Note: Package insert for use with the products listed below.

Multistix PRO® 11 • Multistix PRO® 10LS • Multistix PRO® 7G Reagent Strips

Tests for Protein–High, Protein–Low, Creatinine, Blood, Leukocytes, Nitrite, Glucose, Ketone (Acetoacetic Acid), pH, Specific Gravity, and Bilirubin in Urine.

SUMMARY AND EXPLANATION / INTENDED USE: Bayer MULTISTIX PRO® Reagent Strips for Urinalysis include test pads for protein–high, protein–low, creatinine, blood, leukocytes, nitrite, glucose, ketone (acetoacetic acid), pH, specific gravity, and bilirubin. *Please refer to the carton or bottle label to see which tests are included on the product you are using.*

MULTISTIX PRO Reagent Strips are for professional use in near-patient (point-of-care) and centralized laboratory locations. The strips are intended for use in at-risk patient groups to assist diagnosis in the following areas:[1-3]

- kidney function
- urinary tract infections
- carbohydrate metabolism (e.g., diabetes mellitus)
- liver function

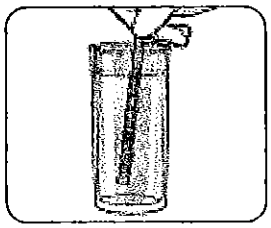
The strips also measure physical characteristics, including acid-base balance and urine concentration. Test results can be used along with other diagnostic information to rule out certain disease states and to determine if microscopic analysis is needed.[1,4]

MULTISTIX PRO Reagent Strips are ready to use upon removal from the bottle and the entire reagent strip is disposable. The strips may be read visually, requiring no additional laboratory equipment for testing. The strips can also be read instrumentally, using the CLINITEK® family of Urine Chemistry Analyzers and the appropriate software; MULTISTIX PRO 11 Reagent Strips are for use on the CLINITEK® 500 Analyzer only. The CLINITEK® 50 and CLINITEK® 100 instruments automatically identify the strip being tested, using the colored ID band(s) near the handle of the strip. Contact your product representative for further information.

MULTISTIX PRO Reagent Strips are for *in vitro* diagnostic use. They have been determined to be nonhazardous under the guidelines issued by OSHA in 29 CFR 1910.1200(d).

SPECIMEN COLLECTION AND PREPARATION: Collect freshly-voided urine in a clean container and test it as soon as possible. The container should allow for complete dipping of all reagent strip areas. A first-morning specimen is preferred but random collections are acceptable. Test the urine within two hours after voiding, sooner if testing for bilirubin. If unable to test within the recommended time, refrigerate the specimen immediately and let it return to room temperature before testing. Work areas and specimen containers should always be free of detergents and other contaminating substances.[5]

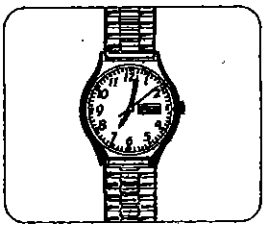
Procedure



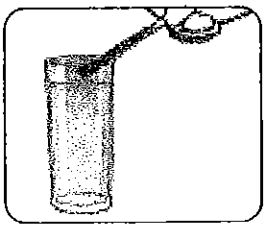
1. • **Collect** a fresh urine specimen in a clean, dry container.
   • **Mix** well just before testing.
   • **Remove** one strip from the bottle.
   • **Replace** the cap.

2. • **Dip** all the test pads of the strip into the urine.
   • **Immediately remove** the strip.
   • If reading the strip visually, **start timing.**

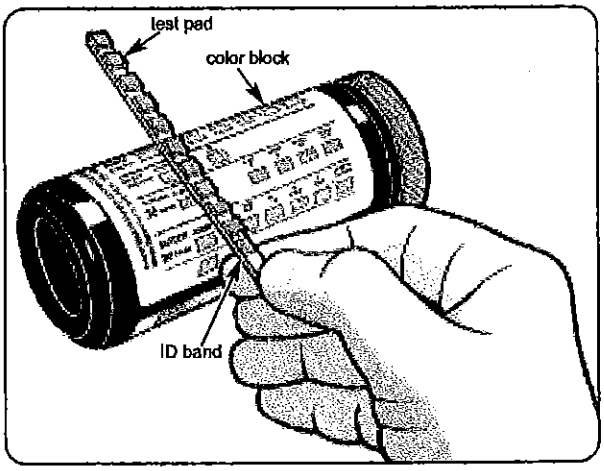
   NOTE: There is no need to wet the colored ID band(s) near the handle.



3. **Drag** the *edge* of the strip against the container rim to remove excess urine.



4. a. **If reading visually:**
   - **Compare** each test pad to the corresponding row of color blocks on the bottle label.
   - **Read** each pad *at the time shown on the label,* starting with the shortest time.
   - **Hold** the strip close to the color blocks and match carefully.
   - **Read** the pads in good light.



b. **If using a CLINITEK Instrument,** carefully follow the directions given in the appropriate instrument operating manual. The instrument will automatically read each test pad at a specified time.

5. Report the results to the lab supervisor or physician.

RESULTS: With visual use, results are obtained in clinically meaningful units directly from the Color Chart comparison. A single protein result should be reported, based on the protein–low and protein–high readings; a protein-to-creatinine ratio can also be determined.

Reporting Visual Protein Result and Ratio (see examples below):

1. Report the **higher** reading of the two pads (Protein–Low and Protein–High) as the protein result. Report "Negative" if both readings are negative.
2. You can determine the protein-to-creatinine ratio using the table below. Locate the square that corresponds to **both** the **reported** protein result, from Step 1 above, and the creatinine result.

Reported Protein Result (mg/dL)	Creatinine Result (mg/dL)				
	10	50	100	200	300
Negative	Recollect*			Normal	
15		Abnormal			
30					
100, 300, or 2000					

*Specimen is too dilute to accurately determine ratio result. Repeat test on new specimen, preferably a first-morning collection.

Examples:

Protein Readings	Reported Protein Result	Creatinine Result	Protein-to-Creatinine Ratio
Protein–High = Negative Protein–Low = 15 mg/dL	15 mg/dL	200 mg/dL	Normal
Protein–High = 30 mg/dL Protein–Low = 15 mg/dL	30 mg/dL	200 mg/dL	Abnormal

With CLINITEK Instruments, the test pads are "read" by the instrument and the results are displayed or printed as soon as they are available. The instrument reports a single protein result, based on the protein–low and protein–high readings, and calculates the protein-to-creatinine ratio.

QUALITY CONTROL: Test known negative and positive specimens or controls whenever a new bottle is first opened. Water should NOT be used as a negative control. Each laboratory should establish its own goals for adequate standards of performance. For information about control manufacturers, contact the Bayer Customer Service Department at 1-800-348-8100 (U.S. only).

STORAGE: All unused strips must remain in the original bottle. Transfer to any other container may cause reagent strips to deteriorate and become unreactive. Store at temperatures between 15°–30°C (59°–86°F). Do not use the strips after their expiration date. 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.

REAGENT PERFORMANCE:

Expected values for the "normal" healthy population and the abnormal population are listed below for each reagent.

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.

