Urinalysis Made Easy: The Complete Urinalysis with Images from a Fully Automated Analyzer

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Ideal conditions for urine sample handling, and potential in vitro artifacts associated with urine storage

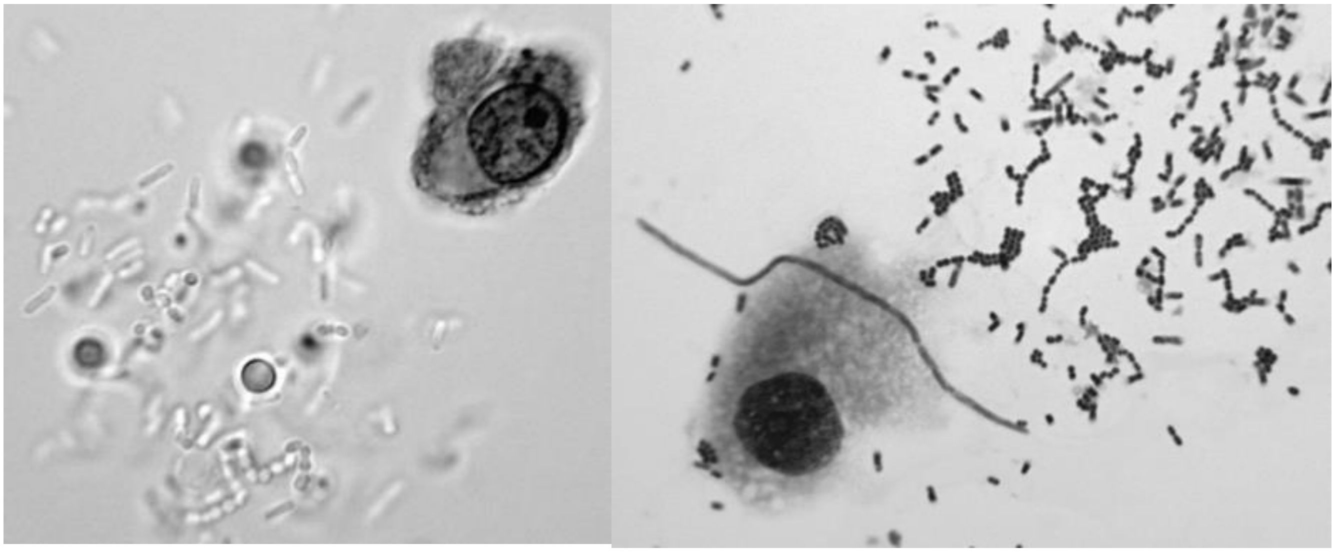
1) Potential artifacts associated with refrigeration:
   a) In vitro crystal formation (especially, calcium oxalate dihydrate) that increases with the duration of storage
      i) When clinically significant crystalluria is suspected, it is best to confirm the finding with a freshly collected urine sample that has not been refrigerated and which is analyzed within 60 minutes of collection
   b) A cold urine sample may inhibit enzymatic reactions in the dipstick (e.g. glucose), leading to falsely decreased results.
   c) The specific gravity of cold urine may be falsely increased, because cold urine is denser than room temperature urine.
2) Potential artifacts associated with prolonged storage at room temperature, and their effects:
   a) Bacterial overgrowth can cause:
      i) Increased urine turbidity
      ii) Altered pH
         (1) Increased pH, if urease-producing bacteria are present
         (2) Decreased pH, if bacteria use glucose to form acidic metabolites
      iii) Decreased concentration of chemicals that may be metabolized by bacteria (e.g. glucose, ketones)
      iv) Increased number of bacteria in urine sediment
      v) Altered urine culture results
   b) Increased urine pH, which may occur due to loss of carbon dioxide or bacterial overgrowth, can cause:
      i) False positive dipstick protein reaction
      ii) Degeneration of cells and casts
      iii) Alter the type and amount of crystals present
3) Other potential artifacts:
   a) Evaporative loss of volatile substances (e.g. carbon dioxide, ketones, urine water)
      i) Avoid this artifact by using an airtight sample container
   b) Photodegradation of light-sensitive chemicals (e.g. bilirubin, urobilinogen)
      i) Avoid this artifact by using a sample container that does not transmit light (i.e. an opaque sample container)

Method to prepare urine sediment for dry-mounting and routine cytologic examination.

