentrations of protein and glucose may affect test results and/or color intensity. Consult product literature for other considerations.

**General handling instructions for dipsticks:**

1. Keep strips in their original containers.
2. Store strips in a cool (room temperature) dry place; with the desiccante.
3. Use strips before expiration date; & verify reactivity using QC specimens.
4. Avoid touching the pad areas.
5. Dip the strip completely in the specimen, allowing all squares to be covered - but avoid over- dipping.
6. Blot off extra urine, & keep strip level to avoid allowing the reagents to run together.
7. Read the reaction pads at the appropriate time, under appropriate light, a report accordingly.

**Sources of Error and General Limitations:**

1. Testing cold samples
2. Inadequate mixing of sample before dipping
3. Over-dipping
4. Not blotting extra sample away - failure to keep strip horizontal
5. Improper timing of tests
6. Improper reading of results due to poor light or misreading chart.
7. Very high specific gravities will suppress reactions.
8. High pH may depress color development.
9. Ascorbic acid - Ascorbic acid (vitamin C) in the urine can result in falsely low or ‘false negative’ reactions for the analyte being tested on the urinalysis test strip. Increased levels (i.e., > 25 - 50 mg/dl) of ascorbic acid will suppress the reactions of bilirubin, glucose, nitrite, leukocytes, blood and urobilinogen on the dipstick while causing a ‘false positive’ reaction on the Clinistest for reducing substances. A patient should submit urine specimens for testing after discontinuing vitamin C therapy for at least 10-12 hours for results to be valid.
10. Samples that have strong red to red-orange coloring due to medication such as phthalein compounds can produce false positive readings on any part of the test strip. In some cases a confirmatory test can be utilized to obtain more useful results.
11. Specimens collected in containers contaminated with strong oxidizing agents or those containing preservatives are unacceptable.

**URINE CONFIRMATORY TESTING**

The alternative methods for the testing urine protein, bilirubin/bile, ketones, glucose, and other reducing substances are collectively known as the urine confirmatory tests. While these tests are not routinely performed, there are instances where they may be called upon to assist in clarifying a dipstick’s result or they may be individually ordered. Explanation of when these tests would be used is provided within the following sections. During this course, each student will have several opportunities to practice these tests in preparation for competency validation.

Students are advised to review the YouTube video listed in the references, in preparation for performing these tests. Course instructor(s) will also demonstrate these tests before students perform them.
• **Protein by Sulfosalicylic Acid (SSA)** – This test is used to confirm a manually read positive protein dipstick result. The test is based on the level of turbidity formed as the result of acid precipitation of urine proteins.
  
  Approximately ½ mL of three percent sulfosalicylic acid (3% SSA) is added to an equal amount of fresh urine supernatant and observed immediately for the formation of a precipitate. If protein is present, the solution will become turbid. The degree of turbidity is graded from trace to 4+. While the protein area of the dipstick is specific for albumin, this test will react equally with all forms of protein.
  
  Reasons to use the protein back-up test:
  1. Specimen has a strong color that masks the dipstick result.
  2. Specimen has a strongly alkaline pH that results in false positive in the dipstick result.
  3. Technician has difficulty deciding between the ‘negative’ and the ‘trace’ or ‘trace’ and ‘1+’ squares on the dipstick.
  
  It is important that any other source of turbidity (i.e., crystals, mucous, cells, etc.) be removed by centrifugation; therefore this procedure must be performed on the supernatant.

• **Bilirubin by Ictotest reagent tablets** – The Ictotest is used to confirm the presence of bilirubin or bile in the urine sample. This reaction is based on the coupling of bilirubin with a unique solid diazonium salt in acid medium to give the blue or purple reaction product. The Ictotest is less subject to the effects of interfering substances and is much more sensitive than is the dipstick counterpart; making it most useful as a confirmatory test for questionable dipstick results. The Ictotest method has sensitivity from 0.05 - 1.0 mg/dL, while dipsticks are only sensitive to 0.5 mg/dL; therefore, it is an excellent choice to detect early stages of liver disease.

