**1.0 Background Information**

The analysis of urine has two purposes. One is to detect body disturbances, such as endocrine or metabolic abnormalities, in which kidneys function normally but excrete abnormal amounts of metabolic structural elements, such as red blood cells and leukocytes, from the urinary tract. Casts from the diseased kidneys may appear in the urine as end products specific for a particular disease. The second purpose is to detect intrinsic conditions that may adversely affect the kidneys or urinary tract. Diseased kidneys cannot function normally in regulating the volume and composition of body fluids, and in maintaining homeostasis. Consequently, substances normally retained by a kidney or excreted in small amounts may appear in the urine in large quantities, or substances normally excreted may be retained. Structural elements, such as red blood cells, leukocytes, cells from the urinary tract, and casts from the diseased kidneys may appear in the urine.

The kidney is a highly discriminating organ, which maintains the internal environment by selectively excreting or retaining various substances according to specific body needs. Approximately 1,200 mL of blood flow through the kidneys each minute. This represents about one-fourth of the total blood volume. The blood enters the glomerulus of each nephron by passing through the afferent arteriole into the glomerular capillaries.

They filter through the capillary walls and the closely adhering membrane of the capillary walls in the glomerulus are highly permeable to water and the low molecular-weight components of the plasma Bowman’s capsule into Bowman’s space. From here the plasma ultra-filtrate passes into the tubule where re-absorption of the substances, secretion of others, and the concentration of urine occur.

Many components of the plasma filtrate, such as glucose, water, and amino acids, are partially or completely reabsorbed by the capillaries surrounding the proximal tubules. In the distal tubules, more water is absorbed and potassium and hydrogen ions are secreted. The loop of Henle and the system of collecting tubules are the principal sites where the urine is concentrated as a mechanism for conserving body water.

**2.0 Purpose**

The Siemens Multistix® 10SG are used as back up for the Siemens AUWI Instrument **(EMC-P)** and Clinitek Status + **(EMC-EP),** the primary system for performing urinalysis in the laboratory.

The urinalysis consists of two major components:

2.1 Macroscopic urinalysis or physiochemical testing: appearance, color, and reagent strip measurements of protein, glucose, ketone, bilirubin, specific gravity, blood, nitrite and urobilinogen.

2.2 The microscopic examination of the urinary sediment for WBCs, RBCs, crystals, casts, epithelial cells, yeast and bacteria.

**3.0 Chemical Principles of the Siemens Multistix®**

3.1 GLUCOSE: This test is based on the 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 oxidize the chromogen to colors ranging from green to brown.

3.2 BILIRUBIN: This test is based on the coupling of bilirubin with diazotized dichloroaniline in a strongly acid medium. The color ranges through various shades of tan.

3.3 KETONE: This test is based on the development of colors ranging from buff-pink, for a negative reading, to purple when acetoacetic acid reacts with nitroprusside.

3.4 SPECIFIC GRAVITY: This test is based on the apparent pKa change of certain pretreated polyelectrolytes in relation to ionic concentration. In the presence of an indicator, colors change from deep blue green in urine of low ionic concentration through green and yellow-green in urines of increasing ionic concentration.

3.5 BLOOD: The test is based on the peroxidase-like activity of hemoglobin, which catalyzes the reaction of diisopropylbenzene dihydroperoxide and 3,3’, 5,5’-tetramethylbenzidine. The results color ranges from orange through green; very high levels of blood may cause the color development to continue to blue.

3.6 pH: The test is based on the double indicator principle that gives a broad range of colors covering the entire urinary pH range. Colors range from orange through yellow and green to blue.

3.7 PROTEIN: This test is based on the protein-error-of-indicators principle. At a constant pH, the development of any green color is due to the presence of protein. Colors range from yellow for “Negative” through yellow-green and green to green-blue for “Positive” reactions.

3.8 UROBILINOGEN: This test is based on a modified Ehrlich reaction, in which p-diethylaminobenzaldehyde in conjunction with a color enhancer reacts with urobilinogen in a strongly acid medium to produce a pink-red color.