This method is useful when more careful microscopic evaluation of cells is warranted, such as when there is concern about **potential infectious organisms** (e.g., bacteria, fungus) or when there is concern about the **presence of atypical cells that might be neoplastic**.

1) Centrifuge the urine as is done for wet-mounting.
2) Use a transfer pipette to remove all of the supernatant urine.
3) Use the transfer pipette to aspirate the sediment pellet from the bottom of the conical centrifuge tube.

4) Place a small drop of the aspirated material onto a clean, glass microscope slide and gently smear material using a second glass slide.
5) Allow the slide to air-dry. Heat fixation is not necessary and will alter cell morphology.
6) Stain as a routine cytology using Diff Quik® or other similar stain. Alternatively, the slide can be stored in a covered container at room temperature and sent to a referral diagnostic laboratory for evaluation. (Figure below illustrates wet mount (left) and air-dried smear (right) of the same urine sediment).



LEFT: Bacteriuria in wet-mounted urine sediment from a glucosuric Miniature Schnauzer.

RIGHT: Bacteriuria in dry-mounted urine sediment from a glucosuric Miniature Schnauzer. This is the same case as on the left; and the slide was prepared using the method described above.

Chemical Analysis of Urine

Urine pH

The normal urine pH values for dogs and cats is 5.0 to 8.5. Herbivorous animals have an alkaline pH, while carnivores and omnivores will vary from acid to alkaline depending on the amount of animal protein in their diet. Some causes of acidic urine are the ingestion of meat, respiratory and metabolic acidosis, severe vomiting with chloride depletion, severe diarrhea, starvation, pyrexia, and administration of urinary acidifiers. Causes of alkaline urine include a recent meal, ingestion of alkali (bicarbonate or citrate), UTI with urease-producing bacteria, renal tubular acidosis, diets rich in vegetables and cereals, and metabolic and respiratory alkalosis.

Proteinuria

Normally, there should be no protein in the urine. However, a trace or 1+ reaction is considered normal with a specific gravity greater than 1.035. Highly alkaline urine (>8) can produce a false positive result. If the proteinuria is significant (does not fall into above circumstances), then a urinalysis with sediment examination is recommended to exclude hemorrhage (hematuria) and inflammation (pyuria) as the cause. The most common cause of inflammatory proteinuria is urinary tract infection (UTI). If these (hematuria and pyuria) can be ruled out, then another urinalysis should be performed to determine if the proteinuria is persistent. Transient proteinuria has many causes, among them strenuous exercise, fever, seizures, and venous congestion of the kidneys, and is rarely of any significance. Persistent proteinuria is usually due to renal glomerular disease: glomerulonephritis or amyloidosis. The urine protein:urine creatinine ratio helps to determine the magnitude and therefore the significance of proteinuria. To obtain this ratio, urine protein and creatinine concentrations are measured spectrophotometrically. The total protein (mg/dl) is divided by the creatinine reading (mg/dl). Ratios greater than 0.5 are abnormal.

Glucosuria

Normal urine should be negative for the presence of glucose. False negative reactions may occur due to a large quantity of ascorbic acid in the urine. Cats with cystitis may give a false positive reaction. A positive reaction for glucose in the urine demands a measuring of the blood glucose levels because the most common cause of glucosuria is hyperglycemia (blood glucose >180 mg/dL in dogs, >280 mg/dL in cats and >100 mg/dL in cattle). Marked hyperglycemia is usually caused by diabetes mellitus, but may be transient and stress-induced in the cat. If the blood glucose level is normal, the glucose level in the urine should be re-evaluated. If glucosuria is still present, proximal renal tubular dysfunction may be the cause (e.g. Fanconi's syndrome).

Ketonuria

Urinalysis results should be negative for ketones. Lipolysis produces ketones and some of the causes are starvation, fasting and diabetic ketoacidosis. If ketonuria and glucosuria are both present, a strong suspicion of diabetes mellitus is indicated (blood glucose should be measured). Ketonuria without glucosuria suggests excessive lipid catabolism and, in an otherwise normal patient, is not significant.