• **Ketones by Acetest reagent tablets** – The Acetest reagent tablet test can be used to detect and semi-quantitatively evaluate the presence and level of ketones in the urine sample. Although Acetest could be used to verify a positive ketone on a urine dipstick result, it is more likely to be used to detect ketones in serum, EDTA plasma and whole blood samples. It also can be used in determining the extent of ketosis by testing on series of dilutions.

  The Acetest reagent tablet contains sodium nitroprusside, glycine, disodium phosphate (creates a strong alkaline environment) and lactose. Two of the three ketone bodies, acetoacetic acid (majority) and acetone will form a purple colored complex with the nitroprusside in the presence of glycine. The reagent lactose enhances the color development.

  **Review of Ketones:**
  1. Acetone - Acetone makes up 2% of the ketones produced. It is volatile (meaning that it easily goes to a gas state) and is often removed by the lungs. Acetone can be detected on the dipstick and by Acetest.
  2. Diacetic Acid / Acetoacetic Acid - Diacetic acid, also frequently called Acetoacetic acid is the first of the three ketone bodies to be formed makes up 20% of the total ketone bodies and is the form most detectable by dipstick or Acetest tablet.
  3. Beta-hydroxybutyric acid makes up the majority of the ketones formed, but is not detected at all by dipstick or Acetest methodology.

• **Clinitest reagent tablets** (detection of glucose and other reducing substances) –

  The Clinitest reaction was historically used as a confirmatory test to measure the level of glucose and other reducing sugars. Because the dipstick test is more sensitive and more specific for glucose, this test is used to detect galactose in patients less than two years old.

  The Clinitest reagent tablet test is based on the classic Benedict’s copper reduction reaction (the earliest test for glucose). Copper sulfate reacts with reducing substances in urine, converting cupric sulfate to cuprous oxide. Colors range from blue through green to orange.

  **Warning:** Clinitest tablets are hydroscopic and will react with any water present; therefore they must be stored at room temperature in their tightly closed bottle and kept away from water sources. Additionally they must be handled with care to avoid contact with skin. See product insert for procedure and additional precautions.
**Action:** The Clinitest® is a chemical test that detects reducing sugars such as glucose, galactose, fructose, and pentose. Other ‘reducing substances’, such as ascorbic acid, penicillin, salicylates, and cephalosporins can also react providing a positive reaction.

**Sensitivity:** The Clinitest method can detect glucose in the urine in the range from 250 mg/dL up to 2g/dL. (Note: dipsticks are specific for glucose and are sensitive down to @ 50 mg/dL.)

When the amount of sugar is over 2 g/dL (2000 mg/dL or 2%), a “pass through” phenomenon occurs. Pass through appears as rapid color changes through green, tan, and orange, and then a reversion in color - back to brownish blue color. This reversion in color indicates levels of reducing substance greater than 2 g/dL. It is vital that you watch the reaction boiling and color changes throughout the entire reaction so that a “pass through” is not missed.

(The procedure provides direction for a “2 drop” method, as well as the “5 drop” method. If the “5 drop” method demonstrates pass through, consider repeating the procedure with the “2 drop” method.) Regardless of method, always follow the manufacturer’s directions exactly and compare the color developed to the appropriate chart.

**Purpose of Testing:** Detection of the non-glucose sugar, GALACTOSE, in infants and young children. Clinitest can also be used to detect carbohydrate reducing substances in loose stool specimens.

| Galactosemia/galactosuria: During the normal metabolism of milk sugar (lactose), galactose and glucose are produced. As the process continues, the galactose molecule is enzymatically converted to glucose. Infants with deficiencies in these enzymes will have excess galactose build up in their blood (galactosemia) and urine (galactosuria). The results of which include mental retardation, cataracts and liver disease. Early detection of the enzymatic problem and appropriate dietary modifications can prevent the devastating outcomes. Because the Clinitest reagent tablet is nonspecific and will detect reducing sugars, such as galactose, it is, recommended that all infants be tested for galactosuria using the Clinitest. |

