Elephant Urinalysis

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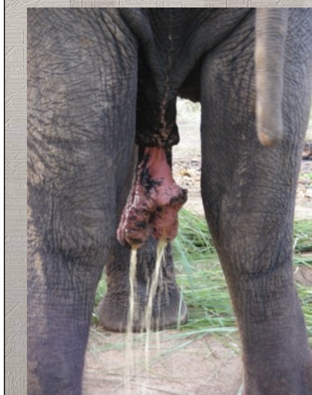
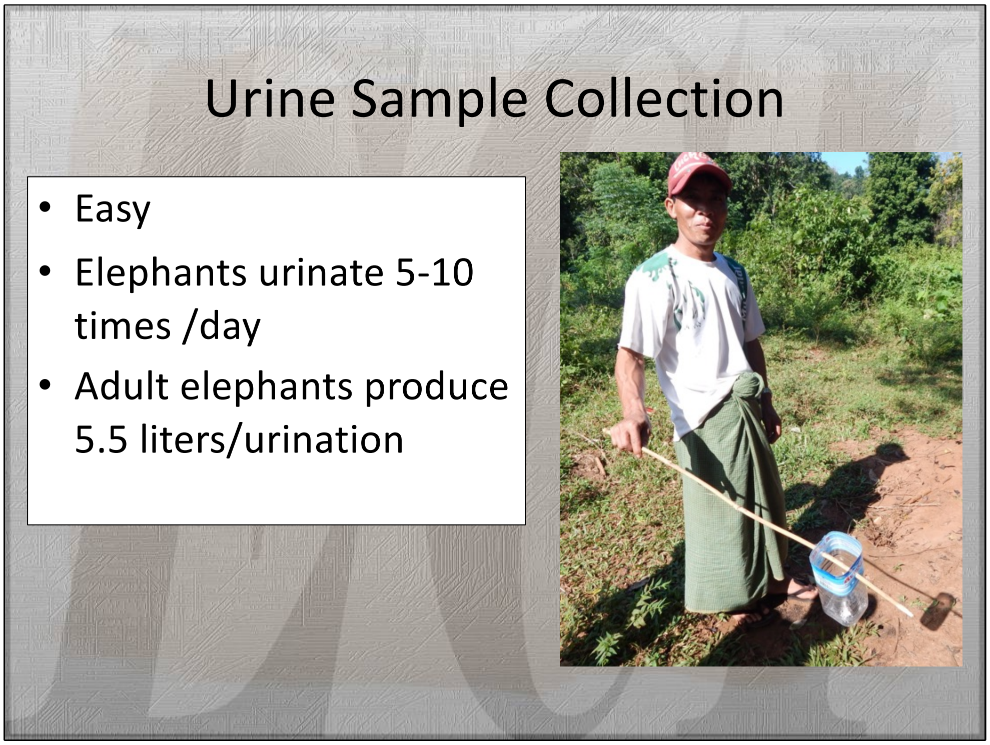
Urine

- Inexpensive
- Minimum of equipment
- Useful for
  - Urinary disease
  - renal disease
  - hepatic disease
- Limited studies in elephants



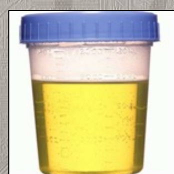
Urine is helpful diagnostically in other species and we should be using it more frequently in elephants. It requires minimal equipment and is inexpensive. Urinalysis can be useful in cases of urinary, kidney, or liver disease. Published data on elephants is limited and there is only one recent study that attempted to formulate reference values from healthy elephants: Wiedner, E., Kiso, W.K., Aria, J., Isaza, R., Lindsay, W., Jacobson, G., Jacobson, K., Schmitt, D., 2017. Vital signs and first occurrences in normal and abnormal newborn Asian elephant (Elephas maximus) calves. J Zoo Wildl Med 48, 997-1015.

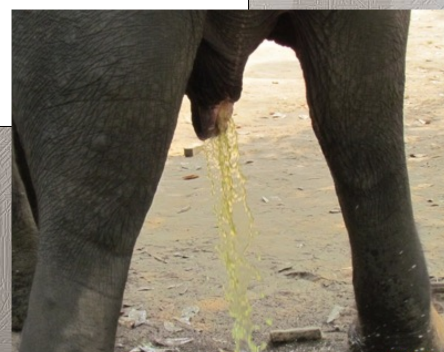


Elephants urinate 5-10 times per day and pass about 5.5 liters each time. So collecting a sample with a simple device like the one pictured here is easy.

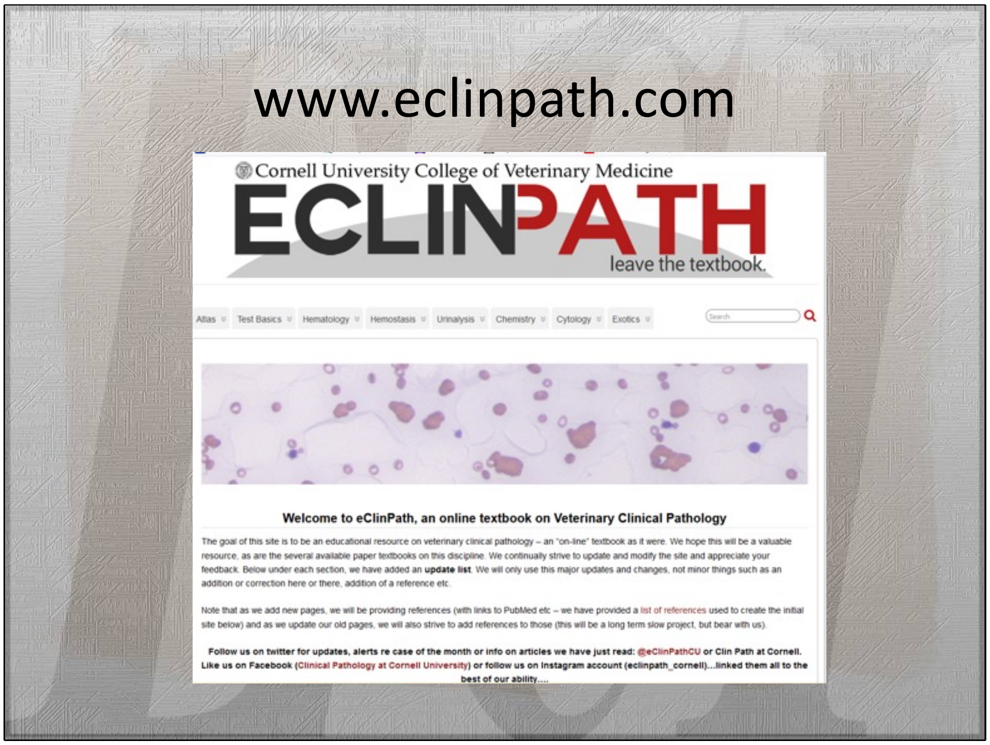
Complete Urinalysis (UA)

