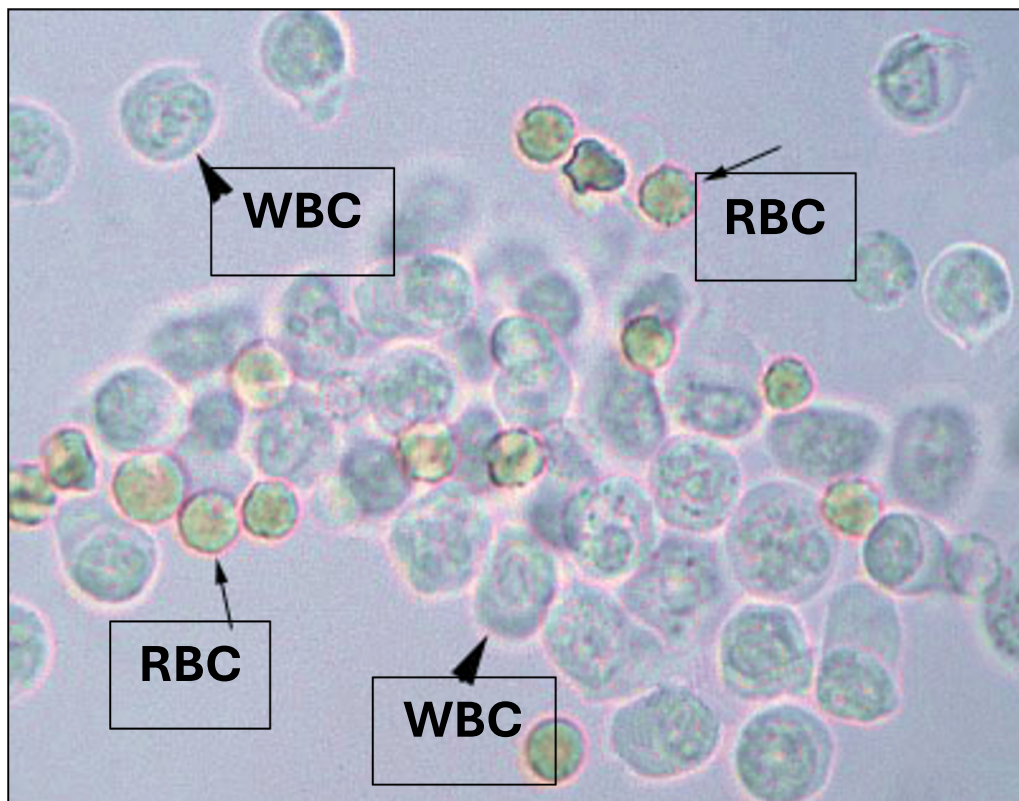


Basic Lab Procedures for Elephant Healthcare



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Introduction

Monitoring health and diagnosing illness in elephants can be challenging. A thorough history and physical examination are essential but may not always yield enough information to establish a diagnosis. While submitting blood to an outside lab for a comprehensive hematology and serum chemistry analysis is convenient, a great deal of information can be obtained in an in-house lab using basic equipment and supplies that are standard in most elephant care facilities. This manual describes the tests that can be performed and how to interpret the results to aid diagnosis. Also see <https://www.elephantmedicine.info/lab-diagnosis>.

Lab Equipment and Supplies

The following table lists equipment and supplies for a basic elephant healthcare lab.

EQUIPMENT	SUPPLIES
Microscope	Blood collection sets
Refractometer	Syringes, needles
Hemocytometer	EDTA and serum tubes
Small counter	Plain hematocrit tubes
Centrifuge	Hematocrit clay
Cell counter (for differentials)	Slides
Slide drying rack	Stains (Dip Quick or Wrights-Giemsa)
	Red blood cell lysing agent
	Gloves

Blood Collection Methods

The auricular and saphenous are the most commonly used veins for blood collection. The auricular (ear) veins may be accessed with the elephant standing. However, the veins are more prominent with the elephant in lateral recumbency. In this position gravity helps the veins to fill. It is also the safest position for the veterinarian should the elephant move suddenly.



The ear veins are most visible on the caudal aspect of the pinnae but can be seen on the rostral aspect of the ear in some elephants. The ear veins have thinner walls than adjacent arteries and will collapse when pressed. Arteries have thicker walls and will not compress when pressed. A pulse can generally be detected in arteries for further differentiation.

The cephalic vein can often be seen on the front leg but is not commonly used for blood collection as there is risk of injury from the elephant's trunk or head.



The saphenous vein on the medial side of the hind leg is also a good site and can be accessed most safely with the elephant in a stretched position. The saphenous vein is deeper than it looks and it is usually necessary to insert a 1-inch needle at a 90-degree angle. Taking blood from the tail vein has been described but is not commonly used.

Blood can be collected with a syringe and needle, with a vacutainer and needle, or with a winged blood collection set.

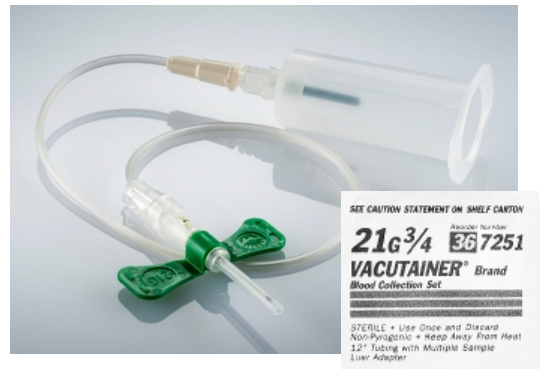


Hemolysis can affect a number of test results so good technique is essential. To prevent hemolysis:

- Always allow the alcohol to dry before inserting the needle.
- Use a large needle – no smaller than 21 gauge; 18 gauge is preferred.
- When transferring the blood from a syringe to the tube, don't force the blood in – allow the suction from the tube to draw the blood in.
- Don't shake the tube.
- Gently invert EDTA tubes so that the EDTA anti-coagulant mixes with the blood sample.
- Don't expose your sample to extreme temperatures.



Winged blood collection sets are useful because they allow you to move with the elephant without dislodging the needle from the vein and blood can be collected directly into multiple vacutainer tubes. The vacuum draws in the proper amount of blood into tubes that have an anticoagulant like EDTA. This method also reduces platelet clumping and clot



formation when collecting samples for CBCs. Even small clots will reduce the platelet count; the hematocrit and WBC count may also be affected. The largest blood collection kit commonly available is 21 ga. 19 ga “winged infusion sets” (also called butterflys) are available but adapters for direct collection into tubes must be purchased separately.

The Complete Blood Count

A complete blood count (also known as a hemogram) includes a red blood cell count, hematocrit, hemoglobin, a white blood cell (WBC) count, and a differential WBC. Automated equipment used in human labs can provide reliable values for all of these parameters except for the differential white blood cell count. For accuracy it is recommended this be done manually.

The hemogram in healthy elephants generally remains constant over time. It is advisable to obtain a baseline for each elephant during health that can be used for comparison if the elephant becomes ill. Using the same lab is also advisable. It is important to consider the physiological status when evaluating the hemogram. Dehydration, for example, can cause falsely elevated values.

The hematocrit, white blood cell count, and differential can be performed by the elephant veterinarian or technician using basic laboratory equipment in an elephant hospital or field lab. Manual RBC counts have a large margin of error and do not provide much information compared to the other tests.

RBC Parameters

The RBC indices – the mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC), provide information about red blood cell size and hemoglobin content. They are mainly used to classify anemias. They are of limited value for elephants because they are affected by reticulocytes and it does not appear that elephants have reticulocytes. However, these values may appear on a report from the lab so you should be familiar with them.

