Methodist Health Services Corporation
& UnityPoint Health Methodist
Department of Pathology
Peoria, IL 61636

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ROUTINE URINALYSIS

Siemens Clinitek

Principle

A routine urinalysis consists of a gross exam, chemical analysis utilizing dipsticks which have various reagent impregnated areas for chemical determinations, and a microscopic exam to identify formed elements.

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 urine of increasing ionic concentration.

pH: The pH test is based on the double indicator principle with the reagent area impregnated with pH indicators, bromthymol blue and methyl red.

Leukocyte Esterase: The test is enzymatic based on granulocytic leukocytes which contain esterases catalyzing the hydrolysis of the derivatized pyrole, amino acid ester to liberate 3-hydroxy-5-phenyl pyrrole. This pyrrole reacts with a diazonium salt to produce a purple color.

Nitrite: This test depends upon the conversion of nitrate to nitrite by the action of Gram negative bacteria in the urine. 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-e-ol to produce a pink color. This test provides a rapid screening test for the presence of urinary tract infection.

Protein: Test is based on the protein error of indicators principle - at a constant buffered pH, the development of any green color is due to the presence of protein.

Glucose: Test is based on a double sequential enzyme reaction. The reaction utilizes the enzyme glucose oxidase to catalyze 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.

Ketones: This test is based on the development of colors ranging from buff-pink, for a negative reading , to purple when acetoacetic acid reacts with nitroprusside.

Urobilinogen: This test is based on a modified Ehrlich reaction, in which p-diethylaminobenzaldehyde in conjunction with a color enhancer reacts with urobilinogen in a strongly acid medium to produce a pink-red color.

Bilirubin: Test is based on the coupling of bilirubin with diazotized dichloroaniline in a strongly acid medium. The color ranges through various shades of tan.

Blood: This test is based on the peroxidase-like activity of hemoglobin and myoglobin, 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.

Clinical Significance

The routine urinalysis is a valuable diagnostic tool used for the detection and evaluation of renal and urinary tract disorders, various endocrine or metabolic abnormalities and in the treatment and monitoring the course of a disease.

Specific Gravity: Conditions causing increased S.G. include dehydration, proteinuria, glycosuria, eclampsia, and lipoid nephrosis. Diseases which can cause decreased S.G. include collagen disease, pyelonephritis, hypertension, protein malnutrition, polydipsia, diabetes insipidus as well as diuretic medications and natural diuretics (coffee & alcohol).

pH: Urinary pH is a reflection of the homeostatic acid-base regulating role of the kidneys.

Leukocyte Esterase: The presence of leukocytes in the urine indicates a possible infection of the urinary tract.

Nitrite: A positive nitrite test indicates bacteriuria caused by cystitis, urethritis, prostatitis or pyelonephritis.

Protein: Protein in the urine is most often the first indication of renal/glomerular/tubular disease such as Glomerulonephritis, Glomerulosclerosis, Nephrotic Syndrome, Pyelonephritis, or Renal Tuberculosis. Protein may also be seen in other pathological and/or physiologic conditions such as Congestive Heart Failure, Gout, Pre-Eclampsia, Hypokalemia, Severe Febrile Infection Nephrotoxic

Drugs, strenuous exercise and stress.

Glucose: Glucose in the urine is most often seen with decreased renal glucose threshold, renal glycosuria during pregnancy, and diabetes mellitus.

Ketones: Diabetes mellitus is the most important disorder in which ketonuria occurs. When diabetes is untreated or inadequately treated, excessive amounts of fatty acids are metabolized, which result in the accumulation of ketone bodies in the blood and are excreted in the urine. Progressive diabetic ketosis is the cause of diabetic acidosis. Ketonuria can also be found in conditions associated with a decreased intake of carbohydrates, digestive disturbances, high fat, low carbohydrate diet, eclampsia, prolonged vomiting, diarrhea, severe prolonged exercise and fever.

Urobilinogen: Elevated levels may occur in liver diseases and hemolytic anemias. Reduced levels or absence of urobilinogen is associated with biliary duct obstruction.