1. Visual exam
2. Biochemical assessment (dipstick)
3. Microscopic evaluation of urine sediment
4. +/- culture



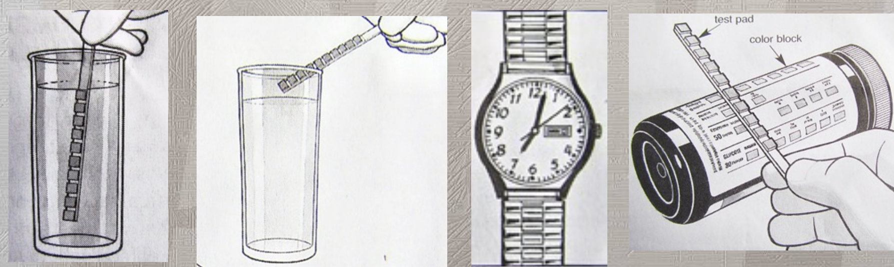


A complete UA includes a visual exam, biochemical assessment using urine dipsticks, and usually a microscopic examination of urine sediment. If infection is suspected a culture may be warranted. Don't forget to ask about or observe the elephant urinating. Any changes in the frequency or volume of urine may be important; also straining or blood.



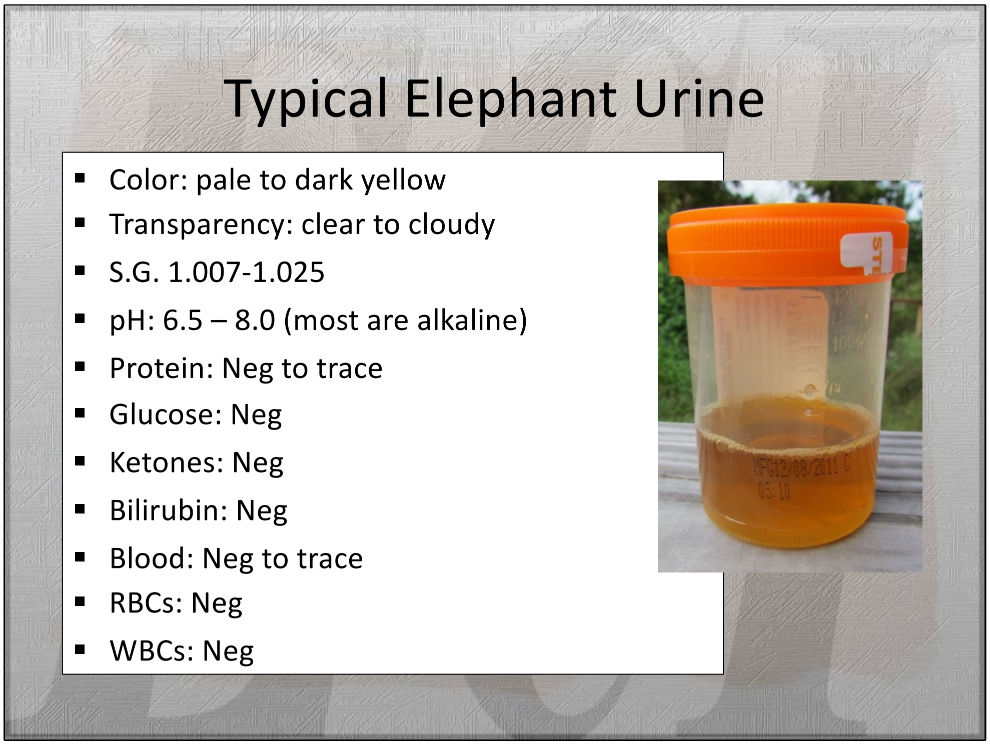
I'd like to acknowledge the Cornel e-Clin Path website. Most of the following information on urine examination came from this website.

Urine Dipstick Analysis



1. Mix the urine
2. Dip the dipstick ; remove extra urine
3. Start timing
4. Compare to chart on bottle in good light

Dipsticks consist of pads containing chemical reagents that provide a color change when a particular analyte is present in urine. Depending on the brand there can be up to 10 different tests in a strip. The procedure is simple, but it is important to read the dipstick at the specified time and in good light. The dipstick analysis is performed on uncentrifuged urine, unless there is marked hematuria (which may affect interpretation of the color changes on the dipstick). If there is marked hematuria, centrifuge the sample and perform the dipstick analysis on the supernatant. After dipping the stick in urine observe the specific times to read and record each test result.

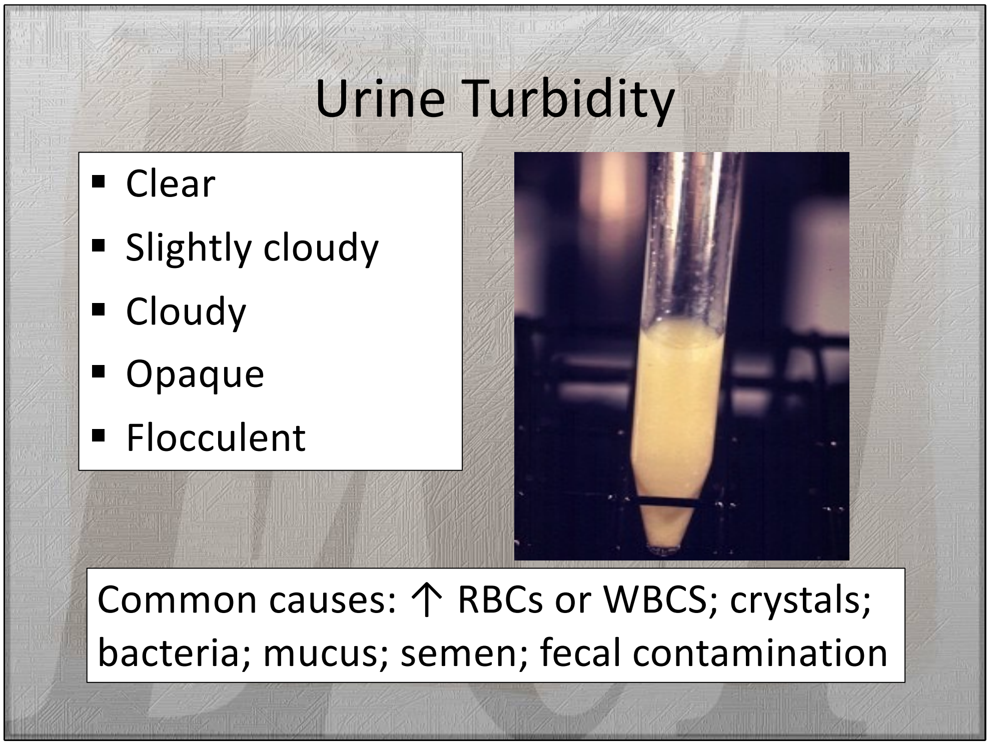


This slide lists some of the things we can look at in elephant urine and what we would expect normal elephant urine to look like. We will look at each of these parameters individually.

