The Intricacies and Subtleties of Microscopy

BY JAMES R. ROBERTS, MD

Emergency physicians order routine urinalyses many times each shift. It's usually a straightforward issue, and most physicians think they are well versed in interpreting the results: Give it a quick glance, and make a decision. The dipstick analysis, the microscopic exam, and other information gleaned from a urinalysis (UA) make their way into decision-making for a variety of diagnostic, therapeutic, and disposition issues. Like most things learned in detail many years ago, the interpretation of the UA should be revisited on a regular basis.

I find myself thinking I know everything about a certain test only to find out that guidelines have changed, technology has advanced, and previously-held dogma is now relegated to myth. It is obvious that the UA is not a simple test when one considers its complexity. The intricacies and subtleties are actually quite amazing.

Urinalysis:
A Comprehensive Review
Simerville J, Maxted WC, Pahira JJ
Amer Fam Physician
2005;71(6):1153

This article contains basic information on the microscopic urinalysis. Unlike the sometimes confusing dipstick, the microscopic UA is relatively straightforward. Overall, it is an indispensable part of the urinalysis when clinical information and the dipstick do not give all of the required answers. The primary task of the microscopic UA is to identify casts, cells, crystals, and bacteria.

The preparation of the sample is important to ensure reproducible results. The proper way to prepare a urine sample for microscopic analysis is to use 10-15 ml of freshly voided urine and centrifuge it at 1,500 to 3,000 rpm for five minutes. The supernatant is then decanted, and the sediment is resuspended in the remaining liquid. Transfer a single drop to a glass slide, apply a cover slip, and examine the urine under low- and high-power magnification.

Cellular Elements: Men generally have fewer than 2 white blood cells (WBCs) per high-powered field (HPF), and women and children normally have fewer than 5 WBCs per HPF. These parameters often are used to define normal limits, but they are not universally agreed upon. Finding large and irregularly shaped squamous epithelial cells with WBCs suggest contamination because these cells are not normally found in the urinary tract. The American Urological Society defines hematuria as 3 or more red blood cells (RBCs) per HPF in two of three urine sediment samples.

Casts: Casts in the sediment can localize disease to a specific portion of the genitourinary (GU) tract. A cast is a coagulum of mucoprotein that traps contents in the tubule lumen or collecting ducts during periods of urinary concentration or urinary stasis. Casts contain hyaline, RBCs, leukocytes, or epithelial cells, or they can be granular, waxy, fatty, or broad. A specific type of cast can be associated with a specific pathologic condition. RBC casts are nearly always diagnostic of glomerulonephritis or vasculitis, for example. WBC casts suggest some type of interstitial renal disease or pyelonephritis, but may be seen with a number of glomerular disorders.

Crystals: Healthy patients can excrete calcium oxalate crystals, uric acid crystals, or triple phosphate crystals. The refractile characteristics of the crystals help the lab technician identify each crystal type. The triple phosphate crystals, although normal, are often associated with alkaline urine (pH 9.0 and above), and may be associated with nephrolithiasis (staghorn calculus) or a proteus or other urea-splitting organism infection.

The use of the HIV-1 protease inhibitor indinavir can produce crystalluria, leading to urolithiasis and obstruction from stones composed of these drug crystals. (It's an unusual piece of trivia, but the sagacious clinician who wants to be a star keeps this in the back of his mind when evaluating AIDS patients with the signs and symptoms of a kidney stone.)

Bacteria: A variety of bacteria can be seen under high-powered magnification. Bacteriuria is usually associated with infection, and specimens contaminated by vaginal flora can contain large amounts of bacteria. When five bacteria per HPF are seen, there are roughly 100,000 colony forming units per ml, the classic diagnostic criteria for true bacteriuria and compatible with UTI. Men rarely have enough contamination to demonstrate bacteria in the urine under the laboratory microscope.

Comment: I could find precious few rigorous data on the technique or interpretation subtleties of urine microscopy, and it seems that everyone just accepts the party line. Urine microscopy is not routine in most hospitals, but most labs have criteria for performing this test. Often microscopy is mandated by abnormal findings on the dipstick, but it can be ordered as a separate test.

I have always been puzzled, even occasionally flummoxed, by results from the laboratory that describe the microscopic findings in the sediment of the urinalysis. I am even more puzzled by the fact that a certain number of cells or elements have been attributed to various diseases. I just can't believe it's possible that every lab technician performs a urinalysis in exactly the same way. Just the fact that 5 or 15 ml of urine can be collected means that different volumes enter the centrifuge. Spinning down 5 ml versus 15 ml would seem to triple the amount of cellular elements in the sediment.