Bilirubinuria

Normal dogs (esp. males) may have small amounts of bilirubin in the urine if the specific gravity is greater than or equal to 1.030. **Normal cats do not have bilirubinuria**. This test pad may give false negative results if exposed to air or light for long periods of time or if urine has a dark color (hemoglobinuria) which may mask any color changes on the strip. To be excreted into the urine, bilirubin must first be conjugated. This normally occurs in the liver, but canine kidneys are also capable of performing this function. The most common causes of bilirubinuria are hepatic disease, posthepatic bile duct obstruction, and hemolytic diseases. Mild bilirubinuria can possibly result from anorexia, especially in the horse.

Urobilinogen

The normal values of urobilinogen are 0.1 to 1.0 Ehrlich units. The reagent strip cannot accurately measure this and the test results are of little significance.

Occult Blood

The reagent strip will detect hemoglobin, myoglobin and/or intact red cells. Normally, hemoglobin and myoglobin will not be present in the urine. Only a few RBC's (five or fewer / hpf) will occur in normal urine, unless the animal is in proestrus, in which case higher numbers of RBC's may be detected in voided urine. Hematuria is the most common cause of a positive reading for blood on the reagent strip. If, upon looking at the urine sediment, RBC's are found, hematuria is confirmed. If there are no RBC's in the urine sediment, the patient's hematocrit and plasma color should be examined. If plasma is red or pink (and proper venipuncture technique was performed) hemoglobinemia is present. This indicates hemolytic anemia (complete CBC should be done). If hematuria is not indicated, a test for myoglobinuria can be performed (ammonium sulfate precipitation test). Persistent hemoglobinuria of unknown cause may indicate some kind of occult urinary hemorrhage.

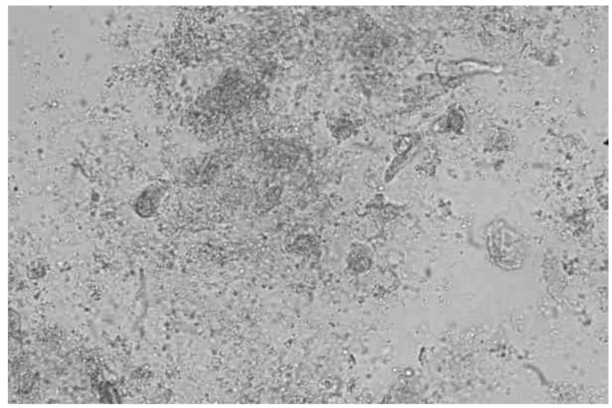
Nitrituria

No nitrites should be present in the urine. However, this test is not reliable due to false negative results.

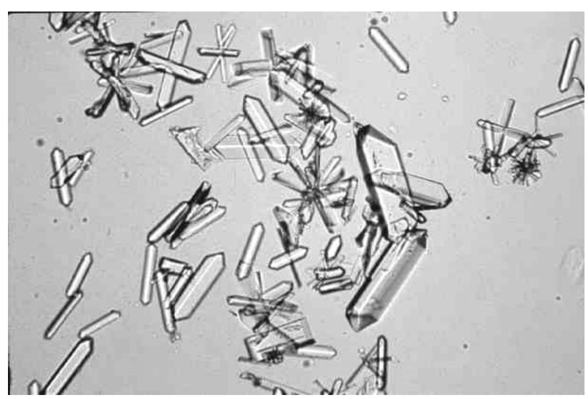
Microscopic Evaluation of Urine Sediment

Note: The microscope condenser or light source should be turned down when examining urine sediments, particularly if unstained.