Performance characteristics are based on clinical and analytical studies and depend upon several factors: the variability of color perception; the presence or absence of inhibitory and matrix factors typically found in urine; and the laboratory conditions in which the product is used (e.g., lighting, temperature, and humidity). The strips should be read in good light, such as fluorescent; do not read in direct sunlight.

Each color block or instrumental result represents a range of values. Because of specimen and reading variability, specimens with analyte concentrations that fall between nominal levels may give results at either level. Results will usually be within one level of the true concentration. Exact agreement between visual results and instrumental results might not be found because of the inherent differences between the perception of the human eye and the optical systems of the instruments.

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.

PROTEIN:

Expected values: Protein in urine can be the result of urological and nephrological disorders. Albumin has been established as an appropriate marker of glomerular damage. Albumin is normally present in urine at concentrations of 0.5–2.0 mg/dL. Increased albumin excretion (2.0 to 30 mg/dL) is indicative of nephropathy in high-risk groups.[2,6,7]

In normal urine, less than 150 mg of total protein is excreted per day (<15 mg/dL), while clinical proteinuria is indicated at greater than 500 mg of protein per day (strip result of ≥30 mg/dL). Positive results may also indicate tubular or overflow proteinuria in the absence of any glomerular abnormality or proteins of renal origin that may be excreted during infection. Urinary protein excretions can be temporarily elevated in the absence of renal abnormality by strenuous exercise, orthostatic proteinuria, dehydration, urinary tract infections, and acute illness with fever.[1,8,9]

Sensitivity: 8–15 mg/dL albumin (Protein–Low)
30–65 mg/dL protein (Protein–High)

Performance characteristics: The Protein–Low test can accurately and specifically determine albumin. A strip result of 15 mg/dL is indicative of clinical albuminuria. The test is not affected by other proteins at concentrations at least nine times greater than the excretion rate considered to be abnormal.[9,10]

The Protein–High test pad is not specific for a particular protein, and proteins other than albumin can cause a positive response. The test is less sensitive to mucoproteins and globulins, which are generally detected at levels of 60 mg/dL or higher.[10]

Limitations: A visibly bloody urine (≥5 mg/dL) may cause falsely elevated results.[12]

CREATININE:

Expected values: The normal creatinine concentration in adults is 0.6 to 2.0 g of creatinine per day (strip results of approximately 50 to 200 mg/dL). Random urines may have strip results that vary from 10 to 300 mg/dL. Concentrated urines from dehydrated individuals or first morning specimens will typically have elevated concentrations (strip results of ≥200 mg/dL); diuresis will typically result in lower concentrations (strip results of ≤50 mg/dL).[1,11]

Performance characteristics: The test will detect creatinine in concentrations as low as 10 mg/dL or as high as 300 mg/dL. The absence of creatinine in a specimen cannot be determined.

Limitations: A visibly bloody urine (≥5 mg/dL) or the presence of cimetidine (Tagamet®*) may cause falsely elevated results.[12]

PROTEIN-TO-CREATININE RATIO:

Expected values: Clinical proteinuria is indicated at a ratio result of 300 mg protein/g creatinine.[3] A "Normal" strip ratio result indicates that the protein-to-creatinine ratio of the sample is below this cutoff.

Performance characteristics: Use of the protein-to-creatinine ratio can assist in the diagnosis of kidney function by minimizing the impact of changes in the protein result due to exercise, diuresis and urine concentration.[5,11] The ratio improves the results for single-void specimens compared to timed specimens in the discrimination of normal and abnormal levels of protein. Normal strip ratio results occur in urines containing less than 80 mg albumin/g creatinine or less than 300 mg protein/g creatinine.

A ratio result of "Normal Dilute" is reported instrumentally when the protein result is below the sensitivity limits and the creatinine result is 10 mg/dL. In this case, consider testing a new specimen, preferably a first morning collection, for greater confidence in the result. Very low creatinine results can be caused by adulteration of the urine specimen or by severe renal failure.[13,14]

Limitations: The ratio cannot be accurately determined if the creatinine result is 10 mg/dL and the protein–low result is Negative, when the strip is read visually. A new specimen, preferably a first-morning collection, should be tested. Both the protein and P:C ratio results should be considered when making a decision about the clinical diagnosis or need for confirmatory testing.

BLOOD:

Expected values: Normally, no hemoglobin is detectable in urine (<0.010 mg/dL or 3 RBC/μL). Occult blood occurs in urine as intact erythrocytes and hemoglobin, which can occur during urological, nephrological and bleeding disorders. Small amounts of blood (0.030–0.065 mg/dL or a strip result of Small) are sufficiently abnormal to require further investigation.

The significance of the Trace reaction may vary among patients, and clinical judgment is required for assessment in an individual case. Blood is often, but not always, found in the urine of menstruating females.[1,16]

Sensitivity: 0.015–0.062 mg/dL hemoglobin

Performance characteristics: The appearance of green spots on the reacted test pad indicates the presence of intact erythrocytes, while green color across the entire test pad indicates free hemoglobin. The test is equally sensitive to myoglobin as to hemoglobin. This test complements the microscopic examination; a hemoglobin concentration of 0.015–0.062 mg/dL is approximately equivalent to 5–20 intact red blood cells per microliter.

Limitations: Capoten®* (captopril) may reduce the sensitivity. Certain oxidizing contaminants, such as hypochlorite, may produce false positive results. Microbial peroxidase associated with urinary tract infection may cause a false positive reaction.

LEUKOCYTES:

Expected values: Normal urine specimens generally yield negative results. An increase in leukocytes (≥10 leukocytes/µL) is an indication of pyuria and is found in nearly all diseases of the kidney and urinary tract; however, pyuria may often be present in non-infective conditions.[1] A strip result of Small or greater is a useful indicator of infection. Trace results may be of questionable clinical significance; however, Trace results observed repeatedly may be clinically significant.

Sensitivity: 5–15 white blood cells/hpf in clinical urine.

Performance characteristics: Leukocyte esterase is a reliable indicator of leukocytes in urine.[1] A positive reaction (Small or greater) at less than the 2 minute reading time may be regarded as a positive indication of leukocytes in urine.

Limitations: Elevated glucose concentrations (≥3 g/dL) may cause decreased test results. The presence of cephalexin (Keflex®*), 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. Positive results may occasionally be due to contamination of the specimen by vaginal discharge.

NITRITE:

Expected values: Normally no nitrite is detectable in urine. Many enteric gram-negative organisms give positive results when their number is greater than 10^5/mL (0.075 mg/dL nitrite ion or greater).[2,15]

Sensitivity: 0.06–0.1 mg/dL nitrite ion.

Performance characteristics: The test is specific for nitrite and will not react with any other substance normally excreted in urine. Nitrite concentration during infection increases with the length of time the urine specimen is retained in the bladder prior to collection. A minimum of four hours of bladder incubation significantly increases the likelihood of obtaining a positive result.

Limitations: Pink spots or pink edges should not be interpreted as a positive result. A negative result does not rule out significant bacteriuria. False negative results may occur with shortened bladder incubation of the urine, absence of dietary nitrate, or the presence of nonreductive pathological microbes.