Bilirubin: Bilirubin excretion in the urine will reach significant levels in any disease process that increases the amount of conjugated bilirubin in the blood In some liver diseases, liver cells are unable to secrete all of the conjugated bilirubin in the bile so that sufficient amounts are returned to the blood. In obstructive biliary tract disease, biliary stasis interferes with the normal excretion of conjugated bilirubin via the intestinal tract, thus causing a buildup in the blood stream.

Blood: Intact red cells in the urine may be indicative of renal infection, inflammation, or injury such a Renal Tuberculosis, Renal Infarction, Calculi, Polycystic Kidneys, Tumors, or Cystitis. Hemoglobinuria is seen with hemolytic conditions and myoglobinuria is seen in muscle diseases and/or crushing injuries.

Specimen

Freshly voided random urine specimen (first morning specimen if possible) placed in a clean container and labeled with two patient identifiers, name, medical record number and date of birth. The urine must be transported to the laboratory immediately and refrigerated if not examined within one hour. The urine can be stored for up to 24 hours at 0-10^{0}C.

Prolonged exposure of urine to room temperature may result in microbial proliferation with resultant changes in pH. A shift to alkaline pH may cause false positive results with the protein

test area. Urine containing glucose may decrease in pH as organisms metabolize the glucose. Bacterial growth from contaminating organisms may cause false positive blood reactions from the peroxidases produced.

All urines must be saved in Microbiology for 24 hours.

Check LIS pending logs for pending specimens.

Criteria for rejection of urine specimens: improper identification or no identification, quantity not sufficient to perform testing procedure, specimen known to have been at room temperature for more than 1 hour, specimen that has been frozen, specimen containing a preservative, specimen contaminated with fecal material, specimen which does not meet collection requirements for a specific test, ie timed and specimens greater than 24 hours old

Reagents & Equipment

1. SIEMENS Multistix 10 SG test strips
2. 10% Sodium hydroxide (10 gms Sodium hydroxide diluted in 100 ml water)
3. 1% Acetic acid (0.1ml concentrated acetic acid in 10ml of water)

Instrumentation/Equipment

1. Siemens Clinitek Analyzer
2. Nikon Microscopy
3. Refractometer
4. Centrifuge
5. Kova Glass Slide

Calibration

Calibration is performed at each readhead immediately before each Reagent Strip is read. The fixed platform contains two white calibration bars that are positioned directly under each readhead. As a strip comes into position under a readhead, the instrument is calibrated for that scanning cycle by reading the calibration bar.

Successful calibration reports are printed off weekly. Attach printouts to preventative maintenance log. . To print:

- Touch [Menu] from the [Ready/Run] Screen
- Touch [Print}
- Select [Calibration confirmation].

Quality Control

Normal and abnormal Quantrimetric (Quantrimetric Corp.) controls are run daily, when a new lot # of strips is opened, or if the instrument has been serviced.

Procedure:

1. Remove controls from the UA refrigerator and allow to come to room temperature (approximately 30 minutes). Invert gently to assure complete mixing of the contents; do not shake.
2. Remove bottle cap, invert and apply control material directly to the dipstick by drawing the tip across all of the reagent pads while gently squeezing sides of the bottle. **Do not blot** the edge of the strip against a paper towel. Touch the [Menu] key symbol from the [Ready/Run] screen, then touch the [Controls] key. This display will change to a numeric keyboard from which the lot identification of the controls can be entered, the sequential number reset, and the control run started.
   - The control sequential number is displayed immediately to the left of the lot #. If you want to reset this number, touch the key labeled C0001. Enter the lot identifiction of the first control to be tested. If alphabetic characters are needed, touch the [A-Z] key. Touch ↲ entering the letters to return to the numeric display. Touch ↲ ; you are ready to test control;
3. Run strips through Analyzer. Repeat step 3 for each control. Controls will print.
4. After all controls have been run, press ↑.
5. Immediately recap the control and return to refrigerator.
6. Record results on **URINALYSIS QUALITY CONTROL LOG SHEET**. "Out-of-range" results are circled and action taken documented.
7. If either control is out, repeat the test. If control value is still out of range, clean instrument and repeat control. If controls are still out of range, obtain a new lot number of multisix, try a new lot # of controls, or call technical support at 1-877-229-3711. Document all "out-of-range" control results and actions taken.
8. Commercially prepared QC material is also run for manual Urine Microscopics, however, it is not always available. When the QC material is available, it will be run, otherwise competency is assessed by CAP Proficiency testing.