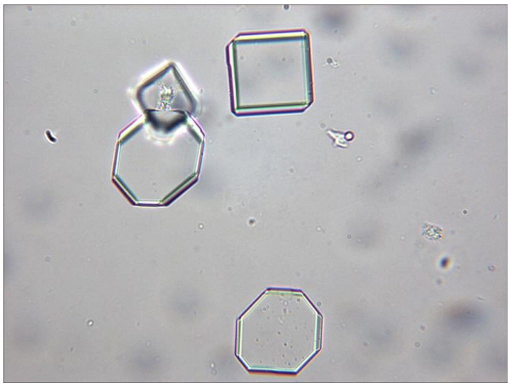
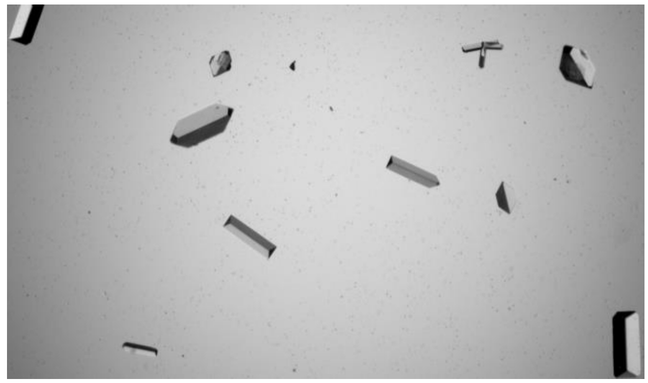
1. <u>Amorphous phosphates and urates</u> - Amorphous phosphates and urates have a similar shape, appearing as amorphous debris or small spheroids. Amorphous phosphates are distinguished from amorphous urates in two ways: phosphates are colorless and precipitate in alkaline urine; while urates are yellow-brown to black and precipitate in acidic urine. Amorphous phosphates are commonly found in urine of clinically normal animals, and are of no known diagnostic significance. However, amorphous urates occur uncommonly in clinically normal dogs and cats. They may be seen in animals with portovascular malformation, severe hepatic disease, or ammonium urate urolithiasis. Amorphous urates are seen commonly in Dalmatians and English bulldogs, and may indicate a predisposition to urate urolithiasis in these breeds.



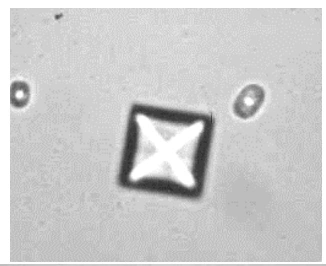
2. <u>Magnesium ammonium phosphate crystals (struvite)</u> - Magnesium ammonium phosphate crystals are referred to as struvite crystals or triple phosphate crystals (a misnomer). They are colorless and frequently form variably sized, coffin lid-shaped crystals. However, they can have a variable appearance and may occur as 3 to 8 sided prisms, needles, or flat crystals with oblique ends. Struvite crystals most commonly form in alkaline urine. **Struvite crystalluria may form in vitro in refrigerated, stored urine samples, or in those that become alkaline with storage.** When they are detected in a stored urine sample, the finding should be verified by examination of a freshly obtained urine sample. They are very commonly seen in dogs and occasionally in cats. When found in significant number, they are most frequently associated with bacterial infection by urease-producing bacteria, such as *Staphylococcus* sp. or *Proteus* sp. However, in cats they can occur in the absence of infection, likely due to ammonia excreted by the renal tubules. **Atypical (4 or 8 sided) struvite crystals can be seen in cats (figure on bottom left)** Struvite crystals may be seen in clinically normal animals that have alkaline urine, animals that have sterile or



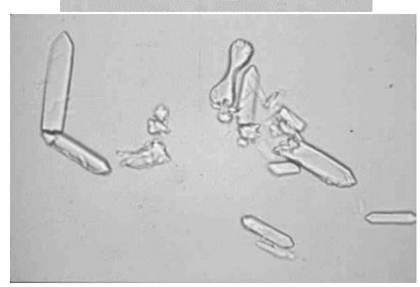
infection-associated uroliths of potentially mixed mineral composition, or with urinary tract disease in the absence of urolithiasis.

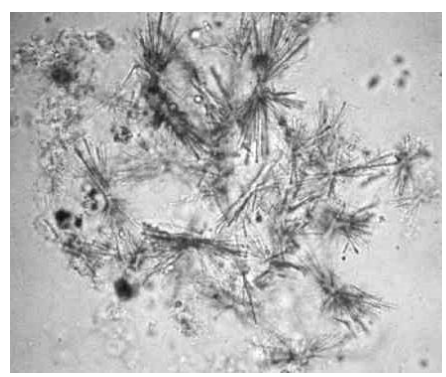
3. Calcium oxalate crystals - Two forms of this crystal are seen.