Urine Color

Color	Possible causes
light to medium yellow	normal
colorless	very dilute urine
very dark yellow	very concentrated; bilirubinuria
red to brownish red	hematuria, hemoglobinuria, myoglobinuria
reddish brown to brown	myoglobinuria, hemoglobinuria, methemoglobin
greenish tint	bilirubinuria

The color of the urine sample is reported as red, brown, yellow, or a combination. Some examples of various urine colors and corresponding common causes are shown above. This is not a comprehensive list - there are many other substances, including drugs, which can alter urine color.



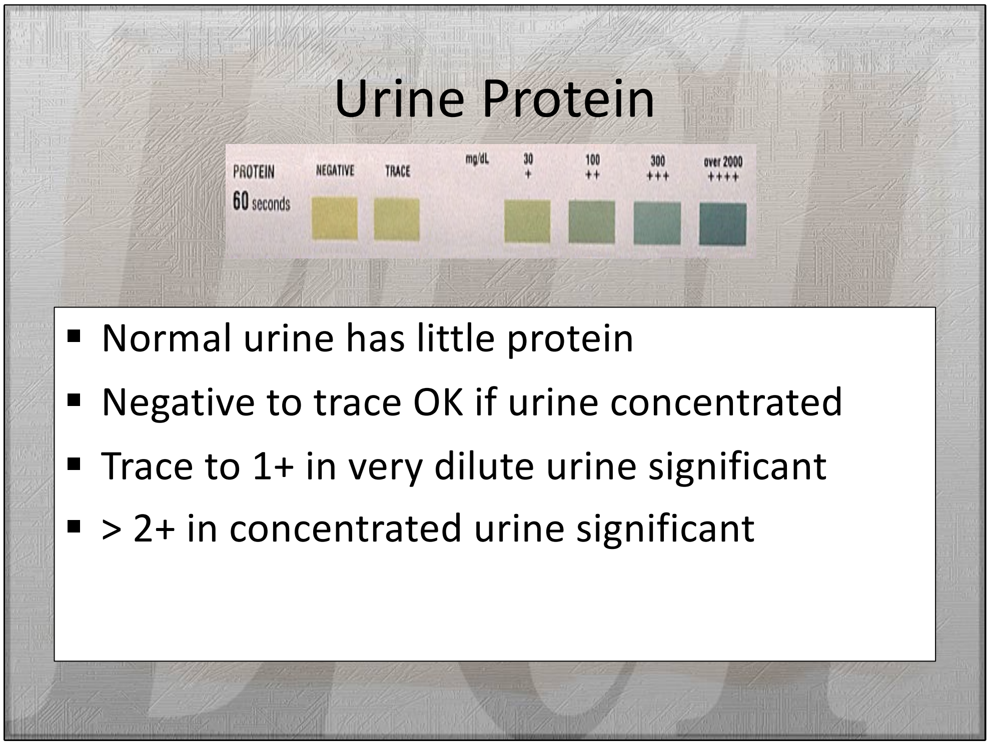
The turbidity of the urine sample is subjective and is reported as: “clear, slightly cloudy, cloudy, opaque, or flocculent.” Normally, fresh urine is clear to very slightly cloudy. Excess turbidity results from the presence of suspended particles in the urine. The cause can usually be determined based on the results of the microscopic urine sediment examination. Common causes of abnormal turbidity include increased RBCs or WBCS; crystals; bacteria; mucus; semen; or fecal contamination.

Specific Gravity

- Ability of kidneys to concentrate urine
- Best done with refractometer; dipstick may not be as accurate

SPECIES	POSSIBLE RANGE	USUAL RANGE
Canine	1.001 – 1.065	1.015 – 1.045
Feline	1.001 – 1.085	1.035 – 1.060
Large animals	1.001 – 1.060	1.015 – 1.030
Elephants	1.004 – 1.033	1.007 – 1.025

Urine specific gravity is a measure of the ability of the kidneys to concentrate urine. This chart compares the specific gravity of various species. There is not much data on elephants but until more studies are done we can use these figures. Based on the studies so far, a urine S.G. > 1.020 in an elephant suggests concentrating capability.



Urine protein should always be interpreted in context with the urine specific gravity and pH. Normal urine contains little protein; negative to trace reactions are usual in concentrated urine. A trace to 1+ reaction in a very dilute urine is suggestive of significant proteinuria. A dipstick protein reaction > 2+ in concentrated or dilute urine indicates significant proteinuria. There are numerous causes of proteinuria, the most common of which are urinary tract inflammation, hematuria, and glomerular disease. False positive and negative results may also occur. The dipstick only provides a subjective measurement of the degree of proteinuria.

Pre-renal proteinuria (also called overload proteinuria) occurs when large concentrations of small proteins are not able to be completely resorbed by the renal tubules. A colostral proteinuria may occur in neonatal animals less than 40 hours old.

Hemoglobinuria will occur in states of intravascular hemolysis. There should be concurrent hemoglobinemia and red urine supernatant (if there is large amounts of hemoglobin) with a concurrent anemia if there is a proteinuria due to hemoglobinuria. Myoglobin will leak into the urine in conditions of severe muscle injury (rhabdomyolysis). Increased values of muscle enzymes (CK, AST) will be seen in chemistry panels from affected animals, although the urine may not be discolored unless the myoglobinuria is severe. Note that both hemoglobin and myoglobin will cause a positive result for heme proteins on the dipstick. However, the protein reaction on the dipstick is less sensitive to hemoglobin and myoglobin than it is to albumin.

Renal proteinuria occurs when disease processes within the kidney cause leakage of serum protein into the urine, e.g. hemorrhage, inflammation.

Glomerular proteinuria can be functional or pathological. Functional proteinuria results in a mild proteinuria and is due to increased hydrostatic pressure or an altered glomerular filtration coefficient, e.g. stress, exercise, fever, excitement, congestive heart failure. Pathological glomerular proteinuria is due to renal disease (e.g. renal amyloidosis, glomerulonephritis).

