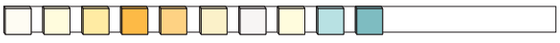
URINE REAGENT STRIPS FOR URINALYSIS
For the Semi-quantitative and qualitative detection of Glucose, Bilirubin, Ketone, Specific Gravity, Blood, pH, Protein, Urobilinogen, Nitrite, Leukocytes and Ascorbic Acid in Urine

ultimed™

SUMMARY

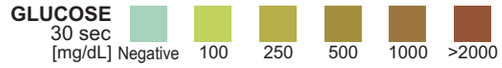
TUP for Urinalysis are firm plastic strips to which several different reagent areas are affixed. Depending on the product being used, **TUP** provide tests for Glucose, Bilirubin, Ketone (Acetoacetic acid), Specific Gravity, Blood, pH, Protein, Urobilinogen, Nitrite, Leukocytes, and Ascorbic Acid in Urine. Test results may provide information regarding the status of carbohydrate metabolism, kidney and liver function, acid-base balance, and bacteriurea.[1,2] Please refer to the outside box and bottle label for the specific test parameters of the product you are using.



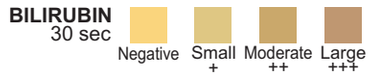
TUP are packaged along with a drying agent in a plastic bottle with a twist-off cap. Each strip is stable and ready to use upon removal from the bottle. The entire reagent strip is disposable. Results are obtained by direct comparison of the test strip with the color blocks printed on the bottle label. No calculations or laboratory instruments are required. **The below color blocks are for information only and do not necessarily match perfectly. Refer to color blocks on vial for a perfect match.**

TEST PRINCIPLE

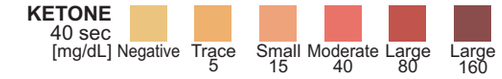
Glucose: This test is based on a double sequential enzyme reaction. One enzyme, glucose oxidase, catalyzes the formation of gluconic acid and hydrogen peroxide from the oxidation of glucose. A second enzyme, peroxidase, catalyzes the reaction of hydrogen peroxide with potassium iodide chromogen to oxidize the chromogen to colors ranging from blue-green to greenish-brown through brown and dark brown.



Bilirubin: This test is based on the coupling of bilirubin with a diazotized dichloroaniline in a strongly acid medium. The colors range from light tan to reddish-brown.

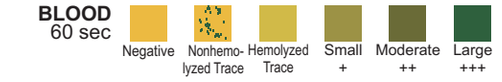


Ketone: This test is based on the reaction of acetoacetic acid with sodium nitroprusside in a strongly basic medium. The colors range from beige or buff-pink color for a "Negative" reading to pink and pink-purple for a "Positive" reading.



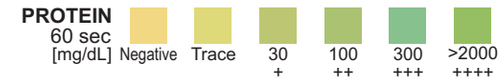
Specific Gravity: This test is based on the apparent pKa change of certain pretreated polyelectrolytes in relation to the ionic concentration. In the presence of an indicator, the colors range from dark blue or blue-green in urine of low ionic concentration to green and yellow-green in urine of higher ionic concentration.

Blood: This test is based on the pseudoperoxidase action of hemoglobin and erythrocytes which catalyzes the reaction of 3,3', 5, 5'-tetramethyl-benzidine and buffered organic peroxide. The resulting colors range from orange to yellow-green and dark green. Very high blood concentration may cause the color development to continue to dark blue.

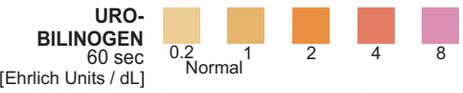


pH: This test is based on the well known double pH indicator method, where bromothymol blue and methyl red give distinguishable colors over the pH range of 5-9. The colors range from red-orange to yellow and yellow-green to blue-green.

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 for a "Negative" reaction to yellow-green and green to blue-green for a "Positive" reaction.



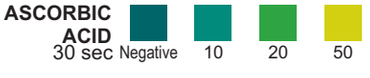
Urobilinogen: This test is based on a modified Ehrlich reaction in which *p*-diethylaminobenzaldehyde reacts with urobilinogen in a strongly acid medium. Colors range from light pink to bright magenta.