Specimen Analysis Procedure

Physical & Chemical Exam

1. Place a barcode label on plastic tube. No numbering is necessary.

2. Mix urine specimen well and pour approximately 10 ml or as much urine as possible into a labeled centrifuge tube. Reject specimens with less than 1 ml of urine.
3. Press the [ID] key on the screen.
4. Scan the barcode on urine tube with the instrument barcode reader. Accession No. will appear at top of screen as ID.
5. Observe the color and appearance of the specimen. Yellow/Clear is the default parameter. If color and clarity is other than yellow/clear, input results into analyzer by touching the color and/or clarity description pads until desired result. Press the arrow key (↲). For a "RUN", repeat steps #4 & 5 until all barcoded specimens have ben scanned. Press the ↑ Key; the pushbar will return to left hand position indicating that the strip reader is ready for a strip and the screen will return to the beginning of the barcode #s for that run.
6. Completely immerse reagent stix in well-mixed uncentrifuged urine. To remove excess urine, run the edge of the entire length of reagent strip against the side of the urine tube when removing the strip. **Do not blot** the edge of the strip against a paper towel.
7. Place the reagent strip, with reagent areas facing up, onto the strip supports of the strip loading station and against the rear wall of the platform.
8. If a problem occurs that requires the run to be stopped before completion of all readings, touch the (▼) key symbol (stop run) in the upper right corner. You will be given the option of canceling the entire run or canceling only the last strip that was placed on the platform. Once strip has been read, the instrument will advance to the next barcode # and you can repeat steps #6 & #7. Continue on until the run has been complete.
9. Results are transmitted to the printer and computer as soon as all reagent areas on the strip have been read. Results will print also.
10. Due to possible interfering substances, perform confirmation testing using following criteria:
    - Bilirubin results showing positivity, perform Ictotest. If Ictotest confirms positive result, report analyzer reading. (See Procedural Notes). If Ictotest is negative, report as negative.
    - Urobilinogen reading of > 2.0 EU/dl, confirm with visual dispstick reading.

Microscopic Exam

1. Centrifuge urine for 5 minutes at 3000 rpms.
2. Decant supernatant to leave approximately 0.5 sediment/supernatant and mix well.
3. Number the wells of a Kova Glass Slide to correspond with tubes. Transfer 1 drop of sediment to a Kova Glass Slide. Mark off well with black marker when exam is completed.
5. Using phase microscopy (Ph1) on low power, examine urine for the presence of casts and epithelial cells. Classify and enumerate elements and input into computer.
6. Change to bright field microscopy and high power. Examine urine for presence of RBCs, WBCs, bacteria, crystals, yeast and parasites. Classify and enumerate elements and input into computer.

Reporting Results

To report out results, input data into the LIS.

Color:	Yellow, orange, red, green, blue, brown
Clarity:	Clear = you can see through it Slightly Cloudy = uniform cloudiness, can read through it Cloudy = uniform cloudiness, cannot read through it Turbid = particulate matter present Milky = thick, heavy cloudiness
Specific Gravity:	<=1.005, 1.010, 1.015, 1.020, 1.025, >=1.030
pH:	5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5 >= 9.0
Protein (mg/dl):	Negative, Trace, 30, 100, >=300
Glucose (mg/dl):	Norm, 100, 250, 500, >=1000
Ketones (mg/dl):	Negative, Trace, 15, 40, >=80
Bilirubin:	Negative, Small, Moderate, Large
Blood:	Negative, Trace, Small, Moderate, Large
Urobilinogen (EU/dl):	0.2, 1.0, 2.0, 4.0, >=8.0
Nitrite:	Negative; positive
Leuko Est (cells/ul)	Negative, Trace, Small, Moderate Large
Casts:	Occ, increments of 5 up to 40/LPF Many = greater than 40/LPF Packed = LPF covered
Nonrenal epithelial cells:	Occ = 1 per 5 LPF Few = 1-10/LPF

