

DEPMEDS LABORATORY PROCEDURES  
DEPARTMENT OF CLINICAL SUPPORT SERVICES  
U.S. ARMY MEDICAL DEPARTMENT CENTER AND SCHOOL  
FORT SAM HOUSTON, TEXAS 78234-6137

MCCS-HC

STANDARD OPERATING PROCEDURE

23 August 2002

URINALYSIS QUALITY CONTROL

1. PRINCIPLE: Quality control is necessary to ensure valid results with dipsticks and confirmatory tests. A regular quality control program will assist in the early detection and correction of problems in a routine and systematic manner.
2. REAGENTS:
  - a. Deionized water -- serves as a negative control.
  - b. Kovatrol I -- the high abnormal level plus urobilinogen.
  - c. Ames 10 Multistix SG.
  - d. Ames Check Stix Control Strips.
3. PROCEDURE:
  - a. Reconstitute Kovatrol as directed by manufacturer by adding 60 mL of deionized water to lyophilized contents of bottle. Transfer contents of urobilinogen additive to control bottle. Immediately replace rubber stopper and gently rotate until all material has dissolved (approximately 20 to 30 minutes). DO NOT SHAKE.
  - b. Obtain deionized water from approved container to serve as negative control; use only deionized water.
  - c. Return reconstituted Kovatrol to storage at 2-8°C, where it is stable for 5 days. When ready to use, remove from refrigerator, gently rotate, remove a test aliquot, and return rest of control to the refrigerator. Aliquot Kovatrol into 2-3 mL portions in screw-top tubes. Cap tightly and freeze. Urobilinogen is light sensitive; therefore, aliquot and freeze quickly. Be sure to label rack with name of control, when prepared, when expires (1 year) and your name.

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- d. Test both the control and deionized water as you would patient specimen, by dipping; read visually (manually).
- e. If expected results (see Kovatrol insert) are not obtained, make new control and repeat. Notify the supervisor if problem is not solved.
- f. Document all results on proper form. If unacceptable, circle out-of-control result, and enter repeat values.
- g. Save all slips for controls.

4. MAJOR USES:

- a. To determine if Ames Reagent Strips are reacting properly.
- b. To confirm user's ability to properly perform and interpret reagent strip tests.
- c. For demonstration and teaching.
- d. To develop proficiency in routine urinalysis.
- e. To provide confidence obtaining good results in routine urinalysis.

5. PRECAUTIONS:

- a. Store dipstick strips only in original, tightly capped bottle, according to manufacturer's instructions.
- b. Do not remove desiccant from bottle.
- c. Store Kovatrol in refrigerator and PROTECT FROM LIGHT. Light breaks down the bilirubin and urobilinogen fractions.

6. CONFIRMATORY TESTS: Perform confirmatory QC once a week. Use of Ames Check Stix control solution is recommended

- a. Each Ames Check Stix urinalysis control strip is a firm plastic strip including six separate analyte areas. Each of these contains one or more materials or synthetic ingredients which, when dissolved out of the analyte areas in a measured quantity of distilled or deionized water, provide defined results with Ames reagent strips used in urinalysis or with the back-up or confirmatory tests such as Acetest, Ictotest, Clinitest, etc. Distilled water serves as a negative control.
- b. Reagents.

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- (1) Check Stix strips.
- (2) Deionized water for negative control.
- (3) Table of expected values.
- (4) Reactive ingredients.
  - (a) Protein -- bovine serum albumin
  - (b) Glucose --glucose
  - (c) Ketone -- sodium methylacetoacetate
  - (d) Bilirubin -- crystalline bilirubin
  - (e) Blood -- bovine hemoglobin
  - (f) Nitrite -- sodium nitrate
- c. Procedure.
  - (1) Place 12 mL of distilled or deionized water in a Urin-Tek tube or 16 x 100 tube. DO NOT USE TAP WATER.
  - (2) Remove a Chek Stix control strip from bottle and replace cap firmly and tightly.
  - (3) Place strip in tube of water. Cap tightly.
  - (4) Gently invert tube back and forth for 2 minutes.
  - (5) Allow tube to stand for 30 minutes at room temperature.
  - (6) Invert one more time.
  - (7) Test the solution as you would a urine and document results. If expected results are not obtained, make new solution and repeat
  - (8) If still in error, obtain new bottle of Check Stix and retest. Notify supervisor if problem is not resolved.
- d. Clinitest.

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- (1) Use the Check Stix urine and test with Clinitest as any urine; perform once weekly.
- (2) Put 5 drops Chek Stix urine in a red top tube.
- (3) Add 10 drops of water and add Clinitest tablet.
- (4) Watch color change carefully to determine if "pass through" occurs. Shake and read 15 seconds after boiling.
- (5) Compare to Clinitest color chart; record and initial results. Expected results for each Chek Stix urine lot number appear on QC chart.

Trace	100 mg/dL
1+	250 mg/dL
2+	500 mg/dL
3+	1,000 mg/dL
4+	2,000 mg/dL

- (6) Repeat if results are not acceptable.

e. Acetest.

- (1) Remove tablet from bottle and recap promptly. Place tablet on clean, dry, white paper and crush to powder.
- (2) Put one drop of Chek Stix urine on powder and observe for purple color development in 30 seconds.
- (3) Compare to Acetest color chart; record and initial results. Expected results for each Chek Stix urine lot number appear on QC chart.

Trace	5 mg/dL
Small	15 mg/dL
Moderate	40 mg/dL
Large	80-160 mg/dL

- (4) Repeat if results are not acceptable.

f. Ictotest.

- (1) Place 10 drops of Chek Stix urine on mat.
- (2) Place Ictotest tablet on clean, moistened mat and flow 1 drop of distilled water over tablet; wait 5 seconds, then place 2nd drop of water onto tablet so that water runs off tablet onto mat.

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- (3) Wait 60 seconds and compare color reaction around the tablet to Ictotest color chart.
  - (4) Record and initial results. Expected results for each Chek Stix urine lot number appear on QC chart.
  - (5) Repeat if results are not acceptable; use new bottle of Ictotest and/or Chek Stix.
- g. Sulfosalicylic acid.
- (1) Place approximately 0.5 mL to 1.0 mL of protein check solution in small, clean glass tube. Kovatrol may be used and is more readily available.
  - (2) Add equal volume of 3% aqueous solution of sulfosalicylic acid and observe for positive reaction (cloudy or milky appearance).
  - (3) Record and initial results. The concentration of protein check solution will be indicated on bottle; expected results appear on QC chart.
  - (4) Repeat if results are not acceptable; make new protein check solution and/or sulfosalicylic acid. Kovatrol may be used as a protein check solution; see insert for expected results.

7. REFERENCES:

- a. Ames Chek Stix Control Package Insert, Miles Laboratories, Inc.
- b. Kovatrol I Package Insert, Miles Laboratories, Inc.