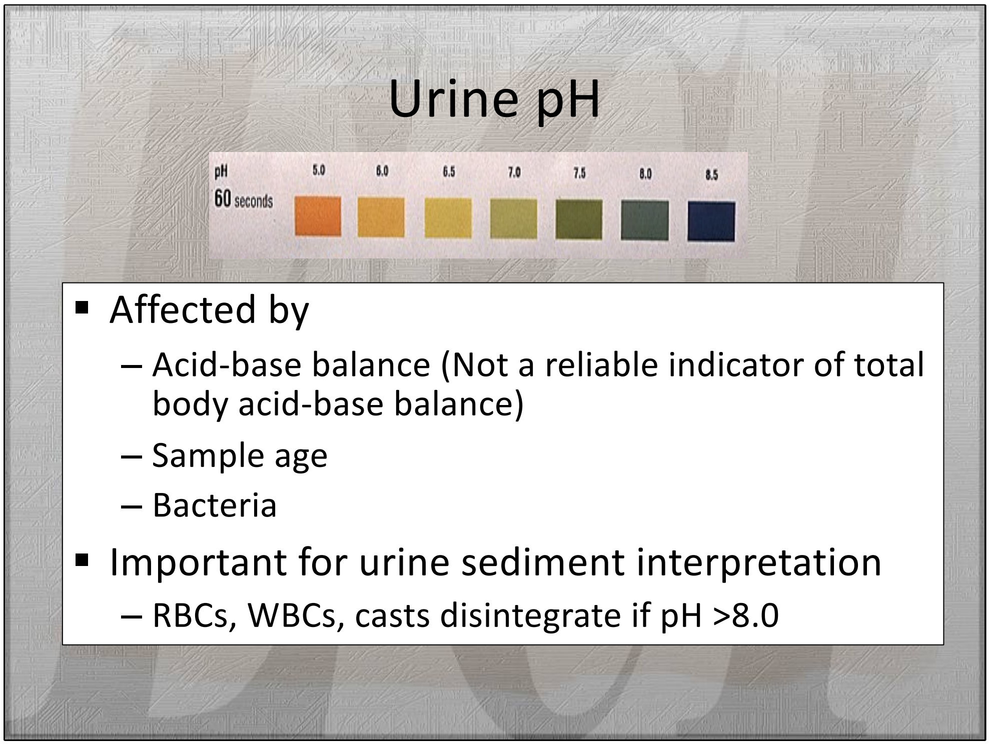
Tubular proteinuria is due to decreased renal tubule function resulting in decreased absorption of filtered low MW proteins or increased excretion of proteins by damaged tubules. This results in a mild to moderate proteinuria, i.e. dipstick readings of 2+ or less. Causes of tubular malfunction are many incluidng renal ischemia and nephrotoxins (such as aminoglycosides).

Interstitial proteinuria is due to hemorrhage or inflammation within the kidney.

Post-renal proteinuria is due to hemorrhage or inflammation in the urinary tract (ureter, bladder, urethra) or in the reproductive tract that causes protein (in the serum that accompanies inflammation or hemorrhage) to enter the urine once it has been formed and entered the renal pelvis.

Inflammation: Inflammation and/or infection anywhere in the distal urinary or genital tracts, e.g. cystitis, prostatitis, will cause proteinuria from leakage of serum protein along with leukocytes or due to increased vascular permeability.

Hemorrhage: In severe hemorrhage, the dipstick pad will be unreadable due to the urine color. Hemorrhage frequently results in proteinuria when serum protein accompanies erythrocytes. Hemorrhage can occur anywhere within the urogenital tract (including the kidney itself, although proteinuria associated with renal hemorrhage would be called renal interstitial proteinuria) but more frequently reflects lower urinary tract (bladder disorders) or reproductive tract disease.

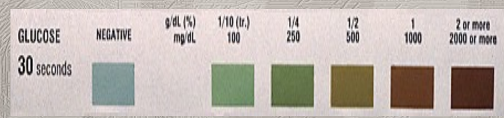


The pH of urine sample can be affected by systemic acid/base balance, the age of the sample, or the presence of bacteria. Knowledge of the urine pH is important in interpreting urine sediment findings. RBCs, WBCs, and casts tend to disintegrate in alkaline urine (pH > 8.0). And elephants typically have alkaline urine.

The age of the sample can also affect the pH – as older samples lose CO_2 to the air the pH increases. Some bacteria convert urea to ammonia, raising pH.

Although the kidneys play a central role in the control of acid/base metabolism, the pH of a random urine sample is not a reliable indicator of total body acid/base status.

Glucose



Species	Threshold
Canine	180-220 mg/dL
Feline	280-290 mg/dL
Bovine	100-140 mg/dL
Equine	150-160 mg/dL

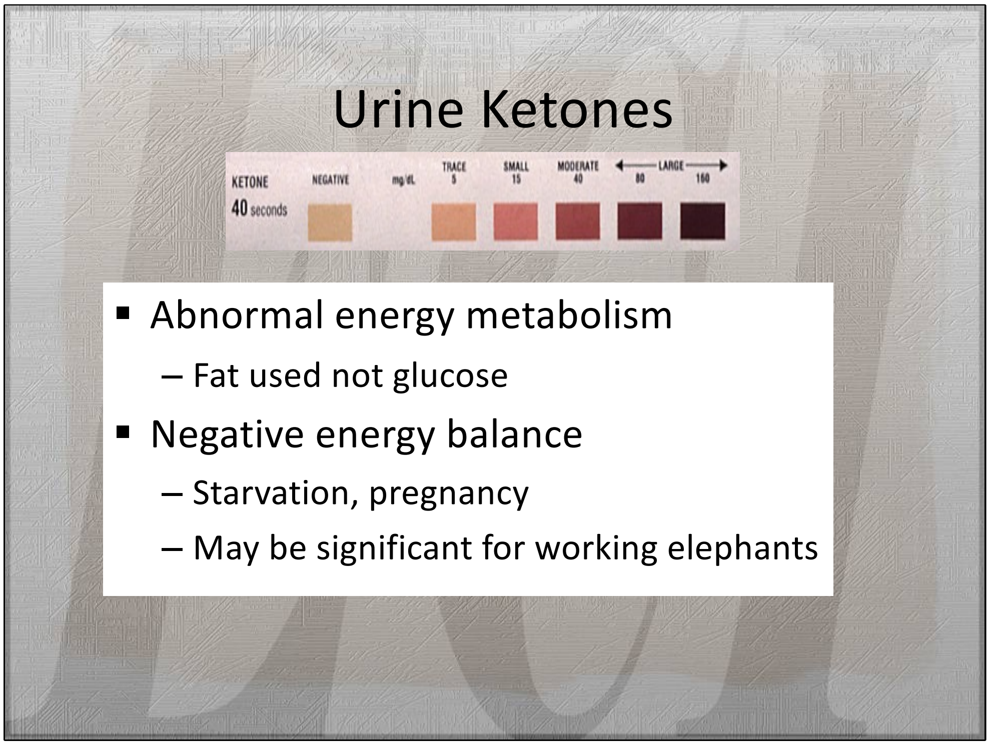
Glucose is an abnormal finding in urine. The threshold is the value above which glucose spills into the urine. The threshold for elephants has never been reported but is probably similar to the horse.