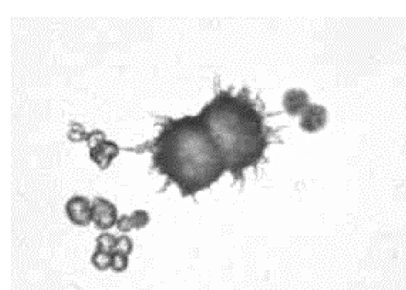
The dihydrate form (top figure) is envelope-shaped, and may be seen in normal dogs particularly in those that have ingested plant material or vegetables high in oxalates. **Calcium oxalate dihydrate crystals may develop after collection in stored urine samples with or without refrigeration or in those that become acidic during storage** due to bacterial overgrowth, for example.



The monohydrate form is an elongated, flat crystal, often with pointed ends (bottom figure). Although either type can be seen in cases of ethylene glycol poisoning, the monohydrate form is more diagnostic since this type is usually only seen in acute cases of ethylene glycol poisoning, and not in clinically normal animals.



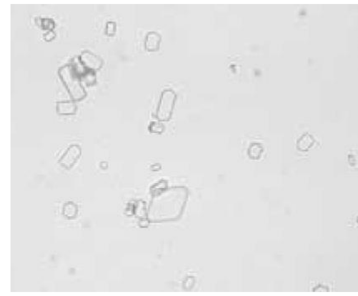
4. Bilirubin crystals - Bilirubin may precipitate as orange to reddish-brown granules or needle-like crystals. They can be found in low numbers routinely in canine urine, especially in highly concentrated samples. When bilirubin crystals are found in other species or repeatedly in significant quantity in the urine of a canine patient, their presence suggests a disorder in bilirubin metabolism, which may be the result of either a prehepatic (hemolysis), hepatic, or posthepatic disorder.



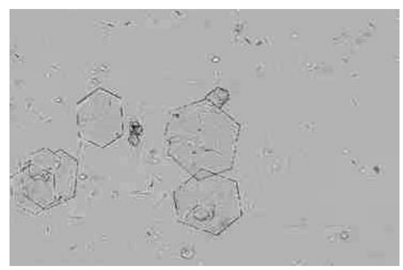
5. Ammonium biurate crystals - Ammonium biurate crystals may also be referred to as ammonium urate crystals. They

are golden to brown and spherical with irregular protrusions, which engenders a thorn-apple or sarcoptic mange-like appearance. In cats, they may occur as smooth aggregates of spheroids. Ammonium urate crystals are seen in animals with portovascular malformation, severe hepatic disease, and uncommonly in clinically normal Dalmatians and English bulldogs.

6. <u>Uric acid crystals</u> - Compared to other breeds, Dalmatians excrete a larger amount of uric acid in their urine and are therefore prone to form uric acid crystals. Uric acid crystals are colourless; flat; variably, but often diamond-shaped; six sided crystals. Most other breeds convert uric acid to a water-soluble compound (i.e. allantoin) for excretion. Dalmatians have defective purine metabolism, preventing this conversion, so that uric acid is excreted in its native form into the urine. Also, Dalmatians have decreased tubular resorption of uric acid compared to other breeds. Uric acid crystals can also occasionally be seen in English Bulldogs. They are rarely seen in other dog breeds or cats and, when observed, have the same significance as amorphous urate or ammonium biurate crystals.



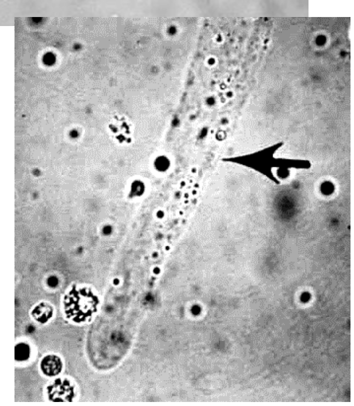
6. <u>Cystine crystals</u> - Cystine crystals are colorless, flat hexagons that may have unequal sides. Cystine crystalluria is not considered a normal finding and is seen in animals that are cystinuric due to an inherited defect in renal tubular transport of the amino acid cystine. Crystals are prone to develop in cystinuric patients that have concentrated, acidic urine. Cystinuria is a predisposition for the development of cystine urolithiasis, though not all cystinuric individuals develop uroliths. Among dogs, male dachshunds, English bulldogs, and Newfoundlands may be affected. (Females and other breeds may also be affected). Among cats, this disease has been recognized in male and female Siamese and domestic shorthairs.