GLUCOSE:

Expected values: Small amounts of glucose (<15 mg/dL or 50 mg/day) are normally excreted by the kidney. These amounts are usually below the sensitivity level of this test but on occasion may produce a result between Negative and 100 mg/dL that is interpreted as a positive result. Results at the first positive level may be significantly abnormal if found consistently.[23]

Sensitivity: 75–125 mg/dL glucose

Performance characteristics: The test is specific for glucose; no substance excreted in urine other than glucose is known to give a positive result. This test may be used to determine whether the reducing substance found in urine is glucose. If the color appears somewhat mottled at the higher glucose concentrations, match the darkest color to the color blocks.

Limitations: Ketone bodies reduce the sensitivity of the test; moderately high ketone levels (40 mg/dL) may cause false negatives for specimens containing small amounts of glucose (75–125 mg/dL) but the combination of such ketone levels and low glucose levels is metabolically improbable in screening.

KETONE:

Expected values: Normally, no ketone is detectable in urine (up to 2 mg/dL acetoacetic acid). In ketoacidosis, starvation or with other abnormalities of carbohydrate or lipid metabolism, ketones may appear in urine at levels of 10 mg/dL or higher before serum ketone levels are elevated. Clinical judgment is needed to determine the significance of Trace re-sults, which may occur during physiological stress conditions such as fasting, pregnancy and frequent strenuous exercise.[1]

Sensitivity: 5–10 mg/dL acetoacetic acid

Performance characteristics: The test reacts with acetoacetic acid in urine. It does not react with acetone or β-hydroxybutyric acid.

Limitations: 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.

pH:

Expected values: The normal pH of urine can range from 4.6 to 8.0. Certain dietary conditions can produce acid or alkaline urines, which can be useful in the treatment of some calculi.[1]

Performance characteristics: The pH test area measures pH values from 5–8.5 visually and 5–9 instrumentally, generally to within one unit of the expected result. pH readings are not affected by variations in the urinary buffer concentration.

Limitations: Bacterial growth by certain organisms in a specimen may cause a marked alkaline shift (pH >8.0), usually because of urea conversion to ammonia.

SPECIFIC GRAVITY:

Expected values: The normal SG of urine ranges from 1.001–1.035. If the specific gravity of a random urine is 1.023 or greater, the concentrating ability of the kidneys can be considered normal.[1]

Performance characteristics: This 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. For increased accuracy, 0.005 may be added to readings from urines with pH ≥6.5. Strips read instrumentally are automatically adjusted for pH by the instrument. The Bayer SG test is not affected by the presence of radiopaque dyes as are the refractive index, urinometer, and osmolality methods.

Limitations: The Bayer 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.

BILIRUBIN:

Expected values: Normal adult urine contains about 0.02 mg/dL of bilirubin, which is not detectable by even the most sensitive methods. Even trace amounts of bilirubin are sufficiently abnormal to require further investigation.[1] Since very small amounts of bilirubin (0.1 mg/dL or greater) 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., in the earliest phase of viral hepatitis), ICTOTEST®* Reagent Tablets should be the method of choice.

Sensitivity: 0.4–0.8 mg/dL bilirubin

Performance characteristics: The test is specific for bilirubin and will not react with any other substance normally excreted in urine.

Limitations: Indican (indoxyl sulfate) can produce a yellow-orange to red color response that may interfere with the interpretation of a negative or positive 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).

HELPFUL HINTS:

- Do not remove the strip from the bottle until immediately before it is to be used for testing. Replace the cap immediately and tightly after removing the reagent strip. Do not touch the test areas of the strip.
- Do not read any test pad after 2 minutes; color changes that occur after this time are of no diagnostic value.
- Discoloration or darkening of the test pads may indicate deterioration. If this is evident, or if test results are questionable or inconsistent with expected findings, the following steps are recommended: (1) confirm that the product is within the expiration date shown on the label;

(2) check performance against known negative and positive control materials; (3) retest with fresh product. If proper results are not obtained, consult your local product representative, or contact the Customer Service Department, by calling 1-800-348-8100 (U.S. only), for advice on testing technique and results.

- Contamination of the urine specimen with skin cleansers containing chlorhexidine may affect protein (and to a lesser extent specific gravity and bilirubin) test results. The user should determine whether the use of such skin cleansers is warranted.
- It is especially important to use fresh urine to obtain optimal results with the test for bilirubin, as this compound is very unstable when exposed to room temperature and light.
- You may notice a "burst" of color on the protein–low pad that fades within several seconds after dipping. This does not indicate a positive result, unless the color remains until the specified reading time.

CHEMICAL PRINCIPLES OF PROCEDURES AND INGREDIENTS:
(based on dry weight at time of impregnation)

Protein–Low (Albumin): This test is based on dye binding using a high affinity sulfonephthalein dye. At a constant pH, the development of any blue color is due to the presence of albumin. The resulting color ranges from pale green to aqua blue. **Ingredients:** 1.9% w/w bis (3',3"-diiodo-4',4"-dihydroxy-5',5"-dinitrophenyl)-3,4,5,6-tetrabromosulfonephthalein; 94.2% w/w buffer; 3.9% w/w nonreactive ingredients.

Protein–High: 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. **Ingredients:** 0.3% w/w tetrabromphenol blue; 97.3% w/w buffer; 2.4% w/w nonreactive ingredients.

Creatinine: This test is based on the peroxidase-like activity of a copper creatinine complex that catalyzes the reaction of diisopropylbenzene dihydroperoxide and 3,3',5,5'-tetramethylbenzidine. The resulting color ranges from orange through green to blue. **Ingredients:** 2.5% w/w copper sulfate; 4.5% w/w diisopropylbenzene dihydroperoxide; 2.0% w/w 3,3',5,5'-tetra-methylbenzidine; 56.4% w/w buffer; 34.6% w/w nonreactive ingredients.

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

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. **Ingredients:** 0.4% w/w derivatized pyrrole amino acid ester; 0.2% w/w diazonium salt; 40.9% w/w buffer; 58.5% w/w nonreactive ingredients.

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. **Ingredients:** 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 nonreactive ingredients.

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. **Ingredients:** 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 nonreactive ingredients.

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

pH: This test is based on a 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. **Ingredients:** 0.2% w/w methyl red; 2.8% w/w bromthymol blue; 97.0% w/w nonreactive ingredients.

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

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. **Ingredients:** 0.4% w/w 2,4-dichloroaniline diazonium salt; 37.3% w/w buffer; 62.3% w/w nonreactive ingredients.

AVAILABILITY: MULTISTIX PRO Reagent Strips for Urinalysis are available as follows:

- MULTISTIX PRO® 11 (#1557, 100 strips/bottle)
- MULTISTIX PRO® 10LS (#1554, 100 strips/bottle)
- MULTISTIX PRO® 10LS (#1566, 25 strips/bottle)
- MULTISTIX PRO® 7G (#1556, 100 strips/bottle)

U.S. PATENT NUMBERS: Refer to the carton of the product you are using for applicable patent numbers.

***TRADEMARKS:**

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Pyridium® is a registered trademark of Warner-Chilcott Laboratories.
Tagamet® is a registered trademark of SmithKline Beecham Pharmaceuticals.

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UrinChek™

Test strips for the rapid determination of Bilirubin, Urobilinogen, Ketones Ascorbic Acid, Glucose, Protein (Albumin), Blood, pH, Nitrite, Leukocytes and Specific Gravity in Urine. The test is intended for use by health care professionals.

