EDUCATIONAL COMMENTARY – RED BLOOD CELLS AND WHITE BLOOD CELLS IN URINALYSIS

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LEARNING OUTCOMES

On completion of this exercise, the participant should be able to
- explain how urinalysis varies depending on characteristics of the specimen.
- describe several ways to perform a urinalysis.
- describe the procedural and commercial methods available to standardize the urinalysis.
- quantitate and report the red and white blood cells observed.
- explain how artifacts and items observed in a urine specimen may impact the red and white blood cell being reported.

Urinalysis is one of the most routinely ordered laboratory tests. Compared with other laboratory test results, findings from urinalysis are reported in highly variable ways. Microscopic results are reported in grades (e.g., 1+, 2+) or as a range of counts (e.g., white blood cells (WBCs) = 5-10/high-power field (hpf), red blood cells (RBCs) = 0-4/hpf). This is partially because of the diurnal changes in hydration as well as the laboratory reporting parameters. Although there are methods to standardize the reporting, not all laboratories have adopted or agreed to use a universal standard for reporting the results.

The kidney primarily filters and removes fluid and compounds from the circulating blood. Urine specimens are highly variable in composition, owing in part to the patient's hydration, renal function, circulation, and time of collection. The kidney "filtrate" is collected in the bladder until urination. Information from analysis of the urine can give the clinician an idea of the patient's general health. Many compounds or analytes can be tested in a urinalysis. The focus of this commentary is on the WBCs and RBCs found in the urine during the microscopic examination.

Small quantities of WBCs and RBCs are considered normal in a urinalysis. However, a moderate to large number is considered abnormal and would suggest that additional testing is needed. The "macroscopic" dipstick portion of a urinalysis detects the presence of RBCs by measuring free hemoglobin, which acts like a peroxidase and produces a color change. Presence of WBCs is estimated by the reaction with leukocyte esterase, which primarily reacts with granulocytes. The amount of RBCs and WBCs in the urine is estimated by observing the intensity of the chemical color change.
A microscopic examination is warranted when a large presence of either type of cell is detected by the chemical tests. The microscopic part of urinalysis involves taking a drop of concentrated urine sediment from a spun aliquot of the specimen and examining it on the microscope at 40X magnification. The amount observed is reported per high-power field. The method of concentrating the urine specimen can introduce variability, depending on the procedure used and the technique of the technologist. The basic steps for concentrating the urine specimen are to transfer a well-mixed urine specimen into a centrifuge tube and spin at a relative centrifugal force of 400 for 5 minutes. The specimen is decanted to a volume of 10 to 1 (10 mL to 1 mL left in the tube) or, in some procedures, to a “dry button.” One can see the variability of these methods.

There are commercial systems that try to standardize the microscopic urinalysis. These systems standardize processing by standardizing the urine concentrated and the amount of urine remaining in the sediment after decanting. The urine is then examined in a standardized volume. Systems such as Kova (ICL Scientific), Quick Read 10 (Globe Scientific), Urisystem (Fisher Scientific), and Count 10 (V-tech, Inc) use specialized centrifuge tubes, pipets, and slides with calibrated chambers that have a set volume. The Cen-Slide (Davstar) uses a specially designed centrifuge tube with a viewing chamber for microscopic examination. Using these commercial systems helps control variables to improve the accuracy of the counts and provide a result that relates to the volume of urine.

Red blood cell and WBC counts are based on the concentrated urine specimen observed using the commercial systems or through a slide and coverslip. It is not practical to use a hemocytometer for urinalysis, owing to the high variability introduced when urine is manually processed. Using a slide and coverslip has an inherent flaw; heavier and larger particles in the liquid specimen are caused to flow out to the edges. Other errors that occur might result from a too-small urine specimen being plated on the slide, resulting in too thin a layer observed, which can give a falsely low count.

When utilizing a standardized commercial system, the manufacturer's instructions and products must be used. Most of these systems concentrate the urine specimen about 10 times and then plate to a slide with chambers of a set volume. The chambers have gridlines to help determine the fields to observe. The counts are averaged after observing 10 fields. With processing variables standardized, the basic formula for calculating the amount of cells observed can be related to the volume.

\[
\text{Cells counted, hpf/mL} = \frac{\text{Urine concentration} \times \text{Number of cells counted} \times \text{Chamber volume, in mL}}{\text{Number of fields observed}}
\]

For the example given, the calculation would be:
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\[
\text{Cells counted hpf/mL} = \frac{10 \times \text{Cells counted} \times \text{Chamber volume in mL}}{10}
\]

Microscopic observation of RBCs and WBCs in urine is usually performed with bright-field microscopy. In certain circumstances, the use of reagents or stains enhances the analysis of the specimen, helping differentiate the cells from other items or artifacts. For example, 2% acetic acid is used to lyse the RBCs and to enhance the nuclei of the WBCs, Sterheimer-Malbin stain is used to help identify WBCs from artifacts or RBCs, and toluidine blue enhances nuclear detail to differentiate WBC from renal tubular epithelial (RTE) cells.

