I. PRINCIPLES:

A. General:

Bayer Multistix 8 SG and Clinitek Atlas reagent pak provide tests for glucose, nitrite, ketone (acetoacetic acid), specific gravity, blood, pH, protein, bilirubin, urobilinogen and leukocytes in urine by means of separate reagent strips affixed to a firm plastic strip. Test results may provide information regarding the status of carbohydrate metabolism, kidney and liver function, acid-base balance and urinary tract infection.

B. Clinical Use:

Dipstick chemical testing is very widely used for screening many different diseases including urinary tract infections, diabetes, intrinsic kidney diseases and cancer of the lower urinary tract. Depending on which dipstick is used there are up to 10 different chemically sensitive pads on the dipstick (the Multistix 8 and the Clinitek reagent pak includes: glucose, ketones, specific gravity blood, pH, protein, nitrite, leukocyte esterase, bilirubin and urobilinogen). Initial follow-up tests would include: urine sediment evaluation and urine culture if infection suspected from the dipstick; sediment evaluation if bleeding is suspected (positive blood on the on the dipstick could also be free hemoglobin or myoglobin Representing hemolysis, or crush or other types of muscle damage); blood sugar if glucose is positive; and 24 hour urine protein alone is positive.

C. Chemical:

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

2. Ketone: The test is based on the development of colors from buff-pink for a negative reading to purple when acetoacetic acid reacts with nitroprusside.

3. Specific Gravity**:** This test is based on the change in apparent pKa of certain pretreated polyelectrolytes with a change in ionic concentration. Colors range from deep blue-green in urine of low ionic concentration through green and yellow-green in urines of increasing ionic concentration. (not included on Clinitek Atlas Pak)

4. Blood: This test is based upon the peroxidase-like activity of hemoglobin, which catalyzes the oxidation of tetramethylbenzidine causing development of color ranges from orange through green. Very high levels of blood may cause the color development to continue to blue.

5. pH: The test uses two different pH indicators. In combination they give a broad range of colors covering the entire urinary pH range. Colors range from orange through yellow and green to blue.

6. Protein: This test is based on the protein-error-of-indicators principle. The development of any green color on the test area is due to the presence of protein. Colors range from yellow for "negative" through yellow-green and green to green-blue for "positive" reactions.

7. Nitrite: This test depends on the conversion of nitrate to nitrite by the action of Gram negative bacteria in the urine. Nitrite in the urine will react with the chemicals present on the test area to form a pink color. A positive finding is significant, but there a several sources of false negatives.

8. Leukocytes: Granulocytic leukocytes contain esterases that catalyze a chemical reaction that liberates a pyrrole. This pyrrole then reacts with a diazonium salt to produce a purple product.

9. Bilurubin: This is based on the Ehrlich reaction which obenzaldehyde in conjunction with a color enhancer reacts with urobilogen in a strongly acid medium to produce pink-red color.

10. Urobilinogen: This is based on the Ehrlich Aldehyde Reaction:
p- Dimethylaminobenzaldehyde + urobiligen= red-colored azo dye.
A color development reaction in which aldehyde or diazonium compounds react with urobilinogen to produce a pink to red color in an acid environment.

II. SPECIMEN COLLECTION AND HANDLING:

Collect clean-catch or catheterized urine in a clean container. A current YNHH nursing manual and specific literature provide instructions of proper urine collection and handling (proper ID labeling) to be analyzed in laboratory. A minimum of 12 ml. in a clear plastic conical tube or swirl top with preservative is recommended to provide for optimal specimen quality for analysis. Specimens(in plastic conical tubes) must be processed within two hours of collection. Preservative tubes can be processed up to 24 hours. Prolonged exposure of the urine in plastic conical tubes to room temperature may result in microbial proliferation which can cause an increase in pH, metabolism of glucose if present and false positive blood reactions from the production of bacterial peroxidases. If testing cannot be performed within 2 hours of collection, the specimen may be refrigerated to prevent bacterial growth. Refrigerated specimens must be warmed to room temperature before analysis and a comment needs to be put in the computer when results are reported.
Unacceptable specimens (QNS, ID errors, improper collection, contamination and extended delay to laboratory beyond 24 hours if preservative tube) are not processed. Proper documentation in the computer is required. This includes reason for not processing samples as well as a follow-up call to caregiver (name and title) explaining unsuitability of specimen. If incorrect data is released, results are immediately amended with a prompt call to caregiver to alert them of changed data.