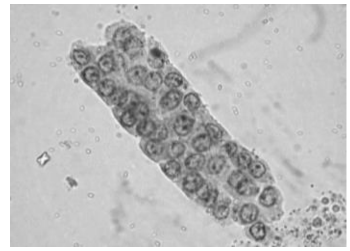
7. <u>Sulfa crystals</u> - Variably shaped, pale yellow crystals, depending on the sulfur drug being administered. May be haystack-like bundles or radiant spheres. Seen in some patients being administered sulfur-containing drugs.

B. **Casts**: Best visualized on unstained preparations, recorded as #/LPF. Casts are typically seen as an early indicator of renal tubular disease.

1. <u>Hyaline cast</u> - Tamm-Horsfall mucoprotein precipitates secreted by the renal tubular epithelial cells. They are usually colorless. Low numbers of hyaline casts are usually insignificant and can be seen in hyperthermic patients or post-exercise.



2. Epithelial cast - May be seen in cases of nephritis or pyelonephritis. See round to polygonal epithelial cells fixed in a tubular arrangement. These cells may undergo variable degrees of degeneration and develop a granular appearance. The cellular or granular nature of the cast gives an indication of how long the cast has been present.

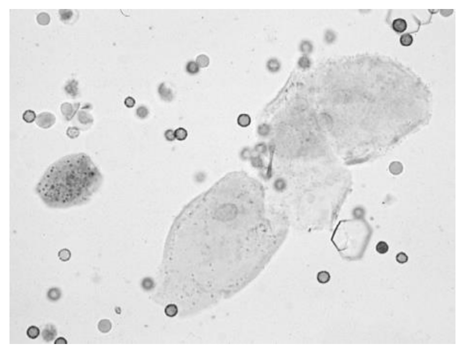


3. Coarse granular cast - Seen initially when epithelial cell casts begin to degenerate. They are primarily an indicator of renal tubular damage. Coarse granular casts will progress to fine granular casts.

4. Fine granular cast - Intermediate stage of degenerating cellular casts. This will eventually progress to waxy casts.

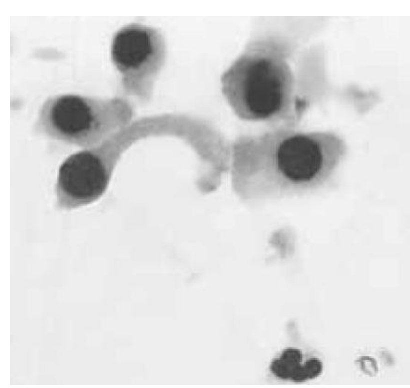
5. Waxy cast - Homogeneous casts with sharp edges and possible cracks. Indicates a chronic tubular lesion.

C. Epithelial Cells: evaluated on stained preparation, recorded as #/LPF



1. Squamous epithelium - Large flat cell with angular sides and small nuclei. Originate from the distal a of the urethra, the vagina, or the prepuce. Usually seen as a result of lower urinary tract contamination from voided or catheterized samples. May rarely be seen with squamous metaplasia or squamous cell carcinoma of the bladder.

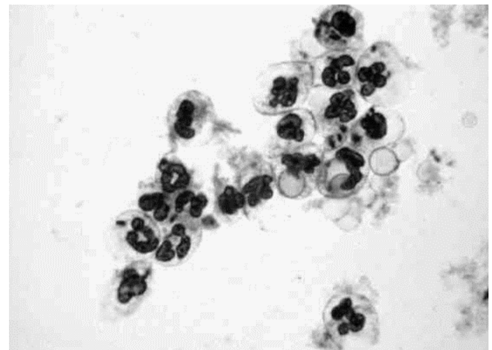
2. Transitional epithelium - Smaller, round to pear-shaped cells with larger nuclei. These cells line the bladder and proximal urethra.



