Evaluation of Urine by Automatic and Manual Methods

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Introduction

Urinalysis is considered one of the most frequently performed tests in the clinical diagnostic laboratory. It is the third major diagnostic screening test in the clinical laboratory, only preceded by serum/plasma chemistry and complete blood count analysis. Laboratory evaluation of urine can provide a significant amount of useful information, as a variety of various disease states may produce abnormal laboratory findings. Routine laboratory tests used to assess urine are generally inexpensive and reliable, when performed by well-trained laboratory professionals. However, manual microscopic sediment examination is quite time consuming, labor intensive, and lacks standardization generally required in most other clinico-pathological diagnostic techniques.

The urinalysis needs to be performed as quickly as possible after urine collection. The time of collection relative to the time of analysis, or even the time needed for analysis, is important because delay can result in:

- Degradation of fragile urinary formed elements (cells and casts)
- Microbial proliferation (Urine contaminants or urinary pathogens)
- Increase in urine pH
- Degradation of certain chemical parameters (bilirubin, ketones)

Optimally, urine should be examined within 30 minutes of collection. If the analysis cannot be performed promptly (<30 min), the urine should be stored at refrigerated temperature to minimize changes in urine physical and chemical makeup, inhibit bacterial growth and maximize cell preservation.[1] Strict recommendations for duration of refrigerated storage cannot be made, since it is directly dependent on the sampled urine components.[2] The general recommendation, however, is to perform the urinalysis within 12 hours. It is very important to bring the sample back to room temperature prior to analysis, since refrigeration can potentially cause in vitro formation of crystals. This may inaccurately indicate the presence or extent of crystalluria in vivo.[3] If crystalluria is a clinical concern, freshly collected urine should be examined immediately.[4] Because urinalysis results may be affected by storage duration and temperature, the time the urine was collected, the time it arrived in the laboratory, and method of storage should be recorded.

Microscopic Examination of Sediment

In human medicine, it was reported that over 30% of urine studies conducted on patients with normal general blood work results had abnormal urine sediment results, supporting the importance of urine sediment microscopic examination. These findings can have important clinical relevance that may otherwise go unreported.[5]

Due to a wide variety of factors, there is tremendous subjectivity in the identification of the various formed elements in urine. In a 2009 study conducted by Wald et al., it was reported that only “slight to moderate agreement” was found on results obtained from shared samples of urine sediment evaluated microscopically, independent of years of experience of the observers.[6]

Traditional Manual Sediment Analysis

Many factors outside the control of the clinician can influence the accuracy of microscopic urine sediment analysis. The manual method traditionally requires review of at least 10 high-powered fields (hpf), the equivalent of 2 µL of unspun urine, which may or may not represent all clinically significant elements.[7]

The typical imprecision/inaccuracy observed in the manual method at clinical decision levels can easily change a normal result to an abnormal result. For example, one slide may show 1-3 RBC/hpf, while another slide prepared on the same sample may show 10-15 RBC/hpf, which is abnormal and may trigger follow-up by the clinician.

Many factors can contribute to the error inherent to manual sediment analysis. Counting cells in low concentrations results in high Coefficients of Variation (%CV), which increase mathematically when lower volumes of sample are examined; one microliter of analyzed sample is associated with 60% CVs, but when analyzing 10 µL it drops to 18%CV.[8]

On cover-slipped samples, any or all of the following distribution errors may occur:

- Formed elements may settle on different focal planes.
- Elements may have a non-random distribution so some rarely formed urinary elements may not be observed.
- In abnormal samples with a high concentration of formed elements, the elements may adhere to one another, to mucous or migrate to the edges of the slide.[9]

The use of stain, or lack thereof, can also impact the imprecision/inaccuracy of visual methods. When stain is not used, it can be difficult to discriminate between look-alike cells. On the other hand, non-specific stains can color the background and may obscure some formed elements such as RBCs or introduce artifactual changes of the sample.[10]

Sample Volume and Handling

The "Old Gold Standard" protocol for urinalysis (See Text Box A) is a sample of 5 mL centrifuged to provide a sediment volume of 0.5 mls. However, some clinicians are often put in the position of having to test a sample and generate results even though the sample quantity was not sufficient.