STAT/ROUTINE URINALYSIS

1. PRINCIPLE:

- a. Urinalysis is one of the most useful screening procedures available to the physician. The results frequently provide valuable information regarding the health or disease status of several metabolic pathways and of the genital-urinary system.
  - b. Routine urinalysis is usually performed as part of a routine physical examination, health screening physical, or initial hospital admission evaluation, and is ordered frequently throughout hospitalization.
  - c. A microscopic examination is performed if dipstick is positive. Multistix and sulfosalicylic acid (protein) are performed on all STAT/Routine specimens.
2. SPECIMEN: The specific procedures used in the collection of a urine sample depend upon the age and physical condition of the patient, in addition to the types of testing procedures the physician requests.
- a. Routine specimen.
    - (1) Most testing can be accomplished on any randomly collected, freshly voided urine specimen. Urines collected under special conditions, such as first morning or post-prandial urines, also provide useful information to the clinician.
    - (2) Nitrite results are optimized by using a first morning void specimen or one that has incubated in the bladder for 4 or more hours.
    - (3) The specimen must always be collected in a clean dry container and ideally should be examined within one hour of collection. Use of fresh urine is especially important for best results with the tests for bilirubin and urobilinogen, as these compounds are very unstable when exposed to room temperature and light.
    - (4) If urine cannot be tested within an hour after voiding, refrigerate at 2-8°C immediately and warm to room temperature before testing.
    - (5) Prolonged exposure of unpreserved urine to room temperature may result in microbial contamination with consequent changes in pH. A shift to alkaline pH may cause false-positive results with the protein test. Urines containing glucose may decrease in pH as organisms utilize the glucose. Bacteria and yeast from contaminating organisms may cause positive blood reactions from the peroxidases produced.

- (6) Preservatives do not prevent deterioration of urine ketones, bilirubin or urobilinogen.
  - (7) Urokeep and the Kingsbury-Clark (Metropolitan Life Insurance Company) urine preservative tablets do not give false-positive results.
- b. Collection.
- (1) The clean-voided midstream catch method of urine collection is a procedure utilized when the physician suspects that a patient has a urinary tract infection. This method of collecting urine requires proper cleansing of the genitalia before voiding, and collection of the urine in a clean or sterile container after the initial stream is passed. The purpose of this method is to prevent contaminating organisms from the external genitalia and distal portion of the urethra from entering the urine specimen and causing misleading results. A bacterial colony count performed on contaminated urine is worthless to the physician.
  - (2) Suprapubic aspiration: Under certain circumstances, it may be desirable or necessary to utilize the catheterization or suprapubic aspiration method of urine collection.
    - (a) Urethral catheterization involves introducing a catheter through the urethral opening so that fluids from the bladder may be withdrawn. Suprapubic aspiration is a procedure in which a needle is inserted through the abdominal wall into the bladder to aspirate urine. When appropriately performed, both of these methods of urine collection avoid contamination of the urine by organisms from the external genitalia and urethra.
    - (b) To ensure proper performance, the examiner must be careful not to introduce bacteria from a contaminated catheter or needle into the urinary tract. Catheterization should be treated as a surgical procedure performed on a limited basis, such as when a patient is unable to pass a specimen due to conditions such as obesity or severe illness. Suprapubic aspiration is a method sometimes used to collect specimens from infants and young children.
- c. Patient instructions and specimen identification.
- (1) The technician will supply patient with a nonsterile or sterile urine container as appropriate, a container label, and towelettes. Container label will include the following information:

Patient's name and last four digits of patient's social security number.

(a) Male patients will be supplied the following instructions:

- Follow these instructions carefully to avoid contaminating your urine.
- Wash your hands well, rinse, and shake-off excess water.
- Wipe your hands using one of the special towelettes provided. Then air dry.
- Using another special towelette, wash the head of your penis from front to back.
- Repeat the wash with two more towelettes.
- Start urinating, allowing the first portion of the urine fall into the toilet. Catch some urine in the specimen cup, without touching the cup to your skin or clothing. Fill cup about one-half full.
- Replace the lid on tightly on the cup and return it to the laboratory immediately.

(b) Female patients will be supplied the following instructions:

- Follow these instructions carefully to avoid contaminating your urine.
- Wash your hands well, rinse, and shake off excess water.
- Wipe your hands using one of the special towelettes provided. Then air dry.
- Spread your labia with one hand and use another antiseptic towel to wash yourself by wiping from front toward back.
- Repeat the washing with two more towelettes.
- Remain spread, start urinating, and let the first portion of the urine fall into the toilet. Catch some urine in the specimen cup, without touching the cup to your skin or clothing. Fill cup about one-half full.
- Replace the lid tightly on the cup and return it to the laboratory immediately.

NOTE: If both a Urinalysis and a Microbiology specimen are requested, label two containers 1 and 2, respectively and instruct patient to fill container #1 first and, without stopping urine stream, fill container #2.

### 3. PRINCIPLES AND CLINICAL SIGNIFICANCE OF EACH TEST.

- a. Color: The color of urine is affected by many components, e.g., concentration, food pigments, dyes, blood, medication, etc. The yellow or amber color of normal urine is due to the presence of yellow pigments, urochrome, urobilin and uroerythrin.

Record the color of the urine using one of the following terms: Colorless, straw, yellow, orange, yellow-green, amber, red, or pink.

- b. Appearance: The appearance of normal urine ranges from clear to slightly hazy. Cloudy urine may be due to a number of factors, which may or may not be pathological.

After mixing well, record appearance on lab slip using one of the following terms: Clear, Hazy or Slightly Cloudy, or Cloudy.

- c. Microscopic examination: The red and white cells may enter urine at any point from the glomerulus to the terminal urethra. Casts are cylindrical masses of protein (hyaline) and various cells (RBCs, WBCs, renal tubular epithelial cells). Casts form inside renal tubules. Crystals represent crystallization of compounds cleared into urine at the glomerulus and/or excreted by renal tubules. There are numerous causes of increased cellular elements in urine. Cellular components and casts are usually present in increased numbers in renal disease, and casts other than hyaline are often present. The presence of crystals may reflect systemic metabolic abnormalities (gout, cystinosis) or drug therapy (sulfonamides).

Precision is a problem with microscopic examinations of urine specimens. When concentrating and standardizing the aliquot of urine being screened, the probability of identifying those urine constituents that occur in low numbers will be significantly increased.

- d. Multistix SG Series: The Multistix SG (Ames) contains 10 reagents affixed to an inert plastic strip, for the determination of pH, Protein, Glucose, Ketone, Occult Blood, Bilirubin, Nitrite, Urobilinogen, Specific Gravity, and Leukocyte Esterase. Results from this disposable, dip-and-read reagent strip may provide information regarding the patient's carbohydrate metabolism, kidney and liver function, acid-base balance,

the presence of bacteria, and concentration or hydration of the urine sample.

