**URINE TESTING USING THE MULTISTICK**

<table>
<thead>
<tr>
<th>PREPARED BY</th>
<th>DATE ADOPTED</th>
<th>SUPERSEDES PROCEDURE #</th>
</tr>
</thead>
<tbody>
<tr>
<td>Janet M. Swaim</td>
<td>2/2004</td>
<td>#2</td>
</tr>
</tbody>
</table>

**DISTRIBUTED TO**

<table>
<thead>
<tr>
<th>DISTRIBUTED TO</th>
<th># COPIES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Torresdale Lab</td>
<td>1</td>
</tr>
<tr>
<td>Hospital Intranet</td>
<td>1</td>
</tr>
</tbody>
</table>
PURPOSE:

Test results provide information regarding the status of carbohydrate metabolism, kidney and liver function, acid-base balance, and urinary tract infection.

PRINCIPLE:

**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 oxidize the chromogen to colors ranging from green to brown.

**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.

**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.

**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 range from deep blue-green in urine of low ionic concentration through green and yellow-green in urines of increasing ionic concentration.

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

**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.

**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.

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

**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-tetrahydrobenzo(h)quinolin-3-ol to produce a pink color.

**Leukocytes:** 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.
SPECIMEN:

**Patient Preparation:**
Urine is tested within an hour after voiding.

**Type:**
Random urine is acceptable.

Nitrite test results are optimized by using a first morning specimen or one that has incubated in the bladder for four hours or more.

| Optimal Amount – 10 ml | Minimum Amount – 1 ml |

**Handling Conditions:**
Collect urine in a clean container and test it as soon as possible. Do not centrifuge. The use of urine preservatives is not recommended. If testing cannot be done within an hour after voiding, refrigerate the specimen immediately and let it return to room temperature before testing.

It is especially important to use fresh urine to obtain optimal results with the tests for bilirubin and urobilinogen, as these compounds are very unstable when exposed to room temperature and light.

Prolonged exposure of urine to room temperature may result in microbial proliferation with resultant changes in pH. A shift to alkaline pH may cause false positive results with the protein test area. Urine containing glucose may decrease in pH as organisms metabolize the glucose. Bacterial growth from contaminating organisms may cause false positive blood reactions from the peroxidases produced. In random urine specimens from females, a positive result for leukocytes may be due to a source external to the urinary tract.

**EQUIPMENT AND MATERIALS**

**Materials:**
Bayer Reagent Strips for urinalysis are firm plastic strips to which are affixed several separate reagent areas.

**Glucose:** 2.2% w/w glucose oxidase (microbial, 1.3 IU); 1.0% w/w peroxidase (horseradish, 3300 IU); 8.1% w/w potassium iodide; 69.8% w/w buffer; 18.9% w/w non-reactive ingredients.

**Bilirubin:** 0.4% w/w 2,4-dichloroaniline diazonium salt; 37.3% w/w buffer; 62.3% w/w non-reactive ingredients.

**Ketone:** 7.1% w/w sodium nitroprusside; 92.9% w/w buffer.

**Specific Gravity:** 2.8% w/w bromthymol blue; 68.8% w/w poly (methyl vinyl ether/maleic anhydride); 28.4% w/w sodium hydroxide.

**Blood:** 6.8% w/w diisopropylbenzene dihydroperoxide; 4.0% w/w 3,3',5,5'-tetramethylbenzidine; 48.0% w/w buffer; 41.2% w/w non-reactive ingredients.

**pH:** 0.2% w/w methyl red; 2.8% w/w bromthymol blue; 97.0% w/w non-reactive ingredients.

**Protein:** 0.3% w/w tetrabromophenol blue; 97.3% w/w buffer; 2.4% w/w non-reactive ingredients.
Urobilinogen: 0.2% w/w p-diethylaminobenzaldehyde; 99.8% w/w non-reactive ingredients.

Nitrite: 1.4% w/w p-arsanilic acid; 1.3% w/w 1,2,3,4-tetrahydrobenzo(h)-quinolin-3-ol; 10.8% w/w buffer; 86.5% w/w non-reactive ingredients.

Leukocytes: 0.4% w/w derivatized pyrrole amino acid ester; 0.2% w/w diazonium salt; 40.9% w/w buffer; 58.5% w/w non-reactive ingredients.

Preparation/Patient Identification:
Patient is identified by asking them to state their name and date of birth. This information is placed on the urine container prior to specimen collection.

Performance Parameters:
Test strips must meet quality control requirements prior to use.

Storage Requirements:
Store at room temperature between 15-30°C (59-86°F). Do no use product after expiration date. Do not store the bottle in direct sunlight.

All unused strips must remain in the original bottle. Transfer to any other container may cause reagent strips to deteriorate and become unreactive. Do not remove desiccant(s) from bottle. Do not remove strip from the bottle until immediately before it is to be used for testing. Replace cap immediately and tightly after removing reagent strip. Do not touch test areas of the reagent strip. Work areas and specimen containers should be free of detergents and other contaminating substances.

CALIBRATION:
None required.

QUALITY CONTROL:
1. Quality control consists of two levels.
2. Quality control must be performed everyday of testing and whenever a new vial is opened. Results of quality control material must be in range prior to patient testing.
3. If quality control is out of range, repeat testing on new strip.
4. If within range, record and proceed with patient testing. If out of range, open a new vial of strips and repeat both levels. If within range, record, discard vial of strips that failed quality control and proceed with patient testing. If out of range, open a new set of quality control material and repeat testing. If still out of range, contact manufacturer for replacement strips and the Point of Care Coordinator. Forward all urine samples to the central lab for processing.