Nitrite: This test depends on the conversion of nitrate to nitrite by the action of Gram-negative bacteria in the urine. The nitrite reacts with *p*-arsanilic acid to from a diazonium compound in an acid medium. The diazonium compound in turn couples with 1,2,3,4-tetrahydrobenzo(h) quinolin to produce a pink color.

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

Ascorbic Acid: This test is based on the action of a complex chelating agent with polyvalent metal ion in its higher state and an indicator dye that can react with the metal ion in its lower state to produce a color change from blue-green to yellow.



REAGENTS (Based on dried weight at time of impregnation)

Glucose: 16.3%w/w glucose oxidase (Aspergillus niger, 1.3IU); 0.6%w/w peroxidase (horseradish, 3300 IU); 7.0% w/w potassium iodide; 76.1% w/w buffer and non-reactive ingredients.

Bilirubin: 0.4% w/w 2,4-dichloroaniline diazonium 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, 69.0%; poly (methyl vinyl ether/maleic anhydride); 28.2% sodium hydroxide

Blood: 6.6% w/w cumene hydroperoxide; 4.0% w/w 3, 3', 5, 5'-tetramethylbenzidine; 89.4% w/w buffer and nonreactive ingredients.

pH: 0.2% w/w methyl red; 2.8% w/w bromothymol blue; 97% w/w nonreactive ingredients.

Protein: 0.3% w/w tetrabromophenol blue; 99.7% w/w buffer and nonreactive ingredients.

Urobilinogen: 2.9% w/w *p*-diethylaminobenzaldehyde balanced with buffer and nonreactive ingredients.

Nitrite: 1.4% w/w *p*-arsanilic acid, balanced with buffer and nonreactive ingredients.

Leukocytes: 0.4% w/w indoxyl ester derivative; 0.2%w/w diazonium salt; 99.4% w/w buffer and nonreactive ingredients.

Ascorbic Acid: 5.8% w/w ferric chloride; 4.9% w/w DTPA; 1.2% dipyridyl; 89.1% w/w buffer and nonreactive 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 use 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 used within 3 months after first opening.

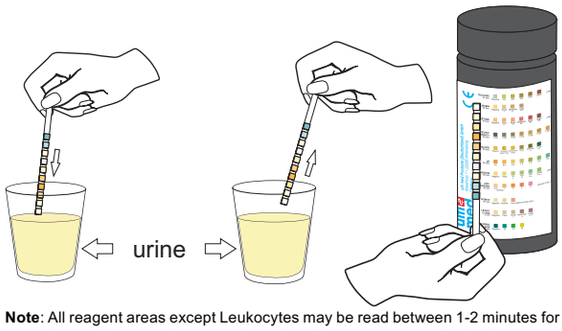
SPECIMEN COLLECTION AND PREPARATION

Collect urine in a clean container and test as soon as possible. Do not centrifuge. The use of urine 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 cap tightly.
2. Completely immerse reagent areas of the strip in fresh, well-mixed urine. Remove the strip immediately to avoid dissolving out the reagent areas.
3. While removing, touch the side of the strip against the 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 running over (contamination from adjacent reagent pads).
4. Compare each reagent area to its corresponding color blocks on the color chart and read at the times specified. Proper read time is critical for optimal results.
5. Obtain results by direct color chart comparison.

TEST PROCEDURE



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

TUP URINALYSIS

LIMITATIONS OF PROCEDURE

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 tested for color blindness.

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 color development in urine containing small amounts of glucose (75-125 mg/dl). However, such concentration of ketone simultaneously with such glucose concentration is metabolically improbable in screening. The reactivity of the glucose test decreases as the SG and/or ascorbic acid of the urine increases. False negative and weak reaction of glucose may be observed if there is more than 50 mg/dl ascorbic acid in the sample. Reactivity may also vary with temperature.[3F]

Bilirubin: Reactions may occur with urine containing large doses of chlorpromazine or rafampen that might be mistaken for positive bilirubin.[3] Indican (indoxyl sulfate) and metabolites of Lodine may cause false positive or atypical color; ascorbic acid (25mg/dL or greater) may cause false negative results.