- (1) Specific gravity. This test is based on the pKa change of certain pretreated polyelectrolytes in relation to ionic concentration. In the presence of an indicator, after 45 seconds colors range from deep blue-green in urine of low ionic concentration through green and yellow-green in urines of increasing ionic concentration. The specific gravity test permits determination of urine specific gravity between 1.000 and 1.030. In general, it correlates within 0.005 with values obtained with the refractive index method.
- (2) pH. This test is based on a double indicator principle, which gives a broad range of colors covering the entire urinary pH range. After 60 seconds, colors range from orange through yellow and green to blue.
- (3) 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" reactions through yellow-green and green to green-blue for "Positive" reactions. Quantitative results are obtained after 60 seconds. Five to 20 mg/dL of albumin may be detected as a "Trace" result. The test area is more sensitive to albumin than to globulin, hemoglobin, Bence-Jones protein, and mucoprotein; a negative result does not rule out the presence of these other proteins.
- (4) Glucose. This test is based on a double sequential enzyme reaction. One enzyme, glucose oxidase, catalyzes the formation of gluconic acid and hydrogen peroxide from the oxidation of glucose. A second enzyme, peroxidase, catalyzes the reaction of hydrogen peroxide with a potassium iodide chromogen to colors ranging from green to brown. Quantitative results are obtained with the glucose test when read at 30 seconds. The test is specific for glucose. The reagent area does not react with lactose, galactose, fructose, or reducing metabolites of drugs. Approximately 100 mg/dL of glucose is detectable. Glucosuria is typically a manifestation of diabetes mellitus. High urinary ascorbic acid (vitamin C ingestion, parenteral tetracycline containing ascorbic acid as a reducing agent), gentisic acid, or homogentisic may cause false-negative results. False-positives may occur with contaminating bleach or peroxide.
- (5) Ketones. This test is based on the development of colors ranging from buff-pink (for a negative reading) to maroon, when acetoacetic acid reacts with nitroprusside. Results are obtained after 40 seconds. Striking ketonuria is characteristic of diabetic

ketoacidosis but also occurs in many other diseases. The test does not detect B-hydroxybutyric acid, which comprises 40-70% of "ketone bodies." The reaction with acetoacetic acid is 10x that with an equal weight of acetone. Acetone is excreted predominantly through the lungs. The reaction will detect 10 mg/dL of acetoacetic acid. Only 1% of normals have detectable urine ketones; up to 20% of hospital patients are positive.

- (6) Bilirubin. This test is based on the coupling of bilirubin with diazotized dichloroaniline in a strongly acid medium. After 30 seconds, the color ranges through various shades of tan. Only conjugated bilirubin enters the urine in significant amounts. Fresh urine should be used because hydrolysis converts conjugated bilirubin to the unconjugated form upon standing. The unconjugated bilirubin reacts poorly with the test reagents. The test is somewhat less sensitive than the Ictotest but this causes few false-negatives. A positive result indicates elevated direct-acting serum bilirubin, seen in liver disease. Large doses of chlorpromazine may cause false-positive results, as with many drug metabolites which have a red color at low pHs (Pyridium and Serenium).
- (7) Blood. Hemoglobin catalyzes the oxidation of orthotolidine to a blue chromogen. Results are obtained after 60 seconds. The test is more sensitive to free hemoglobin than intact red blood cells. The test also detects myoglobin. Oxidizing contaminants such as hypochlorite or microbial peroxidases may cause false-positive results. Hemoglobinuria occurs with intravascular hemolysis. Myoglobinuria occurs with massive muscle damage, as in a crushing injury. There are numerous causes of red blood cells in the urine.
- (8) Nitrite. This test detects the conversion of nitrate to nitrite caused by certain species of bacteria in the urine, and is an indication of a bacterial infection. At the acid pH of the strip, nitrite in the patient's urine reacts with *p*-arsanilic to form a diazonium compound, which in turn couples with 1,2,3,3-tetrahydrobenzoquinolin-3-ol to produce a pink color. Results are obtained after 60 seconds. Pink spots or pink edges should not be interpreted as a positive result. Any degree of uniform pink color development should be interpreted as a positive nitrite test, suggesting the presence of 10<sup>5</sup> or more organisms per mL; but color development is not proportional to the number of bacteria present. A negative result does not in itself prove that there is no significant bacteriuria. Negative results may indicate the urine has not been retained in the bladder long enough (4 or more hours) for reduction of nitrate to nitrite to take place.

- (9) Urobilinogen. Urobilinogen is one of the major compounds produced in heme synthesis and is a normal substance in urine. The test will react with substances known to react with Ehrlich's reagent such as porphobilinogen and *p*-aminosalicylic acid. However, it is not a reliable method for detection of these substances. Drugs containing azo dyes (AcoGantrisin) may mask the color development. The absence of urobilinogen cannot be determined with this test, although the test gives quantitative results after 60 seconds and will detect levels as low as 0.1 Ehrlich units per deciliter. In a healthy population, the normal urobilinogen range with this test is 0.1 to 1.0 Ehrlich unit per dL. In patients with elevated urobilinogen excretion, urobilinogen test results correlate closely with Watson-Schwartz spectrophotometric procedures.
  - (10) Leukocyte Esterases. Granulocytic leukocytes contain esterases that catalyze the hydrolysis of the derivatized pyrrole amino acid ester to liberate 3-hydroxy-5-phenyl pyrrole; this pyrrole then reacts with a diazonium salt to produce a purple product.
- e. Sulfosalicylic acid (protein). Sulfosalicylic acid in proper concentration precipitates all proteins from solution. This test detects globulins and Bence-Jones protein, in addition to albumin, and is a necessary adjunct to dipstick methods based on the protein error of indicators, which detect predominantly albumin.

#### 4. REAGENTS AND CONTROLS:

- a. Multistix SG.
  - (1) pH -- 0.2% w/w methyl red; 2.8% w/w bromthymol blue; 97.0% w/w nonreactive ingredients.
  - (2) Specific Gravity -- 2.8% w/w bromthymol blue; 68.8% w/w poly (methyl vinyl ether/maleic anhydride); 28.4% w/w sodium hydroxide.
  - (3) Protein -- 0.3% w/w tetrabromphenol blue; 97.3% w/w buffer; 2.4% w/w nonreactive ingredients.
  - (4) Glucose -- 16.3% w/w glucose oxidase (1.3 IU); 0.6% w/w peroxidase (3300 IU); 7.0% w/w potassium iodide; 60.7% w/w buffer; 15.4% w/w nonreactive ingredients.
  - (5) Ketone -- 7.1% w/w sodium nitroprusside; 92.9% w/w buffer.
  - (6) Bilirubin -- 0.4% w/w 2, 4-dichloroaniline diazonium salt; 37.3% w/w buffer; 62.3% w/w nonreactive ingredients.

- (7) Blood -- 6.8% w/w diisopropylbenzene dihydroper-oxide; 4.0% w/w 3,3', 5,5'-tetramethylbenzidine; 48.0% w/w buffer; 41.2% w/w nonreactive ingredients.
- (8) Nitrite -- 1.4% w/w *p*-arsanilic acid; 1.3% w/w 1,2,3,4-tetrahydrobenzo(h)-quinoline-3-ol; 10.8% w/w buffer; 86.5% w/w nonreactive ingredients.
- (9) Urobilinogen -- 0.2% w/w *p*-diethylaminobenzaldehyde; 99.8% w/w buffer.
- (10) Leukocytes -- 0.4% w/w pyrrole amino acid ester; 0.2% w/w diazonium salt; 40.9% w/w buffer; 58.5% w/w nonreactive ingredients.

NOTE: Store at temperatures under 30°C, NOT in refrigerator. Do NOT use after expiration date. Do NOT remove desiccants; protection against ambient moisture, light, and heat is essential to guard against altered reagent reactivity.

