Urinalysis – the body fluid of choice for disorders of the urinary tract and more

Collection of urine without contamination (non-urinary chemicals, cells, environmental elements) and without trauma to the urinary tract (which introduces cells and protein into the urine) is critical to the proper interpretation of results. 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. The single most important kidney function test from the urinalysis is the degree of urine concentration as evaluated by urinary specific gravity (USG). Less than maximal urine concentration may provide clues to underlying renal and endocrine disorders. A complete urinalysis should be submitted whenever serum biochemistry and CBC are submitted in order to allow a clearer analysis of the patient’s condition. Two handbooks/manuals of veterinary urinalysis are available as references.1,2

Voided urine

Voided samples are acceptable for evaluation of urinary specific gravity (USG). It is almost never possible to collect mid-stream voided samples from cats. Urine should NOT be expressed from the bladder of cats as trauma from this procedure often adds blood and protein to the sample. Wide fluctuations in USG do not occur throughout the day in cats as occurs in dogs, so timing of sample collection is usually not important. Non-absorbable kitty litter (e.g., Nosorb ®) placed in a cleaned and rinsed litter box may allow the collection of a voided sample from cats. Make certain there is no bleach contamination to the sample as this can give an artificially positive reaction for blood on dipstrip chemical analysis. Contamination from the distal urethra, genital tract, skin, and environment can make interpretation of results from voided urine samples difficult. Voided samples are not acceptable for bacterial culture due to the potential for heavy bacterial contamination of the sample from the distal urethra and genitai tracts, although the degree of this type of contamination is far less in cats than in dogs. Analysis of a voided urine sample is often needed to determine whether blood observed from a previous sample collected by cystocentesis was caused by the cystocentesis needle.

Catheterized urine

It is rarely justified to obtain routine urinalysis by catheter, since the possibility of introducing bacteria is always a threat to create iatrogenic urinary tract infection (UTI). If a urinary catheter is being placed for other reasons, collection of urine through the catheter may be acceptable, but some changes in the urinalysis may be the result of trauma from passing of the catheter. Routine catheterization of male cats should be avoided due to the possibility of causing urethritis and urethral obstruction following the procedure. Culture of catheterized samples may help document urinary infection. Results of urinalysis taken from animals with indwelling urinary catheters are more likely to have blood and protein present, secondary to the presence of the catheter. The initial 1-3 mL of urine from the catheter should be discarded (called a mid-stream catheterized sample), since the first few mL are most likely to be contaminated from the urethra and genitai tracts.

Cystocentesis samples

In general, it is best to evaluate urine collected by cystocentesis (vesicopuncture), since this method bypasses potential contamination of the specimen with cells, protein, or bacteria from the urethra, vagina, prepuce, and perineum. This is unquestionably the method of choice for urine culture and microscopic evaluation of bacteria in sediment, since normal urine directly from the bladder should not contain any bacteria. Some problems with interpretation of results can occur when the tip of the needle has traumatized the bladder or if the bladder wall has inadvertently been aspirated into the needle during sampling (adding RBC or epithelial cells). Cystocentesis should also be avoided if there has been recent major caudal abdominal trauma due to the possibility of bladder wall devitalization from the trauma.

Cystocentesis is readily performed when the urinary bladder is palpable in cats. If the bladder is not palpable, cystocentesis should not be attempted with blind techniques as used with some success in dogs. Urinary urgency and pollakiuria can make it difficult to keep enough urine in the bladder to obtain a sample from a palpable bladder. It may be necessary to give the cat an analgesic and mild tranquilizer to decrease urgency so that the bladder will fill over the next few hours. Removing the litter tray the night before a first morning appointment increases the chances to be able to palpate the bladder and obtain a cystocentesis sample. This method is useful for cats scheduled to be examined for wellness visits or elective pre-operative procedures.