3.9 NITRITE: This test depends upon the conversion of nitrate (derived from the diet) to nitrite by the action of Gram-negative bacteria in the urine. At the acid pH of the reagent area, nitrite in the urine reacts with p-arsanilic acid to form a diazonium compound. This diazonium compound in turn couples with 1,2,3,4-tetra-hydrobenzo(h)quinolin-3-ol to produce a pink color.

3.10 LEUKOCYTES: Granulocytic leukocytes contain esterases that catalyze the hydrolysis of the derivatized pyrrole amino acid ester to liberate 3-hydroxy-5-phenly pyrrole. This pyrrole then reacts with a diazonium salt to produce a purple product.

1. **Reagent Storage**

4.1 Unused strips must remain in the original bottle. Transfer to any other container may cause reagent strips to deteriorate and become un-reactive.

* 1. Store at temperatures between 15°-30°C (59°-86°F).
  2. Do not use the strips after their expiration date.

4.4 Do not store the bottle in direct sunlight and do not remove the desiccant from the bottle.

Important Note: Protection against exposure to light, heat and ambient moisture is mandatory to guard against altered reagent reactivity.

Siemens Reagent Strips are for in vitro diagnostic use only. They have been determined to be non-hazardous under the guidelines issued by OSHA in 29 CRF 1910. 1200 (d)

1. **Specimen Collection and Handling**
   1. **Patient Preparation:** 
      1. Give patient instructions to collect a “clean catch” urine specimen in a clean and/or sterile container. Follow instructions posted in the Guide to Lab Services.
   2. **Specimen Type:**
      1. A freshly voided urine sample collected by the “clean catch” method is the specimen of choice. First morning urine yields the most meaningful results.
   3. **Specimen Rejection Criteria:**
      1. Improperly labeled specimens.
      2. Specimens containing preservatives (e.g.gray top tubes for culture)
      3. Samples older than 24 hours that have not been refrigerated will be rejected.
      4. Insufficient specimen volume.
      5. Leaking or frozen samples
      6. Specimens containing gross contamination with fecal material.
      7. When a specimen is unacceptable, notify the ordering physician or unit with the reason. Request recollect if possible. Document who was notified along with time/date and reason for cancellation in the LIS.
   4. **Specimen Handling/Transport:**
      1. Specimens should be delivered to the laboratory as soon as possible after collection preferably within 2 hours of collection.
      2. Do not add disinfectant or detergent to the specimen.
   5. **Specimen Stability:**
      1. Urines, kept at room temperature, are stable for several hours. Analysis should be made within 4 hours of collection.
      2. If the specimen is not processed within 2 hours after collection, cap the container tightly and store at 2 - 8° C. Refrigeration may cause a change in pH and the formation of crystals in specimens.
      3. Specimens must be at or brought to room temperature before analysis.

## 6.0 Instrumentation

* 1. Refractometer
  2. Microscope

6.3 Centrifuge

## 7.0 Quality Control

Negative and positive urinalysis controls are performed once per 24 hours by the day shift using Siemens Multistix® 10SG on form **UA01-004 Form A3 (EMCP) and form UA01-004 Form C (EMC-EP).** Specific gravity controls of specified value are run by the day shift. Control limits are defined in the LIS and will flag all out of range QC. All corrective action must be documented in the appropriate log.

## 8.0 Procedure for Performing Manual Urinalysis

8.1 COLOR: Examine urine specimen for color. The color is usually light straw to dark amber. Dilute urines tend to be lighter in color while concentrated specimens appear darker. Report findings as: straw, yellow, amber or bloody.

8.2 APPEARANCE: Examine the urine for clarity. Report as: clear, slightly hazy, cloudy or turbid

* 1. DIPSTICK CHEMISTRIES

8.3.1 Collect fresh urine specimen in a clean, dry container. Mix well immediately before testing.

8.3.2 Remove one strip from the bottle and replace the cap tightly. Do not touch the test areas of the strip.

8.3.3 Completely immerse reagent areas of the strip in fresh urine and remove immediately to avoid dissolving out the reagents. Start timing.