III. REAGENTS / MATERIALS:

A. Multistix 10 SG (Siemens)
Clinitek Atlas Reagent Pak (Siemens)

1. Glucose: 16.3% w/w glucose oxidase; 0.6% w/w peroxidase; 7.0% w/w potassium iodide; 60.7% w/w buffer; 15.4% w/w nonreactive ingredients

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

3. Specific Gravity: 2.8% w/w bromthymol blue; 68.8% w/w poly methyl vinyl ether/maleic anhydride; 28.4% w/w sodium hydroxide. (not included on Clinitek Atlas strips)

4. Blood: 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.

5. pH**:** 0.2% w/w methyl red; 2.8% w/w bromthymol blue; 97.0% w/w nonreactive ingredients. Protein: 0.3% w/w tetrabromphenol blue; 97.3% w/w buffer, 2.4% w/w nonreactive ingredients.

6. Nitrite: 1.4% w/w p-arsanilic acid; 1.3% w/w 1,2,3,4-etrahydrobenzo(h)-quinolin-3-01; 10.8% w/w buffer; 86.5% w/w nonreactive ingredients.

7. Leukocytes: 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.

8. Bilirubin: 0.4% w/w 2,4-dichloroaniline diazonium salt; 37.3.% w/w Buffer;62.3%w/w nonreactive ingredients

9. Urobilinogen: 0.2% w/w p-diethylaminbenzaldhyde; 99.8%w/w ` nonreactive ingredients

Warnings and Precautions**:**

Siemens Multistix 10 SG Reagent Strips and Clinitek Atlas Reagent Pak are for in vitro diagnostic use. They have been determined to be nonhazardous under the guidelines issues by OSHA in 29CFR 1910.1200(d). Appropriate precautions for the handling of body fluids according to hospital protocol should be followed.

Storage:

Store at room temperature between 59-86 degrees F. Do not use product after expiration date shown on the package. Do not store in direct sunlight. Keep strips tightly capped when not in use; keep pak sealed until install into instrument.

B. Count-10 Trol I, Myers-Stevens Group. Montebello, California
Count-10 Trol III, Myers-Stevens Group. Montebello, California
Control material stable through outdate when stored at 2-8', reconstituted vials stable for 5 days

C. Digital Hand-held pocket refractometer

D. 5% NaCl (5 gr NaCl in 100ml distilled water)

E. applicator sticks

F. transfer pipets

IV. QUALITY CONTROL:

New Target Range:

Before a new lot of controls is put into use a statistically valid target range must be determined. This is accomplished by running new control lots in parallel with the control lots currently in use. After seven days of analysis the Mean,SD and CV are determined. From this information, the high and low target range is determined for normal and abnormal control ranges. If the ranges of the normal and abnormal controls overlap for SG or pH, contact the manufacturer.

A. Manual "dip"/ Clinitek 500 urinalysis:

Each shift - Count-10-Trol level I and III(Normal and Abnormal) controls must be tested with Multistix 10SG and resulted in Soft Total Q.C.
Any results that do not fall within posted ranges must be repeated. No dipstick or Clinitek urinalysis may be performed if control results are unsatisfactory.

1. Specific gravity:

Each shift – Count-10-Trol level I and III must be tested. Follow digital Refractometer procedure.

a. Remove controls from refrigerator and warm to room temperature

b. Read the specific gravity of distilled water on the refractometer, result must be 1.000, any variances immediately alert a supervisor (every Monday read the specific gravity of 5% NaCl, reading must be 1.023 +/- 0.003)(See Appendix A-reagent preparation)

c. Read the specific gravity of the level I and III controls. Results must

fall within the posted ranges, if not repeat. If specific gravity still unacceptable, notify a supervisor immediately and do not use refractometer.

