URINALYSIS REVISITED: GETTING THE MOST OUT OF LIQUID GOLD
Dennis J. Chew, DVM Diplomate ACVIM (Internal Medicine)
Professor Emeritus
College of Veterinary Medicine, The Ohio State University, Columbus, Ohio

THE BODY FLUID OF CHOICE FOR DISORDERS OF THE URINARY TRACT
A complete urinalysis includes evaluation of physical properties, chemical properties, and urinary sediment microscopy. All 3 components should be performed at the same time on the same sample when possible, otherwise potentially meaningful clinical information will not be evaluated. Acquisition of a very small urine sample volume may not allow the performance of all 3 components of the complete urinalysis, but there is almost always enough volume to analyze the chemical dipstrip and the USG. Most practices that are performing urinalysis in-house do a complete urinalysis, yet as many as 1/3 still perform only the chemistry reagent dipstrip evaluation.

The method by which urine is collected influences the cell and chemical content that will be reported and should be clearly noted on the urinalysis form. Urine may be collected by voiding, catheterization, or cystocentesis; each method has its own advantages and disadvantages.

A urinalysis should be submitted as part of the minimum database for all sick dogs and cats. Results from urinalysis are often integrated with findings from serum biochemistry and urinary tract imaging to gain deeper understanding of the disease process and diagnosis. Urinalysis should always be submitted for animals displaying signs related to the urinary tract that include urine with abnormal color (red or colorless), pollakiuria, dysuria, polyuria, polydipsia, oliguria, urinary incontinence, and those with known azotemia. Urinalysis should be part of the work up for animals with endocrine disorders, as occult UTI and proteinuria are often part of these processes. In addition to diagnostic blood sampling, urinalysis plays a pivotal part of wellness examination consultations for dogs ≥ 6 years and for cats ≥ 8 years of age. Many systemic diseases (chronic infections/inflammation, systemic hypertension) result in protein leaking across the glomerulus into the urine that can be detected early.

Should the UA be performed in-house or shipped to a veterinary referral laboratory? One answer does not fit all practice situations especially depending on technical personnel available and their level of expertise with urinalysis. UA results from fresh urine can differ from those following storage and shipping depending upon time before analysis and temperature conditions of the sample. Samples that sit overnight in the refrigerator before analysis may suffer loss of cells, loss of cellular detail, degradation of casts, and precipitation of crystals that were not there at the time of collection. To lessen the impact of this, an unstained dry mount of urine sediment may be sent along with the urine specimen allowing cellular detail to be preserved but this may not preserve casts or crystals for observation. In-house performance is still the most common method for urinalysis in veterinary medicine but sending samples to reference laboratories has gained popularity in recent years. Results are generated after about 20 minutes when urinalysis is done manually in-house by an experienced technician. Depending on when the urine is collected and sent to a reference lab, results are available in about 5 to 24 hours.

Automated urine microscopy has recently become widely available in veterinary medicine. It takes about 3 minutes to finish automated urine microscopy and generate the results. This technology includes face-recognition technology to accurately identify most elements in the urinary sediment. Multiple digital pictures are captured that allow the DVM or technician to double-check the reported results with the visual inspection and to provide an archive for the medical record if indicated. Automated microscopy removes the variables in technique of sample handling and microscope settings between individual technicians that occurs with use of manual microscopy. Automated microscopy can increase the confidence in results that are generated in house. For difficult cases, digital images taken from urinary sediment can be sent to specialists for further review.

Urinalysis should be performed as quickly as possible following collection of the sample (within 15 to 30 minutes) in order to have the best results. Prolonged exposure of urine to room temperature before analysis can result in dissolution or degradation of delicate casts, change in pH, growth of bacterial contaminants, and loss of cellular detail due to intracellular degeneration. Refrigeration of the specimen is necessary if examination within 15 to 30 minutes after collection is not possible. The diagnostic value of the urinalysis is greatly enhanced when the urine sample is obtained prior to initiation of diuretic or intravenous fluid therapy that may alter urine concentration.
Fresh warm urine is the gold standard for performance of the urinalysis. Several handbooks/manuals of veterinary urinalysis are available as references.1-4