8.3.4 While removing, run the edge of the strip against the rim of the urine container to remove the excess urine. Hold the strip in a horizontal position to prevent possible mixing of chemicals from adjacent areas and/or contaminating the hands (gloves) with urine.

8.3.5 Compare reagent areas to corresponding Color Chart on the bottle label at the appropriate time interval starting with the shortest time. Hold the strip close to the color blocks and match carefully.

8.3.6 Read the pads in good light. Do not read any test pad after 2 minutes: color changes that occur after this time are of no diagnostic value.

* 1. MANUAL MICROSCOPIC

8.4.1 Pour 10 to 12 mL of thoroughly mixed urine sample into a tube and centrifuge for 5 minutes at 1400 rpm.

8.4.2 Pour off the supernatant and re-suspend the sediment in 1 mL of the residual fluid.

8.4.3 If stain is desired add 1 to 2 drops of Sedi-Stain. Mix the sediment by slight agitation.

8.4.4 Place a drop of the sediment suspension on a KOVA slide or use a clean slide and cover with a coverslip.

8.4.5 Using reduced light, examine under low power (10x objective) for epithelial cells, mucous and casts.

8.4.6 Turn the magnification to high dry (40x objective) increasing the light and examine for leukocytes, erythrocytes, bacteria, crystals, yeast and *Trichomonas vaginalis.*

* 1. URINE MICROSCOPIC RECORDING

8.5.1 Casts are reported under low power. Identify and count the number per low power field (e.g. 2-4 hyaline casts/lpf).

8.5.2 WBC’s and RBC’s are counted and reported per high power field (hpf).

8.5.3 Crystals are identified and reported as few, occasional, moderate and many per hpf.

8.5.4 Epithelial cells are reported as the number seen per high power field.

8.5.5 Yeast are reported as few, occasional, moderate or many per hpf.

8.5.6 Trichomonas is reported as few, occasional, moderate and many per hpf.

8.5.7 Bacteria is reported as few, occasional, moderate and many per hpf.

8.6 CONFIRMATORY TESTING

Perform confirmatory testing on the following (refer to specific procedure for instructions)

8.6.1 Refractometer: Used when the AUWI is not operational or when there is insufficient sample for the AUWI specific gravity. Siemens Multistix should not be read manually by the human eye for specific gravity. Use the refractometer for accurate measurement.

8.6.2 Any parameter that might be affected by a color interference should be footnoted with the canned comment abbreviation “COLOR” which will explode out to state “Urine color may cause interference with Chemistry dipstick readings.” (i.e. urine protein and urine bilirubin)

# 9.0 Reference Range

## Chemistry Results

Specific Gravity 1.001-1.035

pH 5.0-9.0

Leukocyte Esterase Negative

Nitrite Negative

Protein, Qualitative Negative

Glucose Negative

Ketones Negative

Urobilinogen 0.1-1.0 mg/dL

Bilirubin Negative

Blood Negative

Color Clear

Clarity Clear

## Microscopy Results

WBC 0-8/HPF

RBC 0-3/HPF

Bacteria Negative

Epithelial Cells:

Squamous Epi’s 0-3/HPF

Transitional Epi’s 0-3/HPF

Renal Epi 0-5/HPF

# Casts:

Hyaline Casts 0-1 /LPF

Broad Casts 0/LPF

Granular Casts 0/LPF

Cellular Casts 0/LPF

WBC Cast 0/LPF

RBC Cast 0/LPF

Waxy Cast 0/LPF

Fatty Cast 0/LPF

Epi Cell Cast 0/LPF

# Crystals:

Calcium Oxalate Cry. none

Amorphous Crystals none

Uric Acid Crystals none

Triple Phosphate Cry. none

Calcium Carbonate Cry. none

Calcium Phosphate Cry. none

Leucine Crystals none

Cystine Crystals none

Tyrosine Crystals none

**Miscellaneous Particles**

Yeast none

WBC Clumps none

Oval Fat Body none

Trichomonas none

Sperm

Mucus

**10.0 Critical Values:** None

**11.0 Calculations:** None

**12.0 Record of results:** Patient results will be recorded using UA01-004 Form B1

**13.0 Expected Values**

Expected values for the typical “normal” urine healthy population are listed below for each reagent.