B. Clinitek Atlas 1000:

A. At the beginning of each shift Count-10 Trol level I and III must be tested. Any results that do not fall within posted ranges must be repeated. No Clinitek Atlas 1000 urinalysis may be performed if control results are unsatisfactory. Follow directions in the Clinitek Atlas 1000 procedure.

At the beginning of each shift, Q.C. is ordered in Soft for specific Atlas being used, Manual/Clinitek 500 urinalysis and MUA(confirmatory urine tests- SSA and Clinitest)
Specific Gravity for both Trol I (# 11-MUA-1) and Trol III (#12-MUA-3) see Procedure for Soft Urinalysis.

C.

Changing Reagent Strips:

a. After reagent change, Atlas must be calibrated and Trol I(#11) and Trol III(#12) must be run. When there is a change in lot #s of Atlas reagent strips, a positive (abnormal) and a negative(normal) patient sample is also run in addition to the QC to assure accuracy. Select a normal and abnormal patient sample which were run within 1 hour of reagent change. Results from these samples are recorded on worksheet with printouts from Atlas attached. Data is evaluated. Results should be within one level of original results reported. Worksheets are then filed in Reagent Change Notebook.

D. Count-10 Trol level I and III preparation:

1. Bring vials to room temperature (stored at 2-8'C)

2. Verify correct lot number and within expiration date
3. Slowly vent cap to avoid reagent loss
4. Reconstitute each vial with 15 ml sterile distilled water
5. Cap and swirl for 20 seconds
6. Allow 20-30 minutes for complete reconstitution
7. Remix reagent vials
8. Label with date made and date expire
9. Aliquot reagent to labeled conical centrifuge vials
10. Return to refrigerator after use.

E. Proficiency Testing:

Proficiency testing is performed twice a year in urinalysis. P.T. kits include samples for macroscopic urinalysis, confirmatory testing and photo images. All staff performs proficiency testing, which is rotated through all shifts and is incorporated in routine patient sampling. Alternative assessment is done on infrequent tests by blinded split sampling between 2 technologists. Results need to be within one range level of each other. These results are assessed, reviewed and recorded in a log. Performance in proficiency testing is also used for technologist competency.

V. TESTING PROCEDURE:

Note: grossly bloody, mucoid and markedly turbid urines should be performed by manual "dip" urinalysis/ Clinitek 500 and specific gravity done by refractometer. Markedly bloody urines, sample should be spun first and then performed by dipstix.

A. Clinitek Atlas 1000: follow Clinitek Atlas 1000 procedure
B. Clinitek 500: follow Clinitek 500 procedure.

C. Manual testing:

1. Multistix:

a. Mix well immediately before testing.
b. Remove one strip from bottle and replace cap.

c. Completely immerse reagent areas of the strip in the urine and remove immediately to avoid dissolving out of reagents.

d. Run the edge of the strip against the rim of the urine container to remove excess urine.

e. Hold the strip in a horizontal position to prevent possible mixing of chemicals from adjacent areas.

f. Compare reagent areas to corresponding Color Chart on the bottle label at the time specified.

HOLD THE STRIP CLOSE TO COLOR BLOCKS AND MATCH CAREFULLY.

Avoid laying the strip directly on the Color Chart as this will result in urine soiling the chart.

NOTE: PROPER READ TIME IS CRITICAL FOR OPTIMAL RESULTS.
Read glucose at 30 seconds.
Read ketone test at 40 seconds
Read specific gravity test at 45 seconds
pH, protein, blood and nitrite at 60 seconds
Read leukocytes at 2 minutes
Read pH and protein immediately up to 2 minutes.
Read urobilinogen at 60 seconds.
Read bilirubin at 30 seconds.

C. Specific gravity:

1. Using a transfer pipette, place min. of 3 drops of urine on measuring prism of digital refractometer.
2. Press start and measurement will be displayed after arrow blinks 3 times.

Note: See full procedure for Digital refractometer.

Note: The digital refractometer is linear to 1.060 specific gravity. If a dilution(1:1) is required, multiply the decimal portion by 2 and comment in computer that specific gravity done by dilution.