Text Box A: Steps for Quantitative Manual Urine Sediment Evaluation

1. Obtain urine sample. If the sample cannot be analyzed within 30 minutes, it should be refrigerated. If the sample needed to be refrigerated, it should be allowed to warm to room temperature. Mix sample well.
2. Using a pipette, transfer a standard volume (5ml is recommended) of urine into a clean tube (12 X 75 mm or conical tip centrifuge tube).
    a. The actual volume used is not critical, as long as a consistent volume is used each time to help the clinician determine a perspective of what is normal and abnormal in that particular volume.
    b. The volume used should be recorded on the urinalysis form.
3. Centrifuge the sample for 5 minutes at 450 X g.
4. If necessary, perform confirmatory chemical tests using the supernatant.
5. Since the relative concentration of formed elements observed by microscopy is significantly influenced by the volume of supernatant with which the sediment is resuspended, the volume with which the supernatant is to be resuspended needs to be standardized. It is recommended to use 0.5ml of supernatant to resuspend the sediment.
6. Resuspend the sediment by gentle agitation.
7. The quantity of resuspended sediment also needs to be standardized. Using a plastic pipette, transfer a drop of the sediment suspension onto a clean glass slide and cover with a 22 X 2 mm glass coverslip. The drop needs to be large enough to be entirely covered by the coverslip, but not large enough that sample spills around the coverslip, since larger elements will likely collect around the coverslip, introducing elements of variability in the results (analytical variability).
    a. The use of a coverslip is important:
        i. Promotes a uniform layer of sediment to be analyzed.
        ii. Allows for sediment evaluation under oil immersion if needed.
        iii. Protects the microscope objective from sample contamination.
        iv. Reduces the rate of sample evaporation.
8. To enhance the visualization of formed elements, reduce the microscope illumination either by lowering the microscope condenser and/or close the substage iris diaphragm.
9. Examine the sediment on 10X magnification to evaluate for larger formed elements (i.e. casts and crystals) and to determine overall cellularity.
10. Examine the urine sediment on 40X to enumerate RBCs, WBCs, and epithelial cells, and identify, enumerate, and characterize bacteria, yeast/fungus, parasites, and smaller crystals.
11. Record results.

If less than the recommended amount is analyzed, the concentration factor may be off and established. reference ranges will no longer apply for result interpretation. In these cases, the deviation from the required quantity should be noted on the report.

Centrifugation

Centrifuges can introduce error into urine sediment evaluation even before the microscopic phase begins. Centrifugation with removal of supernatant is required for sample concentration and analysis. However, it remains an important source of laboratory errors since formed elements may stay in suspension or be accidentally removed when the supernatant is removed. The use of the centrifuge brake is contraindicated since it can cause turbulence and potentially cause the resuspension of lighter formed elements. The strain of centrifugation can also induce lysis of fragile formed urinary elements (e.g. casts).

Cells remaining in the supernatant to be poured down the drain will obviously not be analyzed. This error is inherent in the centrifugation process and varies by cell condition, sample viscosity and specific gravity. In a 2003 study, investigators found uneven urine formed element distribution of erythrocytes, leukocytes, bacteria and casts when samples were centrifuged at 400g / 5 minutes or 500g / 5 minutes.[11]

The resulting dense sediment pellet requires proper resuspension prior to counting. After centrifugation and decanting, the sediment is mixed with the pipette before preparing the slide. Formed elements may stick together especially in samples with elevated protein, introducing another level of variability.

Microscopic Evaluation of the Urine Sediment

Unstained urine sediment is examined under reduced illumination by either lowering the microscope condenser and/or closing the substage iris diaphragm. The lower condenser position provides the necessary contrast to identify formed elements in the urine. The sediment should initially be examined under low-power field (lpf) to assess the general composition of the sediment and to visualize large structures; identification of individual structures is accomplished under high-power field (hpf). When using the conventional glass slide method, larger elements tend to flow toward the perimeter of the cover glass necessitating a scan of this area.

Manual Methods

Numerous procedures have been established for the detection of urinary formed elements. In the traditional manual sediment analysis, the presence of formed elements like red and white blood cells, epithelial cells, urinary casts, spermatozoa, bacteria, yeasts, various artifacts, mucus, lipids and crystals are checked generally microscopically.[12] Despite multiple efforts for standardization, intra-assay coefficients of variation of routine sediment analysis can become as high as 100%, when residual volume of the sediment and centrifugation efficiency are taken into account.[13] Therefore, a sediment analysis method can never be considered as reference of quantitative urinary particle counting.[14]

Typically, microscopy should be used as a rough estimate of the amount of urinary formed elements.