False positive reactions can result from: the presence of hydrogen peroxide, bacterial peroxidases (e.g. cystitis), hypochlorite or chlorine; formaldehyde; or outdated reagents

False negative reactions can result from: high concentrations of ascorbic acid which inhibit the reaction; and drugs such as salicylates, tetracyclines.

Pathologic glucosuria due to hyperglycemia: In nearly all cases, glucosuria is a result of prior (often, continuing) hyperglycemia to a level in excess of the renal threshold for reabsorption.

Transient hyperglycemia: Stress-related hyperglycemia above the renal threshold will result in glucosuria. This is especially true in cats and cattle, which develop marked stress-related hyperglycemias, and where the finding of glucosuria is not diagnostic for diabetes mellitus. Other conditions which produce transient hyperglycemia, e.g. pancreatitis, may induce a mild, transient glucosuria. Note also that a transient glucosuria may be seen 1-2 hours after a heavy meal.



Ketones in the urine indicate abnormal energy metabolism such that fat is used instead of glucose. This can occur in states of negative energy balance, where energy intake does not meet demands. Starvation and pregnancy are two such states. It may also occur in elephants that are working hard.

Fat metabolism can result in production of the ketone bodies in amounts greater than can be metabolized by tissue; filtration into urine in excess of tubular reabsorption then results in ketonuria. Female elephants produce a variety of ketones in their during different stages of their estrous cycle so it is possible that these may cross-react.

Urine Blood

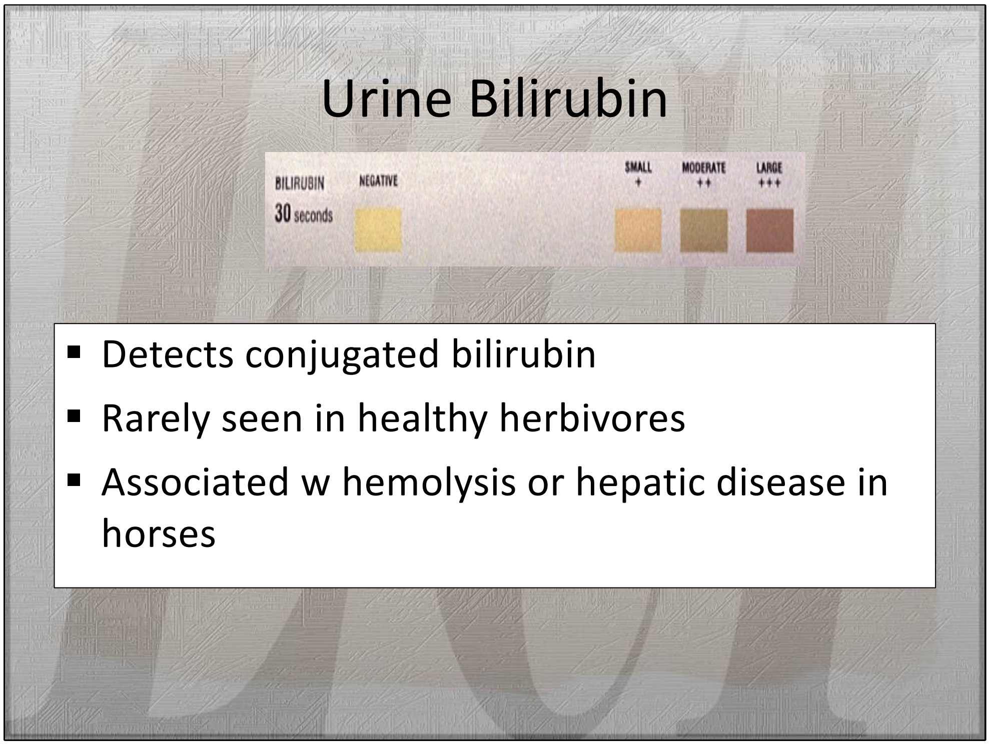
- Very sensitive
- Detects hematuria, hemoglobinuria, myoglobinuria
- Evaluate sediment to differentiate
- Also hct, AST and CK to differentiate hemoglobinuria vs myoglobinuria

The urine blood test is very sensitive and will detect hematuria, hemoglobinuria or myoglobinuria. You have to look at the sediment to differentiate these. RBCs will be seen in the sediment with hematuria but not with hemoglobinuria. If we suspect myoglobinuria – from muscle breakdown – it is a good idea to check the AST and CK values in the blood.

- Hemoglobinuria: There will be no RBC on the urine sediment and the urine supernatant will be red (remember that RBC will lyse in very dilute, alkaline or "old" urine). In general, affected animals have a low hematocrit (in rare cases of intravascular hemolysis, the hematocrit may be normal due to concurrent dehydration or splenic contraction).

- Myoglobinuria: There will be no RBCs on the urine sediment and the urine will have a red supernatant (if there is a lot of myoglobin in the urine). The affected animal will have a very high CK and (usually) high AST, reflecting muscle injury.

- Hematuria: RBC will be present on urine sediment examination (if hematuria is marked, a red precipitate forms after centrifugation of urine). Note that RBCs can lyse in very alkaline or dilute urine or in urine that is stored for some time, so intact RBCs may not be seen in these settings even though there was hematuria.



Detecting bilirubin in urine is an abnormal finding. Hemolysis or liver disease may be the underlying cause.

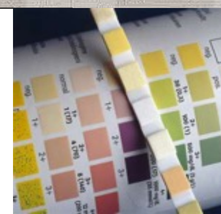
Bilirubinuria generally results when conjugated bilirubin levels in blood are elevated as a result of cholestatic hepatobiliary disease. Bilirubinuria indicates cholestasis.

In some cases of intravascular hemolytic anemia, bilirubinuria may be secondary to the hemolysis without any evidence of cholestasis. The renal tubular epithelium is capable of absorbing free hemoglobin from the glomerular filtrate and converting it to conjugated bilirubin, which is then excreted in the urine. This will only occur with intravascular hemolysis, when free hemoglobin is filtered by the glomerulus.

False negative reactions can occur is the sample is too old (conjugated bilirubin hydrolyzes to unconjugated bilirubin if left at room temperature) or if it is exposed to UV light (UV light converts bilirubin to biliverdin).