PROCEDURE – STEPWISE:
1. Collect FRESH urine specimen in a clean, dry container. Mix well immediately before testing.
2. 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.
3. While removing, run the edge of the entire length 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.
4. 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 laying the strip directly on the Color Chart, as this will result in the urine soiling the chart.

Proper read time is critical for optimal results. Read the glucose and bilirubin test at 30 seconds after dipping. Read the ketone test at 40 seconds; the specific gravity test at 45 seconds; pH protein, urobilinogen, blood, and nitrite at 60 seconds; and leukocytes at 2 minutes. The pH and protein areas may also be read immediately or at any time up to 2 minutes after dipping.

After dipping the strip, check the pH area. If the color on the pad is not uniform, read the reagent area immediately, comparing the darkest color to the appropriate Color Chart. All reagent areas except leukocytes may be read between 1 and 2 minutes for identifying negative specimens and for determination of the pH and SG. A positive reaction (small or greater) at less than 2 minutes on the leukocyte test may be regarded as a positive indication of leukocytes in urine. Color changes that occur after 2 minutes are of no diagnostic value.

**CALCULATIONS:**
None required.

**REPORTING RESULTS:**
All results are recorded on the point of care testing form. Prior to separating the two part form, the operator must double check recorded results against the visual results. All results are reviewed within 24 hours by the nurse manager or designee and forwarded to the point of care coordinator.

**Reference Ranges:**

**Specific Gravity:**
- Newborn 1.012
- Infant 1.002 – 1.006
- Adult 1.002 – 1.030

**pH:**
5.0 – 9.0

**Blood:**
- Erythrocyte excretion up to 5 RBC/uL may be expected in normal urine.
- Normal urine should produce no color reaction and is reported as negative.

**Bilirubin:**
- In normal urine, bilirubin should not be detectable with this test. However, the test is very sensitive to bilirubin (0.5 mg/dl will produce positive results) and any positive reaction indicates that further diagnostic evaluation of the patient is needed.

**Urobilinogen:**
- Values up to 1 mg/dl are usually considered normal.

**Leukocyte, Protein, Nitrate, glucose and Ketones:**
- Normal urine should produce no color reaction and is reported as negative.

**LIMITATIONS OF THE PROCEDURE:**
Substances that cause abnormal urine color, such as drugs containing axo dyes (e.g., Pyridium®, Azo Gantrisin®, Azo Gantanol®), nitrofurantoin (Macrodantin®, Furadantin®), and riboflavin, may affect
the readability of the reagent areas on urinalysis reagent strips. The color development on the reagent pad may be masked, or a color reaction may be produced on the pad that could be interpreted visually as a false positive.

**Glucose:** Ascorbic acid concentrations of 50 mg/dL or greater may cause false negatives for specimens containing small amounts of glucose (75-125 mg/dL). Ketone bodies reduce the sensitivity of the test; moderately high ketone levels (40 mg/dL).

**Bilirubin:** Indican (indoxyl sulfate) can produce a yellow-orange to red color response that may interfere with the interpretation of a negative or positive bilirubin reading. Metabolites of Lodine (etodolac) may cause false positive or atypical results; 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 phases of liver disease, the user must consider whether the sensitivity of Bayer Reagent Strips to bilirubin is sufficient for the intended use. When very small amounts of bilirubin in urine are sought (e.g., earliest phase of viral hepatitis), ICOTEST Reagent Tablets should be the method of choice.

**Ketone:** False positive results (Trace or less) 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.

**Specific Gravity:** The chemical nature of the Bayer SG test may cause slightly different results from those obtained with other specific gravity methods when elevated amounts of certain urine constituents are present. Highly buffered alkaline urines may cause low readings relative to other methods. Elevated specific gravity readings may be obtained in the presence of moderate quantities (100-750 mg/dL) of protein.

**Blood:** Elevated specific gravity may reduce the reactivity of the blood test. Capoten (captopril) may also cause decreased reactivity. Certain oxidizing contaminants, such as hypochlorite, may produce false positive results. Microbial peroxidase associated with urinary tract infection may cause a false positive reaction. Levels of ascorbic acid normally found in urine do not interfere with this test.

**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 of the pH result.

**Protein:** False positive results may be obtained with highly buffered or alkaline urines. Contamination of the urine specimen with quaternary ammonium compounds (e.g., from some antiseptics and detergents) or with skin cleansers containing chlorhexidine may also produce false positive results.

**Urobilinogen:** The reagent area may react with interfering substances known to react with Ehrlich’s reagent, such as p-aminosalicylic acid and sulfonamides. Atypical color reactions may be obtained if formalin is present. Strip reactivity increases with temperature; the optimum temperature is 22-26º C. The test is not a reliable method for the detection of porphobilinogen. The absence of urobilinogen cannot be determined with this test.

**Nitrite:** 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 result suggesting the presence of $10^5$ 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 not significant bacteriuria. Negative results may occur when urinary tract infections are caused by organisms that do not contain reductase to convert
nitrate to nitrite; when urine has not been retained in the bladder long enough (four hours or more) for reduction of nitrate to nitrite to occur; or when dietary nitrate is absent, even if organisms 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 negative results with specimens containing small amounts of nitrite ion (0.06 mg/dL or less).

**Leukocytes:** Elevated glucose concentrations (≥ 3 g/dL) or high specific gravity may cause decreased test results. The presence of cephalexin (Kelfex), cephalothin (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.

**REFERENCES:**