Pouring off the supernatant can lead to tremendous variability unless some standardization is used. It is my conclusion that this technique is not standardized. The amount of remaining fluid used to resuspend the sediment can affect the results under the high-powered field. Different fields under the microscope contained different numbers of cells when I was looking at urine sediment in medical school, allowing different fields to contain varying numbers of RBCs or WBCs. These variables could mean the difference

Standard methodology for performing a microscopic urinalysis: Urine is placed in a centrifuge for 10 minutes at 3000 RPM, left. The device that protects the sediment in a standard volume of urine is used to resuspend the sediment, center. Then the sediment is examined under a high-power microscope. Note that the amount of urine used and the volume of the sediment can cause varying results in the same urine.

between 5 or 10 cells per HPF, a 100 percent difference. One hundred versus 200 WBCs are of little consequence, but if 2 WBCs per HPF is considered normal, then 5 per HPF can suggest a different diagnosis if one uses standard criteria. To my mind, there is no difference between 3, 5, or 8 WBCs per HPF.

In my lab, the technician uses a plastic tube to collect urine for the centrifuge. About 10 ml are used, but the technician usually eyeballs it. Our technicians use a nifty device (the KOVA petter) to standardize a 1 ml volume of remaining supernatant, so at least in our lab that volume is a minimal variable. Fogazzi et al. report on the use of a new plastic 10 ml tube that has a 0.5 ml bottom ball to collect sediment. This has been termed the YX tube. After centrifugation, the bottom of the tube is opened to allow the first few drops of urine onto a glass slide, supposedly giving more reproducible counts in the microscopic field. (*Curr Opin Nephrol Hypertens* 2003;12[6]:625.)

A few other variables seem to be ignored when reporting the microscopy results. Obviously, the specific gravity of urine would alter the number of cellular elements found under the microscope. The first part of the urine void can contain urethral contaminants, and the midstream sample is the one that is generally preferred. Of course, there are many other causes of pyuria that are not related to infection. I had mentioned previously that the presence of an obstructing stone by itself can produce pyuria, so WBCs in the UA do not always mean infection. Tuberculosis is the classic cause of sterile pyuria, but some cancers also can cause it. Appendicitis and endocarditis often put WBCs and RBCs into the urine sediment.

More than 5 WBCs per HPF seem to be a standard definition of abnormal pyuria, but a more scientific definition is at least 8,000 WBCs per ml of uncentrifuged urine. This often corresponds to 2-5 WBCs per HPF in the centrifuged sediments, but the customary determination of pyuria using cells per HPF is not sufficiently accurate to be considered a gold standard. Laboratories and clinicians, however, often use it as one. *Pediatric Emergency Medicine* states that a normal urine sediment for children (no age given) can contain 5-10 WBCs per HPF. (Fleisher G, Ludwig S., 6th Edition, Philadelphia: Lippincott Williams & Wilkins; 2010.) *Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases* also uses greater than 10 WBC per HPF as abnormal. (5th Edition, Philadelphia: Churchill Livingston; 2010.)

Hematuria has been defined as a presence of 3 or more RBCs per HPF in a spun urine sediment. If one field contains 2 RBCs and another contains 4, how is this interpreted? As with WBCs, the difference between 2 and 3 RBCs cannot possibly be clinically significant given the vagaries of the technique of sediment analysis.

Conflicting Textbook Recommendations on Pyuria

The number of WBCs found in a normal urinalysis varies among different authors, and published studies conclude somewhat minimally different numbers. Two examples are listed below:

The most recent edition (2014) of *Mandell, Douglas, and Bennett's Principles and Practice of Infecious Disease* states: "A clean-catch midstream urine specimen is centrifuged for 5 minutes at 2000 rpm, and then the sediment is examined under high power. Each leukocyte seen represents about 5 to 10 cells/mm3 of urine; 10 to 50 white cells/mm3 are considered the upper limit of normal. With this criterion, 5 to 10 leukocytes per high power field in the sediment from a clean-catch midstream urine specimen is the upper limit of normal, as they represent 50 to 100 cells mm3. It should be emphasized that the finding of pyuria is nonspecific, and patients with and without pyuria may or may not have infection."

The most recent version (2010) of *Pediatric Emergency Medicine,* states that a normal urine sediment for children (no age given) can contain 5-10 WBCs per HPF.

Read InFocus and Earn CME!

Earn CME by completing a quiz about this article. You may read the article here, on our website, or in our iPad app, and then complete the quiz, answering at least 70 percent of the questions correctly to earn CME credit. The cost of the CME exam is $10. The payment covers processing and certificate fees.