13.1 COLOR: Normal urine varies widely from colorless to deep yellow. Interpretation is subjective.

Pathologic disease states (renal and metabolic disorders) are associated with abnormal urine colorations. Blood will appear as pink, red or brown depending on the quantity and preservation of the urine. Conjugated bilirubin will produce a yellow foamy urine sample. A pink urine will occur in the presence of excess myoglobin.

13.2 APPEARANCE: Normally freshly voided urine is clear. When urine is allowed to stand, amorphous crystals usually phosphates may precipitate and cause urine to be cloudy. Cloudiness due to phosphates and urates is normal. Cellular material such as leukocytes, large quantities of epithelial cells and mucous strands will also produce cloudiness.

13.3 SPECIFIC GRAVITY: Random urines may vary in specific gravity from 1.003 to 1.040 or higher. Normal adults with normal diets and normal fluid intake will produce urine specific gravity between 1.016 and 1.022 during a twenty-four period. Urine specific gravity may increase in dehydration or uncontrolled diabetes. In renal tubular damage, the concentrating function is frequently lost, resulting in a low specific gravity.

13.4 pH: Both the normal and abnormal urinary pH range is from 5.0 – 9.0.

Acidity (decreased pH) is noted in patients with severe diarrhea, dehydration or fever. Patients with renal tubular acidosis are not able to excrete a very acid urine causing an excess of acid to build in the body resulting in acidosis. High (alkaline) pH may be caused by acute chronic renal disease, severe vomiting or respiratory alkalosis. After a meal urine may be alkaline.

13.5 PROTEIN: Normally no protein is detectable in urine, although the normal kidney excretes a minute amount. A color matching any block greater than a trace indicates significant proteinuria. For a urine of high specific gravity, the protein test block may match the trace color block even if the concentra-tion of protein is less than trace. Clinical judgment is needed to evaluate the significance of trace results. Increased amount of protein in urine is most often one of the first indications of renal disease.

13.6 GLUCOSE: The kidney normally excretes small amounts of glucose. These amounts are usually below the sensitivity of this test but on occasion may produce a color between the negative and the 100 mg/dL color blocks. Results of 100 mg/dL may be clinically significant if found consistently. The appearance of glucose in urine occurs in diabetes mellitus or it may also occur in a benign condition called renal glucosuria. Glucose may also be present in hemachromatosis, glycogen storage disease, obesity as well as the result of drug therapy such a thiozides and corticosteroids.

13.7 KETONE: Normal urine specimens ordinarily yield negative results with this reagent. Detectable levels of ketone may occur in urine during physiological stress conditions such as fasting, pregnancy and frequent strenuous exercise. In keto-acidosis, starvation or with other abnormalities of carbohydrate or lipid metabolism, ketones may appear in urine in large amounts before serum ketone is elevated. Ketonuria can occur in individuals on fasting or starvation diets. It frequently occurs in children in a condition with a febrile state and the child is not eating and/or vomiting. Ketone bodies may occur with carbohydrate metabolism abnormalities, such as diabetes mellitus. Since this test only determines acetone and acetoacid, conditions like diabetic acidosis that produce beta-hydroxybutyric acid will not be detected with this test.

13.8 BILIRUBIN: Normally no bilirubin is detectable in urine by even the most sensitive methods. Even trace amounts of bilirubin are sufficiently abnormal to require further investigation. Atypical colors which are unlike the negative or positive color blocks shown on the color chart may indicate that bilirubin derived bile pigments are present in the urine sample and may be masking the bilirubin reaction. These colors may indicate bile pigment abnormalities and the urine specimen should be tested further with the ICTOTEST.