Mean corpuscular volume (MCV): red cell volume

- $(PCV \times 10) \div \text{RBC count (millions)} = \text{MCV (fl)}$
- Reticulocytosis most common cause of $\uparrow$ MCV
- Iron deficiency most common cause of $\downarrow$ MCV
- Of limited value for elephants

Mean corpuscular hemoglobin (MCH): amount of hgb in an average RBC

- $(\text{Hgb} \times 10) \div \text{RBC count (millions)} = \text{MCH (pg)}$
- Not used in anemia classification
- MCHC is a better measure

Mean corpuscular hemoglobin concentration (MCHC): average hgb concentration per average RBC

- $[\text{Hgb conc (pg)} \times 100] \div \text{Hct (\%)} = \text{MCHC (g/dl)}$
- Most accurate of the RBC indices as it does not require a RBC count
- No true increase
- $\downarrow$ with reticulocytosis, iron deficiency

Hematocrit / Packed Cell Volume

The hematocrit (Hct) and packed cell volume (PCV) are methods to determine the proportion of RBCs to the total blood volume. Technically, the hematocrit is a value *calculated* by automated hematology analyzers whereas the packed cell volume is *measured directly* by centrifuging blood in a hematocrit tube. However, both terms are commonly used synonymously and both are expressed as a percentage.



There are two types of hematocrit tubes – plain (untreated) and heparinized. Heparinized tubes are most often used in humans to collect blood directly from a finger prick. In elephants we typically collect blood into an EDTA tube and then fill the hematocrit tube from the EDTA tube. A plain (non-heparinized) hematocrit tube is preferred because a heparinized tube could add extra anticoagulant to an already treated sample and dilute it, although this may not be clinically significant (Bamberg 2008, Hughes

2011). Filling the hematocrit tube from a large EDTA tube containing only a small amount of blood may also cause dilution and alter the PCV.

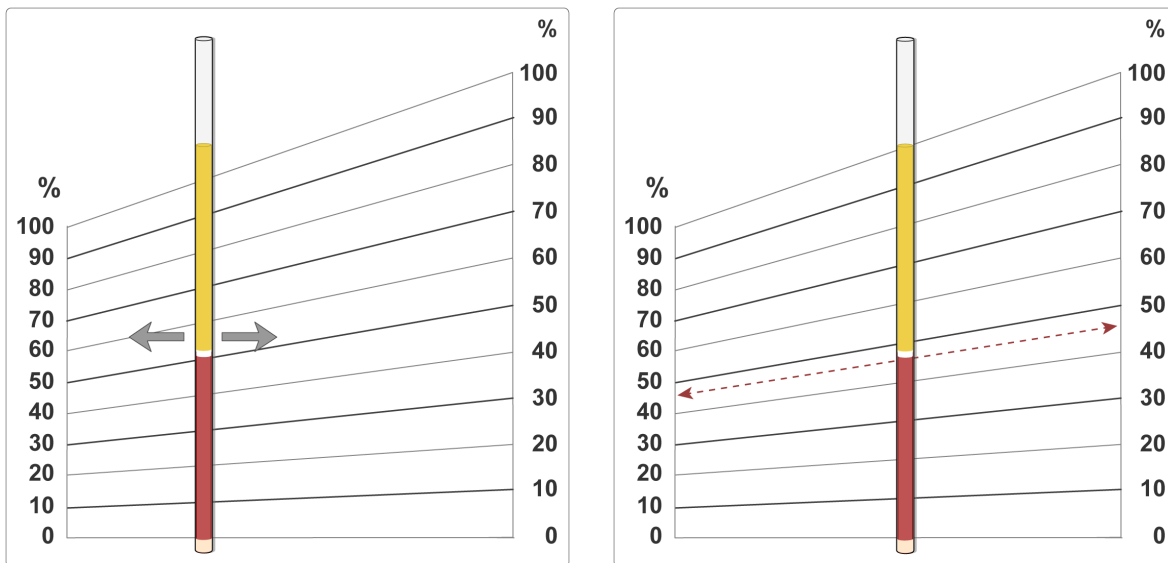
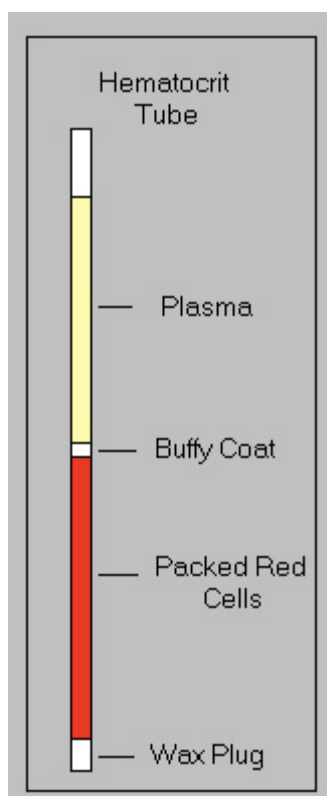
If you are collecting blood directly from an animal using a blood collection set or transferring blood from a syringe, a heparinized tube should be used to prevent clotting. After the tube is filled it is sealed with clay. Soap also works to seal the tube.

To perform a packed cell volume a centrifuge specifically designed for hematocrits or a regular centrifuge that has an adapter to hold the hematocrit tubes is needed. The hematocrit should be spun at 12,000 rpms for 5-10 minutes.



If you don't have access to a lab or a centrifuge there are two Do-It-Yourself versions that you can make (Bhamla 2017; Brown 2011). Instructions for a *Paperfuge* can be found in the Appendix M.

The hematocrit is read using a microhematocrit card reader. To use a microhematocrit card reader, position the hematocrit tube on the left side of the reader card, with the bottom of the RBC column on the 0% line. Then move the tube across the reader card until the top of the plasma column reaches the 100% line. The top of the red blood cell column intersects the correct measurement. Images from <https://humanbiomedia.org/>.



Centrifugation separates the blood into 3 parts – packed red blood cells, a buffy layer composed of WBCs and platelets, and plasma (containing water, electrolytes, proteins, glucose, clotting factors, and mineral ions). Note that serum has the same components as plasma except serum does not contain clotting factors.

The PCV is one of the most simple yet valuable tests that can be performed in the in-house lab.

Examination of the layers above the packed red cells also provide useful information. The size of the “buffy coat” (which contains WBC and platelets) is a very rough indication of the WBC count. The tube can be easily broken and a small drop of plasma used in the refractometer to measure total protein.

<https://eclinpath.com/hematology/tests/hemat>

Increased hematocrit/PCV values can occur with dehydration and the PCV is a good way to monitor fluid balance. False increases can occur if the centrifugation speed is not adequate or if the sample is stored > 24 hours before centrifugation. In either case, the cells won’t pack properly.

Decreased values may indicate anemia or over-zealous fluid therapy (unlikely with an elephant). If the sample is hemolyzed or stored too long, values may falsely decrease. See Appendix A for causes of anemia.

The hematocrit/PCV are not affected by icterus or lipemia.

See Appendix K for a Hematology reporting form.

Plasma Evaluation

1. Evaluate color and transparency.

- Clear and colorless: normal
- Pink to red and clear: hemoglobinemia
- Yellow: icteric
- White to pink and opaque: lipemic

2. Look at the buffy coat (the layer between the RBCs and the plasma); the height is a rough indication of the WBC.

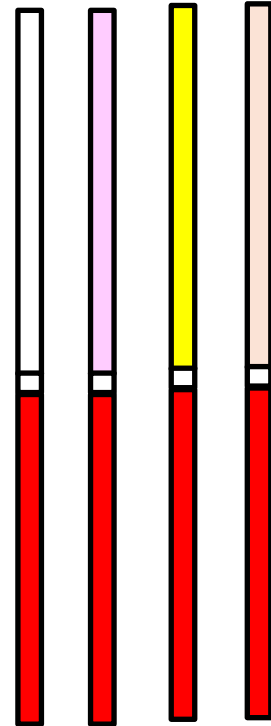
3. Measure the total protein using the refractometer.