- b. Sulfosalicylic acid assay -- 3 g/dL sulfosalicylic acid in 50% methanol (dissolve 3 grams sulfosalicylic acid in 1:1 mixture of distilled water and absolute methanol (or use Kovatrol solution).
- c. Kovatrol solution.
  - (1) Reconstitute bottle with 60 mL of deionized water and let stand at room temperature to dissolve. Mix well and add urobilinogen additive.
  - (2) Mix well and aliquot into plastic 12 x 75 tubes. Cap tightly and freeze.
  - (3) Test dipsticks with Kovatrol and deionized water once per shift; record results. Tolerance limits are listed on each log.
  - (4) Retests with new aliquot if acceptable limits are exceeded. Notify supervisor if unable to resolve.
- d. Check Stix control solution -- use for confirmatory/ backup methods only:
  - (1) Place 12 mL of distilled or deionized water in a centrifuge tube (16 x 100).
  - (2) Remove Stix Control Strip from bottle and place strip in water (replace container lids tightly).

- (3) Gently invert tube back and forth for 2 minutes. Allow tube to stand for 30 minutes at room temperature. Invert one more time; remove and discard Control Strip.
- (4) Test solution as you would urine; document results of confirmatory or backup tests once a week. Backup tests are Ictotest, Acetest, and Clinitest. (See Quality Control procedures.)

NOTE: This solution is stable for up to 8 hours.

5. URINALYSIS PROCEDURE:

- a. Collect fresh urine specimen in a clean, dry container. Mix well immediately before testing.
- b. Remove one strip from bottle and replace cap. Completely immerse reagent areas of the strip in fresh urine and remove immediately to avoid dissolving out reagents.
- c. While removing, run the edge of the strip against the rim of the urine container to remove excess urine. Hold the strip in a horizontal position to prevent possible mixing of chemicals from adjacent reagent areas and/or contaminating the hands with urine.
- d. Interpretation.
  - (1) If reading visually, compare reagent areas to corresponding color chart on the bottle label, at the time specified. Hold strip close to color blocks and match carefully. Avoid placing the strip directly on the color chart, as this will result in the urine soiling the chart.
  - (2) If using automation, press RUN button to enter the RUN mode.
- e. Patients under two years of age must be tested for the presence of reducing substances. (See Clinitest SOP.)

NOTE: Definitive diagnostic or therapeutic decisions should not be based on any single result or method. Drugs may affect the readability of reagent areas on urinalysis reagent strip. Under these conditions, the laboratory is obligated to conduct confirmation testing (Clinitest -- reducing substances, Acetest -- ketones, Ictotest -- bilirubin, specific gravity -- refractometer. See Confirmatory Tests at Appendices C through G.

- f. If you suspect color interference, i.e., all or most of the dipstick chemistry results are positive, urobilinogen is  $\geq 4$  EU/dL, and color is intense (amber, red, dark yellow, etc.), then perform the Acetest, Ictotest, Clinitest, and specific gravity by refractometer and report these results. Results may be invalid due to interfering substance. Perform an Acetest on all Ketone results  $\geq 80$  mg/dL and Clinitest on glucose  $\geq 1000$  mg/dL.
  
- g. Microscopic examination.
  - (1) Pour 12 mL of fresh well-mixed urine into a concentrating urine tube. Centrifuge for 5 minutes at 2500 RPM. Sulfosalicylic acid method can be accomplished at this point. Pour off supernate, saving sediment.
  - (2) Resuspend sediment by flicking the end of the tube with the forefinger or agitate with a transfer pipet and transfer drop to glass slide.
  - (3) Examine with subdued light under low power field for casts, and high power dry (40x) for other elements.
  - (4) Report number of casts/low power field, and number of RBCs and WBCs/high power field. Report bacteria as "-" to "4+". Report other elements as Negative, Slight, Moderate, and Heavy. If oval fat bodies are suspected, use polarized light for confirmation.
  - (5) Report all crystals and mucus threads as Occasional, Few, or Many.

NOTE: If there is any doubt about the exact identification of any element, contact the section supervisor or lab OIC.

NOTE: The accuracy of microscopic identification should be checked by correlation with macroscopic results, such as the presence of protein with casts and positive test for occult blood with red cells.

- (6) If all dipstick tests are negative, eliminate microscopic examination unless physician requests "micro regardless."
- (7) See Appendix A for examples of organized urinary sediment.
  
- h. Protein (sulfosalicylic acid) method.
  - (1) Pour 12 mL well-mixed urine into urine concentration tube. Centrifuge five minutes at 2500 RPM.

- (2) Layer 3% sulfosalicylic acid reagent over urine surface. Record results of turbidity as follows:

Negative	No turbidity
Trace	Faintly visible turbidity
1	Definite turbidity
2+	Heavy turbidity but no floccules
3+	Heavy cloud with floccules
4+	Heavy cloud with heavy floccules

- (3) Sediment may be used for microscopic.

6. NORMAL VALUES:

<u>TEST</u>	<u>EXPECTED VALUES</u>
Clinic-tek glucose	Neg-100 mg/dL
Bilirubin	Neg
Ketone	Neg

<u>TEST</u>	<u>EXPECTED VALUES</u>
Specific gravity	1.003-1.004 (Random sample) 1.016-1.022 (24-hr sample)
Blood	Neg
pH	5-9
Protein	Neg-trace
Urobilinogen	0.2-1.0 mg/dL
Nitrite	Neg
Leukocytes	Neg
Sulfosalicylic Acid	Neg
Protein	Neg

Microscopic:

Leukocytes	0-8 HPF
Erythrocytes	0-3 HPF
Hyaline Casts	0-2 LPF

NOTE: Urine in healthy individuals may contain small number of red cells, occasional hyaline casts, and various crystals. If there is any doubt about the exact identification of any element, contact your supervisor for confirmation or save the specimen in the refrigerator until the next day.

7. PROCEDURAL NOTES:

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- a. Refer to Ames package insert for procedural limitations.
- b. All specimens and reconstituted standards and controls should be considered as a biohazard. Appropriate precautions should be taken when working with these items (see Safety and Infection Control SOPs).

8. REFERENCES:

- a. TM 8-227-6, Jan 64.
- b. Henry, R.J., Clinical Chemistry. 5th ed., New York: Harper Row, 1988.
- c. Miller, B.E., A Text Book of Clinical Pathology. 7th ed., Baltimore: Williams and Wilkins Company, 1966.
- d. Modern Urine Chemistry, A Guide to the Diagnosis of Urinary Tract diseases and Metabolic Disorders. Ames Division, Miles Laboratories, Inc., 1978.
- e. Graff, Sister Laurine., A Handbook of Routine Urinalysis. New York: J.B. Lippincott Company, 1983.
- f. Todd and Sanford, Clinical Diagnosis by Laboratory Methods. 17th ed., Philadelphia: W. B. Saunders Company, 1984.
- g. Clinitest Package Insert, Miles Laboratories, Inc.
- h. Multistix SG Package Insert, Miles Laboratories, Inc.

## Appendix A

### Organized Urinary Sediment

- A. Erythrocytes (RBCs).

Intact Red Blood Cells. Red blood cells may be morphologically intact and have a characteristic shiny surface with a blue-green tint.

Crenated Red Blood Cells. In concentrated urine the red cells are often crenated with the margins displaying numerous sharp angles.

"Ghost" Red Blood Cells. In dilute urine specimens the red cells demonstrate the swollen transparent appearance form of the erythrocyte.

B. Leukocytes (WBCs).

White Blood Cells. Leukocytes will usually appear round to oval in shape and have a granular appearance with no nucleus. If the specimen has an alkaline pH, the WBCs may be swollen.

NOTE: In alkaline urine, red blood cells are small in size or may be absent entirely. To differentiate between erythrocytes and leukocytes, yeast cells, or contaminants, add a drop of 3% acetic acid to the sediment. Red cells, if present, will dissolve while other structures remain unaffected.

