

Microscopic Urinalysis

Note: Students are expected to review the corresponding information in the course textbook(s) as well as the classroom notes in preparation for this lab and to aid in answering the study questions..

Points: Points are awarded for Admission Tickets, Skills, including general lab requirements, as well as successful and timely completion of Study Questions.

Objectives: Using criteria set by the instructor, by the completion of this two session lab the student will:

1. perform microscopic examinations on a minimum of ten urine specimens, obtaining results matching those of the instructor within $\pm 20\%$ accuracy. To meet this objective, the student must be able to identify RBCs, WBCs, squamous and renal epithelial cells as well as normal acid and alkaline crystals, mucous threads, bacteria and yeast at least 4 out of 5 times. At the instructor's discretion, microphotographs or electronic pictures may be used to aid in evaluation. In the evaluation of quantity, the student's results must match that of the instructor's \pm reporting unit. (Using the quantitation of amorphous urates as an example, if the instructor reviews a representative area and determines the report value as 2+, the student's value can be as low as 1+ or as high as 3+ to be acceptable.)
2. bring to the instructor's attention any abnormal / unexpected results including fatty, cellular, or waxy casts, abnormal crystalline structures, oval fat bodies, fat globules, trichomonas, etc.
3. use appropriate recording format to report results.
4. use quality control results to determine the acceptability of test results.
5. answer all pre-test and study questions using related information found in the textbook, lecture guide, and this lab procedure and submit the results to the instructor by the due date.

Equipment & Supplies:

1. Urine specimens
2. Centrifuge tubes racks, marking pencils, Kim-wipes, microscope slides and cover glasses.
3. Centrifuge, AO TS Meter
4. Microscope
5. Color reference pictures of urinary sediment

References Lecture and Lab Guides
Current UA / BF course textbook(s) **Mundt & Shanahan Ch. 5**

Supplemental References: McBride, L.J. (1998). Textbook of Urinalysis and Body Fluids. Lippincott
Strasinger, S. K. & Di Lorenzo, M.S., (2001). Urinalysis and Body Fluids, Chapter 6.
Estridge, etal. (2000) Basic Medical Laboratory Techniques, pp. 36-371.
Ringsrud, K. M. & Linne, J. J., (1995). Urinalysis and Body Fluids A Color Text and Atlas Chapters 5 & 6.

Overview After the urine sample has been evaluated visually and chemically, a portion of the well mixed sample it is centrifuged and the 'sediment' evaluated microscopically to identify insoluble materials including formed elements such as blood cells, epithelial cells, bacteria and other parasites, crystals, casts and mucous. At one time, the urine microscopic examination was routinely performed on each UA ordered. This part of the UA is the most time consuming and may not provide much, if any, diagnostic information. Therefore as a means of making the UA test more cost effective, the microscopic examination is no longer performed unless warranted. The process of determining whether a urine would be selected to undergo microscopic examination is sometimes referred to as 'macroscopic screening' or 'chemical sieving'. When a specimen meets specific criteria during the physical or chemical testing phase, a microscopic evaluation is warranted. Some laboratories call this a 'reflex microscopic'. The goal is to be sure that urines with significant macroscopic (that is physical and or chemical) findings are viewed microscopically.

EXERCISE 3:

Microscopic Urinalysis

Correlating results	<p>When the microscopic is performed, its results should be correlated with the findings of the physical /chemical testing results.</p> <p>Example correlations between physical/chemical analysis and microscopic:</p> <ol style="list-style-type: none">1. Urine that is red in color - hazy to cloudy - has positive blood should demonstrate the presence of RBCs under the microscope.2. Urine that is hazy to cloudy - has positive protein, leukocytes and nitrite should be carefully examined for WBC and bacteria.3. Increased turbidity in a refrigerated urine sample often means the presence of crystals. <p>More information on correlation of physical / chemical results with expected microscopic analysis can be found in your textbook and other resources.</p>
Troubleshooting discrepancies	<p>There are many possible reasons that a specimen's physical / chemical characteristics do not correlate with the microscopic results. Among the first thing to be considered is a mix-up in samples - where the microscopic was not performed on the same sample as the physical and/or chemical analysis. Another possibility to consider is a deterioration in the sample. This is most commonly seen when there is a significant lag period between the different phases of testing or if the sample is very alkaline. Regardless of the reason, the best course of action is to recollect the sample and repeat the testing ASAP.</p>
Principles and related info:	<p>The formed elements suspended in the urine are concentrated by centrifugation and analyzed under the microscope. Careful interpretation of the findings observed in examination of the urine sediment can provide rather accurate localizing information of pathologic processes in the urinary tract, or on occasion, point to a specific diagnosis of clinical problem.</p> <p>Normal urine sediment contains a limited number of formed elements including:</p> <ul style="list-style-type: none">1-2 RBCFew WBCFew epithelial cells (<i>squamous</i> epithelial cells are most frequently identified, especially in females, they originate from the linings of the genitourinary system)Occasional hyaline cast <p>In performing a microscopic examination of the urine, at <i>least</i> 10-15 fields of vision should be examined and averaged to enumerate each cellular element. This method is only a semi-quantitative method, but generally provides enough information to satisfy diagnostic needs.</p> <p>Urine Stains</p> <p>Some laboratories routinely use one or more stains to enhance the visualization of the urine sediment. The most commonly used urine sediment stains are commercial preparations of the supravital stain developed by R. Sternheimer & B. Malbin. The basic ingredients of the Sternheimer - Malbin stain are <i>crystal violet</i> and <i>safranin O</i> in ethyl alcohol. Commercially prepared variations of this stain have added stabilizers to limit precipitation. KOVA Stain and Sedi-Stain are two such examples.</p> <p>Review the textbook for information on additional stains used when examining urine sediment.</p>
Agenda:	<ol style="list-style-type: none">1. Review textbook, lecture notes and other sources that describe urine microscopic structures.2. Review the Urine Standardization Criteria page provided.3. For each of the 2 sessions, perform 5 urine microscopic exams on specimens provided, using the format of the 'Criteria' page.4. Use lecture and textbook materials to aid in answering study questions.

Procedure: **Preparation of Slide for Microscopic Examination**

- *1. Mix urine specimen thoroughly.
 - *2. Pour approximately 10 ml of the urine into a urine centrifuge tube.
 - *3. Centrifuge for at least *5 minutes* at 1500-2000 RPM (low speed). **Note:** make sure the centrifuge is properly balanced.
 4. Decant off all supernatant (this can be used for protein tests).
 5. Resuspend the sediment by one of the methods demonstrated by the instructor.
 6. Using a pipette, place a small drop of sediment onto a clean microscope slide. Coverslip, being careful to avoid bubbles. Or load a Urinalysis Microscopic cartridge. Ex. "Count 10"
- *You need to do these steps on your own specimen (SPECIMEN # 5) only; they have been performed for you on the other specimens.

Examination

1. Place slide on the mechanical stage of the microscope. Use the *low power objective* to get the field into focus.
2. Scan the slide under low power to get an "over-all impression." Then observe 10-15 low power fields, including the coverslip edges and center. Check for casts and mucous threads.
3. Using *high power*, check another 10-15 fields for epithelial cells, red and white blood cells, crystals, sperm, bacteria, yeast and parasites. Check with available pictures and diagrams to aid in identification.
4. Use the Urine Standardization Criteria sheet to aid in quantitation and reporting of urine microscopic sediment. Record all results in appropriate place, using appropriate format for recording patient and performance control results on the report form provided. Result forms not using appropriate format will have a 50% penalty.
 - * Recording of any laboratory result MUST be in black or blue ink.
 - * Acceptable recording for positive results:
Positive / Pos
 - * Acceptable recording for negative results:
Negative / Neg
 - * Other results are to be as indicated on the Urine Standardization Criteria sheet .