A milky appearance of serum or plasma indicates lipemia. It is due to increased concentrations of lipoproteins. Lipemia can be associated with certain diseases but none are common in elephants. In elephants it usually means that they have just eaten.

However, lipemia can interfere with certain lab tests.

Calcium, phosphorus, total bilirubin or hemoglobin may be falsely elevated. If you are using a refractometer to measure the total protein the value may be falsely elevated. Albumin, sodium and potassium may be falsely lower. Lipemia also enhances hemolysis which in turn can affect lab results.

If you have a sample that is lipemic, it can be cooled, ideally in a refrigerator. The lipemic portion will separate and you can use the clear aliquot below the lipid layer.



Total Protein

Total protein is a measure of the overall protein in the blood. Plasma proteins have numerous functions in the body. They maintain osmotic pressure and acid-base balance. Antibodies, enzymes, hormones, and coagulation factors are proteins. Plasma proteins are primarily made up of albumin, globulin, and fibrinogen. Elephants have higher levels of globulins than other mammals. Serum contains all of the plasma proteins except fibrinogen, factor V and factor VIII which are consumed when a clot forms.

Total protein is used to assess nutritional status and hydration. Values outside the reference range may indicate kidney or liver disease, dehydration, or malnutrition. See Appendix F for causes of increased and decreased total protein and Appendix G for how to evaluate total protein and PCV together.

How to Use a Refractometer

A medical refractometer measures light refraction in a liquid sample to determine the concentration of



substances in blood or urine. The path of light is bent (refracted) when it passes through a liquid; the amount of bending depends on the concentration of dissolved substances present. Refractometry can be used to measure plasma, serum, or body cavity protein.

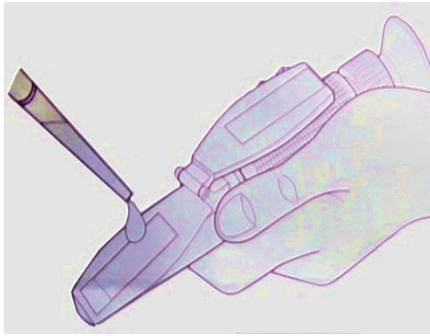
Temperature-compensated, hand-held refractometers are calibrated to read protein directly in g/dl. Most units are temperature-compensated to give accurate readings between 16° C and 38° C (60°F to 100°F).

Abnormally high concentrations of glucose, urea, sodium, or chloride may result in falsely high protein readings. *The serum, plasma, or body cavity fluid must be clear for accurate measurement.*

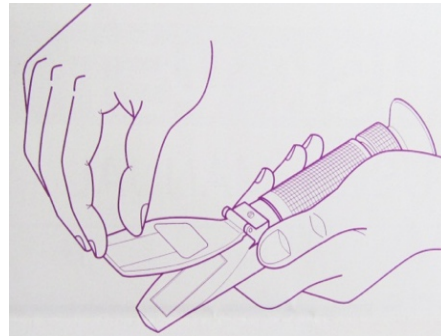
Hemolysis may cause a mild increase in protein concentration. Turbidity from lipemia or cells may result in falsely high readings. Icterus alters the color of the specimen but does not alter the reading.

Quality control consists of calibrating the instrument by measuring the specific gravity of water (1.000) and a 5% w/v sodium chloride solution (1.022).

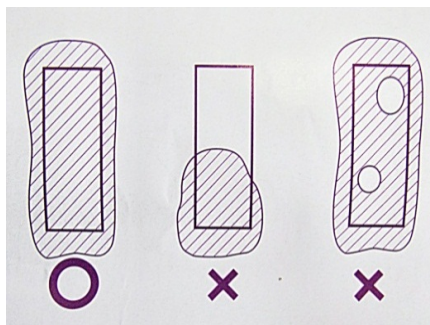
See images on the next page for proper use of the refractometer.



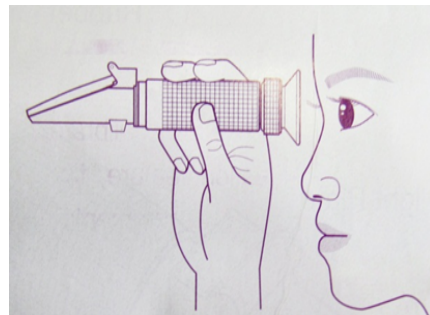
1. Place 1-2 drops of samples on the prism.



2. Close the daylight plate gently.



3. The sample must spread over the prism surface completely, eliminating any air bubbles that may be trapped under the daylight plate. If the sample does not completely cover the prism surface the measured value may not be accurate.



4. View the scale by pointing the unit towards a light source and looking through the eyepiece. Focus the scale by turning the eyepiece until the scale can be read clearly.



5. Read the measured value where the boundary line intercepts the scale.



6. Clean the prism and daylight plate after each use by gently wiping off the sample with a damp, lint-free tissue. Then use another tissue to remove any excess moisture.

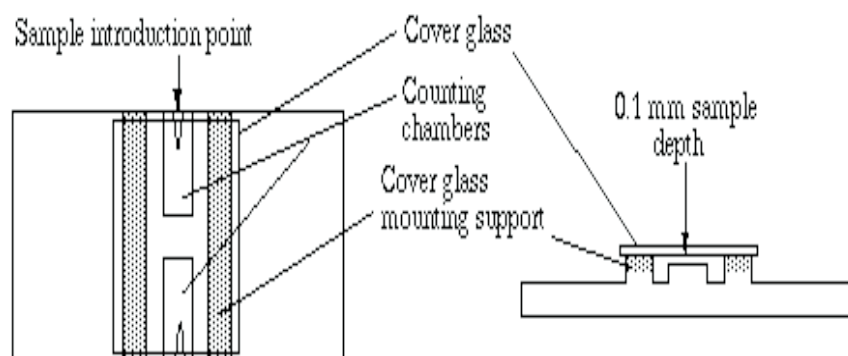
Manual White Blood Cell Count

A manual white blood cell count can be critical for monitoring a sick elephant. Performing sequential counts can determine the course of the disease (improving/worsening) and response to treatment. Practice is essential to ensure accuracy. Causes of increased and decreased WBCs are listed in the Appendix B. WBC and RBC reference values are listed in Appendix C. A reporting form can be found in Appendix K.

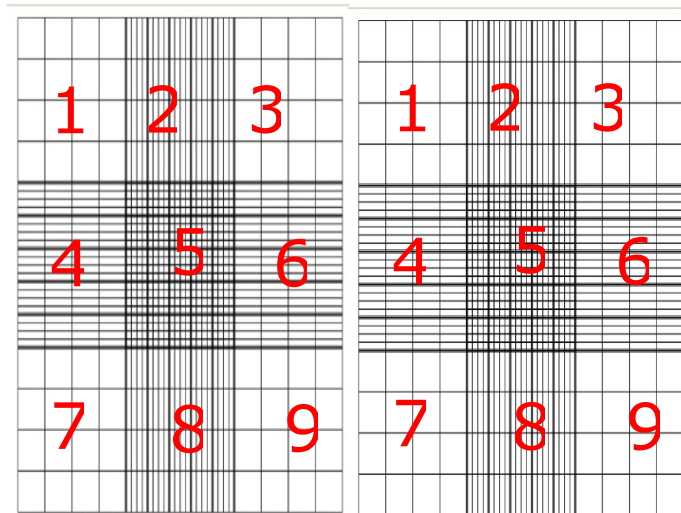
How to Use a Hemocytometer Counting Chamber

A hemacytometer counting chamber can be used to count RBCs, WBCs, or platelets. For a WBC count follow this procedure:

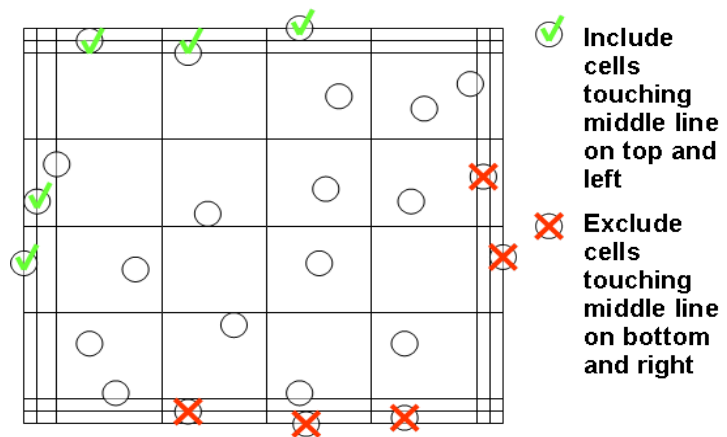
1. Mix the anticoagulated (EDTA) room temperature sample by gently inverting the tube.
2. Dilute the sample 1:20 with a product that lyses the RBCs and stains the WBCs such as Turk's solution (2 ml glacial acetic acid, 1 ml of 1% Gentian violet, 97 ml distilled water). Mix 20 μ L of blood with 380 μ L of Turk's solution in a small test tube. The Leuko-TIC 1:20 Blue Kit provides ready-to-use tubes and pipettes and is a convenient alternative. It is available in the U.S.(Amazon), India (ubuy.co.in), Laos (ubuy.la/en), and other distributors in Asia.
3. Mix the sample and allow 2-3 minutes for lysis and staining.
4. Use 70% alcohol to clean the hemocytometer and coverslip and allow to dry.
5. The hemacytometer has two raised bridges that hold the coverslip and two counting areas surrounded by a moat. These coverslips are specially made and should not used for other purposes.
6. Place the coverslip on top of the counting chamber, ensuring it sits flat on the raised supports.
7. Draw a sample into a micropipette. Charge the chambers by touching the pipette tip to the edge of the over slip to let the diluted sample fill the chamber by capillary action. Avoid air bubbles and overfilling. The amount needed to fill each chamber is $\sim 10 \mu$ L. Allow 2-3 minutes for the cells to settle.



The sample counting area contains a ruled grid, most commonly Neubauer ruling, consisting of 9 large squares, each measuring 1 mm². The depth of the chamber (distance between the grid area and the coverslip) is 0.1mm. One entire grid of Neubauer rulings can be seen at 40x (4x objective).



Count the 4 large squares on both sides. Count the cells that touch the middle line on the top and the left side; don't count the cells that touch the middle line on the bottom and right side.



To calculate the WBC:

General formula:
$$\frac{\text{Number of cells counted} \times \text{Dilution factor} \times \text{Depth factor}}{\text{Area counted (in mm}^2\text{)}}$$

Using these standard values ...

Dilution factor: 20 (from a 1:20 blood dilution pipette)

Depth factor: 10 (constant depth of 0.1mm)

Area counted: 4 large corner squares (4 mm²)

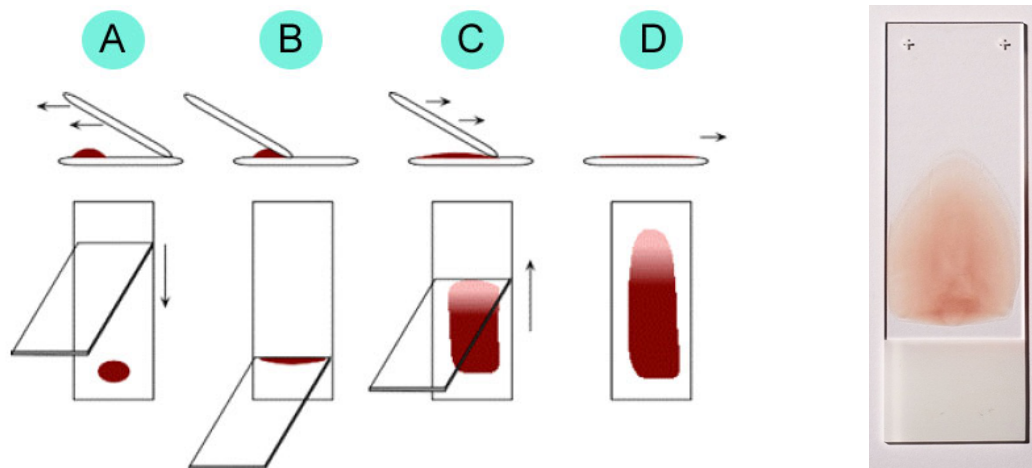
... the general above formula can be simplified to

Total count from 4 large corner squares X 0.05 = WBCs x 10⁹/L blood

Total count from 4 large corner squares X 50 = WBCs /μl blood

Preparing a Proper Blood Smear

A proper blood smear is essential for a differential white blood cell count. The smear should be prepared as soon as possible after blood collection. First clean, dry, and label the slide with the elephant's ID and the date. Use a pencil to label slides as all inks will dissolve on contact with the fixer – the first step of the slide staining process. Apply a drop of blood from the end of the hematocrit tube or use an applicator stick or a toothpick to transfer the blood from an EDTA tube. The drop of blood should be about 2-4 mm in diameter. Spread the drop using another slide held at a 30- 45° angle and **BACKING** it into the drop of blood. As soon as the blood has spread along the edge of this spreader slide quickly push the slide forward. This action pulls the blood.



A proper smear should have a nice feathered edge like the slide on the right. The feathered edge is important because we are striving to create an area where the cells are one cell thick so they are easy to identify and count.

Causes of a Poor Blood Smear

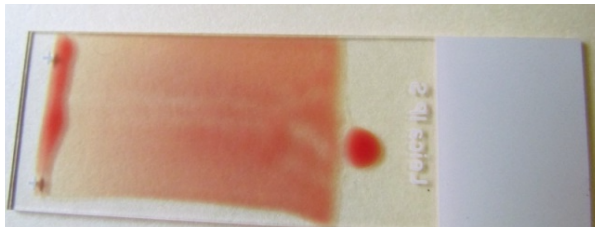
1. Drop of blood too large or too small or containing microclots
2. Jerking the spreader slide
3. Not keeping the edge of the spreader slide against the slide while making the smear
4. Incorrect angle
5. Not pushing the spreader slide completely across the slide
6. Waiting too long after placing the drop of blood on the slide
7. Artifacts See Appendix E



The spreader slide was probably chipped.



The smear has holes because the slide was dirty.



This slide had too much blood.

Staining the Slide

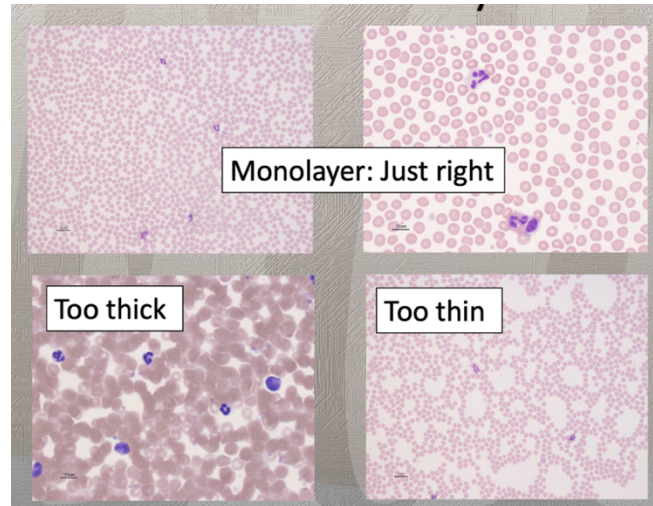
Romanowsky type stains (Wright stain, Wright-Giesma stain and others) are used to stain blood smears to distinguish cells. Wright-Giemsa best differentiates WBC morphology and is preferred. Dip Quick is a rapid stain similar to Wright-Giemsa but may only be available from the U.S. Follow the instructions for whichever stain you are using.