C. Epithelial Cells.

Renal Epithelial

Cells. Cells are slightly larger than leukocytes with a single round nucleus.

Transitional Epithelial

Cells. These cells are round to oval in shape, and are slightly larger than the renal epithelial cell. The nucleus is round to oval and is slightly smaller than the nucleus of the renal epithelial cell.

Squamous Epithelial

Cells. The most common type of epithelial is the squamous variety. These are large, irregularly shaped cells with small distinct nuclei.

D. Casts.

Hyaline Casts.

These casts are composed primarily of

Tamm-Horsfall mucoprotein, albumin, and some globulin that make up the basic cast structure. This is often referred to as the "hyaline matrix." The cast is almost transparent, colorless, and is slightly refractive in low light.

#### White Blood Cell

Casts. These casts are basic hyaline containing WBCs.

#### Epithelial Cell Casts.

These casts are basic hyaline casts containing epithelial cells.

#### Red Blood Cell Casts.

These casts are basic hyaline casts containing RBCs and usually exhibit a slight reddish tint. The age of the RBCs contained in this cast may cause the cells to degenerate, giving them a distorted appearance.

Coarse Granular

Casts. These are basic hyaline casts with degenerated cellular elements comprising their internal structure. They have a very "rough or grainy" appearance.

Fine Granular Casts.

These are basic hyaline casts whose internal structure is comprised of further degenerated cellular elements. These casts have a slightly less granular appearance sometimes referred to as "sand-like."

Waxy Casts.

These are basic hyaline casts that have remained in the acidic distal convoluted tubule an extended period of time. The casts appear to be fragile or brittle, more highly refractive, and homogenous in their internal structure than the granular casts.

Fatty Casts.

These are basic hyaline casts containing fat inclusions. These fatty deposits are round, highly refractile globules that vary in size. The

fatty inclusions are generally larger in size when compared to the granulation in granular casts.

Hemoglobin Casts.

These are basic hyaline casts containing free hemoglobin from degenerating RBCs. These casts demonstrate a red-orange tint due to the hemoglobin pigment.

NOTE: Use subdued light when identifying casts. Casts will dissolve in alkaline urine. It is important to analyze the sediment as soon as possible to avoid degeneration of the cast structure.

E. Miscellaneous Urinary Structures.

Mucous Threads.

These thread-like structures are long, slender, transparent strands of protein excreted by the genitourinary tract. It may be helpful for identification purposes to use lower light.

Spermatozoa.

These structures are characterized as having a small highly refractile head and a whip-like tail. When reporting the presence of spermatozoa, care should be exercised and proper laboratory protocol should be followed. Consult laboratory SOP for proper reporting procedure.

Yeast Cells. These cells resemble RBCs but usually show a characteristic hyphal or budding element. They are insoluble in 3% acetic acid and have no nucleus.

Bacteria. These structures if identified could indicate an infection or inflammation of the urinary tract. Bacteria is found as microscopic rod-shaped bacilli or round cocci. These shapes may vary due to urinary pH.

Trichomonas Species. These parasites are oval to round in shape, however, for identification purposes can ONLY be speciated by demonstrating erratic flagellar movement.

NOTE: Other parasites may be observed when performing a microscopic urinary examination. These parasites should be identified if possible and noted.

F. Normal Crystals Observed in Alkaline Urine Specimens.

Triple Phosphate. This usually rectangular crystal assumes a "coffin-lid" or "gold ingot" shape. It is colorless and sometimes in slightly acidic urine

may be observed in a "leaf-like" or "feathery fan" form.

Calcium Phosphate.

Calcium phosphate crystals are normally observed as plates exhibiting a "broken glass," refractile appearance. They may have fine, irregular granulation and are only found in an alkaline pH.

Dicalcium Phosphate.

These crystals are observed as elongated, rectangular plates, radiating from a central point. This is sometimes called the "starburst" or "rosette" form. This crystal is also colorless.

Amorphous

Phosphates. These crystals are common in alkaline urine specimens and appear as a granular precipitate with no definite shape or arrangement.

Ammonium Urates.

These crystals precipitate in the presence of free ammonia. They often appear as "fuzzy-balls," "dumbbells," or as a "thorn-apple" shape. This crystal exhibits a

yellow-green tint in normally colorless urine sediment.

Calcium Carbonate.

These crystals appear as colorless minute "dumbbell" spheres often confused with budding yeasts. The addition of 3% acetic acid to a portion of the urinary sediment will cause a bubbling effect, indicating the presence of calcium carbonate crystals.

G. Normal Crystals Observed in Acidic Urine Specimens.

Uric Acid. These yellow-brown crystals may be observed as a "football" or "diamond" shape and are often arranged in "rosette" or prism patterns.

Amorphous Urates.

These common crystals appear as a granular precipitate. They exhibit no predominate shape or arrangement, and can be differentiated from amorphous phosphates by noting the pH.

Sodium Urates.

These crystals may be observed as elongated rectangular plates radiating from a central point. This gives a "Chinese-fan" arrangement.

NOTE: Sodium urate and dicalcium phosphate crystals are similar in structure. To differentiate between these two crystals the pH of the urine specimen must also be noted.

Calcium Oxalate.

These crystals are commonly found in acid urine but may be observed in neutral or slightly alkaline urine. They are colorless, demonstrate a square boxed shape, and have a distinctive prismatic "X" in the center.

Calcium Sulfate.

These crystals, rarely observed because of their minute size, are colorless rectangular prisms.

H. Abnormal Crystals Observed in Urine Specimens

Leucine. These crystals are characterized as colorless crystals consisting of concentric circles possessing radial striations. This gives the crystal a characteristic "sea-shell" appearance.

Tyrosine. These crystals appear as very fine needle-like projections arranged in sheaves often constricted in the middle.

Cystine. These colorless crystals appear as hexagonal plates possessing well defined edges. This gives the crystal it's distinctive "stop sign" or "cheese-cracker" appearance.

Cholesterol. These crystals appear as large plates in which corner(s) are missing. This gives the crystal the distinctive "stairstep" effect.

## Appendix B

### Microscopic Urinalysis

- A. Urine microscopic analysis will only be performed on specimens that indicate a positive reaction on the Urine Chemistry Test Strips. These test include:

Bilirubin

Blood

Glucose

Ketone

Leukocytes

Nitrite

Protein

Urobilinogen

- B. Clinicians who want a microscopic on test strip negative urine must write, "Please do microscopic regardless of U/A results" on lab slip.

## Appendix C

### SPECIFIC GRAVITY USING THE REFRACTOMETER

#### (Confirmatory Test)

1. PRINCIPLE:
  - a. The volume of excreted urine and its concentration of solute is varied by the kidney to maintain hemostasis of body fluid and electrolytes. In order to achieve this, the kidneys produce urine much more concentrated than the plasma from which it is derived. The concentration of the urine varies with water and solute ingestion, the state of the tubular cells, and the influence of antidiuretic hormone (ADH) on water reabsorption in the distal tubules.
  - b. In the healthy person, ingestion of large volumes of water will produce dilute urine of large volume, and in water deprivation, the urine volume will be reduced as the kidneys conserve water. The inability to concentrate or dilute urine is an indication of renal disease or hormonal deficiency of ADH.
  - c. The refractive index is the ratio of velocity of light in air to velocity of light in solution. This ratio varies directly with the number of dissolved

particles in the urine and varies similarly with the specific gravity of urine. Therefore, the temperature- compensated refractometer may be used to measure the specific gravity of urine.