Standardization of urine sediment examination consisting of urine volume, speed and time of centrifugation, concentration of urine or sediment volume, volume of sediment examined and result reporting are essential to ensure accuracy and precision. Although these goals are theoretically achievable (See Textbox A above), in practice, they present a different set of challenges. Automation of the process can remove the human factor from the problematic and may yield more consistent, reliable, repeatable results.

Results Reporting

Traditionally, the units used for the determination of the urine formed elements in the urine has been expressed in units counted per low- or high-power field. As previously described, several pre-analytical and analytical factors, such as centrifugation techniques, depth of the urine sediment specimen under the cover slip, and the geometry of the optics for a particular microscope manufacturer, inter-observer variability most probably affect a significant inconsistency in the results obtained among laboratories for routine manual urinalysis.

Since most formed element concentrations in veterinary fluid evaluation (e.g. blood, body cavity fluids, etc.) is expressed in number of cells per unit of volume (cells/ul) of untreated (un-concentrated) fluid, it stands to reason that the same units should be used for the urinalysis. These units should enable a better comparison of results for urinalysis among laboratories.

Traditionally, the units used for the determination of the urine formed elements in the urine has been expressed in units counted per low- or high-power field. The major drawback in using the semi-quantitative method for formed elements data reporting (cells/lpf or cells/hpf) are well known and can potentially give rise to an increased rate of false negative and false positive results, leading to under- or over-diagnosis of renal and/or urinary tract disease.[15] The automated device generates results in quantitative concentration (cells/µL) instead of the semi-quantitative values (cells/hpf). The results expressed in cells/µL are more standardized and easier to interpret, since it is comparable to other cellular quantification assays used in the other areas of clinical pathology (CBC, body cavity fluid analysis, etc.). Furthermore, the use of cells/µL measurement units allows for an evaluation of cellularity of the sample which then can be compared to future samples to evaluate the effects of treatment on a disease process. The quantitative cell concentration results provide more reliable results by avoiding the misinterpretation created by the variable size “fields of view” observed with different microscope brands and objective types, and the ever present variability in the exact number of high-power fields observed by the technician.

The use of quantitative cell concentration reporting by the use of an automated device is in line with all other fluid evaluation and allows keeping the results within the same diagnostic constructs and framework as all other body fluid analysis. It raises the diagnostic importance and reliability of the urinalysis to the same diagnostic level as all other body fluid analysis and is no longer considered the add-on to the CBC and biochemical analysis.

Automated Urine Sediment Analysis

It was previously demonstrated, for urine sediment analysis, a high coefficient of variation exists and is caused by a large number of different pre-analytical and analytical factors in sample handling and microscopic evaluation.[16] The diagnostic value gained by the subjective manual microscopic evaluation of sediment analysis is quite overrated, when interpreted as a quantitative result, since so many factors can interfere with the observed results. This level of variability in urine sediment formed elements result reporting makes an accurate assessment of the importance, diagnostic impact and pathophysiological significance of the presence of these cells in the urine difficult and potentially confusing.

Diagnostic accuracy and diagnostic consistency (repeatability) are essential for a reliable assessment of the patient’s condition. For results to be reliable, they need to be repeatable and accurate. The estimation of repeatability and reproducibility of an assay is necessary to assess the robustness and the potential transferability of the method among laboratories and a uniformity of results obtained between laboratories. Because of the variability observed with microscopic evaluation of the urine sediment, automation of the process can significantly increase their diagnostic consistency.

Some advantages of using automated urine sediment analysis:

- Requires a relatively small volume of urine.
- Walk-away instrumentation.
- Examine high numbers of samples in short time.
- Eliminates the problems caused by centrifugation.
- Good precision and accuracy for certain particles (RBCs, WBCs, squamous epithelial cells).
- Good repeatable and comparable results in cells/µL.
- Supplies quantitative results with small variation coefficients.
- Leave time for the manual examination of the more complex samples if needed.

Automated urinalysis devices available on the market are accurate, reproducible, faster and much less labor intensive (walk-away instruments) than standard manual microscopy. It can be run directly at patient-side without any further sample manipulation (e.g. refrigeration, centrifugation etc.). It has the potential to markedly reduce human factor from interpretation by consistently applying the same algorithm to the analysis of every sample. Lately, most areas of clinical pathology laboratory testing have progressively transferred their process to automation, improving accuracy, precision, repeatability and reliability. It then stands to reason that the next logical step would be to integrate automation technology in the urine sediment analysis and interpretation.

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