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**Specific Gravity by Refractometer:**

Refractometry determines the concentration of dissolved particles in a specimen by measuring the refractive index. Refractive index is the comparison of the velocity of light in air with its velocity in a solution. The concentration of dissolved substances within the solution determines the velocity and angle at which the light beam passes through the solution. Review the refractometer information in your textbook. Procedure varies somewhat based on particular equipment, but basically a small drop of urine is placed on a glass prism/plate. A flap holds the urine in place. Hold the refractometer up towards an area of natural light to view a scale. The specific gravity is read from the scale at the point of contrast line (area where a boundary between light and dark) crosses the scale.
PROCEDURES
After observing the instructor’s demonstration, the student will:

1. Mix specimens well. Visually inspect and classify five (5) specimens for color and clarity. Note any unusual characteristics and record results on the lab report form provided.

2. Determine the following chemical concentrations on each of the five specimens using the reagent strips and following the procedure, as demonstrated by the instructor:
   a. Mix the specimen.
   b. Completely immerse all reagent areas of the strip.
   c. Remove the reagent strip immediately; blot off any excess urine.
   d. Keep the strip in a horizontal position to prevent possible mixing of chemicals and 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.* Record the results on the Lab Report Form.

*NOTE: Always check and follow the timing and other instructions stated by the product manufacturer.

3. Determine the specific gravity of the five (5) specimens using refractometer. Record all results on the form provided.
   a. QC checks. Checking the refractometer’s accuracy:
      1. Measure the specific gravity of a drop of deionized H2O. The reading should be 1.000 ± 0.002.
      2. Measure the specific gravity of a drop of 5% sodium chloride solution (should be 1.022 ± 0.002), OR a commercially prepared control solution (check the control product insert for range of acceptable results). If the refractometer readings of the deionized H2O and the commercial control do not match the expected values, consult the instructor for further directions.
   b. Patient samples. Measure the specific gravity of the five (5) patient specimens using the refractometer. Record the results on the laboratory report form provided.

4. Perform a sulfosalicylic acid test (SSA) for protein on each urine sample showing a positive dipstick protein. *Note: each student must do a minimum of three SSA tests. Contact instructor for specimens.
   a. Into a small test tube, pour about ½ - 1 ml (10 - 20 drops) of the supernatant obtained from 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

5. Perform an Acetest on each specimen showing a positive ketone on the dipstick using the following procedure. *Note: each student must do a minimum of one of these. Contact instructor for specimens.
    a. Place an Acetest table 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 follows:
       Negative
       1+ Small amount of color
       2+ Moderate amount of color
       3+ Large amount of color
6. If currently available, perform an Ictotest on each specimen showing a positive bilirubin on the dipstick using the following procedure. Note: each student must do a minimum of one of these. Contact instructor for specimens.
   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.

7. Clinitest (5 drop method) Perform this procedure on urine specimens of all children under the age of 2 years to check for the reducing sugar, galactose. Because the dipstick is more sensitive and specific for glucose, this procedure is NOT used to quantify urine glucose. Note: each student must do at least one Clinitest procedure. Contact instructor for specimen.
   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."
   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.
**RECORDING RESULTS**

Record all results in appropriate place. Use appropriate format for recording patient and performance control results on the report form provided. Result forms not using appropriate format will have a penalty and may be completely rejected!

- Recording of any laboratory result MUST be in black or blue ink.
  - Acceptable recording for positive results: Positive OR Pos
  - Acceptable recording for negative results: Negative OR Neg
- Other results are to be as indicated on the manufacturer's chart or by the instructor's direction.