3. Caudate epithelium - Spindle, or tadpole-shaped cells. A specific type of transitional epithelium that originate from the renal pelvis and are seen in the urine of patients with disease in the renal pelvis, such as pyelonephritis or calculi in the renal pelvis.

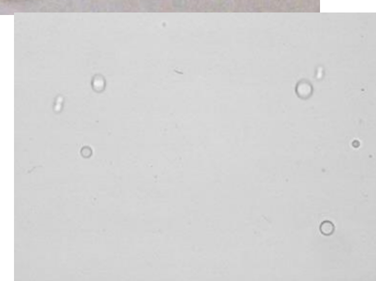
C. **Blood Cells & Bacteria** : Evaluated on the stained preparation and counted as #/hpf. Bacteria, particularly in the absence of inflammatory cells, should be confirmed in the unstained preparation to rule out the potential for stain contamination as the source of bacteria.

Pyuria - Pyuria is the presence of increased numbers of WBC's in the urine sediment (normally, less than 3 WBCs/hpf are present) and can indicate inflammation. Another factor to consider is the presence of preputial or vaginal secretions (but if a cystocentesis-obtained sample is being used these contaminations should be avoided). UTI is the most common cause of pyuria, with calculi and neoplasia being others.

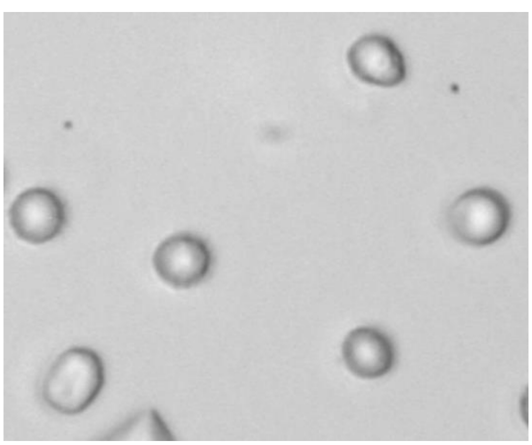


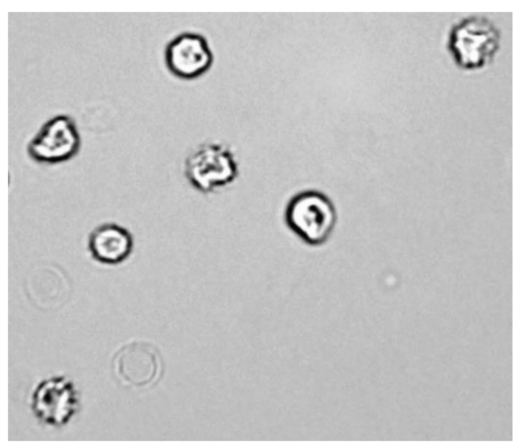
Bactiuria - If obtained from cystocentesis, any bacteria in the urine is abnormal. If bacteria are found and there has been no contamination of the sample (urine remains at room temp. for long period of time or comes into contact with contaminated tubes or staining solutions), UTI may be diagnosed. Intracellular bacteria within neutrophils are the best indicators of a septic process. Urine culture is recommended in all patients with UTI.

Erythrocytes vs. lipid droplets - Erythrocytes are all the same size, and some may be crenated. Lipid droplets are variably sized, refractile spheres. They are produced by the tubular epithelium and are particularly common in cats. They usually have no clinical significance.



Variable morphology of erythrocytes – depends on specific gravity of urine





1. Transitional cell carcinoma - In a patient that has a bladder or urethral mass, the urine sediment finding of atypical transitional epithelial cells in the absence of inflammation is suggestive of transitional cell carcinoma. The cells may exfoliate in cohesive sheets or individually. They will

display various malignant features, such as high nuclear to cytoplasmic (N:C) ratio, variable cell and nuclear size, clumped chromatin with prominent nucleoli and mitotic activity. Other less commonly observed tumors include squamous cell carcinomas, papillomas, and rhabdomyosarcomas to name a few.