Observed RBCs in the urine may have the biconcave shape as seen on a Wright-stained slide. Red blood cells appear round and have slightly “refractile” cell edges. Occasionally, RBCs are crenated or may look microcytic or macrocytic. There are also "ghost" cells, in which the outline of the RBC is seen but very little hemoglobin remains in the structure. Red blood cell size may vary with the concentration or the oncotic pressure caused by the urine on the cells. On average, the RBCs are 6 to 8 µm in diameter.

Interfering substances that may mimic RBCs in microscopic observation are nonbudding yeast, oil droplets, and air bubbles. Micro air bubbles appear highly refractile and vary in size. Oil droplets are seen when the slide or coverslip is contaminated by immersion oil.

![Figure 1. Examples of RBCs.](image_url)
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Yeast is often seen when there is an infection. Budding and Hyphated yeast are easily distinguished from RBCs. However, individual yeast may appear round, slightly oval, or have an elongated, "rice" appearance. Yeast cell sizes vary, and many are in the RBC size range. Nonbudding yeast is more difficult to distinguish from RBCs and may require scanning many more fields to look for possible budding of the yeast. Acetic acid may be used to help determine if the cells in question are RBCs or just rounded nonbudding yeast. Adding equal amounts of concentrated urine and acetic acid will lyse any RBCs present, and if the cells in question are still present, they are most likely yeast. If there is a combination of RBCs and yeast, there would be more than a half-fold of cells after treatment.

Figure 2. Squamous epithelial cells with budding yeasts. The size of the yeast cells approximates the size of RBCs.
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White blood cells in a freshly voided urine specimen are easily identified, because they appear as unstained WBCs on a hematology slide. Neutrophils seen in urine are round with lobular nuclei, and granules are easily seen in a high-power view. In addition, the activity and motion seen in a live WBC makes the cell appear to glitter when it reflects the microscope light. The WBCs seen this way are commonly referred to as “glitter cells”. White blood cells may appear as individual cells or in clumps. If the urine has the right amount of protein in the tubules of the kidney and there is an increased number of WBCs (as in a kidney infection), they may eventually become a WBC cast.

![Figure 3. These are examples of a WBC, budding yeast, squamous epithelial cell clump, and an RBC (however, out of focus).](image)

Neutrophils are the most common WBC present in urines. Other WBCs are present; however, they are seen in much lower frequency. Lymphocytes are small and have a single nucleus. If the nucleus is not observed, the lymphocyte may appear like an RBC and be classified as such. Also, monocytes are larger than neutrophils and may appear like RTE cells.
Renal tubular epithelial cells are lining cells from the tubules that are slightly larger than WBCs and usually appear slightly polyhedral and elongated. They may appear columnar and slightly tapered on one end. However, some RTEs appear round, with lobular-looking, eccentrically located nuclei. These round RTEs are sometimes classified as WBCs because they are difficult to recognize if there are no WBCs in the urine for comparison.

Figure 4. Examples of size difference between WBCs and renal tubular epithelial cells (both polyhedral and round). The background is overgrown with bacteria (appears mixed), indicating an infection or a urine specimen that was contaminated with a time lapse that allowed the bacteria to grow before being processed and observed microscopically.

Summary

For such a frequently ordered laboratory test, urinalysis has many variables, owing to the nature of the urine itself, specimen-processing techniques and procedures, concentration ratios, and the technical skills of the clinical laboratory scientist (CLS). Standardized processing methods have been developed to minimize the volume-concentrating process and the amount of urine examined so that observed items can be quantitated. The CLS must know these variables when observing the RBCs and WBCs and consider their impact on reporting.
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With the wide range of methods, processing and concentration variables, and CLS skills and knowledge, it is essential for each laboratory to develop procedures and methods to minimize these variables to ensure standardization, consistency, and quality of results. Laboratory accreditation processes and staff competency assessments are also ways to ensure that results are as accurate and reliable as possible.

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