Ketone: Color reaction that could be interpreted as "positive" may be obtained with urine specimens containing MESNA or large amounts of phenylketones or L-dopa metabolites.[3]

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. Highly buffered alkaline urine 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: The sensitivity of the blood test is reduced in urine with high specific gravity and/or high ascorbic acid content. Microbial peroxidase, associated with urinary tract infection may cause false positive reactions. False negative and weak reaction of blood may be observed if there is more than 10 mg/dl ascorbic acid in the sample.

pH: If proper procedure is not followed and excess urine remains on the strip, a phenomenon known as "running over" may occur, in which the acid buffer from the protein reagent area run onto the pH area, causing a false lower ingin the pH result.

Protein: False positive results may be obtained with highly alkaline urine. Contamination of the urine specimen with quarternary ammonium compounds may also produce false positive results.[4]

Urobilinogen: The test area will react with interfering substances known to react with Ehrlich's reagent, such as porphobilinogen and *p*-aminosalicyclic acid.[3] This test is not a reliable method for the detection of porphobilinogen. Drugs containing azo-dyes (e.g. Azo Gantrisin) may give a masking golden color. The absence of urobilinogen cannot be determined with this test.

Nitrite: The pink color is not quantitative in relation to the number of bacteria present. Any degree of pink coloration should be interpreted as a positive nitrite test suggestive of 10^5 or more organisms/ml. There are occasional urinary tract infections from organisms, which do not contain reductase to convert nitrate to nitrite.

Leukocytes: Highly colored urine and the presence of the drugs cephalexin (Keflex) and gentamicin have been found to interfere with this test. High urinary protein of 500 mg/dl or above diminishes the intensity of the reaction color. Elevated glucose concentration or high specific gravity may cause decreased results.

Ascorbic Acid: There are no chemicals known in urine that affect the Ascorbic Acid parameter.

EXPECTED VALUES

Glucose: Small amounts of glucose are normally excreted by the kidney.[5] Concentrations as little as 0.1 g/dl glucose, read either 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 method. Even trace amounts of bilirubin are sufficiently abnormal to require further investigation. Atypical colors (colors produced which are different than 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 are possibly masking the bilirubin reaction.

Ketone: Normally, no ketones are present in urine. Detectable levels of ketone may occur in urine during physiological stress conditions such as fasting, pregnancy, and frequent strenuous exercise.[6-8] In starvation diets, or in other abnormal carbohydrate metabolism situation, ketones appear in the urine in excessively large amounts before serum ketones are elevated.[9]

Specific Gravity: Random urine may vary in specific gravity from 1.003-1.040+. Twenty-four hour urine from normal adults with normal diets and normal fluid intake will have a specific gravity of 0.016-1.022[10] in severe renal damage the specific gravity is fixed at 1.010, the value of the glomerular filtrate.

Blood: Any green spots or green color developing on the reagent area within 40 seconds is significant and the urine should be examined further. Blood is frequently, but not invariably found in the urine of menstruating females.

pH: newborn: 5-7 thereafter: 4.5-8 average: 6.[3]

Protein: In 24-hour urine, 1-14 mg/dl of protein may be excreted by the normal kidney.[4] A color matching any color block greater than trace indicates significant proteinuria. For urine with high specific gravity, the test area may most closely match the trace color block even though only normal concentrations of protein are present. Clinical judgment is needed to evaluate the significance of trace results.

Urobilinogen: In a healthy population, the normal urine urobilinogen range obtained with this test is 0.2-1.0 Ehrlich Unit/dl. A result of 2.0 EU/dl may be of clinical significance and the same patient sample should be evaluated further.

Nitrite: Normally no detectable amount of nitrite is present in urine.[3] The nitrite area will be positive in a proportion of cases of significant infection, depending on how long the urine specimens were retained in the bladder prior to collection. Retrieval of positive cases with the nitrite test range from as low as 40%, in instances where little bladder incubation occurred, to as high as 80% in instances where a minimum of 4 hours incubation occurred.

Leukocytes: Normal urine specimens generally yield negative results with this test. A trace result may be of questionable clinical significance and it is recommended that the test be repeated using a fresh sample from the same patient. Repeated trace and positive results are of clinical significance.

Ascorbic Acid: The daily urinary output of ascorbic acid varies with the intake: it approximately half of the intake. The average urinary output ranges from 20-30 mg/day. If detect ascorbic acid in urine, stop taking ascorbic acid for 24 hours and retest.