13.9 BLOOD: The significance of the trace reaction may vary among patients and clinical judgment is required for assessment in an individual case. Development of green spots (intact RBC’s) or a green color on the reagent area within 60 seconds indicates the need for further investigation. This test is highly sensitive to hemoglobin (it is slightly less so to intact RBC’s) and thus complements the microscopic examination. Hematuria occurs in patients with urinary tract bleeding and is also found in patients with renal disease.

13.10 NITRITE: Normally no nitrite is detectable in urine. The proportion of positive nitrite tests in cases of significant infection depends on how long the urine was retained in the bladder prior to collection. Identification of known positive cases with the nitrite test ranges from as low as 40% when little bladder incubation occurred, to as high as approximately 80%, when a minimum of four hours of bladder incubation occurred.

13.11 UROBILINOGEN: In a healthy population, the normal urobilinogen range with this test is 0.1 – 1.0 Ehrlich unit per dL. Elevated results can occur in liver disease. Increased amounts are also noted in hemolytic disease. In patients with biliary duct obstruction, urobilinogen may be reduced or absent from the urine.

13.12 MICROSCOPIC: The appearance of excess cellular elements such as WBCs and RBCs usually indicates some disease of the renal tract such as cystitis. Large quantities of casts indicate a disruption of the renal glomerular system.

**14.0 Limitations**

Limitations given for the reagents include specific substances and conditions that may affect the test results. As with all laboratory tests, definitive diagnostic or therapeutic decisions should not be based on any single result or method.

Substances that cause abnormal urine color may affect the readability of test pads on urinalysis reagent strips. These substances include visible levels of blood or bilirubin and drugs containing dyes (e.g., Pyridium, Azo Gantrisin, Azo Gantanol), nitrofurantoin (Macrodantin, Furadantin), or riboflavin. Levels of ascorbic acid normally found in urine do not interfere with these tests.

14.1 SPECIFIC GRAVITY: The Siemens SG test is dependent on ions in urine and results may differ from those obtained with other specific gravity methods when certain nonionic urine constituents, such as glucose, are present. Highly buffered alkaline urines may cause low readings, while the presence of moderate quantities of protein (100-750 mg/dL) may cause elevated readings.

14.2 pH: If proper procedure is not followed and excess urine remains on the strip, a phenomenon known as “runover” may occur in which the acid buffer from the protein reagent will run onto the pH area, causing a false lowering in the pH. Bacterial growth by certain organisms in a specimen may cause a marked alkaline shift (pH >8.0), usually because of urea conversion to ammonia.

14.3 PROTEIN: False positive results may be obtained with highly buffered or alkaline urines. Contamination of the urine specimen with quanternary ammonium compounds (some antiseptics and detergents) or with skin cleansers containing chlorhexidine may also produce false positive results. A visibly bloody urine may cause falsely elevated results.

14.4 BLOOD: Elevated specific gravity or elevated protein may reduce the reactivity of the blood test. Certain oxidizing contaminants, such as hypochlorite, may produce false positives. Microbial peroxidase associated with urinary tract infection may cause false positives. Ascorbic acid concentrations of 5 mg/dL or greater may cause false negatives at the trace level.

14.5 GLUCOSE: Ascorbic acid concentrations of 50 mg/dL or greater may cause false negatives for specimens containing small amount of glucose (100 mg/dL). Ketone bodies reduce the sensitivity of the test; moderately high ketone levels (40 mg/dL) may cause false negative results for specimens containing small amounts of glucose (75-100 mg/dL) but the combination of such ketone levels and low glucose levels is metabolically improbable in screening. The reactivity of the glucose test decreases as the specific gravity of the urine increases.

14.6 KETONE: False Trace results may occur with highly pigmented urine specimens or those containing large amounts of levodopa metabolites. Compounds such as mesna (2-mercaptoethane sulfonic acid) that contain sulfhydryl groups may cause false positive results or an atypical color reaction.