Urinalysis Reporting Standardization Guide (CRg 7/1/09)**Use & Purpose:**

Use this form to guide as you evaluate urine in the MLAB laboratory. When at clinical rotations, be sure to consult with your mentor on that facilities reporting criteria / style. The purpose of this guide is to standardize the way urinalysis results are to be reported in our labs.

Urine Colors:

Use only the following terms: colorless, light yellow, yellow, dark yellow, amber, orange, red, brown, black, blue, or green. Consult with your lab instructor if you encounter an unusual color.

Appearance / Clarity :

Note: clarity is different from color. Clarity is an evaluation of how light passes through the urine sample. It provides an indication of the amount (and possibly the type) of sediment you should expect to see under the microscope.

Use the following terms to describe appearance / clarity: clear, slightly hazy, hazy, slightly cloudy, cloudy, turbid (a thick obvious sediment that settles to the bottom of the cup upon standing). 'Bloody' is also sometimes uses as a clarity term.

Microscopic Elements:

Use the following table as you quantatify the urine sample's sediment.

- Directions:
1. Locate the element in the far left column.
 2. Note whether the element is counted / quantitated using low power (10X / LPF) or high power (40X / HPF). Casts require special consideration; they are counted using 10X, but you may have to go to 40X to identify them.
 3. Use the block of information under the "Enumerated As" to report the urine sediment.

Example 1: Evaluating WBCs - using high power / 40X; looking at 5-10 distinct high power fields, you see 7 in one field, 5 in another, 9 in the third, etc. - you would report: 6-10 WBC /HPF.

Example 2: A red colored sample with positive blood having more than 200 RBC / HPF would be reported as "TNTC" (too numerous to count). A quick way of determining whether there are more than 200 RBC/field is to mentally divide the visual field into quarters - count only what is in one representative quarter - if it is at least 50, there are more than 200 cells.

Example 3: A refrigerated urine sample has developed so many amorphous urate crystals that everything else in the microscopic fields is hidden. The appropriate report: PKD amorphous urates (PKD meaning 'packed')
Comment about Example 3: The problem of not being able to read the presence of other elements due to the amorphous urates could have been avoided IF the sample had been tested when it was fresh. Refrigeration temperature often resulted in the precipitation of the crystalline salts. By the way, these are considered normal.

Example 4: A random sample from a female patient is NOT collected using the 'clean catch' method. The LPF microscopic field has @ 15 squamous epithelial cells in each field. Report '11-20" / LPF. Notes about Example 4: 1. Many labs would reject this sample as the squamous epithelial cells indicate vaginal contamination and the evaluation of bacteria would also be compromised. 2. Lab protocol may vary on how epithelial cells are evaluated. Some use low power while others use high power. Be sure to follow the protocol of the specific facility.

Urinalysis Reporting Standardization Guide (CRg 7/1/09)

Element	Counted on		Enumerated As								
	LPF	HPF									
Casts*	✓		Rare	Occ	0-2	3-5	6-10	11-20	21-50	51-100	TNTC
WBC		✓	Rare	Occ	0-2	3-5	6-10	11-20	21-50	51-100	TNTC
RBC		✓	Rare	Occ	0-2	3-5	6-10	11-20	21-50	51-100	TNTC
Squamous Epithelial Cells	✓		Rare	Occ	0-2	3-5	6-10	11-20	21-50	51-100	TNTC
other Epithelial Cells		✓	Rare	Occ	0-2	3-5	6-10	11-20	21-50	51-100	TNTC
** Crystals**		✓	Trace	1+	2+	3+	4+	Pkd	** Note: When reporting urine Crystals you <i>must</i> identify the type of crystal in addition to its enumeration. See textbook or other reference materials for descriptions of acid, alkaline, and pathological crystals.		
Bacteria		✓	Trace	1+	2+	3+	4+	Pkd			
Yeast		✓	Trace	1+	2+	3+	4+	Pkd			
Mucous	✓		Trace	1+	2+	3+	4+	Pkd			
Amorphous		✓	Trace	1+	2+	3+	4+	Pkd			
Sperm		✓	Rare	1+	2+	3+					
Trichomonas		✓	Rare	1+	2+	3+					
WBC clumps		✓	Rare	1+	2+	3+					
Epithelial cell clumps		✓	Rare	1+	2+	3+					