SUMMARY AND EXPLANATION

UrinChek 10+ SG and UrinChek 8+ urine test strips comprise a battery of chemical tests that help to screen for urinary tract infection, renal and liver disease, and metabolic disorders. Changes in the composition of urine occur very early in many disease processes, often before the patient is aware of any symptoms. As such, the chemical analysis of urine is one of the most frequently ordered laboratory tests. Though it is one of the simplest analyses to perform, it maintains the sophistication and accuracy of more complicated laboratory tests.

The table below describes which analytes are included with each UrinChek product.

	Bilirubin	Urobilinogen	Ketones	Ascorbic Acid	Glucose	Protein	Blood	pH	Nitrite	Leukocytes	Specific Gravity
UrinChek 10+ SG	•	•	•	•	•	•	•	•	•	•	•
UrinChek 8+	•	•	•	•	•	•	•	•	•		

PRINCIPLE OF THE TEST

Bilirubin: This test is based on the coupling of bilirubin with diazonium salt in an acid medium. A pinkish-tan color proportional to bilirubin concentration is produced.
Urobilinogen: This test contains a stable diazonium salt and a buffer. In a coupling reaction, urobilinogen reacts with this field to produce a pink to red color.
Ketones: This test is based on Legal's method in which the test field contains glycine and sodium nitroprusside in an alkaline buffer. The presence of methylketones results in a violet discoloration of the test field.
Ascorbic Acid: The test involves the decolorization of Tillmann's reagent. The presence of ascorbic acid causes the color of the test field to change from gray-blue to orange.
Glucose: This test is an enzymatic test using glucose oxidase, peroxidase and a chromogen. The intensity of the green or blue color formed in the reaction is proportional to the concentration of glucose present. Other sugars are not detected.
Protein: This buffered test is impregnated with a yellow indicator that turns green in the presence of protein. This change in color is based on the "protein error" of the pH indicator and is particularly strong for albumin.
Blood: This buffered test contains an organic peroxide and a chromogen. The peroxidase activity of hemoglobin and myoglobin results in a green color.
pH: This test contains a mixed indicator which assures a marked change in color between pH 5 and pH 9 (orange → yellowish green → turquoise). The indicators are unaffected by protein.
Nitrite: This test indirectly detects the presence of nitrite-forming bacteria in urine. The buffered test pad for nitrite is impregnated with an amine and a coupler. Nitrite present in the urine diazotizes the amine. The subsequent coupling reaction produces a pink color.
Leukocytes: This test contains an indoxyl ester and a diazonium salt. Granulocyte esterases split the ester, and as a result the free indoxyl can react with the diazonium salt to produce a violet color.
Specific Gravity: This test contains a detergent and Bromthymol blue that indicates the presence of ionic constituents in the urine by changing color from green to yellow. The test pad for specific gravity is impregnated with a reddish dye so that the color produced is yellow-brown tan.

REAGENTS

Composition

The reagents in the individual test fields are formulated to contain:

Bilirubin:
2,4 dichlorobenzene diazonium salt 3.1%
Urobilinogen:
Fluorodiazonium tetrafluoroborate 0.4%
Ketones:
Sodium nitroprusside 2.0%
Glycine 68.9%
Ascorbic Acid:
2,6-dichloro-phenol-indophenol 0.7%
Glucose:
Glucose oxidase 2.1%
Peroxidase 0.9%
Tolidine hydrochloride 5.0%
Protein:
Tetra-bromophenol blue 0.2%
Blood:
Cumene hydroperoxide 25.0%
Tetramethylbenzidine dihydrochloride 0.2%

pH:
Bromthymol blue 10.0%
Cresol red 3.0%
Methyl red 2.0%
Nitrite:
4-arsanilic acid 8.2%
N-(naphthyl)-ethylenediammonium dihydrochloride 2.6%
Leukocytes:
Indoxylcarbonic acid ester 0.4%
Diazonium salt 0.2%
Specific Gravity:
Bromthymol blue 3.6%

Concentrations given are based on reagent composition (w/w) at time of manufacture and may vary within manufacturing tolerances.

Warnings and Precautions

UrinChek 10+ SG and UrinChek 8+ urine test strips are for *in vitro* diagnostic use. The test strips have been determined to be nonhazardous under the guidelines issued by OSHA in 29 CFR 1910.1200(d). Use appropriate precautions in the collection, handling, storage and disposal of specimens and used test strips. Do not touch the test strip fields. Discard any discolored strips that may have deteriorated.

Storage and Stability

Store the container at 2°C to 30°C (36°F to 86°F) under dry conditions. Do not freeze. Protect the strips against light and moisture. Remove only the number of test strips required and then immediately reseal the container tightly with the original cap that contains a drying agent. Unused strips that remain in the original capped container are stable until the expiration date. Do not use test strips after the expiration date printed on the outside of the vial.

SPECIMEN / COLLECTION AND PREPARATION

Collect a clean-catch/midstream sample in a clean, dry and clearly labeled container. Do not add preservatives. Analyze as soon as possible after collection because most urine elements deteriorate at room temperature within an hour. If testing cannot be performed within one hour after collection, the specimen should be refrigerated at 2°C to 8°C immediately and returned to room temperature prior to testing (≤ 4 hours). The use of a fresh first-morning urine specimen is the most concentrated and is best for protein, nitrite and bilirubin. It is particularly important to test using a fresh urine specimen when making bilirubin and urobilinogen determinations, as these compounds are very unstable when exposed to room temperature and daylight. Unpreserved urine at room temperature may undergo pH changes due to microbial proliferation, which may interfere with protein determination. If cleanly voided specimens are not collected from females, positive results for leukocytes may be found due to contamination from outside the urinary tract. Skin cleansers containing chlorhexidine may affect protein test results if specimen contamination occurs.

PROCEDURE

Materials Provided

Each box of QuickVue UrinChek 10+ SG and UrinChek 8+ comes with 150 reagent strips and a package insert. In addition, a flat laminated color chart and 150 report sheets are provided with catalog number 20104.

Method

1. Dip the test strip into the urine specimen so that all the test fields are completely immersed for about 1 second.
2. Remove the test strip from the specimen by drawing its edge across the rim of the container to remove excess urine (Keep the test strip horizontal to prevent possible cross-contamination between adjacent test fields.).
3. After 30 to 60 seconds (or 60 to 120 seconds for Leukocytes) compare against the color chart. Color changes occurring after 2 minutes, and discoloration at the edges, are without significance. The compensation pad located between the Specific Gravity and Leukocyte test pads is for instrument measurements and is used to compensate for the intrinsic color of urine.

QUALITY CONTROL

Good Laboratory Practice principles suggest that performance of reagent strips should be confirmed by testing known positive and negative solutions (controls) to assure reactivity of all portions of the reagent strip whenever a new vial is first opened. It is up to each laboratory to establish its own goals for acceptable standards of performance.

RESULTS

- Results are determined visually by direct comparison of reacted test fields with the color chart on the container label or the lot specific laminated color chart. Visual color determinations represent nominal test values for each test and may vary around the nominal values.
- The leukocyte and red blood cell tests are not quantitative determinations, but serve as screening methods for the presence of leukocytes and red blood cells in urine. Microscopic examination of specimens positive for leukocyte or blood test results should be performed if quantitative results are required.
- Ascorbic acid may interfere with the glucose, nitrite, bilirubin, and blood test results (see limitations below). If a positive ascorbic acid result is found, either repeat the test at least 10 hours after discontinuation of vitamin C administration or use a photometric test unaffected by ascorbic acid.