Sudden collapse following/during cystocentesis has been very uncommonly encountered in cats, probably a result of a vagal-vagal response. Though sometimes dramatic, this effect is quite transient. We have observed this in some male cats with urethral obstruction in which decompressive cystocentesis was very rapidly accomplished. A 22 gauge needle or smaller should be used for puncture of a palpable bladder using dorsal or lateral recumbency. A one-inch needle should be used for thin animals; up to a two inch needle can be used for large or obese cats. The needle should be pointed toward the pelvic inlet to allow collection of a sample as the bladder collapses without needle trauma during aspiration. Although cystocentesis can be performed in cats using dorsal recumbency, it is safer and easier in most cases to perform the procedure with the cat restrained in lateral recumbency. The bladder can be palpated and
isolated using one hand to position the bladder away from the bowel. With four fingers under the cat pull up lightly on the abdomen, using the thumb to isolate the bladder within the abdomen in the ideal position. With the other hand, direct the syringe and needle perpendicular to the body wall, through the abdomen, and into the bladder. Ultrasound (ULS) guidance usually allows cystocentesis of enough urine from a small bladder that could not be sampled during bladder palpation. Even with ULS the bladder may be too small to successfully obtain a sample. In these instances, waiting for the bladder to fill with more urine is advised. In some practices, all urine samples are obtained with ULS guidance whether the bladder is palpable or not. The advantage to this method is that it allows a brief structural evaluation of the bladder to exclude the presence of cystic calculi or bladder masses.

Performing the urinalysis
A complete urinalysis that includes evaluation of physical properties, chemical properties, and urinary sediment microscopy should always be performed 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. In some instances all of the small volume will be prioritized to submit for urine culture instead of components of the UA.

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 (Dr. Maxey Wellman personal communication) but this will not preserve casts or crystals for observation.

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 to 10 mL is 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 recent veterinary abstract but differences in the number of reported of casts were found.

Urinalysis should be performed as quickly as possible following collection of the sample (within 15 to 30 minutes). 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 urine sediment evaluation is likely to be most valuable/revealing in cats that are systemically ill or in the hospital receiving treatment.

USG is the weight of urine compared to that of distilled water. Highly concentrated urine is expected in the urine of healthy cats. USG is the only indicator of renal function in the urinalysis and consequently is very important. 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. USG most often exceeds 1.035 in cats with normal renal tubular function. It is not acceptable to report USG values as “Greater than 1.035” or “Off the Scale,” as potentially valuable quantitative information is lost regarding renal function and risk for idiopathic cystitis or urolithiasis. The refractive index for urine differs between dogs, cats, and humans, so it is best to use a veterinary refractometer that displays different scales to record the refractive index (estimate of USG) for dogs and cats. Both digital and optical refractometry correlate well to urine osmolality, but digital methods remove the variability of subjective interpretation.

Dipstrip reactions for urine chemistry are graded 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. 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.
Evaluation of urinary sediment
The goal of centrifugation is to concentrate otherwise undetectable abnormal urinary elements for microscopic evaluation. A pellet at the bottom may or may not be macroscopically visible following centrifugation. Sedi-Stain® may be added to the sediment to enhance contrast of cellular elements; although this is optional, it is recommended. Sedi-stain sometimes causes mucus strands to look like casts or precipitates to look like bacteria. 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. It is now easy to capture digital images of urinary sediment using a smart phone and an inexpensive adapter to the microscope eyepiece. This allows a more permanent record to be captured and stored for part of the patient’s medical record and also provides a means to send images to specialists for further identification of abnormal elements.

Urinary sediment from healthy animals contains very few cells or casts and no bacteria, but can contain certain crystals. The ability to properly identify red blood cells, white blood cells, and bacteria is most important. 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. 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 cats. Transitional epithelial cells vary widely in size, and are usually rounded, 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.

Bacteria
When urine samples from healthy animals are properly collected and examined in a timely manner, none or very few bacteria should be seen. Particles of debris, stain precipitates, and very tiny crystals may look like cocci when subjected to Brownian motion in urine sediment, resulting in a false positive for bacteria to be reported by the laboratory. It is easier to be confident that bacteria are present when rod-shaped organisms are seen. Specimens which are reported positive for bacteria should be Gram stained or stained with Diff-Quick® for confirmation, and a quantitative urine culture should be performed. The absence of microscopically visible bacteria does not ensure that bacteria are absent; at least 10,000 rods/mL or 100,000 cocci/mL of urine must be present to be visible during wet-mount microscopy.