Differential White Blood Cell Count

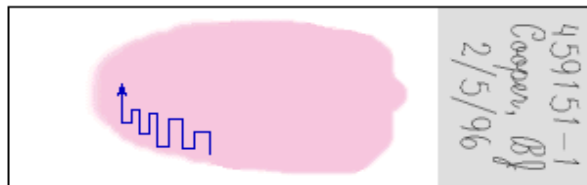
As the size of the elephant's monocytes differ significantly from other mammals, automated machines do not properly identify elephant WBCs. The differential must be performed manually. The differential WBC is used to establish a baseline during health, compare to changes during illness, and monitor changes over the course of an illness.

The accuracy of the differential count depends on a well-prepared blood smear (see section on making a blood smear). If too thick, the cells are difficult to differentiate; if too thin the distribution may not be even.

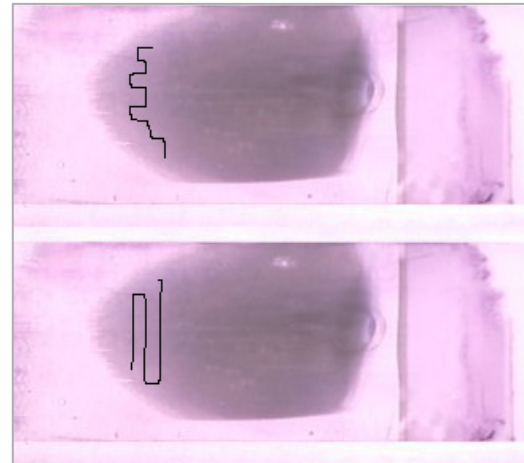


1. After staining, scan the smear at low magnification (10X). Select a thin area where the cells are evenly distributed and observe the relative numbers of WBCs.
2. Next examine at high dry (40X). At this magnification it is easier to see the WBCs and you can estimate whether they are normal in number or increased or decreased. If the morphology of the cells is clear you can perform the differential count at this magnification. If not then perform the differential under oil (100X). Under oil you can also evaluate whether the numbers of platelets seem adequate.
3. Examine using immersion oil (100x) to identify cell features (toxic changes for example).
4. Begin the differential count by moving back and forth across the smear in a pattern that avoids covering the same area.
5. Identify each WBC that is encountered until 100 cells have been counted and sorted by type. Use the cell counter (below) to place each cell in the proper category.

The percentage of each cell type is the relative differential count. The percentage of each WBC type multiplied by the WBC count (acquired using the hemocytometer) gives the absolute number of that particular cell type. The cumulative total of all the absolute cell counts should equal the total WBC.



Patterns for differential counting (above and right)



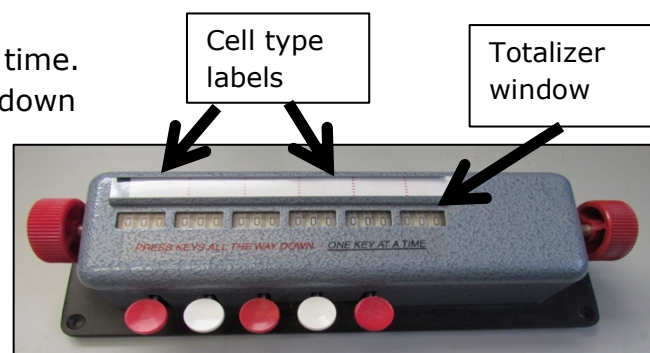
Platelet Counting

If the monolayer and staining are of optimal quality, manual cell differentiation also gives an estimation of the number of platelets and the presence of microthrombi. This is of particular interest in the diagnosis of EEHV-hemorrhagic disease. The average microscopic field of view (100x magnification) should contain between 7 and 20 platelets. (Webb 2004). Final confirmation of the number of platelets must be obtained by using an automated blood cell counter.

How to Use the Cell Counter

1. Set all the counter units to 000 by rotating the end knobs one time towards the operator. Mark the cell types to be counted below each window.

2. Press only 1 key down at a time. Press each key all the way down then release completely. This is critical because each single key stroke records the count for that cell type and also for the total count.



3. The totalizer bell rings when you have counted 100 cells. The totalizer is the last counter on the right with no key beneath the window. The number shown in each window directly corresponds to the % of that cell type counted.

For more precise counts, continue until the totalizer bell rings again, at a total of 200 strokes. Divide the total shown on each individual cell counter by two to determine the % of each cell counted.

After each use, re-set the counters to 000. This assures the longest counter life, as it reduces tension on springs and other moving parts. Cover the cell counter when not in use to protect from dust. Never lubricate the counter.

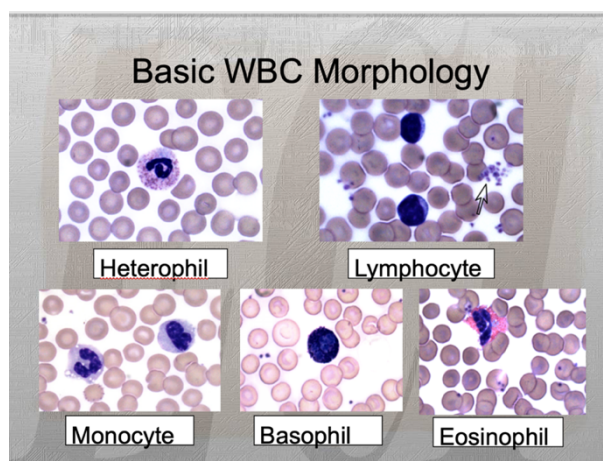
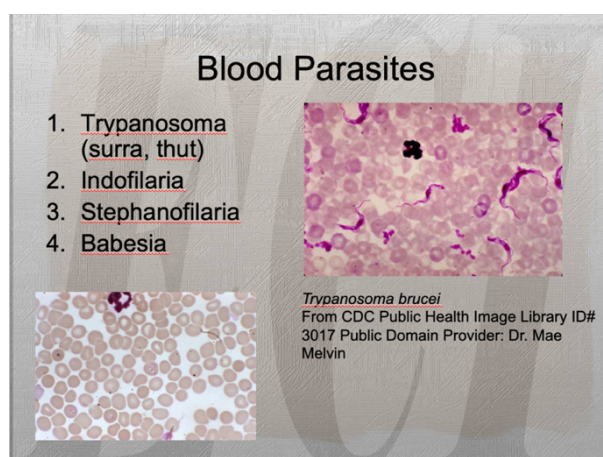
WBC Morphology

This chart illustrates the WBCs we typically see in elephants. The lymphocyte is usually the predominant cell. Elephants have unusual monocytes which has caused confusion with identification. There are two types of monocytes – a typical unsegmented monocyte and a segmented type that has either a bi- or tri-lobed nucleus. Segmented monocytes occur more frequently than unsegmented monocytes.

Neutrophils are also described as heterophils because of the presence of reddish cytoplasmic granules with Leishman staining. I use the terms neutrophil and the term heterophil interchangeably.

Basophils occur but in low numbers. Eosinophilia, associated with parasite infections in other species, does not appear to be common even in free-ranging elephants that would be expected to have high parasite loads or in elephants positive for microfilaria. Decreases in lymphocytes and eosinophils have been noted in pregnant Asian elephants. See additional images in the Appendix.

Blood parasites that have been reported in elephants are pictured in the image to the right. Babesia is rare.



Urinalysis

The routine urinalysis is a quick and relatively inexpensive test that can be readily performed in a field laboratory. Ideally, urine should be collected at the same time as blood is collected for hematology and clinical chemistry and before any treatment is administered.

Urine should be examined as soon as possible after collection, because artifacts will occur in the urine over time (cells lyse, crystals form *in vitro*). If a delay is anticipated before analysis, the urine should be refrigerated. Refrigerated urine should always be brought to room temperature before testing.

