iChem 10 SG Urine Chemistry Strips
Beckman Coulter, IRIS Diagnostics Division

Technical Procedure 3312T

Principle

Intended Use

iChem™ 10 SG Urine Chemistry Strips, when used with the iChem™ 100 Urine Chemistry Analyzer, a semi-automated benchtop urine chemistry analyzer, is intended for the *in vitro* measurement of the following analytes: glucose, protein, bilirubin, urobilinogen, pH, specific gravity, blood, ketones, nitrite, leukocyte esterase, ascorbic acid, and color. At UCDHS, specific gravity will not be reported by the iChem™ analyzer. Specific Gravity will be reported by refractometer.

Clinical Significance

These measurements are useful the evaluation of renal, urinary and metabolic disorders.

Methodology

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 is based on the coupling reaction of urobilinogen with a stable diazonium salt in buffer. A pinto red color proportional to urobilinogen concentration is produced.

Ketones:

This test is based on the coupling of methyl-ketone with glycine and sodium nitroprusside in alkaline buffer. A violet color proportional to ketone concentration is produced.

Ascorbic Acid:

The test is based on the decolorization of Tillmann's reagent. The presence of ascorbic acid causes the color of the test pad to change from gray-blue to orange. (not reported at UCDHS)

Glucose:

This test is based on the enzymatic reaction of glucose with 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 test is based on the "protein error" of the pH indicator on the green color formed in the presence of protein. The reaction is particularly strong for albumin.

Blood:

This buffered test contains an organic peroxide and a chromogen. The preoxidase 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 iin the urine diazotizes the amine. The subsequent coupling reaction produces a pink color.

Leukocytes:

This test is based on the enzymatic reaction of granulocyte esterases with an indoxyl ester in the presence of 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.

NOTE: Specific Gravity is not reported from the iChem SG pad; Specific Gravity is reported by refractometer.

Reagents

Catalog Number: iChem™ 10 SG Urine Chemistry Strips: 800-7004

Composition:

Strip Pad	Constituents Present
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

iChem™ 10 SG Urine Chemistry 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. This product should not be disposed in general waste, but should be discarded with infectious medical waste. Do not touch the test strip pads. Discard any discolored strips as they may have deteriorated.

Storage and Stability

Store 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. Unused strips that remain in the original capped container are stable until the expiration date. Do not use test strips after the expiration date.

Specimen Collection and Preparation

Acceptable Sample Containers

Sterile collection bottles
BD yellow top urinalysis tubes
BD tiger top urinalysis tubes with preservative.

Gray Top culture tubes are not acceptable.

Sample Collection

A clean freshly voided midstream specimen should be collected in a clean container for routine analysis, and a sterile container for UACII requests. Infant bag collections are acceptable for children ≤ 2 years of age. Other acceptable specimens include catheterized specimens, suprapubic and ostomy collections, as well as kidney or bladder collections from the operating room.

BD tiger top urinalysis preservative tubes must be filled to a level between the marked minimum and maximum lines on the tubes (7-9 mL). Under-filled or over-filled tubes are unacceptable.

For best results, BD yellow top urinalysis tubes without preservative require eight (8) mL for UA or UACII.

Sample Stability and Handling

1. Urine collected without preservative at room temperature must be delivered to the lab within 1 hour of collection.
2. Urine collected without preservative and immediately placed on ice must be delivered within 4 hours of collection.
3. Urine collected in BD urinalysis preservative tubes will be accepted up to 48 hours after collection.

All specimens should be handled using the principles of Universal Precautions, and must be capped tightly.

Specimens that leak are unacceptable for analysis.

Calibration

Prior to each measurement, the optics assembly is calibrated using a "fixed standard" and it permits a one-point calibration. The iChem100 is also capable of performing an internal, automated two-point calibration if the one-point calibration done before each specimen determination falls outside of the acceptable limits. A secondary "movable standard" is automatically moved into place and readings from it in conjunction with the "fixed standard" are used to perform a two-point calibration. No external calibration strips or procedures are required.

Quality Control

At least two levels of control material should be analyzed daily. Parallel testing between the old shipment or lot number and the new shipment or lot number will be done to assure acceptable strip performance.

The following controls should be prepared and used in accordance with the package inserts. Allow controls to come to room temperature and mix well for several minutes before testing. Quality Control results should be evaluated and handled with respect to the Clinical Chemistry Quality Control Procedure #3000.T. Strip lot changes are documented on the IRIS reagent log sheet.