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 common in 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 lining of the renal tubule sloughs 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 consequently require the longest intrarenal time for their development. 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. Increased concentration of THP favors its precipitation – this can occur in highly concentrated urine and from increased tubular secretion. Decreased tubular flow rate and the presence of myoglobin in the tubular fluid favor precipitation of THP.

**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 like 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.15,16

Struvite crystals are common in both normal and abnormal small animals and their presence in urinary sediment does not mean by this finding alone that the animal has urolithiasis due to struvite. Struvite crystals are the most common type encountered in small animals. The presence of struvite crystals is commonly encountered in urinalysis from normal dogs and cats. Struvite is easily identified when they assume the "coffin-lid" appearance but they can also assume amorphous forms. Struvite crystals form more often in alkaline urine and are commonly encountered as an artifact following storage and refrigeration.

Calcium oxalate crystals can be helpful in establishing a diagnosis of ethylene glycol (radiator fluid) poisoning in the appropriate clinical setting, but they can also be seen in the urine of healthy animals. So-called "hippurate" crystals also help to support a diagnosis of ethylene glycol poisoning, but they are really not hippurates as was once thought.17,18 There are many different morphological appearances for calcium oxalate crystalluria, some of which are not easy to identify. These crystals are more often found in acid urine. The dihydrate form of calcium oxalate is relatively easy to recognize due to its rhomboid shape with internal Maltese cross pattern. Oxalate crystals may be an artifact of storage and refrigeration or may be associated with urolithiasis, hypercalcemia, or ethylene glycol ingestion.

The presence of cystine crystals is abnormal and in animals with urolithiasis does help to confirm their chemical composition. They are usually noted in acid urine. These hexagonal crystals are never normal and are associated with cystinuria or cystine urolithiasis. These crystals may be confused with struvite crystals, but cystine crystals are flat and display little internal architecture.

Urate crystalluria is never normal in the cat. In the presence of confirmed urolithiasis their presence suggests the chemical composition of the urinary stone. The presence of ammonium biurate, leucine, or tyrosine crystals can be seen in animals with liver disease, but are not commonly observed.

Bilirubin crystalluria is never normal in the cat and should prompt further evaluation of liver function.

**Pseudocasts/artifacts**

Sometimes elements within urinary sediment will resemble casts when they are really artifacts, called pseudocasts. The presence of mucus in urine can trap debris in such a way that the resulting structure appears very similar to a cast. The pseudocast can be quite long and its diameter quite variable. Sometimes packing of crystals or many bacteria during centrifugation can produce structures that resemble casts. In these instances, examine a fresh drop of unspun urine for comparison. Squamous epithelial cells have a tendency to roll on themselves and can look like casts, but they are much larger than casts. Degenerated lower urinary tract epithelial cells can produce pseudocasts that resemble granular casts; however, usually these pseudocasts, unlike true casts, have rounded ends and walls which are not parallel.

Vegetative matter such as straw and fiber is observed frequently in specimens collected by voiding. Ova of Capillaria plica can occasionally be encountered in urine sediment of cats with and without signs of lower urinary tract disease.

**Special tips - urinalysis:**

- Evaluate fresh sediment- everything is easier to identify
- Crystals from refrigerated urine may be artifacts– note if refrigerated
- Describe if WBC are clumped
• Look closely at clumped WBC for possible organisms
• Describe “bacteria” as cocci or rods
• Don’t rely on dipstrip pads for WBC in dogs or cats
• Don’t rely on dipstrip pads for USG
• If you see things that look like fungal elements, make sure they are not elongate bacteria.
• If fungal elements are seen, make sure they are not in the stain
• Consider Gram-stain of urine when “bacteria” are noted in the urinary sediment.
• Get pH by meter if it is important to know precise values
• Make sure you have the “real” specific gravity – not “off scale”
• Perform dispsticks on urine that has been warmed to room temperature if samples have been stored in the refrigerator
• Be careful to distinguish lipid droplets from RBC in urine from cats
• Quantitate the number of crystals, note if they are aggregating or not, and make sure to report if they were discovered in refrigerated urine

References