	Moderate = 10-40/LPF Many = > 40/LPF Packed = LPF covered & layered
Renal epithelial cells:	Occ = 1 per 5 LPF Few = 1-10/LPF Moderate = 10-40/LPF Many = > 40/LPF Packed = LPF covered & layered
WBC:	None, increment of 5 up to 40/HPF Many = >40/HPF Packed = HPF covered & layered
RBC:	None, 0-1/HPF, 2-3/HPF, 4-5/HPF; increments of 5 up to 40/HPF Many = >40/HPF Packed = HPF covered
Bacteria:	Occ = 1-10% Few = 10-25% of HPF Moderate = 25-50% of HPF Many = 50-100% of HPF Packed = HPF covered/layered
Crystals:	Few = 1-10/HPF Moderate = 10-30/HPF Many = >30/HPF Packed = HPF covered
Yeast &/or Parasites:	Present

Before verifying results:

UA-Dip Test order 12931, verify if negative. The following are the criteria for reflexing microscopic test on any positive results: Leukocyte Esterase; Nitrites, Protein > (trace), and Blood.

UA Test order 12925 (Dr. request) automatic order of microscopic classification and enumeration of elements

- check for input errors, completeness of test and accuracy of results.
- correlate microscopic results with Chemstrip results such as crystals correlated with pH, RBCs correlated with blood, WBC correlated with protein & leukocyte esterase, casts correlated with protein, bacteria correlated with nitrites.
- perform confirmation testing:
  - Positive test for Bilirubin on the Multistix must be confirmed using the Ictotest (when available).
  - Urobilinogen readings of greater than 2.0 EU/dl on the analyzer need to confirmed visually.
- Perform visual evaluation of dipsticks on any urines suspected of having erroneous results.
- Computer will transmit the word "recheck" as suppose to the value on all positive results on the bilirubin from the Clinitek . Technologist must perform Ictotest procedure to confirm testing and enter appropriate result.
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When results have been correlated, confirmed and/or retested and control performed and in-control, verify results.

Reference Values

Color/Clarity	Pale Yellow to Amber/Clear
Specific Gravity	1.005 - 1.030
pH	5.0-8.5
Protein	< 30mg/dl
Glucose	Norm (0mg/dl)
Ketones	0 mg/dl
Bilirubin	0 mg/dl
Blood	0 Ery/UL
Leukocyte Esterase	0 Leu/UL
Nitrite	0 mg/dl
Urobilinogen	< 1 EU/dl
RBC	< 4/HPF
WBC	0-5/HPF
Nonrenal Epithelial Cells	0-Few/LPF
Renal Epithelial Cells	0/LPF
Bacteria	0-Few/LPF
Amorph Urates	0-Few/HPF
Amorph Phosphates	0-FEW/HPF
Hyaline Casts	<2/LPF

Procedural Notes/Problem-Solving Tips

General notes:

- Do not report the presence of spermatozoa in any urine specimen, unless specifically ordered by physician. If spermatozoa are seen in female younger than 14 years, have a Pathologist review slide.
- If sediment is packed with a specific type of element, make a 1:2 dilution with saline (1 drop of sediment plus 1 drop of saline) and enumerate elements. Multiply # seen by 2.
- If amorphous urates or phosphates are indistinguishable from bacteria, make a 1:2 dilution with either 10% sodium hydroxide for urates or dilute acetic acid for phosphates. Enumerate bacteria and multiply by 2.
- Unusual microscopic elements must be referred to a pathologist.
  - Urines that contain phenazopyridine fluoresce orange and will cause interference in the macroscopic portion of the urinalysis. These specimens should be canceled and a microscopic exam only reordered and performed
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  The criteria for re-flexing to a UA-Culture are:

  1. Any positive test for Leukocyte Esterase or Nitrite,
  2. **And/Or** WBC >5/HPF.

  Regardless of whether or not a Culture needs to be set up all urines with a CULT HOLD need to be delivered to Micro. When the testing does indicate a Culture needs to be set up, mark the cup with a "+" sign (or write POS). If the testing does NOT indicate a Culture needs to be set up, mark the cup with a "—" sign (or write NEG).

  3. Result in LIS as "POS" or "NEG"

Procedural Notes/Problem-Solving Tips Continued

Specific Gravity:

- The chemical principle of the test may cause slightly different results compared with other specific gravity methods when elevated amounts of certain urine constituents are present. Glucose and urea concentration greater than 1% may cause a low specific gravity reading relative to other methods. In the presence of moderate amounts of protein (100-500 mgs/dl) or ketoacidosis, readings may be elevated.

pH:

- If excess urine remains on the strip, "runover" may occur in which the acid buffer from the protein reagent area runs onto the pH area causing a false lowering of the pH.