SPECIFIC PERFORMANCE CHARACTERISTICS

The performance characteristics of TUP have been determined both in the laboratory and in clinical tests. 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 PROCEDURE)

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 excreted in urine other than glucose is known to give a positive result. The reagent area does not react with lactose, galactose, fructose, or reducing metabolites of drugs; e.g. salicylates and nalidixic acid. This test may be used to determine whether the reducing substances found in urine is glucose. Approximately 100 mg/dl glucose in urine is detectable.

Bilirubin: The test has a sensitivity of 0.4-0.8 mg/dl bilirubin in urine. The test is considered specific for bilirubin in urine.

Ketone: The ketone test area provides semi-quantitative results and reacts with acetoacetic acid in urine. This test does not react with beta-hydroxybutyric acid or acetone. The reagent area detects as little as 5-10 mg/dl acetoacetic acid in urine.

Specific Gravity: The specific gravity test permits determination of urine specific gravity between 1.000 and 1.030. In general, the specific gravity test correlates within 0.005 with values obtained with the reflective index method.

Blood: At the time of reagent manufacture, this test when read as instructed has a sensitivity to free hemoglobin of 0.015 mg/dl or 5-10 intact red blood cells/µL urine. This test is slightly more sensitive to free hemoglobin and myoglobin than to intact erythrocytes.

pH: The pH test area permits quantitative differentiation of pH values to one unit within the range of 5-9. pH reading is not affected by variation in the urinary buffer concentration.

Protein: The test area is more sensitive to albumin than to globulin, hemoglobin, Bence-Jones proteins, and mucoprotein; a negative result does not rule out the presence of these other proteins. The test area is sensitive to 15 mg/dl albumin. Depending on the inherent variability in clinical urine lesser concentration may be detected under certain conditions.

Urobilinogen: This test will detect urobilinogen in concentrations as low as 0.2 EU/dl in urine. The absence of urobilinogen in the specimen being tested cannot be determined with this test.

Nitrite: At the time of reagent manufacture, this test has sensitivity to sodium nitrite of 0.075 mg/dl. Comparison of the reacted reagent area on a white background may aid in the detection of low levels of nitrite ion, which may otherwise be missed. This test is specific for nitrite and will not react with substances normally excreted in the urine.

Leukocytes: This test can detect as low as 10-15 WBC/µL. This test will not react with erythrocytes or bacteria common in urine.

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

BIBLIOGRAPHY

1. Free, A.H and Free, H.M.: Urinalysis, Critical Discipline of Clinical Science. *CRC Crit. Rev. Clin. Lab. Sci.* 3(4): 481-531; (1972).
2. Yoder, J.Adams, E.C., and Free. H.M.: Simultaneous Screening for Urinary Occult Blood, Protein, Glucose and pH. *Amer. J. Med Tech.* 31:285; (1965).
3. Tietz, N.W.: Clinical Guide to Laboratory Tests; W.B. Saunders Company, (1976).
4. Burtis, C.A. and Ashwood, E.R.: Tietz Textbook of Clinical Chemistry 2nd Ed. 2205; (1994).
5. Shchersten, B. and Fritz, H.: Subnormal Levels of Glucose in Urine. JAMA 201:129-132; (1967).
6. McGarry, J.D.: Lilly Lecture, 1978: New Perspectives in the Regulation of Ketogenesis. Diabetes 28: 517-523 May, (1978).
7. Williamson, D.H.: Physiological ketoses, or Why Ketone Bodies? Postgrad. Med. J. (June Suppl.): 371-375: (1971).
8. Paterson, P. et al.: Maternal and Fetal Ketone Concentrations in Plasma and Urine. Lancet: 862-865; April 22, (1967).
9. Fraser, J. et al.: Studies with a Simplified Nitroprusside Test for Ketone Bodies in Urine, Serum, Plasma and Milk. Clin. Chem. Acta II: 372-378; (1965).
10. Henry, J.B. et al.: Clinical Diagnosis and Management by Laboratory Methods, 16th Ed. Philadelphia: Saunders; (1979).

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LGA InterCert Certified Quality Assurance System DIN EN ISO 13488:2001

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