2. SPECIMEN:

The sample should be a fresh, "clean catch" urine specimen. If urine can not be examined within one hour, refrigerate specimen. Allow to come to room temperature before testing. Do not test if sample is over 8 hours old unless it is part of a 24-hour specimen, has been refrigerated, and properly preserved.

3. MATERIALS:

- a. Pasteur pipets.
- b. American Optical Company Refractometer with specific gravity and total protein scale properly calibrated (see refractometer insert). The instrument is temperature- compensated between 60-100°F.

4. QUALITY CONTROL:

- a. The calibration of the refractometer must be checked on each day of use with distilled water; should read zero.
- b. Check a solution of known specific gravity each day of use; use a 5% NaCl solution. Reading should be 1.022  $\pm$  0.001.
- c. Since specific gravity by refractometer is a backup procedure, verify function weekly (or when used) and document on QC chart.

5. PROCEDURE:

- a. Clean surface of the cover and prism with a dry, soft, nonabrasive cloth or lens paper and close the cover.
- b. Apply a drop of urine at the notched (bottom) closed portion of the cover so that it flows over the prism surface by capillary action.
- c. Point the instrument toward a fluorescent light source, rotate the eyepiece until the scale is in focus, and read, on the specific gravity scale, the sharpest dividing line between the light and dark contrast.

6. RESULTS:

a. Normal specific gravity normally remains between 1.010-1.025. Most healthy adults produce urine of a specific gravity of 1.016-1.022. First morning urines are usually more concentrated. Further interpretation requires correlation of results with the clinical status of the patient. Impaired renal function, diabetes insipidus, hypercalcemia, or hypokalemia may prevent concentration of urine even when water intake is restricted. A specific gravity of 1.023 or above in a random urine specimen indicates normal concentrating ability.

b. Low specific gravity.

Diabetes insipidus, a disease caused by the absence of, or impairment to, the normal functioning of the antidiuretic hormone, ADH. This can indicate the loss of the ability to effectively concentrate in the kidney. Specific gravity in such cases usually ranges between 1.001-1.003.

c. High specific gravity.

(1) Specific gravity is high in patients with diabetes mellitus, adrenal insufficiency, hepatic disease and congestive cardiac failure.

(2) It is also elevated in dehydration whenever there has been excessive loss of water, as with sweating, fever, vomiting, and diarrhea.

(3) Abnormally high amounts of some of the urinary constituents, in particular glucose and protein, increase the specific gravity, producing measurements up to 1.050 or more in the urine of some patients with diabetes mellitus or nephrosis.

d. Fixed specific gravity.

Urine with a fixed low specific gravity (approximately 1.010), which varies little from specimen to specimen, is known as isosthenuria. This condition is indicative of severe renal damage with disturbance of both the concentrating and diluting abilities of the kidney.

7. MAINTENANCE:

a. Perform maintenance procedures as outlined in refractometer insert and document on maintenance chart.

b. Notify supervisor if function checks do not produce expected results.

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- c. If refractometer is being used daily, perform function (calibration) checks and instrument maintenance tasks daily. If not in use, perform weekly or when used.

8.. REFERENCES:

- a. TM 8-227-6, Jan 1964.
- b. Freeman, J.A.and Beeler, M.F., Laboratory Medicine/ Urinalysis and Medical Microscopy. Philadelphia: Lea and Febiger. 2d ed., 1983.
- c. Free, A.H. and Free, H.B., Urodynamics. Ames Division, Miles Laboratories, Inc., Elkhart, Indiana, revised edition 1979.
- d. Henry, J.B., Clinical Diagnosis and Management By Laboratory Methods. Philadelphia: W. B. Saunders Co., 1991.

## Appendix D

### ICTOTEST

#### (Confirmatory Test)

#### 1. PRINCIPLE:

- a. Ictotest is a reagent tablet composed of several ingredients which is used to test for the relative amount of bilirubin in urine. The presence of bilirubin is an important finding in the evaluation of liver function. The test is based on the diazotization reaction and was first made available in 1953. Ictotest will detect as little as 0.05-0.1 mg bilirubin/dL urine; it is a rapid simple test.
- b. Chemical Principles of the Procedure: The reaction is based on the coupling of a unique solid diazonium salt with bilirubin in acid medium (sulfosalicylic acid) to give the blue or purple reaction product.

#### 2. SPECIMEN COLLECTION AND PREPARATION:

Bilirubin is rapidly decomposed once excreted, particularly in the presence of light or heat. Urine preservatives do not prevent this decomposition. Consequently, it is important that the Ictotest be used with a fresh specimen. If this is not possible, the urine should be refrigerated immediately and tested as soon as possible.

#### 3. REAGENTS AND CONTROLS:

- a. Tablets are 0.46% w/w 2,4-dichlorobenzenediazonium tetrochlorozincate, 87.45% w/w sulfosalicylic acid. 12.09% w/w nonreactive ingredients. Protection against exposure to light, heat, and ambient moisture is mandatory.

- b. Use tablets that are within expiration date on bottle and have no tan-brown discoloration.
- c. Check with known positive and negative controls weekly or when used. Log results on urine QC charts. If expected results are not obtained, discard tablets and retest with fresh sample.
- d. Avoid contact with skin, use deionized water as negative control and Ames Chek Stix urine for positive control. See Urinalysis QC procedure for Chek Stix urine.

4. PROCEDURE:

- a. Place 10 drops of urine onto center of one square of the absorbent test mat supplied with Ictotest.
- b. Remove one Ictotest reagent tablet, recap the bottle promptly, and place the tablet in the center of the moistened area. Do not touch tablet with fingers. Recap the bottle promptly.
- c. Flow one drop of water onto the tablet. Wait 5 seconds, then place second drop of water onto tablet so that the water runs off tablet onto the mat.
- d. Observe the color of the mat around the tablet at the end of 60 seconds.
  - (1) Normal urines can display a slight pink or red color.
  - (2) The presence of a blue or purple color on the mat indicates that bilirubin is present.

NOTE: See package insert for photographs, which are intended to show two typical positive test results with urine that contains different concentrations of bilirubin.

5. RESULTS:

- a. Results with Ictotest are negative if no blue or purple color develops on the mat within 60 seconds. If a blue or purple color develops on the mat within 60 seconds, the result is positive; the intensity of the color is proportional to the amount of bilirubin present. Pink or red colors should be ignored.
- b. The test will reliably detect between 0.05-0.1 mg bilirubin/dL urine, which is just above the upper limit of normal.

6. PROCEDURAL NOTES:

- a. False-positive/false-negative reactions are rare.
- b. Pyridium and Serenium metabolites give bright red-orange colors which may mask the reaction of small amounts of bilirubin. Chlorpromazine in large amounts may give a false-positive. Elevated concentrations of urobilinogen do not mask the reaction of small amounts of bilirubin. Rifampin and metabolites of the anti-inflammatory drugs Mefenamic and Flufenamic may give false-positive reactions.
- c. The tablets will not react with aspirin, salicylates, or urobilin (a breakdown product of urobilinogen).
- d. The tablets are effervescent and hygroscopic and should be protected from moisture. The diazonium will decompose in light, so store in a brown bottle. The tablets will retain usefulness even if they darken, as long as they are kept dry.