Note: Different laboratories may use different criteria when reporting urine sediment. We have chosen to use this system to standardize reporting in our labs. When at a clinical site, you *must* adopt and use the reporting system of that site.

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Urine Microscopic Report Sheet - Session 1

/20 points

Name _____

Date _____

Students have the option of using their own urine specimen as their unknown # 5

Instructions: Use the Urine Standardization Criteria page to quantitate the urine sediments and determine proper reporting style.

For example: if you find an average of 4 RBCs in each of 10 or more HPF (high power fields), report it as 3-5 RBCs /hpf.

Items reported under casts, crystals or other must be identified as well as quantitated. *For example: 1+ mucous threads, 0-2 hyaline casts / lpf, 2+ CaOx, rare Trichomonas seen.*

Microscopic	1	2	3	4	5 - your spec.
Name & ID #					
Mucous (LP)					
Casts (LP)					
Epithelial cells (HP)					
RBC (HP)					
WBC (HP)					
Crystals (HP)					
Bacteria (HP)					
Other					

EXERCISE 3:

Microscopic Urinalysis

Urine Microscopic Report Sheet - Session 2

Name _____ Date _____

Instructions: Use the Urine Standardization Criteria page to quantitate the urine sediments and determine proper reporting style. *For example: if you find an average of 4 RBCs in each of 10 or more HPF (high power fields), report it as 3-5 RBCs /hpf.*

Items reported under casts, crystals or other must be identified as well as quantitated. *For example: 1+ mucous threads, 0-2 hyaline casts / lpf, 2+ CaOx, rare Trichomonas seen.*

Microscopic	1	2	3	4	5
Name & ID #					
Mucous (LP)					
Casts (LP)					
Epithelial cells (HP)					
RBC (HP)					
WBC (HP)					
Crystals (HP)					
Bacteria (HP)					
Other					

EXERCISE 3:

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___/30 points

Name _____

Date _____

Study Questions

Unless otherwise noted, each question is worth one point. Using lecture notes, reading assignments and information presented in this lab, answer the following questions.

1. What is the primary reason of performing 'macroscopic screening' on urine specimens?
 - A. Allow staff to spend time on specimens with more pathological structures and increase the cost effectiveness of the procedure.
 - B. Provide the results to the physician quicker.
 - C. To better predict the types of casts and other structures in the sample.
 - D. To decrease the need for polarized microscopy.

(14 pts)

2. Complete the following chart.

Grading level	1 pt	1 pt
Microscopic structure	quantitated under high or low power?	Describe its microscopic appearance
RBCs		
WBCs		
Squamous epithelial cells		
Renal tubular epithelial cells		
Oval Fat Bodies		
Hyaline Casts		

3. List two structures that are frequently confused with RBC?

(2 pts)

4. What type of epithelial cell is most frequently seen in a randomly collected urine specimen and from where do they originate?
5. What microscopic structure(s) would you expect to see in a specimen that has a positive nitrite?
6. A specimen collected from a known diabetic patient is positive for glucose. What microscopic structure, in particular, should you look for?
7. What reagents are used in the Sternheimer-Malbin stain?

(9 points)

8. From the list of Sediment Stain Characteristics in your textbook, pick three (3) and complete the following chart. (Do not use Sternheimer-Malbin stain to answer this question.)

Stain	Action	Function
1.		
2.		
3.		