LIMITATIONS OF THE PROCEDURE

Note: Diagnostic or therapeutic decisions should not be based on any single result or method.

Bilirubin: Some urine constituents (medicines, urinary indicants) may produce a yellowish or reddish discoloration of the test pad

that may interfere with interpreting the result. Elevated concentrations of ascorbic acid and nitrite may have an inhibitory effect on the reaction. Bilirubin is light sensitive and prolonged exposure of urine to light may result in diminished or false negative values. Elevated urobilinogen concentrations may slightly intensify the response of the bilirubin test.

Urobilinogen: This test is inhibited by elevated concentrations of formaldehyde. Excreted pigments and medicaments that have a red intrinsic coloration in acidic medium may produce false positive results (phenazopyridine, red beets, azo dyes, p-aminobenzoic acid). As with bilirubin, prolonged exposure to light is to be avoided. This test is not suitable for determining the complete absence of urobilinogen.

Ketones: ß-Hydroxybutyric acid does not react with this test pad. Raised concentrations of phenylpyruvic acid interfere with the reaction and may produce a variety of colors. Phthaleins and anthraquinone derivatives exhibit a red color in alkaline medium and this may mask the response.

Ascorbic Acid: No interferences are known.

Glucose: The main source of interference on this test is from ascorbic acid that may appear in the urine of a patient after: ingestion of high doses of vitamin C, consumption of fruit juice or treatment with antibiotics. If the ascorbic acid test pad is positive, either the glucose test should be repeated 10 hours after discontinuing vitamin C administration or a photometric test that is unaffected by ascorbic acid should be used to obtain reliable information. Other factors that may inhibit color formation are high specific gravity, gentisic acid and acidic pH values (pH < 5), particularly in association with ketonuria. False positive reactions may be caused by hypochlorite or peroxide (cleaning agents).

Protein: False positive results may occur in alkaline urines (pH > 9) with high specific gravity as a result of interference with its buffer's equilibrium as well as due to disinfectants, wetting agents and blood substitutes (polyvinylpyrrolidone, quartenary ammonium compounds, chlorohexidine). Highly alkaline urines (pH > 9) should be acidified with dilute acetic acid before testing. Masking of the color may occur if the specimen contains therapeutic dyes (methylene blue, pyridium) or red beet pigment.

Blood: Non-specific oxygen acceptors such as uric acid, glutathione, gentisic acid and ascorbic acid may interfere by reducing the sensitivity of the test. Formalin can cause false positive reactions, as is the case with hypochlorite or peroxide containing cleaning agents. Very high levels of nitrite or a high specific gravity can delay the response.

pH: No interferences are known.

Nitrite: A negative response with the presence of bacteriuria can be caused by the following: microorganisms without the capacity to reduce nitrate, antibiotic therapy, low-nitrate diets, strong diuresis, high levels of ascorbic acid, high specific gravity or insufficient urinary retention time in the bladder. False positive responses can be caused by dyes excreted in the urine (e.g. pyridium, red beets).

Leukocytes: False positive reactions may be caused by formaldehyde (preservative). Protein concentrations ≥ 500 mg/dL may diminish the color response. Bacteria, trichomonads and red blood cells do not, however, react with this test. High daily doses of cephalexin or gentamicin may diminish the color response. Very high concentrations of glucose or a high specific gravity may diminish the color response. Leukocyte esterase results may be positive in the absence of observable cells if the leukocytes have lysed.

Specific Gravity: Highly acidic urines (pH < 5) yield slightly elevated results whereas highly alkaline urines (pH ≥ 8) yield diminished results. This test is not affected by glucose or urea.

Expected Values

Bilirubin: Detectable amounts of bilirubin are not normally present in urine and therefore any reddish orange discoloration should be interpreted as a warning of the presence of bilirubin. The presence of bilirubin in the urine is very important in the early diagnosis of obstructive and hepatic jaundice and should be investigated.

Urobilinogen: Unlike bilirubin, urobilinogen is normally present (up to 1 mg/dL Urobilinogen) in urine. A 2.0 mg/dL result is at the transition from normal to abnormal, and further investigation is recommended.

Ketones: Normal urine specimens (up to 2 mg/dL ketones) should not be positive at the detectable limits of this test. Detectable levels of ketones may be expected in the following conditions: starvation, diabetes mellitus, digestive disturbances, dietary imbalance, eclampsia, prolonged vomiting and diarrhea.

Ascorbic Acid: Urinary ascorbic acid concentrations as low as 5.0 mg/dL may diminish the color development for glucose and red blood cells in specimens with low concentrations of these analytes. Concentrations ≥ 20.0 mg/dL can be expected to cause strong interference.

Glucose: A small amount of glucose (up to 20 mg/dL glucose) may be present in normal urine, but should fall below the detectable sensitivity of this test. Therefore, due to the sensitivity level established for this test, any positive reaction should be investigated.

Protein: Normally, no protein is detectable in the urine. Therefore, a color change from yellow to green (≥ 30 mg/dL protein) is considered pathological and should be investigated, especially when persistent.

Blood: Generally, red blood cells are not present in normal urine sediment, but the finding of 1–2 red blood cells per high power field (corresponds to 0.3 µL) should not be considered pathological. As such, the practical sensitivity of this test has been set at 5–10 RBCs/µL and all positives should be investigated. Intact red blood cells react as diffuse patches on the pad and the last three color blocks should be used for evaluation. Hemoglobin and myoglobin are indicated by a uniform green coloration, and color blocks 2 through 4 should be used for evaluating.

pH: Normal first morning urines have an average pH value of pH 5 to 6, with the values spread in the range of pH 4.8 to pH 7.5.

Nitrite: This test responds to urinary nitrate levels of 0.05 to 0.1 mg/dL by developing a faint pink color. Normally, no nitrite is detectable in urine and therefore, any positive response should be considered indicative of a urinary tract infection of bacterial origin. However, a negative result does not rule out an infection of the urinary tract.

Leukocytes: Normal urine often contains from 0–2 leukocytes per high power field. As such, the practical sensitivity of this test has been set at 10–20 leukocytes/µL urine. Partial cytolysis intensifies the color response, particularly in the region of the maximum ana-

lytical sensitivity. Discolorations that no longer match the negative color block are to be classed as positive.

Specific Gravity: The normal range for randomly collected urines is 1.003 to 1.035, with the exception in cases of excess hydration where the readings may be as low as 1.001. The normal range for a 24-hour urine collection is 1.015 to 1.025.

Specific Performance Characteristics

The performance characteristics of the QuickVue UrinChek 10+ SG and UrinChek 8+ test strips are based on both clinical and analytical studies. Sensitivity is dependent upon the color perception of the reader, the presence or absence of interfering specimens, and the lighting conditions for visual reading. Each color block on the chart corresponds to a range of analyte concentrations.

Bilirubin: In 90% of urines tested, bilirubin concentrations of 0.5 mg/dL produced a positive result.

Urobilinogen: The practical sensitivity S90 of this test lies at 1 mg urobilinogen/dL, which is the upper limit for a normal concentration in urine based on the work of Kutter[10].