7. REFERENCES:

- a. Freeman, J.A. and Beeler, M.F., Laboratory Medicine/ Urinalysis and Medical Microscopy. Philadelphia: Lea and Febiger, 2d ed., 1983.
- b. Graff, Sister Laurine., A Handbook of Routine Urinalysis. New York: J.B. Lippincott Company, 1983.
- c. Henry, J.B., Clinical Diagnosis and Management By Laboratory Methods. Philadelphia: W. B. Saunders Co., 1991.
- d. Ictotest Package Insert, Miles Laboratories, Inc.

Appendix E

CLINITEST

(Confirmatory Test)

1. PRINCIPLE:

The N-Multistix SG glucose test is specific for glucose and does not measure other clinically significant reducing substances (lactose, galactose, fructose, homogentisic acid, maltose, pentose). In selected cases (for screening for other reducing substances in infants, or when specifically requested by a doctor) it is necessary to perform a semiquantitative test for reducing substances, e.g., the Clinitest by Ames. This test is based on the reduction of cupric ions of copper sulfate to cuprous oxide, with change of color from blue through green to orange. Sodium carbonate and citric acid act as an effervescent, causing rapid solution of reagents. Sodium hydroxide provides alkaline pH and generates heat for the reaction as it enters solution, and as it reacts with citric acid and water.

2. SAMPLE:

Specimen is any well mixed fresh urine sample. If urine cannot be tested within one hour, refrigerate at 2-8°C. Warm to room temperature before testing. All pediatric specimens will be tested with Clinitest as well as dipsticked for glucose if patient is 2 years old or younger.

3. REAGENTS AND CONTROLS:

a. Reagents.

- (1) Clinitest reagent tablets for urinalysis (Ames Division, Miles Laboratories, Inc.). Test for urine sugar for "in vitro" diagnostic use.

NOTE: Clinitest has prolonged stability in unopened container stored at room temperature. Recap bottle immediately after removing tablets. Protect tablets from light, heat and moisture; moisture causes tablets to turn a deeper shade of blue. If tablets darken or control fails to give expected results, discard tablets and get new bottle.

- (2) Test tubes.
- (3) Distilled water.
- (4) Dropper.
- b. Control.

Chek Stix urine may be prepared and used to check adequacy of Clinitest tablets. This should be done weekly or when used, and documented on QC chart. Post expected results on QC charts. Prepare Chek Stix urine by adding Chek Strip to 12 mL of distilled water in capped Uri tube; invert gently back and forth for 2 minutes, and allow to stand for 30 minutes. Use as a control for Clinitest tablets; test the same as sample. If expected results are not obtained, discard tablets, repeat test using new bottle, and document before testing patients.

- c. Reactive ingredients.

1 part copper sulfate, 12 parts sodium hydroxide, 4 parts sodium carbonate, 15 parts citric acid, plus filler and binder.

**POISON: CAUSES SEVERE BURNS.**

**WARNINGS: Contains sodium hydroxide (caustic soda). Avoid contact with skin, eyes, mucous membranes and clothing. Not for internal use. CLINITEST tablets are highly sensitive to moisture from air or water. Excessive moisture may cause chemical reaction and a bottle explosion may occur, never transfer tablets from original container.**

**FIRST AID: INTERNAL -- Do not induce vomiting. Drink large quantities of water or milk and call physician immediately.**

**EXTERNAL -- Flood with water. EYES -- flush with water for 15 minutes; get prompt medical attention.**

4. PROCEDURE:

- a. Perform Clinitest on urines of children under 2 years old, in addition to the dipstick. Perform Clinitest when "Reducing Substances" are requested.
- b. Place 5 drops urine and 10 drops water in test tube. (5-Drop Method.)

- c. Add one Clinitest tablet. WATCH REACTION! If color progresses through orange to brown during this step (pass through), greater than 2 g/dL sugar is present. Do not shake tube during boiling.
- d. Shake tube gently and compare color to color chart 15 seconds after boiling stops. Results vary from negative (blue) up to 2 g/dL (4+, orange).
- e. If "pass through" is observed in the 5-Drop Method, perform the 2-Drop Method.

- (1) Place 2 drops of urine in test tube and add 10 drops of water.
- (2) Drop one tablet into test tube. Watch while boiling is taking place but do not shake test tube during boiling or for 15 seconds after boiling has stopped.
- (3) Compare to color chart and record result.

5. RESULTS:

Clinitest should give negative results with urines from healthy subjects. Test results are obtained in percents (grams per deciliter) directly from comparison to the color chart. These results in percentages have replaced the plus (+) system formerly used at the request of the American Diabetes Association. It should be noted that urines with a sugar concentration greater than 2% will cause a very rapid color change during boiling and 15 second waiting period. This color change will "pass through" the chart colors, and when this happens, results should be reported as greater than 2% or the test should be repeated using a greater dilution, such as 2 drops urine to 10 drops of water.

6. PROCEDURAL NOTES:

- a. Tablets are stable indefinitely if stored at room temperature in unopened bottles. They are hygroscopic and absorb water after opening. Avoid exposure to heat. If tablets show dark blue discoloration in large spots or overall, discard.
- b. Sensitivity is 200 mg/dL glucose (0.2%). This test is less sensitive than the N-Multistix SG. If Clinitest is positive and N-Multistix SG negative, a nonglucose- reducing substance is present. Possibilities include galactose (galactosemia, neonatal liver disease), lactose (pregnancy, lactose intolerance), fructose (diabetes, genetic fructosuria), pentose (ingestion of certain fruits, genetic pentosuria), maltose, ascorbic acid in large quantities, and homogentisic acid (alcaptonuria); Ames claims homogentisic acid does not react with Clinitest tablets. It does react in Benedict's Reaction, which is essentially the same reaction.

- c. A number of substances found in urine, such as penicillin and salicylates, react positively with Clinitest but are usually not present in large enough amounts to interfere. Ascorbic acid, nalidixic acid, cephalosporins, and probenecid in large quantities may cause false-positive results. Metabolites of some sulfa drugs and methapyriline compounds may interfere with the 2-Drop Method at levels below 0.5 g/dL.
- d. Failure to observe the reaction at all times can lead to falsely low results for sugars if "pass through" occurs. Low specific gravity urines containing glucose may give slightly elevated results.

7. REFERENCES:

- a. Graff, Sister Laurine., A Handbook of Routine Urinalysis. New York: J.B. Lippincott Company, 1983.
- b. Henry, J.B., Clinical Diagnosis and Management By Laboratory Methods. Philadelphia: W. B. Saunders Co., 1991.
- c. Clinitest Package Insert, Miles Laboratories, Inc.

Appendix F

ACETEST

(Confirmatory Test)

1. PRINCIPLE:
  - a. Ketone bodies are the products of incomplete fat metabolism; their presence is indicative of acidosis. Ketonuria is commonly seen in uncontrolled diabetes mellitus. In children, ketonuria commonly occurs in a variety of conditions such as febrile disease and toxic states accompanied by vomiting or diarrhea.
  - b. Acetest is a reagent tablet primarily used to test for the presence of ketones (acetoacetic acid and acetone) in urine. Serum, plasma, or whole blood may also be tested.
  - c. The test is based on the formation of a colored complex (purple) between ketones in the urine and the sodium nitroprusside and glycine present in the tablet.
  