A complete urinalysis consists of 1) evaluation of physical characteristics, 2) measurement of specific gravity, 3) dipstick analysis and 4) examination of sediment. See the Appendix H for Reference Values.

Some of the information below may have been downloaded from an earlier version at eClinPath.com, Cornell University.

Equipment and Materials

- Microscope / Centrifuge
- Refractometer
- Slides / Cover slips
- Urinalysis dipsticks / Urine sediment stain
- Report form

Physical Characteristics of Urine

1. Evaluate and record urine color

Normal mammalian urine is yellow to amber. Urine volume and concentration affect the depth of the color.

Examples of urine colors and possible causes are found on the next page.



COLOR	POSSIBLE CAUSES
light to medium yellow	normal
colorless	very dilute urine
very dark yellow	extremely concentrated; bilirubinuria
red	hematuria; the urine appears cloudy and usually clears after centrifugation
red to brownish red	hemoglobinuria or myoglobinuria
reddish brown to brown	myoglobinuria, hemoglobinuria, methemoglobin
greenish tint	bilirubinuria
orange	administration of rifampin

2. Evaluate and record urine turbidity

Fresh urine is clear to very slightly cloudy. Urine may become more cloudy if left standing or if refrigerated. The turbidity of the urine sample is reported as: clear, slightly cloudy, cloudy, opaque, or flocculent. 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 cells (RBC, WBC)
- numerous crystals
- bacteria
- lipiduria (lipids often rise to the surface)
- mucus (especially in horses)
- semen
- fecal contamination

3. Odor

Ammonia is formed from urea by bacterial action. An ammonia smell may be prominent in retained or old urine samples. An acetone smell may suggest ketosis. Some drugs may impart a characteristic odor.

4. Volume

Urine volume may be estimated from urine specific gravity. In general, volume and specific gravity (S.G.) are inversely related in health and in most diseases. Exceptions include:

- Diabetes mellitus. Polyuria and high S.G. coexist because of glucosuria.
- Acute and chronic renal disease. Oliguria ($\downarrow$ urine volume) may be accompanied by a lack of renal concentrating ability.

5. Measure specific gravity (S.G.)

Specific gravity is the ratio of the refractive index of urine compared to water. Refractometry is the easiest method to measure urine S.G. Dipsticks are not as accurate. Glucose and protein may falsely $\uparrow$ urine S.G.

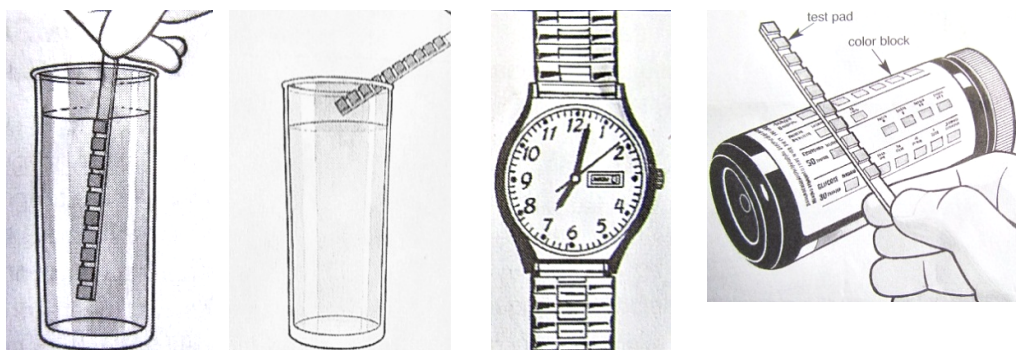
Knowledge of the hydration status is necessary to interpret the S.G. Urine S.G. can vary from 1.001 to 1.065 in most healthy animals but this range also includes values associated with renal abnormalities. S.G. has been inadequately studied in elephants and obtaining a baseline during health and evaluating sequential samples during illness is recommended.

Isosthenuria (fixed S.G.) is the constant maintenance of urine S.G. in the range of the glomerular filtrate (1.008-1.012). Isosthenuria means the kidney is neither concentrating nor diluting urine.

Hyposthenuria is a S.G. < 1.008 . In hyposthenuria the kidneys still have some water-balance function.

6. Perform dipstick analysis and record results (see charts in Appendix I).

- Mix the urine
- Dip the dipstick; remove extra urine (or use a pipette to transfer urine)
- Start timing
- Compare to chart on bottle in good light



7. Urine sediment examination (see images in Appendix J)

1. Centrifuge a fresh urine sample for 5 minutes at about 1500 RPM
2. Remove supernatant
3. Add 1-2 drops of sediment stain to sediment
4. Tap the bottom of the tube to mix
5. Transfer one drop to a slide and place a coverslip over the sample
6. Examine the entire coverslip at 10X and 40X
 - Low magnification (10X): casts, large crystals, debris, parasitic ova are visible
 - High magnification (40X): leukocytes, erythrocytes, epithelial cells, fat droplets, small crystals, sperm, debris and bacteria are visible

A urinalysis reporting form can be found in Appendix L.

Appendices

A. Causes of Anemia in Elephants

Anemia is defined as an absolute decrease in the hematocrit, hemoglobin, or RBC count. There are 3 basic categories of anemia: blood loss, blood destruction, and decreased or ineffective RBC production.

Blood Loss

The slide below lists possible causes of anemia due to blood loss. The history can usually rule out some of these causes such as trauma or exposure to toxins. We can check the urine to rule out hematuria, and we can do fecal exams to rule out parasites. DIC is disseminated intravascular coagulation – a condition where there is clotting in the body followed by hemorrhage when clotting factors are used up. It can occur with very serious infections or significant trauma.

Causes of Blood Loss Anemia

<u>Acute</u>	<u>Chronic</u>
<ul style="list-style-type: none">▪ trauma/surgery▪ toxin (e.g. rat poison)▪ GI ulceration / hemorrhage▪ disseminated intravascular coagulation (DIC)	<ul style="list-style-type: none">▪ parasites(<u>strongyles</u>, liver flukes)▪ GI ulcers / hemorrhage▪ hematuria (blood in urine)▪ <u>neoplasia</u>▪ ↓ platelets▪ vitamin K deficiency



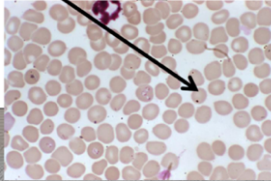
Blood Destruction

Infections from bacteria such as clostridium, E. coli, or leptospirosis or certain blood parasites can result in blood destruction. Snake bite, liver failure, and certain toxicities may also be causes. We can usually determine from the history if there has been a toxin or snake bite exposure or if certain drugs have been given. We can check for blood parasites, and we can do a WBC count to see if it is elevated which we would expect with infection. We can

check for fever. All of this information helps us to “rule-in” or “rule-out” the possible etiologies.

Causes of Blood Destruction Anemia

- Infections
 - Clostridium
 - E. coli
 - Leptospirosis
- RBC parasites
 - Babesia spp.
 - Anaplasma
 - Theileria
 - Trypanosoma
- Snake bite
- Liver failure
- Toxicity
- Cephalosporins
- Penicillin
- DIC



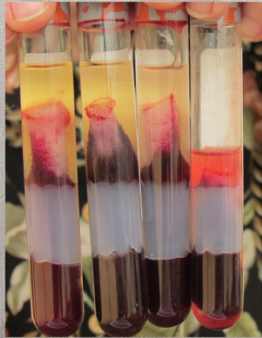
CDC/ Dr. Mae Melvin

Decreased Blood Production

Causes of decreased blood production are a little more challenging to diagnose but we can test for some of these. We can perform a urinalysis and blood urea nitrogen test to evaluate kidney function. We can perform a screening test for TB or culture trunk wash samples. We might suspect neoplasia if our patient is an older elephant. We should be able to rule out lead exposure by our history and we will know whether or not

Causes of Decreased Blood Production

- Chronic renal disease
- Other chronic disease (TB)
- Neoplasia
- Lead poisoning
- Copper or iron deficiency
- Toxic bone marrow damage (i.e. phenylbutazone)
- Metabolic or endocrine disease



phenylbutazone has been given. Endocrine diseases are harder to diagnose but these are not very common. Although all of the problems on this slide can result in anemia, chronic disease is probably the most common cause in elephants. This may be renal disease, TB or any other long-standing disease. We refer to this as "anemia of chronic disease."

