**URINE REAGENT STRIPS FOR URINALYSIS**

**For the Semi-quantitative and qualitative detection of Glucose, Bilirubin, Ketone, Specific Gravity, Blood, pH, Protein, Urobilinogen, Nitrite, Leukocytes and Ascorbic Acid in Urine**

**SUMMARY**

TUP for Urinals are firm plastic strips to which several different reagent areas are affixed. Depending on the product being used, TUP provide tests for Glucose, Bilirubin, Ketones (Acetone-acid), Specific Gravity, Blood, pH, Protein, Urobilinogen, Nitrite, Leukocytes and Ascorbic Acid. Each strip is stable and ready to use upon removal from the bottle. The entire color block is disposable. Results are obtained by direct comparison of the color block with the color blocks printed on the bottle label. The color blocks are information only and do not necessarily match perfectly. Refer to color blocks on vial for a perfect match.

**TEST 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 the hydrogen peroxide with tetramethylbenzidine chromogen to oxidize the chromogen to colors ranging from blue-green to green-brown through brown and dark brown.

**Ketone:** This test is based on the formation of acetoacetic acid with sodium nitroprusside in a strongly alkaline medium. The color range from beige to buff/buff color for a "Negative" reading to pink and purple for a "Positive" reading.

**Protein:** This test is based on the protein error-of-indicator principle. At a constant pH, the development of any green color is due to the presence of protein. Colors range from yellow to blue-green for a "Negative" reaction to yellow-green and green for a "Positive" reaction.

**Urobilinogen:** This test is based on a modified Ehrlich reaction in which p-dimethylaminobenzaldehyde reacts with urobilinogen in a strongly acid medium. Colors range from light tan to reddish-brown.

**Ascorbic Acid:** This test is based on the action of a complex chelating agent with polyvinylpyrrolidone to form a red color in the test strip. In the presence of ascorbic acid, the color changes from light tan to reddish-brown.

**Specific Gravity:** This test is based on the apparent pKa change of certain inorganic ions. The ionic concentration is determined by the reaction of hydrogen peroxide with potassium iodide chromogen to oxidize the chromogen to continue to dark blue.

**pH:** This test is based on the well known double pH indicator method, where phenolphthalein and methyl red give pink or dyeable colors over the pH range 5.9-9. This test provides a qualitative measure of the acid-base balance.

**Bilirubin:** This test is based on the coupling of bilirubin with diazotized dinitrophenyl hydrazine in an acid medium. The color range from beige to buff/buff color for a "Negative" reaction to pink-pink-purple for a "Positive" reaction.

**Ketone:** This test is based on the action of esterase present in leukocytes, which catalyzes the hydrolysis of an indoxyl ester derivative. The indoxyl ester bleed react with a diazonium salt to produce a beige-pink to purple color.

**Betahematin:** This test is based on a chemical cleavage agent with polyvinylpyrrolidone in a highly acid and an indicator dye reaction with metal ion in its lower solution to produce a color change from blue-green to yellow-green.

**REAGENTS (Based on dried weight at time of impregnation)**

- **Glucose:** 16.35% w/w glucose oxidase (Aspergillus niger, 1.3IU); 0.6% w/w peroxidase (horseradish, 3300 IU); 7.0% w/w potassium iodide, 7.61% w/w buffer and non-reactive ingredients.
- **Bilirubin:** 0.4% w/w 2,4-dihydroxyanilinonic acid diazol salt, balanced with buffer and non-reactive ingredients.
- **Ketone:** 7.7% w/w sodium nitroprusside balanced with buffer and non-reactive ingredients.

**Specific Gravity:** 2.8% w/w bromothymol blue, 65.0%, poly (methyl vinyl ether/maleic anhydride); 28.2% sodium hydroxide.

**Blood:** 6.6% w/w urohemoglobin, 4.0% w/w zinc chloride, 5.6% w/w polyvinylpyrrolidone, 1.4% w/w 2,4-dichloroaniline diazonium salt, 99.9% w/w buffer and non-reactive ingredients.

**Nitrite:** 0.3% w/w triphenyltetrazolium chloride, 99.8% w/w buffer and non-reactive ingredients.

**Leukocytes:** 2.8% w/w hematocrit, 3.7% w/w dialyzed dinitrophenyl hydrazine, 99.3% w/w buffer and non-reactive ingredients.

**Acetate Acid:** 5.8% w/w ferric chloride; 4.0% w/w DTA, 1.2% dapsyl, 98.1% w/w buffer and non-reactive ingredients.

**WARNINGS AND PRECAUTIONS**

TUP are for in vitro diagnostic use. Do not touch reactive fields.

**STORAGE**

Store at room temperature between 15°-30°C and out of direct sunlight. Do not freeze after expiration date.

**RECOMMENDED HANDLING PROCEDURES**

All unused strips must remain in the original bottle. Transfer to any container may cause reagent strips to deteriorate and become nonreactive. Do not remove desiccant from bottle. Do not open container until ready to use. Opened bottles should be resealed 3 months after first opening.