URINE SPECIFIC GRAVITY (USG) ESTIMATED BY REFRACTOMETRY
USG is the weight of urine compared to that of distilled water. USG is the most important indicator of excretory renal function and degree of renal mass in the urinalysis. USG is estimated by refractometric methods that depend on the bending of light in proportion to the number of molecules dissolved in solution. Refractometers designed for analysis of human urine are often used in veterinary practices, but these have a limited range for the upper scale (1.001 to 1.035). Refractometers designed for veterinary use are more appropriate to use since the scale is calibrated from 1.001 to 1.060. The refractive index for urine differs between dogs, cats, and humans, so it is best to use a veterinary refractometer that display different scales to record the refractive index (estimate of USG) for dogs and cats.5

CHEMISTRY REAGENT DIPSTRIP PADS
Dipstrip reactions for urine chemistry are semi-quantitative measurements. They are scored on a subjective scale from 0 to 4 plus, with 1 plus being a trace reaction and 4 plus being the most intense reaction possible. It is important that urine be at room temperature for dipstrip testing, as some color reactions are temperature-dependent. Urine should be well-mixed prior to exposure to the dipstrip to ensure that all constituents of the urine will contact the reagent pads. Color reactions should be read in good light, as some of the reactions have subtle color changes, particularly notable for protein content. Color reactions need to be read at specific time intervals as listed by the manufacturer as they are not all the same. Highly pigmented urine (obviously bloody or dark with bilirubin) can make it difficult or impossible to accurately determine the degree of color reaction in some instances. Human dipstrip testing for WBC is very unreliable in urine from cats (many false positives). Similarly, dipstrip testing should not be used to determine USG. Automated devices to read the colorimetric reactions from dipstrips are becoming increasingly available in private practice and can remove some of the inherent subjectivity to reading the color reactions with the naked eye.

Protein reagent pads detect mostly albumin and generally become positive at ≥ 30 mg/dL albumin in urine. Highly concentrated urine can result in a trace to +1 color reaction in some normal dogs and cats, whereas dilute urine can conceal proteinuria, reporting it as negative when more protein was actually there. A reaction of ≥ 2+ protein is usually considered pathological regardless of the USG. It is essential to evaluate the urine sediment in those with + color reactions on the reagent pad. In those with an active sediment (increased RBC and or increased WBC), it is likely that the positive protein reaction is from an inflammatory process, most often from the lower urinary tract. Renal origin proteinuria is characterized by positive protein color pad reactions in the face of an inactive sediment.

URINARY SEDIMENT MICROSCOPY
A standard quantity of urine should be centrifuged to allow semiquantitative comparison of any abnormal findings between animals or from the same animal over time. Usually 6 to10 mL has been recommended for routine urinalysis, but smaller volumes are often analyzed. The volume of urine subjected to analysis should be specifically noted as used in your practice or sent to a referral laboratory. Comparison of urinary sediment results between large and small urinary volumes that were centrifuged at either high or low speed suggested minimal differences in a veterinary abstract but differences in the number of reported casts were found.6

Stain may be added to the sediment to enhance contrast of cellular elements but its use can cause mucus strands to look like casts or precipitates to look like bacteria at times. The microscopic slide is first examined under low power to count casts and to detect areas of interest that need examination under high power. At least 10 high-dry microscopic fields are then evaluated to quantitate white blood cells, red blood cells, epithelial cells, and bacteria, and to examine crystals that might be present. Casts are counted per low-dry power field. It is a good idea to bias the examination to include the coverslip margins as elements often accumulate there.

Urinary sediment from healthy animals contains very few cells or casts and no bacteria, but can contain certain crystals. Do not expect cells in urine to look like they do on a blood film due to the widely varying effects of urinary osmolality on the cells as well as that from urinary pH and urinary toxins. Highly concentrated urine will cause cells to shrink and very dilute urine will cause cells to swell.
RBC & WBC
The presence of up to 5 red and 5 white blood cells per high-dry microscopic field is considered normal when the sample is obtained atraumatically by catheterization or cystocentesis. Some labs include up to 10 RBC per HPF to be “normal”. Slightly higher numbers of cells (up to 8 red or white cells per HPF) may still be considered normal when a voided sample is examined. The presence of clumps of white blood cells increases the probability that an organism is the cause of pyuria, and clumps should be so noted on the form. Lipiduria is normal in cats – lipid droplets are highly refractile and vary greatly in size. Lipid droplets are often confused with RBC (and sometimes with crystals) but can be differentiated with more certainty following staining with Sudan stain.