### SAMPLE

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<tr>
<td>Glucose</td>
<td>¼ (250\text{mg/dL})</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>1+</td>
</tr>
<tr>
<td>Ketones</td>
<td>Trace (5\text{mg/dL})</td>
</tr>
<tr>
<td><strong>Specific Gravity</strong></td>
<td>1.022</td>
</tr>
<tr>
<td><strong>Blood</strong></td>
<td>Negative</td>
</tr>
<tr>
<td>pH</td>
<td>6.0</td>
</tr>
<tr>
<td>Protein</td>
<td>1+ (30\text{mg/dL})</td>
</tr>
<tr>
<td><strong>Urobilinogen</strong></td>
<td>Normal</td>
</tr>
<tr>
<td><strong>Nitrite</strong></td>
<td>Negative</td>
</tr>
<tr>
<td><strong>Leukocyte Esterase</strong></td>
<td>1+</td>
</tr>
<tr>
<td><strong>Back-up / Confirmatory Tests</strong> (performed as directed by the instructor)</td>
<td></td>
</tr>
<tr>
<td>3% SSA (for protein)</td>
<td>1+</td>
</tr>
<tr>
<td>Acetest (ketones)</td>
<td>Trace</td>
</tr>
<tr>
<td>Ictotest (bilirubin)</td>
<td>Trace</td>
</tr>
<tr>
<td>Clinitest (reducing substances)</td>
<td>N/A*</td>
</tr>
</tbody>
</table>

* The Clinitest is not warranted in this case. The dipstick is more sensitive and is specific for glucose. The patient is an adult, so no need to check for reducing substances.

**Testing performed by:**  
Instructor, MT(ASCP)  
**Date:**  
August 1, 2016
# QC Lab Report Form

**XYZ Medical Clinic**  
2243 Round Rock Road  
Austin, Texas 78701

<table>
<thead>
<tr>
<th>Control 1 Lot #</th>
<th>Control 1 Exp Date</th>
<th>Control 1 expected results</th>
<th>Control 2 Lot #</th>
<th>Control 2 Exp Date</th>
<th>Control 2 expected results</th>
<th>Within Range?</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Yes or No</td>
</tr>
</tbody>
</table>

*Within Range?  
(If No, must bring to instructor's attention and add a comment - as to course of action.)  
Whether yes or no, you must include your initials!

## Specific Gravity

<table>
<thead>
<tr>
<th>Refractometer</th>
<th>Specific Gravity</th>
<th>1.010 - 1.025</th>
<th>1.005 - 1.020</th>
</tr>
</thead>
</table>

## Multistix:

<table>
<thead>
<tr>
<th>Test</th>
<th>Lot#</th>
<th>Exp Date</th>
<th>Expected Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td></td>
<td></td>
<td>Negative 100-1000 mg/dL</td>
</tr>
<tr>
<td>Bilirubin</td>
<td></td>
<td></td>
<td>Negative Small - Large</td>
</tr>
<tr>
<td>Ketones</td>
<td></td>
<td></td>
<td>Negative 5 - 160 mg/dL</td>
</tr>
<tr>
<td>Sp. Gravity</td>
<td></td>
<td></td>
<td>1.010-1.025 1.005 - 1.020</td>
</tr>
<tr>
<td>Blood</td>
<td></td>
<td></td>
<td>Negative 10 - 200 cells/uL</td>
</tr>
<tr>
<td>pH</td>
<td></td>
<td></td>
<td>5.0-6.5 7.0 - 9.0</td>
</tr>
<tr>
<td>Protein</td>
<td></td>
<td></td>
<td>Negative Trace-&gt;= 300 mg/dL</td>
</tr>
<tr>
<td>Urobilinogen</td>
<td></td>
<td></td>
<td>0.2 mg/dL 2 - 8 mg/dL</td>
</tr>
<tr>
<td>Nitrite</td>
<td></td>
<td></td>
<td>Negative Positive</td>
</tr>
<tr>
<td>Leukocyte Esterase</td>
<td></td>
<td></td>
<td>Negative Trace - Large</td>
</tr>
</tbody>
</table>