**SPECIMEN COLLECTION AND PREPARATION**

Collect urine in a clean container and test as soon as possible. Do not centrifuge. The use of preservatives is not recommended. If testing cannot be performed within one hour after voiding, refrigerate the specimen immediately. Allow refrigerated specimen to return to room temperature before testing.

**TEST PROCEDURE**

1. Remove from the bottle only enough strips for immediate use and replace tightly.
2. Completely immerse reagent areas of the strip in fresh, well-mixed urine. Remove the strip immediately to avoid dissolving the reagent areas.
3. While removing, touch the side of the strip against the inner rim of the urine container to remove excess urine. Blot the lengthwise edge of the strip on an absorbent paper towel to further remove excess urine and avoid carrying over contamination from adjacent reagent pads.
4. Compare each reagent area to its corresponding color block on the color chart and note the time the specimen was collected. Proper read time is critical for optimal results.
5. Observe results by direct color chart comparison.

**TEST RESULTS**

<table>
<thead>
<tr>
<th>Component</th>
<th>Negative</th>
<th>Trace</th>
<th>Small</th>
<th>Moderate</th>
<th>Large</th>
<th>++</th>
<th>+++</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Glucose</strong></td>
<td>0.000</td>
<td>0.050</td>
<td>0.150</td>
<td>0.250</td>
<td>0.350</td>
<td>0.450</td>
<td>0.550</td>
</tr>
<tr>
<td><strong>Ketone</strong></td>
<td>0.000</td>
<td>0.050</td>
<td>0.150</td>
<td>0.250</td>
<td>0.350</td>
<td>0.450</td>
<td>0.550</td>
</tr>
<tr>
<td><strong>Blood</strong></td>
<td>0.000</td>
<td>0.050</td>
<td>0.150</td>
<td>0.250</td>
<td>0.350</td>
<td>0.450</td>
<td>0.550</td>
</tr>
<tr>
<td><strong>pH</strong></td>
<td>5.0</td>
<td>6.0</td>
<td>7.0</td>
<td>7.5</td>
<td>8.0</td>
<td>8.5</td>
<td>9.0</td>
</tr>
<tr>
<td><strong>Protein</strong></td>
<td>0.000</td>
<td>0.050</td>
<td>0.150</td>
<td>0.250</td>
<td>0.350</td>
<td>0.450</td>
<td>0.550</td>
</tr>
<tr>
<td><strong>Urobilinogen</strong></td>
<td>0.000</td>
<td>0.050</td>
<td>0.150</td>
<td>0.250</td>
<td>0.350</td>
<td>0.450</td>
<td>0.550</td>
</tr>
<tr>
<td><strong>Ascorbic Acid</strong></td>
<td>0.000</td>
<td>0.050</td>
<td>0.150</td>
<td>0.250</td>
<td>0.350</td>
<td>0.450</td>
<td>0.550</td>
</tr>
</tbody>
</table>

**Note:** All reagent areas except Leukocytes may be read between 1-2 minutes for screening positive urine from negative urine. Changes in color after 2 minutes are of no diagnostic value.

**QUALITY CONTROL**

For best results, performance of reagent strips should be confirmed by testing known negative and positive specimens or controls whenever a new test is performed or whenever a new bottle is first opened. Each laboratory should establish its own goals for adequate standards of performance and should question handling and testing procedures if these standards are not met.

**RESULTS**

Results are obtained by direct comparison of the color blocks printed on the bottle label. The color blocks represent nominal values; actual values will vary around the nominal values.

**Revision H / 2004-08**
LIMITATIONS OF COMPARISON

Comparison to the color chart is dependent on the interpretation of the individual. It is therefore, recommended that all laboratory personnel interpreting the results of these strips be familiar with the color chart.

As with all laboratory tests, definitive diagnostic or therapeutic decisions should not be based on any single test result or method.

Glucose: Moderate amounts of ketone bodies (40mg/dL or greater) may decrease the test result in the containing small quantities of glucose (75-125 mg/dL). However, such concentration of ketone simultaneously with such glucose concentrations (250mg/dL or greater) may cause false negative reactivity of the glucose test decreases as the BOD and/or ascorbic acid content of the urine increases. False negative and weak reactivity of glucose may be observed if there is more than 50 mg/dL of ascorbic acid in the sample. Reactivity may also vary with temperature.

Bilirubin: Reactions may occur with urine containing large doses of citrurate, oxalate and urate. Normal levels of bilirubin are unlikely to produce a "blue" (indigo sulfylate) and metabolites of Lobeline may cause false positive or atypical color.

Specific Gravity: The chemical nature of the specific gravity test may cause slightly different results from those obtained with the specific gravity methods when elevated amounts of certain urine constituents are present. High buffered alkaline urine may cause the reagent zones to become relatively large. Elevated specific gravity readings may be obtained with the presence of moderate amounts of uric acid or citric acid in the urine.