Dipstick – other tests

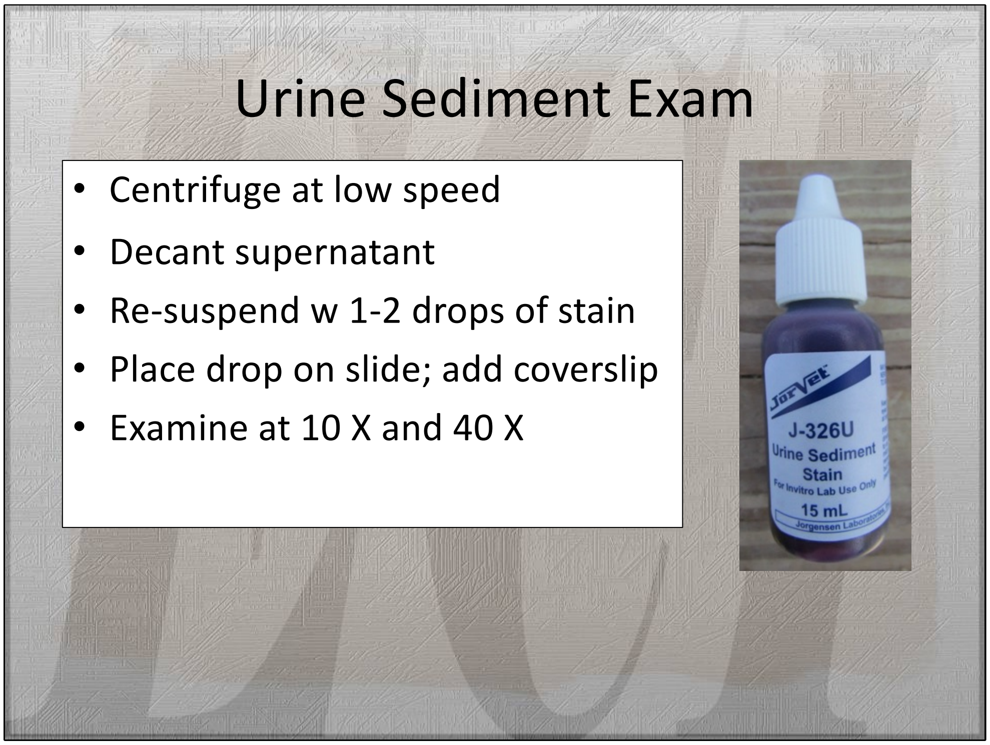
- Nitrite
- Urobilinogen
- Leukocytes – if positive check urine sediment for WBCs
- Specific gravity – best to check with refractometer



There are four other tests that may be on the dipstick that are not quite as useful as the ones we have already discussed. Nitrite and urobilinogen are unreliable. The leukocyte test is detects WBCs which may indicate that infection is present. If this test is positive on the dip stick it is best to check a urine sediment and see if WBCs are also present microscopically. Specific gravity measurement can be done by dipstick, however measurement of specific gravity using a refractometer is more accurate.

Nitrite is used to screen for certain bacteria based on the principle that nitrate which is normally found in urine is reduced to nitrite by nitrate-reducing bacteria. This test has not been shown to be reliable in animals.

Urobilinogen is formed in the intestine by bacterial action on conjugated bilirubin. It is reabsorbed from the intestine into portal blood where it is recycled through the liver into bile. A small amount passes into the urine. If urobilinogen is present it indicates a patent (open) bile duct. The absence of urobilinogen may indicate bile duct obstruction however urobilinogen is unstable and there is a diurnal excretion so there can be false negative results. Increased amounts may occur in hemolytic diseases.



To perform a urine sediment examination the first step is to centrifuge the urine at low speed. The supernatant is decanted (poured off) and the urine is re-suspended using 1-2 drops of stain. A drop of the re-suspended urine is placed on a slide, covered with a coverslip, and examined using the 10x and 40x objectives.

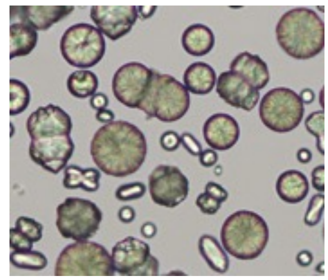
Use low magnification (10X) first and examine the entire coverslip. At this magnification, casts, large crystals, debris, parasitic ova are semi-quantified.

Next use high magnification (40X): At this higher magnification, leukocytes, erythrocytes, epithelial cells, fat droplets, small crystals, sperm, debris and bacteria are semi-quantified.

If examining un-stained urine sediment, subdued lighting is necessary to increase refractility of the urine elements. Lowering the condenser and/or closing down the substage iris diaphragm helps.

Typical Elephant Urine Sediment

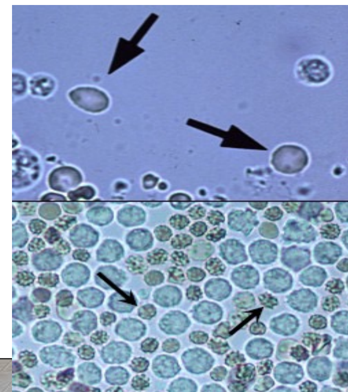
- Crystals common
  - Calcium carbonate most frequent
  - Struvite
  - Calcium oxalate
  - Amorphous phosphate
- Epithelial cells common
- Occasional WBC or RBC
- Sperm
- Mucous threads



Typical urine sediment from healthy elephants often contains crystals. Calcium carbonate are the most common type. Crystals are subjectively quantified as few, moderate and many. Calcium carbonate and struvite crystals are large so you can see them with low power; you will need to use the 40X objective for smaller crystals.
Epithelial cells, WBCs and RBCs may also be seen as well as sperm.

RBCs in Urine

- Reported as number seen per high power field
  - None seen; <5, 5-20, 20-100, or >100/HPF.
- ↑ RBCs (hematuria)
  - hemorrhage,
  - inflammation
  - necrosis
  - trauma
  - neoplasia

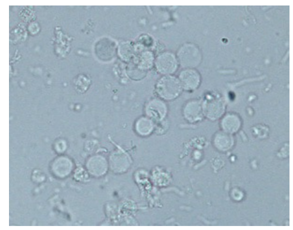


RBCs are reported as the number / high power field. Hematuria can be due to hemorrhage, inflammation, necrosis, trauma, or neoplasia somewhere along the urinary or urogenital tract.

The appearance of red blood cells in urine depends largely on the concentration of the specimen and the length of time the red cells have been exposed. In fresh urine, RBCs are round smooth cells and are slightly red-tinged (from hemoglobin). In fresh samples with specific gravity of 1.010-1.020, RBCs may retain their normal disc shape. In more concentrated urines (>1.025), red cells lose their smooth texture, tend to shrink and appear as small, crenated cells. In more dilute samples, they tend to swell. At urine specific gravity <1.008 and/or highly alkaline pH, red cell lysis is likely. Lysed red cells appear as very faint "ghosts" or may be virtually invisible.

WBCs in Urine

- Reported as the number seen per high power field
  - None seen; <5, 5-20, 20-100, or >100 / HPF
- < 5/HPF normal
- > 5 /HPF
  - Pyuria
  - Urinary tract infection
  - Check for bacteria in sediment

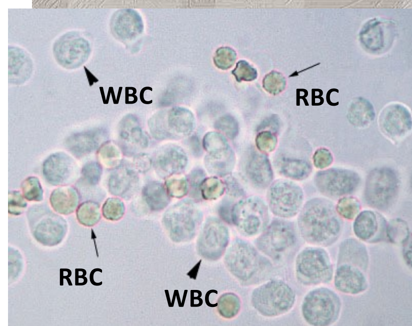


White blood cells are also reported as the number / HPF. Less than 5 WBC/HPF is commonly accepted as normal. Greater numbers (pyuria) may indicate the presence of an inflammatory process somewhere along the course of the urogenital tract.

