Effective Date: January, 2010	Policy No: UA120.01
Cross Referenced:	Origin: Urinalysis
Reviewed Date: 6/2012	Authority: Laboratory Director
Revised Date:01/12	Page: 1 of 4

PRINCIPLE: To describe procedure for analyzing urine specimens.

PROCEDURE: As follows.

Urinalysis is performed to screen for abnormalities in urine. It includes visual examination for color and clarity, semi-quantitative screening for pH, protein, glucose, ketone, bilirubin, blood urobilinogen, nitrite, and leukocyte. If indicated a microscopic examination for cells, casts, crystals, bacteria, yeast and/or may other abnormal elements is done.

Each of the tests included on the MultiStix 10 SG has its own principle. These are summarized below:

<u>Glucose</u>: the formation of gluconic acid and hydrogen peroxide from the oxidation of glucose is catalyzed by glucose oxidase. The reaction of hydrogen peroxide with a potassium iodide chromogen is catalyzed by peroxidase to form colors ranging from green to brown, depending on glucose concentration. <u>Bilirubin</u>: bilirubin couples with diazotized dichloraniline in a strongly acidic medium, resulting in various shades of tan.

Ketone: acetoacetic acid reacts with nitroprusside to form colors from buff-pink to purple.

<u>Specific Gravity</u>: certain pretreated polyelectrolytes go through an apparent pKa change in relation to ionic concentration. Colors range from a deep blue-green in urine of low ionic concentration to yellow-green in urines of increasing ionic concentrations in the presence of an indicator.

<u>Blood</u>: hemoglobin's peroxidase-like activity catalyzes the reaction of cumene hydroperoxide and 3,3', 5,5'-tramethylbenzadine with resulting colors range from orange through green to dark blue.

<u>pH</u>: this test uses a double indicator principle to cover the entire range of urinary pH with color ranging from orange through yellow and green to blue.

<u>Protein</u>: at a constant pH, the development of any green color is due to the presence of protein. Colors range from yellow, yellow-green, and green-blue.

<u>Urobilinogen</u>: p-diethylaminobenzaldehyde with a color enhancer reacts in strongly acidic urine with urobilinogen to produce a pink-red color.

<u>Nitrate</u>: nitrate, derived from the diet, is converted to nitrite by the action of gram-negative bacteria in urine. A diazonium compound is formed from the reaction of urine with p-arsanilic acid at the acid pH of the reagent area. This compound couples with 12, 3, 4-tetrahydrobenzo (h) quinolin-3-ol to produce a pink color.

<u>Leukocyte</u>: 3-hydroxy-5-penyl pyrrole is liberated from the derivatized pyrrole amino acid ester esterases contained in granulocytic leukocytes. The pyrrole reacts with a diazonium salt to produce a purple color.

SPECIMEN REQUIREMENTS

Ten to twelve ml of urine should be collected in a clean container. Less than two ml may limit the extent of procedures that can be performed. A voided specimen is usually sufficient. If contamination is likely from vaginal discharge or hemorrhage, a clean catch specimen is desirable. Do not centrifuge for macroscopic screening portion of urinalysis. The use of urine preservatives is not recommended. Specimen should be tested as soon as possible after collection. If testing is to be delayed for more than two hours, refrigerate the specimen and return to room temperature before testing.

Effective Date: January, 2010 Cross Referenced: Reviewed Date: 6/2012 Revised Date:01/12

_____ _____ Policy No: UA120.01 **Origin:** Urinalysis Authority: Laboratory Director Page: 2 of 4

REAGENTS AND EQUIPMENT

Ames MultiStix 10 SG strips Clinitek 500 **Centrifuges Tubes Click Stick**

CALIBRATION

The Clinitek 500 autocalibrates at two readheads immediately before each Reagent Strip is read.

QUALITY CONTROL

Both positive and negative controls are run daily for each screening test contained on the MultiStix strip. In addition, both controls are run whenever a new bottle of reagent strips is opened. Bio-Rad qUAntify controls Level 1 (neg) and 2 (pos) are used as a positive control and negative control (see specific procedure).