B. Causes of Increased and Decreased White Blood Cells in Elephants

↑WBC (leukocytosis)	↓WBC (leukopenia)
Inflammation	Viral infection
Infection	Endotoxemia
Tissue necrosis	Acute bacterial infection
Hypersensitivity	Blood parasites
Endotoxemia	Viral infection
Poisoning	
Malignancy	

↑Neutrophils (Heterophils)	↓Neutrophils (Heterophils)
Acute bacterial infections	Viral infections (most common)
Inflammation secondary to tissue necrosis (wounds)	Rickettsial infections
Hypersensitivity reactions	Bacterial infections
Hemorrhage	Phenothiazines
Hemolysis	Phenylbutazone
Stress	
Neoplasia	

↑ Lymphocytes	↓ Lymphocytes
Acute or chronic infections – usually viral	May follow steroid treatment

↑ Eosinophils	↑ Monocytes	↓ Monocytes
Parasite infections	Chronic infections (TB)	Specifically characteristic of EEHV-HD
Hypersensitivity reactions	Chronic inflammatory bowel disease	
Leukemia (not reported in elephants)	Other granulomatous diseases	

C. RBC and WBC Reference Values

Disclaimer: The reference values suggested in the following tables are derived from reported studies with preference given to data from clinically healthy elephants. These suggested ranges are to be used as a general guideline rather than as specific cut-offs to define normal vs abnormal. Laboratory values are affected by numerous factors and may vary between individual elephants. Establishing a baseline hematological and serum chemistry profile for each elephant during health is recommended.

Red Blood Cell (RBC) Reference Values ^a

Parameter	Units	Reference Range
PCV	%	28-40
Hb	g/dl	8-15
RBC	$\times 10^6/\mu\text{l}$	2.5-5.0
MCV	fl	80-160
MCH	pg	35-50
MCHC	g/dl	25-40
Platelets	$10^3/\mu\text{l}$	200-600
Reticulocytes	%	0.00
RDW	%	28-32
ESR	mm/hr	65-150

White Blood Cell (WBC) Reference Values

Parameter	Absolute Range ($\times 10^3/\mu\text{l}$)	Relative Range (%)
WBC	10-18	-
Heterophils	2-4	25-30
Lymphocytes	5-8	30-40
Monocytes	2-4	25-30
Eosinophils	0.1-1	< 5
Basophils	0.00-.03	< 1

Coagulation parameters in elephants and horses

Test	Horses ^a	Elephants
Platelet count ($\times 10^3/\mu\text{l}$)	100-600	200-600 ^b
Prothrombin time (sec)		9.6 ± 0.7 ^c (8.2-10.4)
Activated partial thromboplastin time (sec)	9.5-13.5	65.6 ± 9.2 ^c (52.1-83.5)
Fibrinogen (g/dl)	39-64	461 ± 49 ^c (401-546)
	200-400	

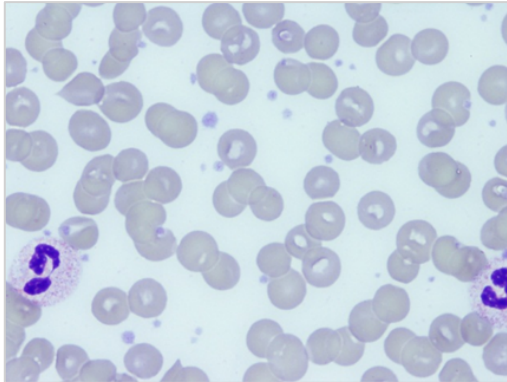
The values in the tables below are from a study conducted in India (Muliya 2025). Consult the article for values for males vs females and other statistical analyses. These values can be used as a guideline but it is best to establish your own baseline normal values with whatever lab you work with. A similar study has been conducted in Thailand (Janyamethakul 2017).

Parameter	Sex	n	Mean	Min	Max	Reference *Interval
Hbg (g/dL)	M/F	86	11.91	7.8	16.9	8.62-12.78
RBC ($\times 10^{12}/L$)	M/F	77	2.91	1.8	4.9	1.77-4.9
PCV (%)	M/F	85	33.03	19.0	50.	21.73-41.34
MCV (fl)	M/F	67	121.90	110.5	133.2	112.49-131.39
MCH (pg/cell)	M/F	82	45.94	39.1	62.5	39.3-62.39
MCHC (g/dL)	M/F	80	36.8	33.3	41.4	33.40-41
Platelets (lakhs/cumm)	M/F	86	477.00	150.0	991	171.57-947.1

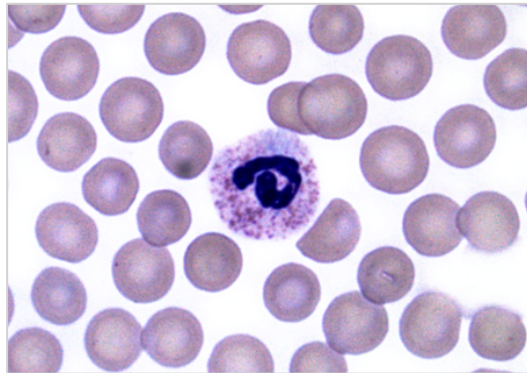
* The reference interval is set of values considered normal for a healthy population.

Parameter	Sex	n	Mean	Min	Max	Reference Interval
Total WBC (cell/cumm)	M/F	84	17.796	9,900	32,800	9,9122.5-29,475
Neutrophils (heterophils)	M/F	84	44.78	26.6	88.6	27.09-80.71
Lymphocytes	M/F	86	35.60	5.8	62.4	7.19-60.48
Monocytes	M/F	70	22.30	4.0	79.5	5.24-69.42
Eosinophils	M/F	17	10.35	3.01	15	-
Basophils	M/F	87	0.00	0.00	0	-