2. SPECIMEN:
  - a. Specimen is a fresh urine sample. If testing cannot be performed within one hour, refrigerate the urine sample. Do NOT use preservatives; they may affect test results.
  - b. Urines containing bromsulphalein and very high quantities of phenylketones may give false-positive results; urines preserved with 8-hydroxyquinoline may also produce high results.
  - c. L-dopa metabolites may give an atypical reaction, which could be interpreted as a false positive.
  
3. REAGENTS AND CONTROLS:
  - a. Acetest test tablets -- 1 part sodium nitroprusside, 9 parts glycine, plus buffer, filler, and binder.

- b. Clean, dry, white paper.
  - c. Droppers.
  - d. Acetest has prolonged stability in unopened container if stored at temperatures between 15-30°C. Do NOT store bottle in direct sunlight.
  - e. Once opened, stability is decreased by exposure to moisture. Recap bottle promptly.
  - f. Deterioration may be noted by a tan to brown discoloration or darkening of the tablet. Protect tablet against exposure to light, heat, and moisture.
4. QUALITY CONTROL:
- a. Proper functioning of the tablet must be verified by the use of Ames Chek Stix urine controls.
  - b. Tablets are tested weekly or when used, and results are documented on QC chart.
  - c. Deionized water is a negative control.
5. PROCEDURE:
- a. Remove tablet from bottle and recap promptly. Place tablet on clean, dry, white paper.
  - b. Put one drop of urine, serum, plasma, or whole blood directly on top of tablet.
  - c. For urine testing -- compare color of tablet to color chart at 30 seconds after application of specimen.
  - d. For serum/plasma testing -- compare color of tablet to color chart at 2 minutes after application of specimen.
  - e. For whole blood testing -- 10 minutes after application of specimen, remove clotted blood from tablet and compare color of tablet to color chart.
6. RESULTS:
- a. Results are negative if no purple color is apparent on the tablet at the appropriate reading time. Disregard any pink, tan, or yellow color.

- b. Positive results are recorded as Small, Moderate, or Large on comparison with color chart.
- d. No calculations are necessary.

7. PROCEDURAL NOTES:

- a. When a drop of urine is put onto a tablet, the drop should be absorbed within 30 seconds. If absorption takes longer than 30 seconds, the tablets are not functioning properly.
- b. Acetest will detect as little as 5 mg of acetoacetic acid/dL of urine.
- c.. The small color block corresponds to approximately 20 mg, acetoacetic acid/dL, the moderate color corresponds to 30-40 mg/dL, and the large color block corresponds to 80-100 mg/dL.

8. REFERENCES:

- a. Graff, Sister Laurine., A Handbook of Routine Urinalysis. New York: J.B. Lippincott Company, 1983.
- b. Henry, J.B., Clinical Diagnosis and Management By Laboratory Methods. Philadelphia: W. B. Saunders Co., 1991.
- c. Acetest Package Insert, Miles Laboratory, Inc.

Appendix G

URINE PROTEIN (SULFOSALICYLIC ACID METHOD)

(Confirmatory Test)

1. PRINCIPLE:
  - a. Sulfosalicylic acid in proper concentration precipitates all proteins from solution. This test detects globulins and Bence-Jones protein, in addition to albumin, and is a necessary adjunct to dipstick methods based on the protein error of indicators, which detects predominantly albumin. The amount of turbidity produced can be estimated visually.
  - b. Changes in urinary solute concentration affect the reagent strip results but not the sulfosalicylic acid (SSA) method.
2. SPECIMEN:
  - a. A freshly voided, "clean catch" urine in a clean container. If testing urine cannot be performed within one hour, refrigerate until ready to test. Bring to room temperature before testing.
  - b. If urine is cloudy or turbid, centrifuge and test clear supernatant.
3. REAGENTS AND CONTROLS:
  - a. 3 gm% sulfosalicylic acid in 50% methanol -- dissolve 3 grams sulfosalicylic acid in 1:1 mixture of distilled H<sub>2</sub>O and absolute methanol, qs to 100 mL. Solution is stable for 6 months. Store at room temperature with warning "CONTAINS ACID."
  - b. Controls.
    - (1) A control to check the adequacy of the sulfosal solution may be prepared by diluting an aliquot of assayed control or calibrator from chemistry (with a known protein content) to 300 mg% in distilled water. This should give a reading of 3+ protein with the sulfosal solution.
    - (2) Check sulfosalicylic acid solution once daily for adequacy and document on quality control chart. If 3+ reading is not obtained, discard sulfosal solution and make fresh solution, and/or control

solution. Do not release test results until expected results are obtained with sulfosalicylic acid solution.

- c. Kovatrol is used daily to check the reaction of sulfosalicylic acid. Check insert for acceptable results and document on QC chart.

4. PROCEDURE:

- a. Pour 12 mL well-mixed urine into Uri tubes or any tube for standardizing urine microscopics.
- b. Centrifuge for 5 minutes at 2500 RPM in tabletop centrifuge.
- c. If urine is alkaline or neutral, add few drops of 10% acetic acid until specimen is just acid.
- d. Layer a few drops of 3% sulfosalicylic acid reagent over surface.
- e. Record results.

Negative	No turbidity
Trace	Faintly visible turbidity
1+	Definite turbidity
2+	Heavy turbidity but no flocculus
3+	Heavy cloud with flocculation
4+	Heavy cloud with heavy flocculation

- f. If in doubt as to result, use the following confirmatory procedure.
  - (1) Fill 10 mL tube with 2.5 mL of urine specimen. Add 7.5 mL of 3% sulfosalicylic acid. Invert several times, let stand for 10 minutes; compare to rack of 8 Cargille Kingsbury-Clark standards.
  - (2) The standards represent the following concentrations of protein suspended in a gel: 5, 10, 20, 30, 40, 50, 75, and 100 mg of albumin; convert to the following reporting system.

Trace	5 and 10 mg
1+	20, 30, 40 mg
3+	50, 75, 100 mg
4+	over 300 mg

- g. Sediment may be used for microscopic examination after supernatant is decanted.

5. RESULTS:

The test is semiquantitative. Normals are negative.

6. PROCEDURAL NOTES:

- a. Be careful not to add too much sulfosalicylic acid reagent; it can contaminate sediment with sulfa crystals.
- b. X-ray contrast media and metabolites of tolbutamide (orinase) are insoluble at acid pH and may give false- positive results.
- c. False-positive tests may occur in the presence of mucin, high concentration of uric acid, high concentrations of penicillin, and *p*-aminosalicylic acid.
- d. Highly buffered alkaline urine causes false-negative results by neutralizing the acid.

7. REFERENCES:

- a. Freeman, J.A. and Beeler, M.F., Laboratory Medicine/ Urinalysis and Medical Microscopy. Philadelphia: Lea and Febiger. 2d ed., 1983.
- b. Graff, Sister Laurine., A Handbook of Routine Urinalysis. New York: J.B. Lippincott Company, 1983.
- c. Henry, J.B., Clinical Diagnosis and Management By Laboratory Methods. Philadelphia: W. B. Saunders Co., 1991.
- d. Modern Urine Chemistry, A Guide to the Diagnosis of Urinary Tract diseases and Metabolic Disorders. Ames Division, Miles Laboratories, Inc., 1978.

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