EPITHELIAL CELLS
Zero to occasional transitional epithelial cells should be present in urine from healthy dogs and cats. Transitional epithelial cells vary widely in size, and are usually round, but only small ones (approximately 1.5 to 2 times the size of white cells) are derived from the kidney. Unfortunately, small transitional epithelial cells can also originate from the lower urinary tract. Small transitional epithelial cells with a tail-like configuration (caudate cells) are thought to arise from the renal pelvis and consequently their presence may suggest upper urinary tract localization of disease. The presence of sheets or clumps (rafts) of transitional epithelial cells strongly suggests neoplasia but may also occur with severe inflammation. A dry mount cytological preparation of urine should be examined for morphology of these epithelial cells if rafts are consistently identified in the urinary sediment. Squamous epithelial cells can be observed in voided specimens. These cells are of no particular significance in urine as they arise from non-urinary tract tissue.

CASTS
Casts are molds of proteins and cells that form within the lumen of the distal tubule and should be rarely encountered in urine from healthy animals. Cellular casts in urine are always considered pathologic regardless of their quantity. Cellular casts are easily disrupted and can undergo rapid cellular degeneration, so it is essential to examine fresh urinary sediment if cellular casts are to be identified. The presence of cellular casts localizes a pathological process to the kidneys.

Cellular casts may consist of red blood cells, white blood cells, or renal tubular epithelial cells. Red blood cell casts are occasionally observed in acute glomerulitis and following severe renal trauma or renal biopsy. Acute glomerular disease is not commonly recognized in dogs or cats. White blood cell casts (pus casts) are indicative of renal inflammation and are often thought to be caused by bacterial infection. Epithelial cell casts result as the renal tubular epithelium desquamates following a variety of injuries to the kidney – indicating severe tubular injury. It is easy to identify the type of cellular cast when the morphology of the cells within the cast is well preserved. When cellular degeneration has occurred it can be difficult to tell the difference between white blood cell and epithelial cell casts. Where cell type cannot be accurately determined, the cast is referred to as a degenerating cellular cast. Since even a single cellular cast is of great diagnostic significance, it is important to note their presence. Cellular casts are especially fragile and their presence is easily missed if urine is stored too long prior to examination.

Granular casts are more commonly encountered in animals with renal disease than cellular casts. According to the classic theory of Addis, granular casts develop from degenerating renal epithelial cells, white cells, and red cells that have remained within the renal tubular lumen. Granules can also originate from precipitation of filtered serum proteins into tubular fluid.

Waxy casts are translucent and sometimes take up stain intensely. They tend to be brittle, often with visible fractures and sharp, broken off ends. They are not fragile casts and are stable for some time in alkaline or acid urine. Since it takes more intrarenal time to form this cast, their presence implies local nephron obstruction and often indicates advanced renal disease.

Hyaline casts are pure precipitates of matrix (Tamm-Horsfall) mucoprotein. Hyaline casts are transparent and have low optical density. They can be missed during brightfield microscopy if lighting intensity is not reduced. The presence of persistent hyaline casts usually indicates increased filtration of serum proteins which does not happen in healthy animals. Increased filtered proteins can occur from glomerular disease, passive congestion, and fever.
CRYSTALS
The presence of crystals in urine is often more confusing than helpful in providing meaningful information. Many amorphous crystals cannot be definitively identified based on morphology alone. Urinary pH can suggest which types of crystals are more likely to precipitate out of solution at a particular pH. Crystals can be identified in those without stones, in those with stones, and sometimes in those with stones of another crystal composition, so their clinical significance is questionable in many instances. It is VERY IMPORTANT to remember that crystals can come out of solution after collection of the sample, especially during storage and even more so during refrigeration. Crystals that are reported may not have been there at the time the sample was collected.\textsuperscript{7,8}

References