Visit http://CME.LWW.com for more information about this educational offering and to complete the CME activity. This enduring material is available to physicians in all specialties, nurses, and other allied health professionals. Lippincott Continuing Medical Education Institute, Inc., is accredited by the Accreditation Council for Continuing Medical Education to provide continuing medical education for physicians. Lippincott Continuing Medical Education Institute, Inc., designates this enduring material for a maximum of 1 *AMA PRA Category 1 Credit*™. Physicians should only claim credit commensurate with the extent of their participation in the activity. This activity expires June 30, 2016.

Learning Objectives for This Month's CME Activity: After participating in this CME activity, readers should better understand the nuances of urinalysis microscopy and apply those to interpreting urinalysis results to aid decision-making for a variety of diagnostic, therapeutic, and disposition issues.

Does This Woman Have an Acute Uncomplicated Urinary Tract Infection?
Bent S, Nallamothu BK, et al.
JAMA
2002;287(20):2701

The Journal of the American Medical Association frequently publishes articles in a format that helps clinicians evaluate everyday clinical scenarios. I chose this article as an example of how one can diagnose uncomplicated UTI in women with clinical history and without laboratory investigation. The authors base their conclusion on a literature review of more than 400 studies, few of which had enough scientific rigor to be included.

The executive summary is that women who present with one or more symptoms of UTI (dysuria, frequency, urgency, hematuria, suprapubic or back pain) have a 50-90 percent chance of having a UTI on history alone. Further history, examination, dipstick analysis, or microscopy adds little additional statistical value in ruling out the diagnosis when these symptoms are present. Of course, other risk factors should be considered, such as sexual activity, immune status, prior UTI, and the patient's own past experience to swing the pendulum toward or away from the diagnosis of an uncomplicated UTI. The presence of vaginal discharge leads the diagnosis away from UTI, but the absence of discharge is a strong indication that symptoms alone define an uncomplicated UTI.

The authors conclude that empirical antibiotic treatment without dipstick analysis, microscopy, or urine culture is an appropriate algorithm in women who have one or more symptoms of a UTI. Note that this defines an uncomplicated UTI, such as cystitis. Importantly, according to these authors, a microscopic urinalysis is not even considered in the algorithm, and the urine dipstick is not required if one or more elements of the history are positive. Women with dysuria, frequency, urgency, and hematuria without back pain and without vaginal discharge have a 96 percent probability of having an uncomplicated UTI. This algorithm negates the use of urine culture or urine dipstick analysis for such individuals.

Bottom line: A urine collection for at least dipstick analysis seems to be a general standard of care in men and women who present with urinary tract symptoms or undiagnosed abdominal or vaginal complaints. There also appears to be a consensus that urine microscopy and culture are not required unless the patient has an abnormal dipstick analysis or some reason to have an unusual or bizarre condition (weight loss, HIV, unusual family history, atypical presentation). It seems rather silly to diagnose UTI if the WBC count is 6 WBCs per HPF or to rule it out if the microscopy demonstrates only 2 WBCs per HPF. Likewise, for hematuria, basing your ED plan on a microscopy that has one or two extra cells or that lacks one or two cells per HPF seems rather unscientific. We have all seen the appendicitis that causes pyuria, the aortic dissection that causes hematuria, and the uncomplicated kidney stone that causes both.

Urinalysis Microscopy: The microscopic report itself defines pyuria

Continued on next page

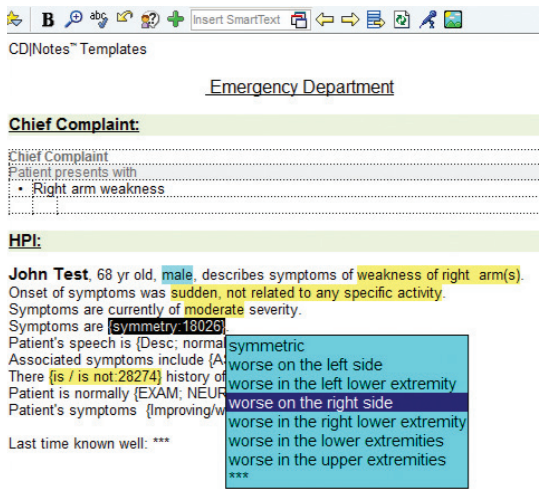
Templates Save the Day with a Workaround for EMRs

BY ALEX MOHSENI, MD

EMRs have solved certain problems, such as legibility and automatic allergy and drug interaction warnings, but it's no surprise that enterprise EMRs have failed dramatically in one important aspect: usability. Usability is so poor and productivity has been impaired so much that most providers have never returned to pre-EMR productivity levels and have been forced to seek workarounds, such as scribes.