Protein: The protein reagent area run onto the pH area, causing a false lowering in the pH result.

Blood: The nitrous oxide test is not reliable for the detection of porphobilinogen. Drugs known to cause nitrous oxide in urine (such as chlorpromazine or ranitidine) that might be mistaken for positive bilirubin. Indican may also be present in urine from the same patient. Random urine may yield positive results with the nitrous oxide test.

Specific gravity: The specific gravity test permits determination of urine specific gravity. Within 0.005 with values obtained with the reflective index method.

Nitrite: This test is specific for nitrite. Nitrous oxide ion present in urine is detectable by the nitrous oxide test.

Ketone: The nitrous oxide test is specific for ketones present in small amounts in urine or other body fluids. The nitrous oxide test is specific for ketone bodies present in urine.

Leukocytes: This test is specific for leukocytes present in urine.

Peroxidase: This test is specific for peroxidase present in urine. The peroxidase test is specific for the constituent to be measured with the exception of interferences listed above.

Expected Values

Glucose: Small amounts of glucose are normally excreted by the kidney. Concentrations as low as 0.1 g/dL glucose, readable at 10 or 30 seconds, may be significantly abnormal if found consistently. At 10 seconds, results should be interpreted qualitatively, for semi-quantitative results, read at 30 seconds only.

Bilirubin: Normally, no bilirubin is detectable in urine by even the most sensitive methods. Even trace amounts of bilirubin are sufficient to produce a positive result. The test area is sensitive to 15 mg/dL of bilirubin. The bilirubin test is specific for bilirubin in urine. The bilirubin test is specific for bilirubin in urine.

Ascorbic Acid: The daily urinary output of ascorbic acid  varies with the intake: it is therefore, recommended that all laboratory personnel interpreting the results of these strips be familiar with the color chart.

Blood: The nitrous oxide test is not a reliable method for the detection of porphobilinogen. Drugs known to cause nitrous oxide in urine (such as chlorpromazine or ranitidine) that might be mistaken for positive bilirubin. Indican may also be present in urine from the same patient. Repeated trace and positive results are of clinical significance.

Peroxidase: This test is specific for peroxidase present in urine. The peroxidase test is specific for the constituent to be measured with the exception of interferences listed above.

SPECIFIC PERFORMANCE CHARACTERISTICS

The performance characteristics of TUP have been determined both in the laboratory and in clinical trials. Parameters of importance to the user are sensitivity, specificity, accuracy, and precision. Generally TUP have been developed to be specific for the constituent to be measured with the exception of interferences listed above. (See LIMITATIONS OF COMPARISON)

For visually read strips, accuracy is a function of the manner in which the color blocks on the bottle label are determined and the discrimination of the human eye in reading the test. Precision is difficult to assess in a test of this type because of the variability of the human eye. It is for this reason that users are encouraged to develop their own standards of performance.

Glucose: This test is specific for glucose; no substances excepted in urine other than glucose is known to give a positive result. The nitrous oxide test does not react with lactic, galactose, fructose, or reducing metabolites of drugs: e.g. salicylates and naldixic acid. This test may be used to determine whether the reducing substances in urine are glucose, galactose, fructose, or lactose.

Protein: The protein test is specific for protein. The urinary protein test may be used to detect low levels of protein, in urine; urine cannot be detected on the specific gravity methods.

Ketone: The nitrous oxide test is specific for ketones present in small amounts in urine or other body fluids. The nitrous oxide test is specific for ketone bodies present in urine.

Leukocytes: This test is specific for leukocytes present in urine.

Phenol: The nitrous oxide test is specific for phenol. Phenol is detectable in urine at concentrations 0.075 mg/dL.

Blood: As with all laboratory tests, definitive diagnostic or therapeutic decisions should not be based on any single test result or method.

Specific Gravity: The specific gravity test is specific for the constituent to be measured with the exception of interferences listed above. (See LIMITATIONS OF COMPARISON)

Ascorbic Acid: The daily urinary output of ascorbic acid exerts a significant effect on the results obtained. Urine may vary in specific gravity from 1.013 to 1.040. Comparison of the reacted reagent area on a white background may not yield a true reading relative to other testing used.

Leukocytes: This test will detect leukocytes in concentrations as low as 0.2 EU/ml. The absence of leukocytes in the specimen being tested cannot be determined with this test.

Bilirubin: Normally, no bilirubin is detectable in urine by even the most sensitive methods. Even trace amounts of bilirubin are sufficient to produce a positive result. The test is specific for bilirubin and will not react with substances normally excreted in the urinary tract.

Leukocytes: This test will detect leukocytes in concentrations as low as 0.2 EU/ml. The absence of leukocytes in the specimen being tested cannot be determined with this test.

Bilirubin: The bilirubin test is specific for bilirubin in urine. The bilirubin test is specific for bilirubin in urine.

Ascorbic Acid: This test can detect ascorbic acid in concentrations as low as 10 mg/L.