E. Color and clarity:

1. Examine clarity of urine and report as clear or cloudy
2. Examine and report color of urine. Color should be reported as yellow, none, brown, green, amber.

Note: Any darkly colored urines may obscure actual color reactions on the test strip. In such cases report specific gravity, 3% SSA for protein and Clinitest tablet for glucose. Note in computer that color interferes with accurate specimen analysis. Tests affected by color of urine and possibly falsely elevated are glucose, bilirubin, urobilinogen, protein, blood, nitrite, pH, ketones and leukocytes.

VI. CONFIRMATORY TESTING

A. Protein:
Any positive protein in an alkaline urine >ph 7.0 must be confirmed by sulfosalicylic acid protein method. (refer to SSA protein procedure)

B. Specific gravity: >1.035 should be tested with Clinitest tablet to check for the presence of contrast dye.

VII. REFERENCE RANGE:

Test	Normal Range
Glucose	Negative
Ketone	Negative
Specific Gravity	1.005-1.030
Blood	Negative
pH	5.5-7.5
Protein	Negative
Nitrite	Negative
Leukocytes	Negative
Bilirubin	Negative
Urobilinogen	<= 2.0

The use of published data and medical literature are the basis of normal ranges pertaining to urinalysis. This has been validated by 20 random negative macroscopic patient results.

VIII. PROCEDURAL NOTES:

A. Recommended procedure for handling Multistix 10 SG:

All unused strips must remain in the original bottle. Transfer to any other container may cause reagent strips to deteriorate and become unreactive. Do not remove desiccants from bottle. Do not remove strip from the bottle until immediately before it is to be used for testing. Replace cap immediately and tightly after removing reagent strip. Do not touch the test areas of the reagent strip. It is important to protect the reagent strips from ambient moisture, light and heat to guard against altered reactivity. Discoloration or darkening of reagent areas may also indicate deterioration. New Multistix bottles needs to be validated by normal and abnormal controls and noted on bottle.

Because this test is visually read and requires color differentiation it should not be interpreted by people who are color blind or visually impaired.

VIII. SPECIFIC PERFORMANCE CHARACTERISTIS:

A. Glucose:

The test is specific for glucose. No substance excreted in urine other than glucose is known to give a positive result. If the color appears somewhat mottled at higher glucose concentrations match the darkest color to the color blocks. The sensitivity of the reagent area is 75-125 mg/dl glucose.

B. Ketone:

The test reacts with only acetoacetic acid in urine. Some high specific gravity/low pH urines may give reactions up to and including trace. Clinical judgment is needed to determine the significance of reactions up to and including trace. The sensitivity of the reagent area is 5-10 mg/dl acetoacetic acid.

C. Specific Gravity:

The specific gravity test permits determination of urine specific gravity between 1.000 and 1.030. The test does not respond to certain non-ionic urine constituents such as glucose or to radiopaque dye, and may substantially underestimate the specific gravity of specimens containing these substances. **Note: laboratory practice is to perform specific gravity using digital refractometer.**

D. Blood:

The sensitivity of the test may be reduced in urines with high specific gravity. The test is equally sensitive to myoglobin and hemoglobin. The appearance of green dots on the reaction area indicates the presence of intact erythrocytes in the urine. The sensitivity of the reagent area is 0.015-0.062 mg/dl hemoglobin.

E. pH:

The pH test area measures pH values generally to within 1 unit in the range of 5-8.5.pH readings are not affected by variations in the urinary buffer concentration.

F. Protein:

The reagent area is more sensitive to albumin than to globulins, hemoglobin, Bence - Jones protein and mucoprotein; a negative result does not rule out the presence of these other proteins. The sensitivity of the reagent area is 15-30 mg/dl albumin.

G. Nitrite:

Comparison of the nitrite reagent area against a white background may aid in the detection of nitrite ion. The test is specific for nitrite and will not react with any other substance normally excreted in urine. The sensitivity of the reagent area is 0.006-0.1 mg/dl nitrite ion.