Ketones: In 90% of urines tested, an acetoacetic acid concentration of 8 mg/dL produced a positive result. The test reacts less sensitively with acetone. Hydroxybutyric acid is not detected.

Ascorbic Acid: In 90% of urines tested, an ascorbic acid concentration of 20 mg/dL yielded a positive result.

Glucose: The maximum sensitivity is 20 mg/dL. However, the color scale for this test has been adjusted to 50 mg/dL with a practical sensitivity S90 equal to 45 mg/dL to permit good semi-quantitative evaluation in the diagnostically important decision range.

Protein: In 90% of urines tested, an albumin concentration of 12 mg/dL produced a positive result. The test is more sensitive to albumin than to globulin, Bence-Jones proteins and mucoproteins. A negative result does not exclude the presence of these other proteins.

Blood: This test permits the differentiation of intact red blood cells from hemoglobin/myoglobin. The practical sensitivity of the test lies between 5–10 red blood cells/µL (≈ 0.015 mg hemoglobin/dL). A study of 625 fresh urine specimens, comparing results with those using another test strip for blood demonstrated a clinical specificity of 90.2% and sensitivity of 81%.

pH: pH values are determined to within 1 unit over the range from 5 to 9.

Nitrite: The maximum sensitivity for this test is 0.05 mg/dL, which is equivalent to about 100,000 bacteria/mL of urine. Up to 90% of all patients with a urinary tract infection can be detected by the testing of a first-morning urine specimen. Results depend on the ability of the bacteria to reduce nitrate to nitrite, the number of bacteria, the nitrate content and the retention time of the urine.

Leukocytes: In 90% of urines tested, specimens with a concentration of 20 leukocytes/µL produced positive results. When 822 fresh urines were tested using this method and a comparative test strip method for leukocytes, clinical specificity of 80% and sensitivity of 89.2% were determined.

Specific Gravity: In 86% of 102 urines tested, the specific gravity results obtained from the color chart were found to be within +/- one color increment (0.005) when compared to the determinations of the reference refractometer.

Assistance

If you have any questions regarding the use of this product, please call Quidel's Technical Support number, 800-874-1517 (toll-free in the U.S.) or 858-552-1100, Monday through Friday, between 7:00 a.m. and 5:00 p.m., Pacific Time. Outside the United States, contact your local distributor.

Literature

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Catalog Numbers:

20104 UrinChek 10+ SG -	150 Tests (includes laminated color chart and report sheets)
20116 UrinChek 10+ SG -	150 Tests
20117 UrinChek 8+ -	150 Tests

Chemstrip®
6, 7, 9, 10 with SG

(Eng) *Urine Test Strips For Specific Gravity, pH, Leukocytes, Nitrite, Protein, Glucose, Ketones, Urobilinogen, Bilirubin, Blood, Hemoglobin*
Catalog Nos. 417145, 417109, 417126, 417135

Instructions for use with Chemstrip® 10 with SG, Chemstrip® 9, Chemstrip® 7, Chemstrip® 6 Urine Test Strips. The Chemstrip® urine testing system is a multi-parameter test strip to measure certain constituents in the urine. These measurements are useful in the evaluation of renal, urinary, and metabolic disorders.

Summary:

Chemstrip® urine test strips are inert plastic strips to which are attached different reagent pads for determining specific gravity, pH, indication of leukocytes, nitrite, protein, glucose, ketones, urobilinogen, bilirubin, and blood and hemoglobin in urine. *Please refer to the outside box and vial label for the specific parameters of the product you are using.* The test pads are uniquely attached to the strip with a nylon mesh which holds the reagent pad in place, protects the pad, and provides for rapid and even wetting of the entire test pad. To prevent urine runover, certain test pads have an inert absorbent paper located between the test pads and the strip.
The Chemstrip® urine test strips are packaged in a vial with a tightly fitting cap which contains a drying agent. Each test strip is stable and ready for use when removed from the vial. No additional instrumentation is required.

Test Principle:

A brief discussion of each test principle follows.

Specific Gravity: In the presence of cations, protons are released by a complexing agent in the test and produce a color change of the indicator bromthymol blue from blue to blue-green to yellow.

pH: The method of determining the pH (in urine) by means of pH indicators is well-known. The test pad contains the indicators methyl red and bromthymol blue. These give clearly distinguishable colors over the pH range of 5-9. Colors range from orange through yellow and green to blue. The application of the indicators used in this test pad was first described by Felton in 1921 and transferred to test pads for urinalysis in 1960.[4,5]

Leukocytes: Leukocytes in urine are detected by the action of esterase, present in granulocytic leukocytes, which catalyzes the hydrolysis of an indoxylcarbonic acid ester to indoxyl. The indoxyl formed reacts with a diazonium salt to produce a purple color.

Nitrite: The detection of nitrite is based upon a reaction discovered by Griess in 1879. Wettmann (1922) was the first to recommend this reaction for the detection of urinary tract infections. The first application to a test strip is described by Fuchs and Gutensohn. The nitrite test used in this test strip is a refinement of previous methods and exhibits increased sensitivity. Nitrite, if present, reacts with an aromatic amine to give a diazonium salt, which by coupling with sulfanilamide, yields a red-violet azo dye.[1,2,3]

Protein: The detection of protein is based on the so-called "protein error of pH indicators" (Sörensen, 1909). The indicator 3',3'',5',5''-tetrachlorophenol-3,4,5,6-tetrabromosulfophthalein used in this test is a more recent development. A positive reaction is indicated by a color change from yellow to light green/green.[6,7,8]

Glucose: Glucose detection is based on the enzymatic glucose oxidase/peroxidase (GOD/POD) method. This method was first described by Keston in 1956 for the determination of blood glucose and applied by Comer to a test pad for glucose in urine. The glucose test of this strip is a further development of this test principle. The reaction utilizes the enzyme glucose oxidase to catalyze the formation of gluconic acid and hydrogen peroxide from the oxidation of glucose. In turn, a second enzyme, peroxidase, catalyzes the reaction of hydrogen peroxide with the chromogen tetramethylbenzidine to form a green dye complex. A positive reaction is indicated by a color change from yellow to green.[8,10]

Ketones: The detection of ketone bodies in urine is based on a well-known method attributed to Legal. The application of the method to a dip test was first described by Chertack and Sherrick. The test pad used in this test strip corresponds closely to this method. Based on the principle of Legal's test, sodium nitroprusside and glycine react with acetoacetate and acetone in an alkaline medium to form a violet dye complex. A positive result is indicated by a color change from beige to violet.[11,12]

Urobilinogen: For many years, the detection of urobilinogen in urine has been by the Ehrlich aldehyde reaction. In recent years, more modern biochemical techniques have demonstrated that this reaction can be unreliable, giving both false-positive and false-negative results. The urobilinogen test pad on Chemstrip® 9 and Chemstrip® 10 with SG urine test strips is a more recent development first reported by Kutter. Urobilinogen is coupled with 4-methoxybenzene-diazonium-tetrafluoroborate in an acid medium to form a red azo dye.[13]

Bilirubin: The detection of bilirubin is based on the coupling reaction of a diazonium salt with bilirubin in an acid medium. The application of 2,6-dichlorobenzene-diazonium-tetrafluoroborate, however, which is used in the test strip is unique. This yields a pink to red-violet color proportional to the total bilirubin concentration.[14]

Blood: The chemical detection of blood is based on the strong pseudoperoxidase action of erythrocytes and hemoglobin. There are numerous methods described in the literature, which in addition to various substrates (peroxides), mention various chromogens. Leonards (1962) was the first to report the use of a test strip based on o-tolidine for the detection of blood in the urine. The blood test of this strip is a further development of this test principle in that the sensitivity has been extended into the physiological range of erythrocyte excretion. Hemoglobin and myoglobin, if present, catalyze the oxidation of the indicator by the organic peroxide contained in the test pad. Intact erythrocytes hemolyze on the test pad, and the liberated hemoglobin produces a green dot. Since the test pad absorbs several µL of urine, more erythrocytes become visible than would correspond to 1µL.[15-18]

Separate sets of color blocks are given for erythrocytes and hemoglobin. Scattered or compacted green dots on the yellow test pad are indicative of intact erythrocytes. A uniform green coloration of the test is indicative of free hemoglobin, myoglobin, or hemolyzed erythrocytes in the urine.