Leukocyte esterase:
- Specimens should not be collected in containers that have been cleaned with strong oxidizing agents and do not use preservatives.
- Leukocyte esterase test - the drugs cephalexin and gentamicin have been found to interfere with test. Nitrofurantoin colors the urine and thus causes interference with visual interpretation of the test strip. High levels of albumin (>500mg/dl) may interfere with the test results.

Nitrite:
- Large amounts of ascorbic acid decreases 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.

Protein:
- False positives may be obtained with strongly basic urine (pH 9 or higher) phenazopyridine therapy, or infusion of blood substitutes and residues of disinfectants.

Glucose:
- False positive readings may be produced by strongly oxidizing cleaning agents used in the urine container.
- False negatives may be caused by high concentrations of ascorbic acid (vitamin C) retained in the urine.

Ketones:
- False positive results may occur when urine specimen is highly pigmented or contains large amount of levodopa metabolites.

Procedural Notes/Problem-Solving Tips Continued

Urobilinogen:
- False positive readings may occur in patients being treated with phenazopyridine.
- False negative results may be obtained if formalin is present or nitrite concentrate above 5 mg/dl.

Bilirubin:
- False positive readings may be produced by medications that color the urine red, or turn red in an acid medium. Check all positive results with Ictotest.
- Large amounts of ascorbic acid present in urine lower the sensitivity of the test.

Blood:

- False positive results can be produced by residues of strongly oxidizing cleaning agents in the urine container.
- False negative results are obtained when formalin is used to preserve the urine.
- Nitrite in excess of 10 mg/dl delays the reaction.

- · All UA aliquot specimens must be poured off and labeled with the initials of the person doing so and distributed to the appropriate depts..
- Finished urine specimens will go to the Micro Dept. for storage for 3 days.

Reportable Parameters

The Chemstrip Urine Analyzer is programed with a set of reflectance ranges specific for each chemistry on the test strip. The following is the acceptable ranges:

Parameters	Ranges
Sp Gr	1.005 -1.030
pH	5 - 9
Leukocyte Esterase	Neg - 2+
Protein	Neg - 500 mg/dl
Glucose	Norm - 1000 mg/dl
Ketones	Neg - 3+
Urobilinogen	Norm - 12 mg/dl
Bilirubin	Neg - 3+
Blood	Neg - 250 Ery/µL

If a measured value fall outside these limits, an ERROR message appears, repeat with a different strip. If ERROR message still appears, perform tests with visual reading and trouble shoot instrument if needed.

References

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Date: 5/15/02
Date: 2-1-10

MEDICAL DIRECTOR		
DATE	NAME	SIGNATURE
January 19, 2017	Elizabeth A. Bauer-Marsh, M.D.	Elizabeth A. Bauer-Marsh MD
SECTION MEDICAL DIRECTOR		
March 4, 2012	Andrew Armstrong, MD	Andrew Armstrong
December 3, 2013	Katherine Kasper, M.D.	Katherine A. Kasper MD

REVISION HISTORY			
Rev	**Description of Change**	**Author**	**Effective Date**
1	Changed on specimen storage to microbiology and reporting entry process due to new LIS	N. Rutledge	10/27/11
2	New Medical Director reviewed	N. Rutledge	1/26/12
3	New Medical Director reviewed, system name updated, formatting changes	T. Lanan/ K. Kasper	12/3/13
4	Basic language change and the addition/deletion of procedural notes	Kim Paige	9/16/16
5	Removed reference to performing Clinitest on all children under 4 yrs of age. Added pour off labeling and where stored until disposal.	Kim Paige	10/15/17

Reviewed by

Lead	Date	Coordinator/Manager	Date	Medical Director	Date
			2/4/10		2/3/10
			4/5/12		4/4/12
		Kathy L. Turpin	9/10/14	Katherine A. Kasper MD	9/10/14
K. Paige	9/16/16	Kathy L. Turpin	9/16/16	Katherine A. Kasper MD	9/16/16
K. Paige	10/15/17	June Bembenek Kathy L. Turpin	11/27/17 11/7/17	Katherine A. Kasper MD	11/20/17