Quality Control Material

Control	Storage
MAS Liquid UA Abnormal Control 1	+2°C to +8°C*
MAS Liquid UA Normal Control 3	+2°C to +8°C*

*Urine controls are received and stored at 2°C to 8°C. Bottles of controls in use are stored at +2°C to +8°C and are good for 30 days

Procedure

Materials Provided

iChem™ 10 SG Urine Chemistry Strips come with 100 reagent strips and a package insert.

Method

1. Dip the test strip into the urine specimen so that all the test pads 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). Blot the test strip with an absorbent paper.
3. Place the strip on the iChem™100 Urine Chemistry Analyzer immediately after blotting. Results are available after 120 seconds.

Refer to Iris Diagnostics *iChem 100 Urine Chemistry Analyzer Operators Manual 1.0* for detailed instructions.

Results

Analyte	Interpretation								
Bilirubin	Semi-Quant	Neg	+	++	+++				
	Conc. mg/dL		1	2	4				
	Conc. µmol/L		17	35	70				
Urobilinogen	Semi-Quant	Norm.	+	++	+++	++++			
	Conc. mg/dL		2	4	8	12			
	Conc. µmol/L		35	70	140	200			
Ketones	Semi-Quant	Neg	+	++	+++				
	Conc. mg/dL		25	100	300				
	Conc. mmol/L		2.5	10	30				
Ascorbic Acid	Semi-Quant	Neg	+	++					
	Conc. mg/dL		20	40					
	Conc. mmol/L		1.14	2.28					
Glucose	Semi-Quant	Neg	+	++	+++	++++			
	Conc. mg/dL		50	150	500	≥1000			
	Conc. mmol/L		3	8	28	≥56			
Protein	Semi-Quant	Neg	+	++	+++				
	Conc. mg/dL		30	100	≥500				
	Conc. g/L		0.3	1	≥5				
Blood	Semi-Quant	Neg	+	++	+++				
	Conc. mg/dL		0.03	0.2	≥1				
	Conc. RBCs/µL		5-10	50	300				
pH	Value	5	6	7	8	9			
Nitrite	Semi-Quant	Neg	+						
Leukocytes	Semi-Quant	Norm.	+	++	+++				
	Conc. WBCs/µL		25	75	500				
SG	Value	**Reported by Refractometer**							

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:

Excreted pigments and medications that have an intrinsic red coloration in acidic medium may produce false positive results (phenazopyridine, red beets, azo dyes, p-aminobenzoic acid).

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 interferent is ascorbic acid. 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. Other factors that may inhibit color formation are high specific gravity, gentisic acid and acidic pH values (pH 4), particularly in association with ketonuria. False positive reactions may be caused by hypochlorite or peroxide (cleaning agents).

Protein:

False positive results may be caused by highly alkaline urines (pH > 9), high specific gravity, disinfectants, wetting agents and blood substitutes (polyvinylpyrrolidone, quartenary ammonium compounds, chlorohexidine). Therapeutic dyes (methylene blue, pyridium) or red pigment may mask the color.

Blood:

Non-specific oxygen acceptors such as uric acid, glutathione, gentisic acid and ascorbic acid may cause false negative results. Formalin, hypochlorite or peroxide containing cleaning agents can cause false positive reactions. 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 non-nitrite producing microorganisms, 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 = 300 mg/dL, cephalexin, gentamicin, very high concentrations of glucose or a high specific gravity may diminish the color response. Leukocyte results may be positive in the absence of observable cells if the granulocytes have lysed. The test can be negative in the presence of visible leukocytes if they have not lysed and/or are not granulocytes.

Specific Gravity:

pH < 5 yield slightly elevated results, whereas pH = 8 yield lowered results.

Expected Values

Bilirubin:

No bilirubin is detected in normal urine.

Urobilinogen:

Urobilinogen is normally present (up to 2 mg/dL) in urine. A 2 mg/dL result is at the transition from normal to abnormal, and further investigation is recommended.

Ketones:

Ketones are not normally detected in the urine of healthy individuals. 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:

Concentrations greater than or equal to 20 mg/dL can be expected to cause strong interference in the reactions test- ing for glucose, nitrite and blood.