Reagent Composition per cm² for each test pad

See the outside of the test strip box for reagent composition.

Warnings and Precautions:

For *in vitro* diagnostic use.

Warning: Avoid contact with skin and mucous membranes; flush affected areas with copious amounts of water. Get immediate medical attention for eyes or if ingested. Exercise the normal precautions required for handling all laboratory reagents.

Gloves: The "universal precautions" recommended by the Centers for Disease Control and Prevention should be followed whenever blood or body fluids are handled. These precautions include wearing gloves.

Storage and Stability: Store at temperatures under 30°C (86°F). Do not freeze. Chemstrip® urine test strips are stable in the original capped vial until the listed expiration date. In order to avoid exposure to moisture, the vial must be closed immediately after removal of a strip, using the original stopper which contains a drying agent.

Specimen Collection and Preparation:

Chemstrip® urine test strips may be used on any freshly voided urine specimen or on urines collected under special conditions, such as first-morning specimens and post-prandial urines. The urine must be collected in a clean container and should be tested as soon as possible after collection (do not centrifuge or use preservatives). It is of particular importance to use fresh urine to obtain the best results with the test for urine bilirubin and urobilinogen as these compounds are very unstable when exposed to room temperature and daylight. If testing cannot be performed within one hour after collection, the specimen should be refrigerated at 2–8°C immediately and returned to room temperature before testing. *Mix urine thoroughly before testing.* Urine should be collected in a container which allows complete immersion of the reagent pads on the test strip. If a cleanly voided urine is not collected, a positive test result for leukocytes or blood may be due to a source of leukocytes or blood external to the renal-urinary system.

Procedure:

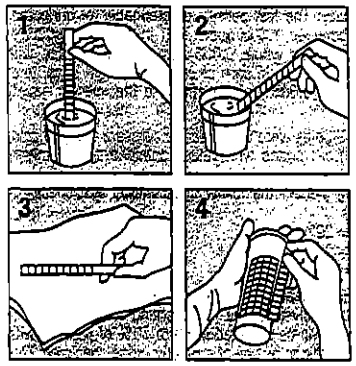
Materials Provided: 1 vial containing 100 Chemstrip® urine test strips. A visual comparison color scale for reading test results is printed on the vial label.

Material Required, But Not Provided: A timer and a clean specimen collection container. It is also recommended that commercial control products be used for quality control checks.

Assay:

1. Briefly (no longer than 1 second) dip test strip into the urine. Ensure that the chemically impregnated pads on the test strip are totally immersed.
2. Draw the edge of the strip along the rim of the specimen container to remove excess urine.
3. Turn the test strip on its side and tap once on a piece of absorbent paper to remove any remaining urine, and to prevent the possible mixing of chemicals.
4. After the appropriate time read the test as follows:
   Hold strip close to color blocks and match carefully, ensuring that the strip is properly oriented to the color chart on the vial label.

Specific Gravity	60 seconds	Glucose	60 seconds
pH	60 seconds	Ketones	60 seconds
Leukocytes	60–120 seconds	Urobilinogen	60 seconds
Nitrite	60 seconds	Bilirubin	60 seconds
Protein	60 seconds	Blood	60 seconds



All test pads should be read at 1 m[illegible] Leukocytes pad indicates a trace result, it should be re[illegible] at 2 minutes.
Color changes that occur after 2 minutes [illegible] immersion are not of clinical value. Color changes that occur only along the edge of the test pad should be ignored. Careful removal of excess urine (steps 2 and 3) should eliminate this effect.

Calibration:

Calibration of the Chemstrip® 6, 7, 9 and 10 SG urine test strips by the user is not required.

Quality Control:

Quality control for this procedure consists of following good laboratory techniques and ensuring that reagents have been properly stored and specimens handled according to instructions. The analyst should be aware of the sources of error outlined under *Limitations*. Each laboratory should establish its own goals for adequate standards of performance.
Commercially prepared control solutions should be used on a regular basis, as established by the institution's quality control protocols.
If the expected results are not obtained and repetition of the assay excludes errors in technique, the following steps should be taken:

1. Check the expiration date stamped on the vial label.
2. To verify that the Chemstrip® urine test strip has not been exposed to heat extremes or moisture, open a new vial of test strips and retest.
3. For further information, contact Roche Diagnostics Technical Service Center, 1-800-428-4674, 7 days a week, 24 hours a day.

Results:

Results are obtained by direct visual comparison with the color scale printed on the vial label. No calculations are necessary. The visual color chart is not intended to represent quantitative findings and serves only as a screening mechanism. If quantitative results are desired, it is recommended that further testing of the urine be carried out utilizing a reference procedure.

Limitations:

The limitations including interfering substances for each reagent are shown below.

Specific Gravity: Results may vary between urine concentration measuring methodologies due to their differing principles and limitations.[23] Urines above 1.025 are not reliably measured with current relative ionic concentration methodology. Test samples with results above 1.025 should be retested with a refractometer or urinometer. The chemical principle of this test may also cause slightly different results compared with other urine concentration measuring methods when elevated amounts of certain urine constituents are present. Glucose and urea concentrations greater than 1% may cause a low specific gravity reading relative to other methods. In the presence of moderate amounts of protein (100–500 mg/dL) or ketoacidosis, readings tend to be elevated.

pH Test: No known interferences when handled according to instructions.

Leukocyte Test: This test is not affected by erythrocytes in concentrations up to 10,000/µL or by bacteria common in urine. Specimens should not be collected in containers that have been cleaned with strong oxidizing agents. Do not use preservatives. The drugs cephalexin and gentamicin have been found to interfere with this test. In addition nitrofurantoin colors the urine and this effect interferes with visual interpretation of the test strip. High levels of albumin (≥ 500 mg/dL) in the urine may interfere with the test results. Studies show that formaldehyde (stabilizer) and medication with imipenem, meropenem and clavulanic acid may cause false-positive reactions.[24]

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Nitrite Test: Large amounts of ascorbic acid (see under glucose) decrease the sensitivity of the test. False-positive readings may be produced by medication that colors the urine red or which turns red in an acid medium (e.g., phenazopyridine).