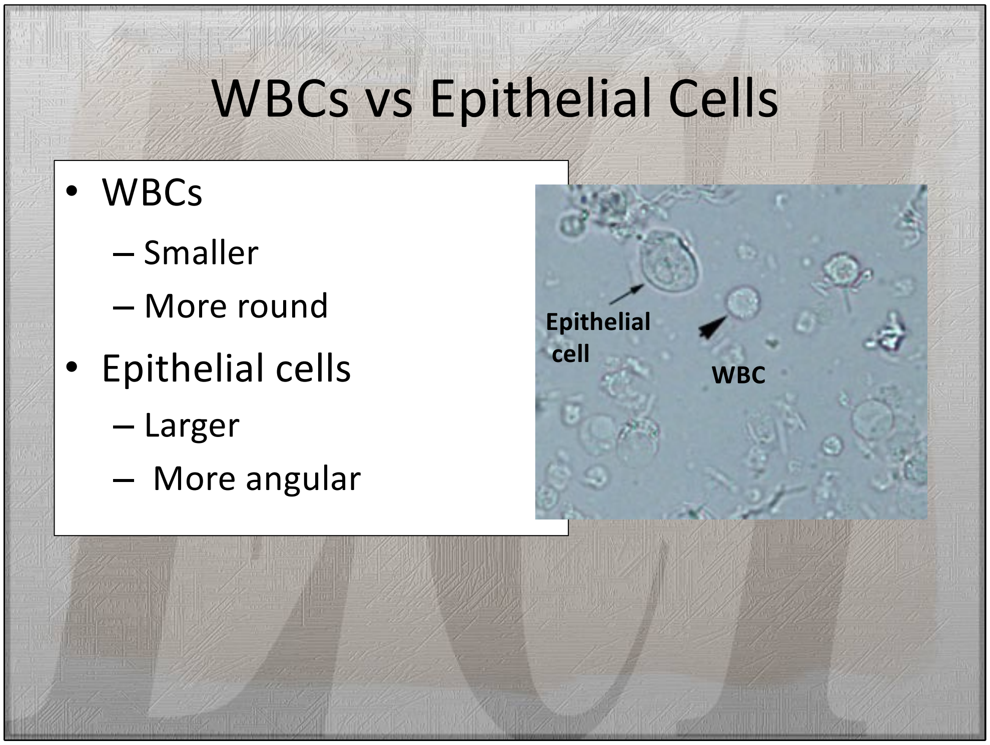
Pyuria often is caused by urinary tract infection, and many times bacteria can be seen on sediment preps. Depending on clinical signs, pyuria may be an indication to culture the urine even if no bacteria are seen. Non-septic causes of inflammation, such as uroliths and tumors, also must be considered.

WBCs vs RBCs

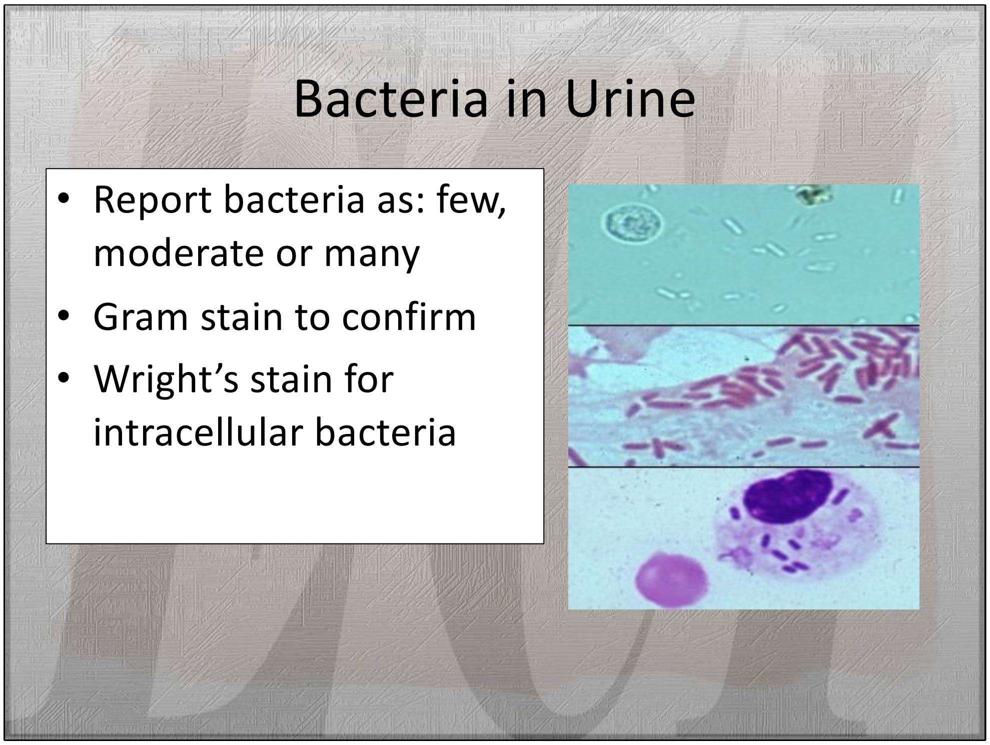
- WBCs
  - Larger
  - Colorless
  - More granular
- RBCs
  - Smaller
  - More red
  - No internal texture



This slide shows the difference between WBCs and RBCs. WBCs are more grainy and RBCs are more smooth. WBCs are also colorless whereas RBC are slightly red-tinged (from hemoglobin).