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:

Any positive reaction should be investigated.

pH:

Typical first morning urines from healthy individuals have an average pH value of pH 5 to 6. The full range of normal and abnormal urines may be 5.0 – 9.0

Nitrite:

This test responds to urinary nitrite levels of 0.1 mg/dL by developing a faint pink color. Normal urine contains no nitrites. However, a negative result does not rule out a urinary tract infection.

Leukocytes:

Normal urine specimens should not produce a positive result. Cell lysis intensifies the color response, particularly in the region of the maximum analytical sensitivity.

Specific Gravity:

Specific gravity is reported out by refractometer. Refer to Technical Procedure 3355, *Refractive Index - Urine*.

Standard Reporting Format

Bilirubun	**LIS result**	Negative	Small	Moderate	Large	
	Semiquantitative result	Neg	1+	2+	3+	
Blood	**LIS result**	Negative	Small	Moderate	Large	
	Semiquantitative result	Neg	1+	2+	3+	
Glucose	**LIS result (mg/dL)**	Negative	50	150	500	>=1000
	Semiquantitative result	Neg	1+	2+	3+	4+
Ketones	**LIS result (mg/dL)**	Negative	25	100	300	
	Semiquantitative result	Neg	1+	2+	3+	
Leukocytes	**LIS result**	Negative	Small	Moderate	Large	
	Value(Leu/µL)	Neg	25	75	500	
Nitrite	**LIS result**	Negative	POSITIVE			
	Semiquantitative result	Neg	+			
pH	**LIS result**	5	6	7	8	9
Protein	**LIS result (mg/dL)**	Negative	30	100	>= 500	
	Semiquantitative result	Neg	1+	2+	3+	
Urobilinogen	**LIS result (mg/dL)**	< 2.0	2.0	4.0	8.0	12.0
	Semiquantitative result	Norm	1+	2+	3+	4+
Specific Gravity	**LIS result**	Reported by Refractomter				

Method Comparison

The clinical performance of the iChem™100 Urine Chemistry Analyzer was evaluated in comparison with a commercially available semi-automated urine chemistry analyzer. Random urine samples were obtained from a local medical center without a pre-screening procedure. Overall agreement between the iChem100 and comparable analyzer is shown in the table below. For pH and specific gravity, the overall agreement was reported within plus or minus one concentration grid. For ascorbic acid, the comparison was measured against another urinalysis system with the capacity of reporting ascorbic acid concentration semi-quantitatively.

Analyte	Number of Samples	Overall Agreement (%)
Bilirubin	188	89.9
Urobilinogen	188	92.0
Ketones	188	97.3
Ascorbic Acid*	188	97.2
Glucose	188	98.9
Protein	188	91.5
Blood	188	95.7
pH	188	99.5
Nitrite	188	98.4
Leukocytes	188	89.9
Specific Gravity	188	70.7[a]

[a]Specific Gravity is resulted by Refractometer. iChem Strip results for SG are not reported.

Validation Studies performed at UCDHS

See attached validation method comparison sheet.

Precision

The within-run precision of the iChem™100 Urine Chemistry Analyzer was evaluated by performing the measurement of twenty replicates of a commercially available control material within one single run. The total precision of the iChem™100 Urine Chemistry Analyzer was evaluated by performing the measurement of twenty-three runs of a commercially available control material within a thirteen-day period. Results were reported by the system in the reflectance values format. Both data are shown in the tables below.

Within-Run Precision

Control at Normal Levels

Analyte	Control Range	% Reflectance	Stamdard Deviation	% CV	95% Confidence Interval for CV
Bilirubin	Neg	65.1	1.7	2.6	[2.0 - 3.8]
Urobilinogen	Norm	59.9	1.4	2.3	[1.8 - 3.4]
Ketones	Neg	60.6	1.5	2.5	[1.9 - 3.6]
Ascorbic Acid	20 - 40 mg/dL	55.4	0.7	1.3	[1.0 - 1.8]
Protein	Neg	60.7	0.4	0.66	[0.5 - 1.0]
pH	5 - 9	65.4	1.0	1.5	[1.2 - 2.2]
Nitrite	Neg	67.8	1.8	2.6	[2.0 - 3.9]
Leukocytes	Neg	64.8	1.2	1.8	[1.4 - 2.7]
Specific Gravity	1.000 - 1.035	26.1	0.8	3.1	[2.3 - 4.5]
Blood	Neg	65.0	0.6	0.9	[0.7 - 1.3]
Glucose	Neg	79.0	1.4	1.8	[1.3 - 2.6]