H. Leukocytes:
The sensitivity of the reagent area is 5-15 cells/hpf in clinical urine.

I. Bilirubin:
Normally no bilirubin is excreted. Positive results >1.0mg/dl are clinically significant.

J. Urobilinogen:
This test will detect urobilinogen in concentrations as low as 0.2 mg/dl in urine.

IX. LIMITATIONS:

Substances that cause abnormal urine color such as drugs containing azo dyes, nitro furantoin and riboflavin may affect the readability of the reagent areas. Color development on the reagent pad may be masked or a color may be produced that could be interpreted as a false positive.

A Glucose:

The reactivity of the glucose test decreases as the specific gravity of the urine increases. Reactivity may also vary with temperature.

B. Ketone:

False positive results (trace or less) may occur with highly pigmented urine specimens or those containing large amounts of levodopa metabolites.

C. Specific Gravity:

Highly buffered alkaline urine may cause low readings relative to other methods. Elevated specific gravity readings may be obtainable in the presence of moderate quantities (100-750 mg/dl) of protein. The test does not respond to certain non-ionic urine constituents such as glucose or to radiopaque dye, and may substantially underestimate the specific gravity of specimens containing these substances.

D. Blood:

Elevated specific gravity may reduce the reactivity of the blood test. Microbial peroxidase associated with urinary tract infection may cause a false positive reaction pH: If proper procedure is not followed and excess urine remains on the strip a phenomenon known as "runover" may occur, in which the acid buffer from

the protein reagent will run onto the pH area, causing a false lowering of the pH result.

E. Protein**:**

False positive results may be obtained with highly buffered or alkaline urines. Contamination of the urine specimen with quaternary ammonium compounds (from some antiseptics or detergents) or with skin cleansers containing chlorohexidine may also cause false positive results.

F. Nitrite:

Pink spots or pink edges should not be interpreted as positive. Any degree of uniform pink color development should be interpreted as a positive nitrite test. A negative result does not in itself prove there is no significant bacteriuria. Sensitivity of the nitrite test is reduced for urines with high specific gravity.

G. Leukocytes:

Elevated glucose concentrations (>3g/dL) or high specific gravity may cause decreased test results.

H. Bilirubin:

False negatives if formalin present. Reactivity increases with temperature.

I. Urobilinogen:

False negatives if formalin present. Reactivity increases with temperature.

X. EXPECTED VALVES:

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

A. Glucose:
Small amounts of glucose are normally excreted by the kidney. These amounts are usually below the sensitivity of the test but on occasion may produce a color

between the Negative and the 100 mg/dl color blocks. Results at the first positive level may be significant if found consistently.

B. Ketone:
Normal urine specimens ordinarily yield negative results with this reagent. Detectable levels of ketone may occur in urine during physiological stress conditions such as fasting, pregnancy and frequent strenuous exercise.

C. Specific Gravity:
Random urines may vary in specific gravity from 1.005-1.029.

D. Blood:
The significance of the Trace reaction may vary among patients, and clinical judgment is required for assessment in individual cases. Development of green spots (in-tact erythrocytes) or green color (free hemoglobin/myoglobin) on the reagent area within 60 seconds indicates the need for further investigation.

E. pH:
The urinary pH range is from 5 to 9. The kidney is incapable of producing urine outside this range. Results outside this range suggest the presence of acid or base added as preservatives. Protein: Normally no protein is detectable in urine, although a minute amount is excreted by the normal kidney. A color matching any block greater than Trace indicates significant proteinuria. Nitrite: Normally no nitrite is detectable in urine.

F. Leukocytes:
Normal urine specimens generally yield negative results. Positive results (small or greater) are clinically significant. Positive results may occasionally be found with random specimens from females due to contamination of the specimen by vaginal discharge.

G. Bilirubin:
Normal urine specimens yield negative results.

H. Urobilinogen:
Normally present in urine at concentrations <= 2.0 EU/DL.

XI. RESULTS:

Reported results are not always the same as the values indicated on the color chart. Use the table below to determine the values to report.