14.7 LEUKOCYTES: Elevated glucose concentrations (≥3 g/dL) may cause decreased test results. The presence of cephalexin (Keflex), cepalothin (Keflin), or high concentrations of oxalic acid may also cause decreased test results. Tetracycline may cause decreased reactivity, and high levels of the drug may cause a false negative reaction. Positive results may occasionally be due to contamination of the specimen by vaginal discharge.

14.8 NITIRITE: Pink spots or pink edges should not be interpreted as a positive result. Any degree of uniform color development should be interpreted as a positive nitrite test suggesting the presence of 5-10 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 bacteruria. Negative results may occur when urinary trace infections are caused by organisms that do not contain reductase to convert nitrate to nitrite: when urine has not been in the bladder long enough (four or more hours) for reduction of nitrate to nitrite to occur; or when dietary nitrate is absent, even if organisms containing reductase are present and organism containing reductase are present and bladder incubation is ample. Sensitivity of the nitrite test is reduced for urines with high specific gravity. Ascorbic acid concentrations of 25 mg/dL or greater may cause false negatives results with specimens containing nitrite ion concentrations of 0.03 mg/dL or less.

14.9 BILIRUBIN: Indican (indoxyl sulfate) can produce a yellow-orange to red color response that may interfere with the interpretation of a negative or a positive bilirubin reading. Metabolites of Lodine (etodolac) may cause false positive or atypical results. Atypical colors (colors that are unlike the negative or positive color blocks shown on the Color Chart) may indicate that bilirubin-derived bile pigments are present in the urine sample and may be masking the bilirubin reaction. These colors may indicate bile pigment abnormalities and the urine specimen should be tested further (e.g., Ictotest Reagent Tablets). Ascorbic acid concentrations of 25 mg/dL or greater may cause false negatives. Since very small amounts of bilirubin may be found in the earliest stages of liver disease, the user must consider whether the sensitivity of Siemens Multistix to bilirubin is sufficient for the intended use. When very small amounts of bilirubin in urine are to be detected the Ictotest should be used.

14.10 UROBILINOGEN: The reagent area will react with interfering substances known to react with Erlich’s reagent, such a p-amino salicylic acid and sulfonamides. Atypical color reactions may be obtained in the presence of high concentrations of p-aminobrnzoic acid. False negative results may be obtained if formalin is present. Strip reactivity increases with temperature: the optimum temperature is 22º-26ºC (72º-79ºF). The test is not a reliable method for the detection of porphobilinogen. Drugs containing azo dyes (Azo Gantrisin) may give a masking golden color. The absence of urobilinogen cannot be determined with this test.

**15.0 Sensitivity**

Sensitivities listed for each reagent are the generally detectable levels of the analytes in contrived urines; however, because of the inherent variability of clinical urines, lesser concentrations may be detected under certain conditions. The percentage of clinical specimens correctly detected as positive increases with analyte concentration.

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| **Analyte** | **Sensitivity** |
| Protein | 15-30 md/dL albumin |
| Blood | 0.015-0.062 mg/dL hemoglobin |
| Leukocytes | 5-15 white blood cells/hpf in clinical urine |
| Nitrite | 0.06-0.1 mg/dL nitrite ion |
| Glucose | 75-125 mg/dL glucose |
| Ketone | 5-10 mg/dL acetoacetic acid |
| pH | 5.0 |
| Specific Gravity | n/a |
| Bilirubin | 0.4-0.8 mg/dL bilirubin |
| Urobilinogen | n/a |

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**Approval Signatures:**

|  |  |  |
| --- | --- | --- |
| **Date** | **Printed Name** | **Signature** |
| 11/21/2016 | Jennifer Lore, MFS, MT  Chemistry Supervisor |  |
| 11/21/2016 | Vanessa Rawlings, MHA, MT  Laboratory Supervisor Elkins Park |  |
| 11/21/2016 | Nancy A. Young, M.D., FCAP  Medical Director |  |

**History Review**

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| **Date Reviewed** | **Reviewed By** | **Revisions** |
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