Control at Abnormal Levels

Analyte	Control Range	% Reflectance	Standard Deviation	%CV	95% Confidence Interval for CV
Bilirubin	1-4 mg/dL	30.5	1.7	5.6	[4.2-8.2]
Urobilinogen	2-12 mg/dL	40.2	1.8	4.5	[3.4-6.5]
Ketones	25-300 mg/dL	8.8	0.5	5.7	[4.3-8.3]
Ascorbic Acid	Neg	8.1	0.3	3.7	[2.8-5.4]
Protein	30 - ≥500 mg/dL	29.0	0.4	1.4	[1.0-2.0]
pH	5-9	38.6	1.2	3.1	[2.4-4.5]
Nitrite	Pos	47.9	1.2	2.5	[1.9-3.7]
Leukocyte	25-500 WBCs/µL	55.5	0.8	1.4	[1.1-2.1]
Specific Gravity	1.000-1.035	21.1	1.4	6.6	[5.0-9.7]
Blood	0.03-1.0 mg/dL	3.8	0.1	2.6	[2.0-3.8]
Glucose	50 - ≥1000 mg/dL	20.1	1.5	7.4	[5.7-10.9]

Total Precision

Control at Normal Levels

Analyte	Control Range	% Reflectance	Stamdard Deviation	% CV	95% Confidence Interval for CV
Bilirubin	Neg	63.6	2.1	3.3	[2.6 - 4.7]
Urobilinogen	Norm	57.7	1.9	3.3	[2.6 - 4.7]
Ketones	Neg	57.9	2.3	4.0	[3.1 - 5.6]
Ascorbic Acid	20 - 40 mg/dL	56.2	1.2	2.1	[1.6 - 3.0]
Protein	Neg	61.9	1.2	1.9	[1.5 - 2.7]
pH	5 - 9	66.6	1.0	1.5	[1.2 - 2.1]
Nitrite	Neg	65.1	2.1	3.3	[2.5 - 4.6]
Leukocytes	Neg	62.5	1.9	3.0	[2.4 - 4.3]
Specific Gravity	1.000 - 1.035	27.9	1.3	4.5	[3.6 - 6.6]
Blood	Neg	65.4	0.8	1.2	[0.9 - 1.7]
Glucose	Neg	78.9	1.4	1.8	[1.4 - 2.5]

Control at Abnormal Levels

Analyte	Control Range	% Reflectance	Standard Deviation	%CV	95% Confidence Interval for CV
Bilirubin	1-4 mg/dL	28.5	2.5	8.8	[6.8-12.5]
Urobilinogen	2-12 mg/dL	38.2	1.9	5.0	[3.8-7.0]
Ketones	25-300 mg/dL	7.5	0.7	9.3	[7.2-13.3]
Ascorbic Acid	Neg	7.9	0.7	8.9	[6.8-12.6]
Protein	30 - ≥500 mg/dL	29.0	0.9	3.1	[2.4-4.4]
pH	5-9	39.8	1.3	3.3	[2.5-4.6]
Nitrite	Pos	46.4	1.5	3.2	[2.5-4.6]
Leukocyte	25-500 WBCs/µL	54.3	1.3	2.4	[1.8-3.4]
Specific Gravity	1.000-1.035	19.8	1.5	7.6	[5.8-10.8]
Blood	0.03-1.0 mg/dL	4.1	0.1	2.4	[1.9-3.4]
Glucose	50 - ≥1000 mg/dL	20.8	1.3	6.2	[4.8-8.9]

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Prepared By	Date Adopted	Supersedes Procedure #
Michael Inn	07/09/2008	New

Revision Date	Type of Revision	Revised by	Review/Annual Review Date	Reviewed By
	Edited from iChem strips insert		07/09/2008	G. Kost
			09/15/2009	G. Kost
			10/12/2010	G. Kost
			11/16/2011	G. Kost
10/05/2012	Updated from iChem strips insert	M.Inn	11/20/2013	G. Kost
03/20/2015	Control change to MAS Urine Controls and Discontinue reporting SG from iChem strips	kdagang	04/15/2015	J. Gregg