Protein Test:
False-positive results may be found:
1. In strongly basic urine (pH 9 or higher).
2. During therapy with phenazopyridine.
3. When infusions of polyvinylpyrrolidone (blood substitutes) are administered.
4. When residues of disinfectants containing quaternary ammonium groups or chlorohexidine are present in the urine container.

Glucose Test: The effect of ascorbic acid (vitamin C) retained in the urine due to ingestion of vitamin tablets, antibiotics or fruit juices has been eliminated at glucose concentrations of 100 mg/dL and above so that false-negative readings may only rarely occur, even at high concentration of ascorbic acid. False-positive readings may be produced by strong oxidizing cleaning agents in the urine container.

Ketone Test: Red-orange to red color shades, which are, however, readily distinguished from the colors obtained with ketone bodies, can be produced by phenylketone or phthalein compounds that may be administered for liver and kidney function tests. 2-Mercaptoethane sulphonate sodium (MESNA) or other sulfhydryl-containing compounds may cause false-positive results.[20]

Urobilinogen Test: The total absence of urobilinogen cannot be detected. Most normal urines give a slight pink reaction. The test gives the same color reaction with urobilinogen as with stercobilinogen; however, the differentiation is not of diagnostic importance. Urine from patients who are being treated with phenazopyridine may show a false-positive reaction. Nitrite concentrations above 5 mg/dL or formalin concentrations above 200 mg/dL (as a preservative) may cause a decrease in the color reaction.

Bilirubin Test: Large amounts of ascorbic acid present in urine following the ingestion of medication containing vitamin C or fruit juices lower the sensitivity of the test. In case of doubt, the test should be repeated on urine voided at least 10 hours after the last administration of vitamin C. Elevated concentrations of nitrite, as in urinary tract infections, may result in lower bilirubin values. Large amounts of urobilinogen in the urine affect the color change of the bilirubin test, but not enough to give a positive result. False-positive readings may be produced by medication that colors the urine red, or which turns red in an acid medium (e.g. phenazopyridine).

Blood Test: False-negative readings are obtained when formalin is used to preserve the urine. Nitrite in excess of 10 mg/dL in the urine (which is rare in urinary-tract infections) delays the reaction. False-positive results can be produced by residues of strongly oxidizing cleaning agents in the urine container. Urine from menstruating females will occasionally yield a positive result. This test has not been found to be affected by the ingestion of reasonable quantities of ascorbic acid.

Expected Values:

Specific Gravity: Random urines vary from 1.001–1.035. Twenty-four hour urines from normal adults with normal diets and fluid intake will have a specific gravity of 1.016–1.022.[21]

pH: Urine pH values generally range from 5 to 9 units. The most frequent pH values for the first morning specimens in healthy subjects are between pH 5 and 6.

Leukocytes: Normal urines should produce no color reaction. A "trace" finding indicates a possible borderline situation, and it is recommended that the test be repeated on a fresh urine sample from the same patient. Positive and repeated trace findings indicate the need for further testing of the patient and/or urine sample in accordance with the medically accepted procedures for pyuria.

Nitrite: A concentration as low as 0.05 mg/dL of nitrite will produce a slightly pink coloration of the test pad. This indicates a positive result.

Protein: A color change from yellow to light green/green will occur if protein is present in urine. The concentrations given on the vial label correspond favorably with the albumin concentration in urine. Pathological proteinuria will usually produce persistent values above 30 mg/dL. Clinical significance of the trace result should be determined by additional testing.

Glucose: Due to the test's sensitivity, glucose should not be detectable in normal urine. Therefore, any positive reaction should be followed by further diagnostic evaluation of the patient, such as a quantitative blood glucose or a glucose tolerance test.

Ketones: Ketone bodies should not be detected in normal urine with this test. Fasting or starvation diets may cause positive indications. In known pathological conditions such as diabetes, the presence of ketones may be useful as an index of metabolic status.

Urobilinogen: Concentrations are usually greater in the afternoon than during the remainder of the day. Values up to 1 mg/dL are usually considered normal.[13]

Bilirubin: In normal urine, bilirubin should not be detectable with this test. However, this 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.

Blood: A trace result is equivalent to 5–10 Ery/µL. Erythrocyte excretion up to 5 Ery/µL may be expected in normal urine.[17,18] Levels above these certainly warrant further diagnostic evaluation of the patient.

Performance Characteristics:

The performance characteristics of the Chemstrip® products have been determined both in the laboratory and in clinical tests. Parameters of importance to the user are sensitivity, specificity, accuracy, precision, and stability. Generally, the tests have been developed to be specific for the constituent to be measured with the exception of interferences listed previously. The stability data has been developed by testing at various temperatures and environmental conditions.

For visually read strips, accuracy is a function of the manner in which the color blocks on the vial label are determined and the discrimination of the human eye in reading the tests. 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 each user is encouraged to develop his own standards for performance.

Specific Gravity: The test permits determination of urine specific gravity between 1.000 and 1.030 in steps of 0.005. In general, it correlates within 0.005 with values obtained with refractometric methods. In case of urines with a pH equal to or greater than 7.0, 0.005 may be added to the specific gravity readings.

Leukocytes: Studies were conducted to compare test pad color development from urines with values obtained by the microscopic method.
Clinical testing yielded the following sensitivity and specificity data:

n	= 203
Sensitivity	= 97.2%
Specificity	= 90.1%

Nitrite: Up to 90% of all patients with urinary-tract infections can be detected by analysis of the first-morning urine specimen.[22] A positive result will be detected in 50 to 70% of patients with urinary-tract infections by use of a random urine specimen. This is dependent on the number of bacteria, nitrite content and retention time of the urine in the bladder. The frequency of false-positive results in normal patients is negligible (less than 1%).

pH: Values from pH 5 to pH 9 may be read to within 1 unit.

Protein: In 90% of urines tested, albumin concentrations of 6 mg/dL or greater produced a color change. The test pad is more sensitive to albumin than globulin, Bence-Jones proteins and mucoproteins.

Glucose: In 90% of urines tested, glucose concentrations of 40 mg/dL or greater produced a positive result. Sugars other than glucose that may be found in urine were tested and found not to react with the reagent. Reducing substances will not give positive results.

Ketones: In 90% of urines tested, acetoacetate at 9 mg/dL or acetone at 70 mg/dL will produce a positive reaction. Beta-hydroxybutyric acid does not contribute to the color development.

Urobilinogen: The sensitivity of the urobilinogen test pad is approximately 0.4 mg/dL; therefore, most normal urines give a slight pink reaction.

Bilirubin: In 90% of tested urines, bilirubin concentrations as low as 0.5 mg/dL produced a positive result.

Blood: Differentiation of hemoglobin from erythrocytes can be determined by the color comparison chart on the vial label. In 90% of urines tested, concentrations of 5 Ery/µL and hemoglobin content corresponding to 10 Ery/µL produced a positive result.[16,19] A field study of 637 freshly voided urine specimens in routine diagnosis produced no false-negative results and in only a small percentage of cases, recorded a higher erythrocyte concentration than the ten-field sediment method.[17]

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This product is covered by the U.S. Patent Nos. 3,853,476; 3,880,588; 3,853,471; 3,853,472; 3,853,466; 3,712,853; 3,897,214; 3,802,842; 4,013,416; 4,385,114.

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Manufactured for: Roche Diagnostics Corporation
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