If you are paying $18 per hour for a scribe and seeing two patients per hour, that means you are paying $9 per patient. We should be considering that this ongoing cost of enterprise EMRs has been transferred onto the physicians. When is the last time you received a survey from an enterprise EMR asking for your feedback on usability?

Thankfully, emergency physicians are an innovative bunch, and workarounds abound. One specific solution that is particularly well done is CD Notes (www.CDNotes.org), the brainchild of Chris Davison, MD, an emergency physician. Dr. Davison has created a series of more than 100 templates that can be integrated into Epic, making documentation simpler, faster, and more thorough. Each template is thoughtfully designed with an intuitive layout and rapid and easy movement among input fields.



Most fields have common answer choices already written that are selectable as dropdown fields. Templates include important elements critical for risk management and accurate coding, each specific to the complaint. Several videos demonstrating the product are available at http://www.cdnotes.org/product-demo.

Using CD Notes, physicians can document a complicated chart in less than three minutes, which is significantly better than doing it from scratch, which takes about six to eight minutes. If you're not currently using scribes, CD Notes is a great option. If you are using scribes, you will have to calculate the value of three minutes of doctor time versus the cost of a scribe. Kudos to Dr. Davison for giving us a great workaround for the usability crisis in which we're stuck. **EMN**

Dr. Mohseni *is an emergency physician in the Washington, D.C., metropolitan area and the chief innovation officer of Emergency Medicine Associates. He is the editor of his own blog, http://CreativeHealthLabs.com. Follow him @amohseni, and read his past columns at http://bit.ly/MohseniDocAPProvED.*

Microscopy

Continued from previous page

and hematuria by some criteria. It essentially cannot be rigorously interpreted, and must be correlated with the clinical scenario. It is likely that it does not define a UTI in a patient with only vague abdominal pain (or perhaps it does if there is ureteral obstruction by a calculus.) Such a urinalysis can be seen with appendicitis, endocarditis, tumor, and a plethora of other conditions. Of course, it can merely represent a contaminated sample.

Presumptive Diagnosis of Urinary Tract Infection: As the authors write in *Principles and Practice of Infectious Disease,* "A clean-catch midstream urine specimen is centrifuged for 5 minutes at 2000 rpm, and then the sediment is examined under high power. Each leukocyte seen represents about 5 to 10 cells/mm3 of urine; 10 to 50 white cells/mm3 are considered the upper limit of normal. With this criterion, 5 to 10 leukocytes per high power field in the sediment from a clean-catch midstream urine specimen is the upper limit of normal, as they represent 50 to 100 cells mm3. It should be emphasized that the finding of pyuria is nonspecific, and patients with and without pyuria may or may not have infection."

The exact number of WBCs per HPF that represent significant pyuria varies among sources in the literature and from lab to lab. More commonly quoted upper limits are 0–3 WBC per HPF for men and 0–5 WBC per HPF for women. This prestigious source uses 5–10 WBC/HPF as being normal. **EMN**

Urinalysis Microscopy

WBCs	4-6	0-5/HPF
RBCs	2-4	0-2/HPF
Bacteria	Occ	Neg, Occ
Crystals	Moderate	
	Amorphous Phosphates	
Epithelial cells	Occ	

The microscopic report defines pyuria and hematuria by some criteria. It essentially cannot be rigorously interpreted, and must be correlated with the clinical scenario. Likely it does not define a UTI in a patient with only vague abdominal pain (or perhaps it does if there is ureteral obstruction by a calculus). Such a urinalysis can be seen with appendicitis, endocarditis, tumor, and a plethora of other conditions. Of course, it can merely represent a contaminated sample.

Share this article on Twitter and Facebook.
Access the links in *EMN* by reading this on our website or in our free iPad app, both available at www.EM-News.com.
Comments? Write to us at emn@lww.com.

Reader Feedback: Readers are invited to ask specific questions and offer personal experiences, comments, or observations on InFocus topics. Literature references are appreciated. Pertinent responses will be published in a future issue. Please send comments to emn@lww.com.

Dr. Roberts *is a professor of emergency medicine and toxicology at the Drexel University College of Medicine in Philadelphia. Read the Procedural Pause, a blog by Dr. Roberts and his daughter, Martha Roberts, ACNP, CEN, at http://bit.ly/ProceduralPause, and read his past columns at http://bit.ly/RobertsInFocus.*