## Confirmatory Tests

<table>
<thead>
<tr>
<th>Test</th>
<th>Control 1 Expected results</th>
<th>Control 2 Expected results</th>
<th>Comments: Within Range?</th>
</tr>
</thead>
<tbody>
<tr>
<td>3% SSA</td>
<td>Neg</td>
<td>Turbid</td>
<td></td>
</tr>
<tr>
<td>Acetest</td>
<td>Neg</td>
<td>Small-Large</td>
<td></td>
</tr>
<tr>
<td>Ictotest</td>
<td>Neg</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>Clinitest</td>
<td>Neg</td>
<td>¼ - 1%</td>
<td></td>
</tr>
</tbody>
</table>

## Controls performed by:

| Date: |  

---

*Reminders: 1. In the 'Comments' box, you must state 'Yes' or 'No' whether or not the controls have given expected results and include your initials.  
2. If at any time, a control sample does not give the expected result, you must note it under 'Comments' and bring it to the instructor's attention.
<table>
<thead>
<tr>
<th>Specimen</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Patient Name</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Patient ID #</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Physical Properties</strong></td>
<td>Color</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Transparency</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Specific Gravity</td>
<td>Refractometer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Multistix:</strong></td>
<td>Glucose</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bilirubin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ketone</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Specific Gravity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Blood</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>pH</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Protein</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Urobilinogen</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Nitrate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Leukocyte</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Confirmatory Tests</strong></td>
<td>3% SSA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(performed as directed by the instructor; record the lot#/exp date of each test performed)</td>
<td>Acetest</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Icotest</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Clinitest</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Testing performed by:** | **Date:** |
Laboratory Exercise #2: Study Questions

Student Name ______________________ Date _____________________  _____ / 35 points

Instructions: Answer the following questions using lecture notes, reading assignments, and information presented in the laboratory. Each question is worth one point unless otherwise stated. The laboratory study questions are due by the end of the following lab period, unless otherwise stated by the course instructor.

1. (5 points) According to the references, list ten (10) changes that begin to occur in an unpreserved urine specimen if it is not examined or preserved within two (2) hours of collection.
Note: Only answers that state the direction or what is happening to the analyte will receive full credit.

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
</tr>
<tr>
<td>4</td>
<td>9</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
</tr>
</tbody>
</table>

2. (3 points) What effect do high levels of ascorbic acid have on the following urinalysis tests?

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

3. (2 points) List two (2) reasons for evaluating a urine’s physical properties.

4. (3 points) List at least three (3) precautions that should be taken in handling urinalysis reagent dipsticks.

1. 
2. 
3. 

5. (2 points) Explain the principle for determining specific gravity by
   a. refractometer
   b. dipstick
6. (3 points) What three (3) urine dipstick tests are used together to support a diagnosis of a urinary tract infection (UTI)?

7. Of the routine chemical tests performed on urine, which is the most indicates the presence of a renal disease?

8. What term is used to describe glucose in the urine?

9. What can cause a false positive glucose dipstick reaction?

10. (4 points) List the 3 ketone bodies and circle the one that is NOT detected by a reaction with sodium nitroprusside (i.e., Dipstick or Acetest).

   1.
   2.
   3.

11. (3 points) List a reason for a false negative ketone reaction and two (2) reasons for false positive reactions. Put your answer in the following table.

<table>
<thead>
<tr>
<th>False negative reaction:</th>
<th>1.</th>
</tr>
</thead>
<tbody>
<tr>
<td>False positive reactions:</td>
<td>1.</td>
</tr>
<tr>
<td></td>
<td>2.</td>
</tr>
</tbody>
</table>

12. Why should the SSA (sulfosalicylic acid precipitation test) be performed on supernatant from centrifuged specimens?

13. What is the most clinically significant substance detected by the Clinitest procedure?
14. Describe the effect light has on bilirubin and urobilinogen in the urine sample.

15. Why is the bilirubin testing an important part of the routine urinalysis?

16. What are the proper storage requirements for Clinitest tablets?

17. The term “pass-through” is sometimes used when discussing Clinitest procedure. When does it occur and how can it be prevented?

18. What is myoglobin and what urine dipstix test will detect it?