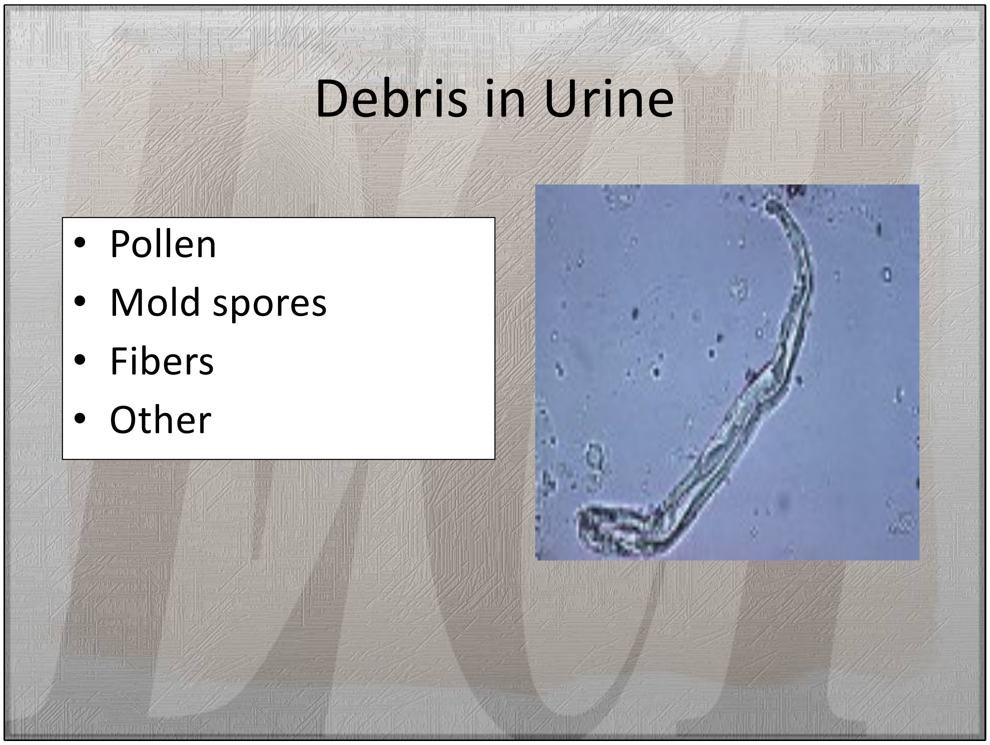
WBCs must also be differentiated from small epithelial cells. WBCs are generally smaller than epithelial cells and are more round. Epithelial cells have more angular borders and round to oval nuclei.



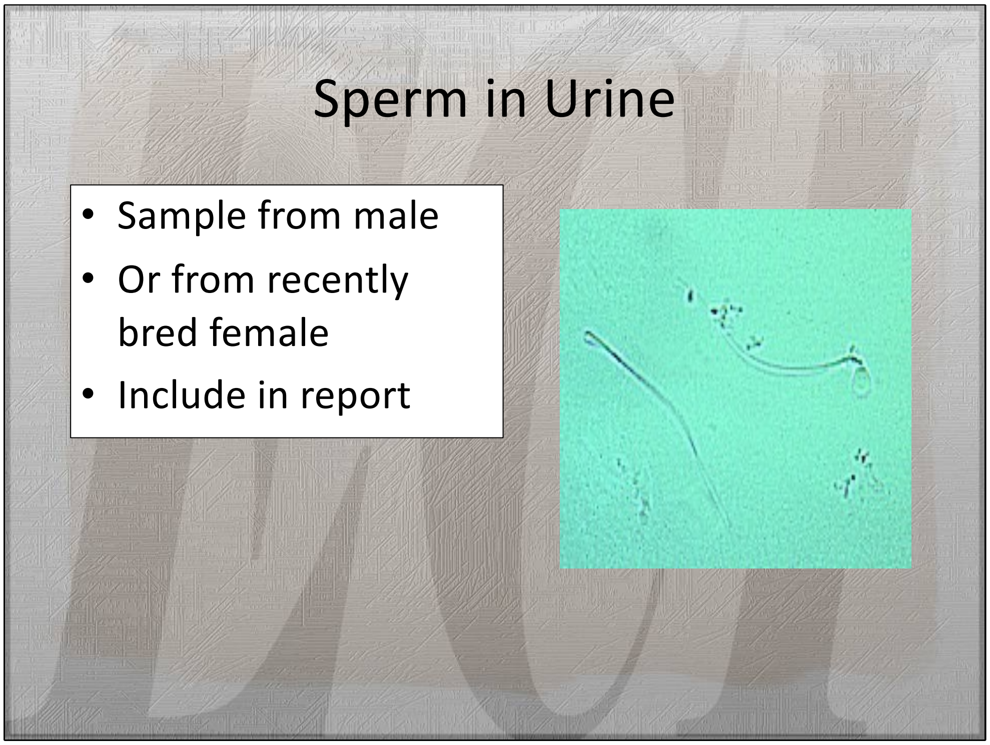
Rod-shaped bacteria and chains of cocci are often readily identifiable in urine sediment. However, small amorphous crystals, cellular debris, and small fat droplets can either mask or mimic round bacteria. If there is any doubt about the presence of bacteria, a Gram-stained smear of urine sediment should be examined. This is illustrated on the middle panel.

Only extracellular bacteria can be visualized on an unstained urine sediment. Intracellular bacteria can only be identified by using staining a sediment sample with a Wright's stain. The bottom panel demonstrates phagocytized bacteria within a neutrophil. The neutrophil's nucleus is swollen as a storage-related artifact.

Bacteria may be insignificant contaminants or important pathogens. Since urine in the bladder of healthy animals is sterile, bacteria are not normally seen in urine, however this does depend on the method of collection. In elephants we can only obtain urine samples from voided, mid-stream free catches. Bacterial contamination is therefore probable. The presence of bacteria in elephant urine does not necessarily mean infection is present. The elephant should be observed for clinical signs such as changes in urination and the sediment should be further examined for WBCs.



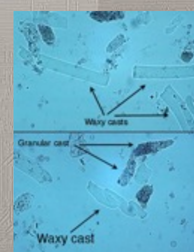
A variety of contaminants can be observed during the microscopic examination of the urine sediment. Pollens, mold spores, and fibers of various types are among the more common sorts of materials encountered. Generally, such material is significant only as an indication of the condition of collection and/or handling of the specimen prior to analysis. Debris is frequently seen in voided urine samples. On occasion, confusion may arise if these are mistaken for findings of significance (casts, parasites, etc.).



Seeing sperm in urine is not significant and really only indicates that the sample was from a male elephant. Rarely, they may be observed in voided urine from a recently-bred female. The presence of sperm is reported as part of the complete sediment examination, the goal of which is to report all microscopic findings.

Casts

- Molds of proteins / cells from distal renal tubules
- Pathologic
  - Renal infection
  - Renal inflammation
  - Renal trauma
  - Renal disease
- Dissolve quickly in alkaline urine
  - Examine soon after collection



Casts are seen with renal disease. Casts are reported as the number per low power field and they are classified by type.

An absence of casts does not rule out renal disease. Casts may be absent or very few in cases of chronic, progressive, generalized nephritis. Even in cases of acute renal disease, casts can be few or absent in a single sample since they tend be shed intermittently. Also, casts are unstable in urine and are prone to dissolution with time, especially in dilute and/or alkaline urine. Although the presence of numerous casts is solid evidence of generalized (usually acute) renal disease, it is not a reliable indicator of prognosis. If the underlying cause can be removed or diminished, regeneration of renal tubular epithelium can occur (provided the basement membrane remains intact).

Thank-you!