PROCEDURE

- 1. Mix room temperature urine well immediately before testing.
- 2. Pour an aliquot of the specimen into a labeled centrifuge tube.
- If the specimen is in a BD Collection tube from the ER, retain a small sample in the tube for 3. potential add on testing. (ex preg or drug screen).
- 4. Perform visual exam for color and clarity of specimen.
- Perform chemical analysis using the Clinitek 500 per SOP (see Clinitek 500 Operation procedure). 5.
- When the Clinitek 500 results download to Cerner, review the results, and edit any results as 6. necessary (ex. color if you do not agree)
- 7. Upon verification additional testing such as Icto, Clinitest, or microscopics may be added on.
- Perform Icotest if indicated by a positive bilirubin (see Icotest procedure). 8.
- Perform Clinitest on al children one year of age and younger (see Clinitest procedure). 6.
- A microscopic examination will be done only if any of the following screening tests are positive: 7.
 - Protein _
 - Blood -
 - Nitrate
 - Leukocytes

The procedure for a microscopic exam is as follows:

- Centrifuge approximately 12 ml of urine at 2000 rpm for 5 minutes in a conical a. centrifuge tube.
- b. Decant all but 1 ml of urine and resuspend the sediment in the remaining 1 ml of

Effective Date: January, 2010	Policy No:	UA120.01
Cross Referenced:	Origin:	Urinalysis
Reviewed Date: 6/2012	Authority:	Laboratory Director
Revised Date:01/12	Page:	3 of 4

urine (a click-stick should be used for this purpose).

c. Place a drop of resuspended sediment on a slide with a coverslip.

- d. Scan under low power (100x) for casts and/or crystals. Casts can usually be found along edge of coverslip. Identify types found under high power (440x).
- e. Under high power, count RBCs and WBCs per field. Examine for the presence of bacteria, yeast, trichomonas, and sperm. Report as listed in Reporting Results section.
- f. If tube was <3/4 filled, add coded comment (54). If tube was low volume, may yield < quantity in sediment.

REPORTING RESULTS

Results from the Clinitek 500 are downloaded in reportable form. Microscopic results are reported as follows:

WBC and RBC: enumerate using the Cerner Drop down menu

Casts: type and approximate number per lpf

Crystals: type of crystals as present

Yeast: as present

Trichomonas: as present (NOTE: trichomonas may resemble WBCs. Tail motion

should be confirmed before reporting).

Bacteria & epithelia: grade as Few (<25% of field), 1+(25%), 2+(50%), 3+(75%), 4+(100%) using drop down

Expected results are as follows:

Specific Gravity: 1.001 – 1.035	Urobilinogen: 0.2 - 1.0 EU/dl
pH: 4.6 – 8.0	RBCs: $0 - 3/hpf$ (male) $0-5/hpf$ (female)
Protein: negative	WBCs: $0 - 5/hpf$
Glucose: negative	Casts: 0 –4 hyaline/lpf
Ketones: negative	Bacteria: negative on spun urine
Bilirubin: negative	Crystals: may be interpreted by a
	pathologist if unsure of identification

All microscopic results (casts, RBS, WBC, etc) must be correlated with macroscopic findings (protein, positive blood, leukocyte esterase, etc.).

Prolonged exposure to light or room temperature may affect the results of pH, protein, blood, bilirubin and urobilinogen. See Multi-Stix 10 SG product insert for specific interferences.

A change in urine color due to azo dyes (such as Pyrodium, AzoGantrise, etc.) needs to have a disclaimer noted on report (i.e., color of urine may cause false positive reports).

If large amounts of amorphous crystals are noted on microscopic exam, the sample can be heated in a 37° C waterbath to remove them.

Effective Date: January, 2010	Policy No:	UA120.01
Cross Referenced:	Origin:	Urinalysis
Reviewed Date: 6/2012	Authority:	Laboratory Director
Revised Date:01/12	Page:	4 of 4

All urines are to be saved for 24 hours Urines will be saved in one of two pink bins in the urine refrigerator after testing. The bins will be labeled "today" and "yesterday" on opposite sides. After midnight, the night tech will empty the bin labeled "yesterday" and turn it around to have "today" face out. The bin other bin labeled "today" will be rotated around to have "yesterday" facing out.

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Henry, John Bernard, "Clinical Diagnosis and Management by Laboratory Methods," 1979, WB Saunders Co., Philadelphia, PA. pp. 612-613.

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