Test	Color Chart Value	YNHH Report
pH	5.0 - 8.5	5.0 - 8.5 (same)
Protein	Negative	Negative
	Trace	Negative
	30 mg/dl (+)	1+
	100 mg/dl (++)	2+
	300 mg/dl (+++)	3+
	2000 or more mg/dl (++++)	3+
Glucose	Negative	Negative
	100 mg/dl	Trace
	250 mg/dl	1+
	500 mg/dl	2+
	1000 mg/dl	3+
	2000 or more mg/dl	3+
Ketones	Negative	Negative
	5 mg/dl (trace)	Negative
	15 mg/dl (small)	Small
	40 mg/dl (moderate)	Moderate
	80 mg/dl (large) .	Large
	160mg/dl (large)	Large
Blood	Negative	Negative
	Trace (Non-hemolyzed)	Small
	Moderate (Non-hemolyzed)	Moderate
	Trace (Hemolyzed)	Small
	Small (+)	Small
	Moderate (++)	Moderate
	Large (+++)	Large

Leuk. Esterase	Negative	Negative
	Trace	Positive
	Small	Positive
	Moderate	Positive
	Large	Positive
Nitrite	Negative	Negative
	Positive	Positive
Bilirubin	Negative	Negative
	Small(+)	Small
	Moderate(++)	Moderate
	Large(+++)	Large
Urobilinogen	0.2 E.U./dl	0.2 E.U./dl
	1.0 E.U./dl	1.0 E.U./dl

XII. REFERENCES:

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4. Henry, J.B. et a1.: *Clinical Diagnosis and Management by Laboratory Methods*, 18th ed. Philadelphia: Saunders; 1991; pp. 396-397,415.
5. Bayer Diagnostic Division. Elkhart, IN. Multistix 8 SG. 1994
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8. Count-trol system package insert, Myers-Stevens Group, Commerce, Calif

XIII. HISTORY:

H-1 This procedure was written by N. Ortoli-Drew on 9-27-02
H-2 This procedure was revised by D. Fico on 4/12/10.
H-3 This procedure was revised by D. Fico on 11/5/2010.
H-4 This procedure was revised by D. Fico on 3-10-2011.
H-5 This procedure was revised by D. Fico on 10/2011.

H-6 This procedure was revised by D.Fico on 2/2012.
H-7 This procedure was revised by D.Fico on 3/2013.
H-8 This procedure was revised by D. Fico on 9/2013
H-9 This procedure was revised by D. Fico on 12/2013.

FACTORS AFFECTING MACROSCOPIC URINALYSIS

FINDING	FALSE POSITIVE	FALSE NEGATIVE
SPECIFIC GRAVITY		
GLUCOSE	PEROXIDE, HYPOCHLORIDE	PENICILLIN, CEPHALOSPORIN, MONOBACTAMS, >50mg ASCORBIC ACID, >2g SALICYLATES
BILIRUBIN	CHLORPROMAZINE	EXPOSURE TO LIGHT, >25mg ASCORBIC ACID
KETONES	ATY COLOR L-DOPA, SALICYLATES, CAPTOPRIL	
PROTEIN	CHLOROHEXIDINE, ANTISEPTICS, HIGHLY ALKALINE URINE	
BLOOD	CAPOTEN, >25mg ASCORBIC ACID, CHLOROHEXIDINE, ANTISEPTICS, MYOGLOBIN	
PH		OLD URINE
UROBILINOGEN	SULFONAMIDES, ALDOMET	OLD URINE, EXPOSED TO LIGHT
LEUKOCYTES	VAGINAL CONTAMINATION, FORMALIN	NON-GRANULOCYTES, KEFLEX, KEFLIN, TETRACYCLINE, INCREASED OXALIC ACID
NITRITE		>25mg ASCORBIC ACID, DIET LACKING IN NITRATE, URINE <4hr IN BLADDER

Package insert Bayer Multistix
Ravel, Richard: Clinical Laboratory Medicine Clinical Application of Laboratory Data. Mosby 1995

Appendix A

Preparation of 5% Sodium Chloride for Specific Gravity:

Preparation: 5 gm NaCl
Dissolve in 100 ml of distilled water

Stability: Indefinitely in refrigerator

Final solution should have specific gravity of 1.0225 +/- 0.0001 and properly labeled.