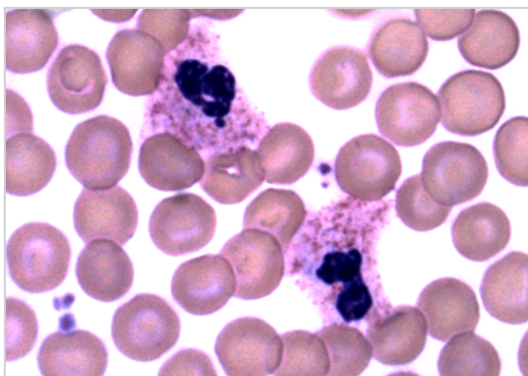
D. Elephant WBC Identification



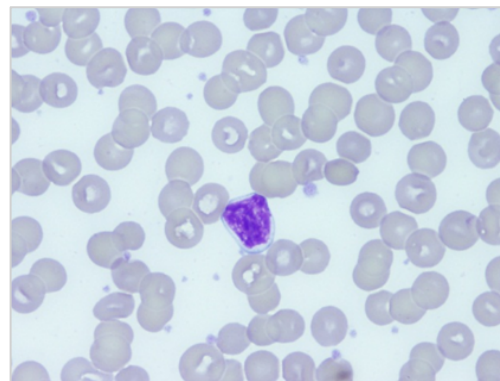
Heterophils (Neutrophils)¹



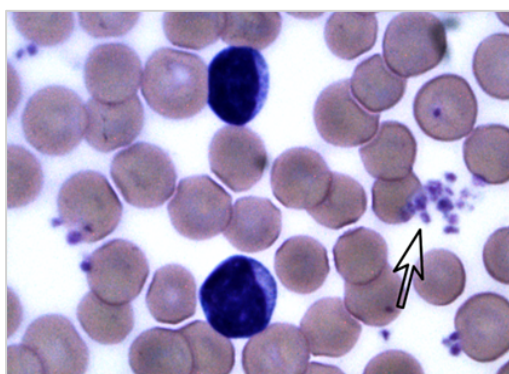
Heterophils (Neutrophils)²



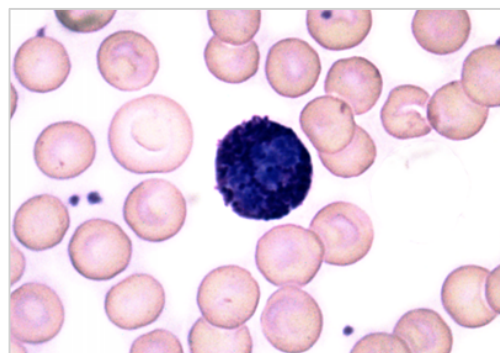
Two Heterophils²



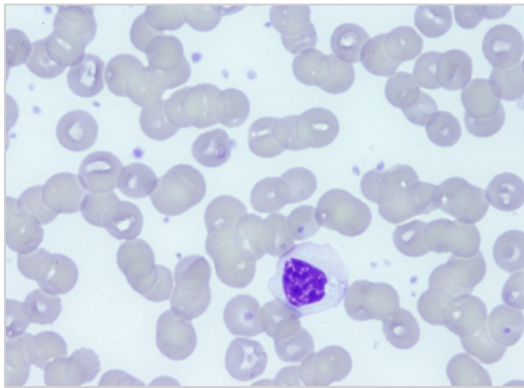
Lymphocyte¹



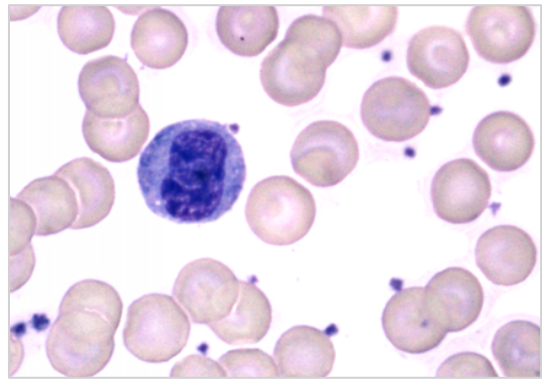
Two lymphocytes; platelets at arrow²



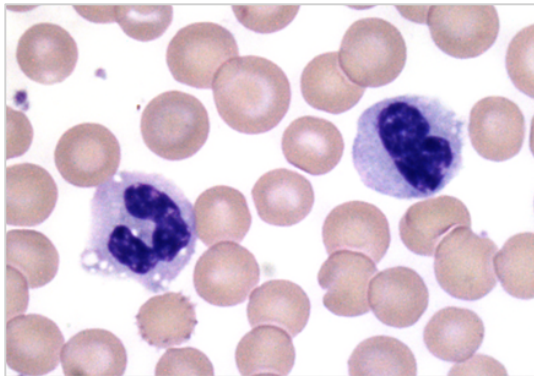
Basophil²



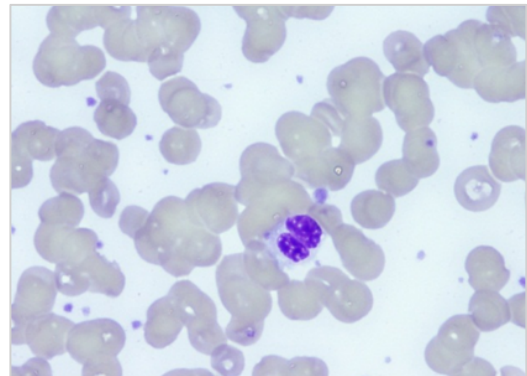
Unsegmented monocyte¹



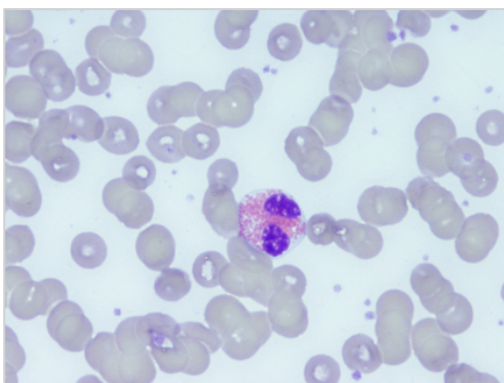
Unsegmented monocyte²



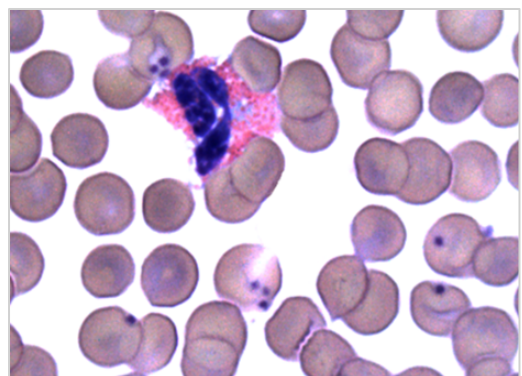
Heterophil (left) and unsegmented monocyte (right)¹



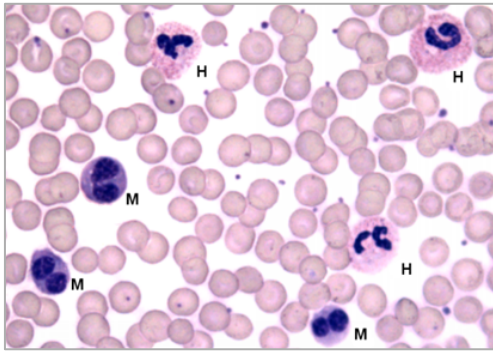
Segmented bi-lobed monocytes²



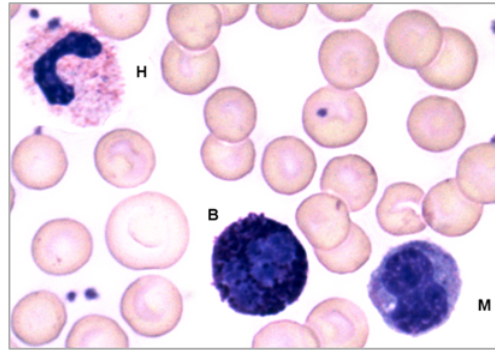
Eosinophil¹



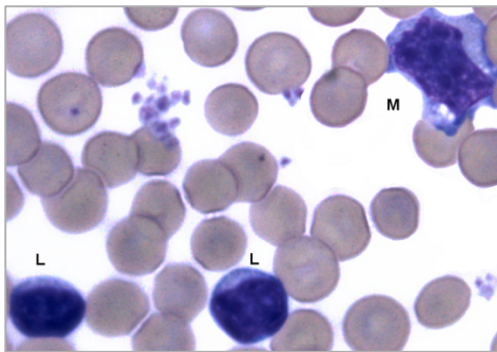
Eosinophil²



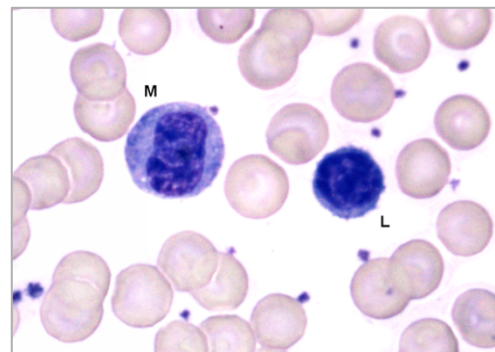
Three heterophils (H) and three bilobed monocytes (M)²



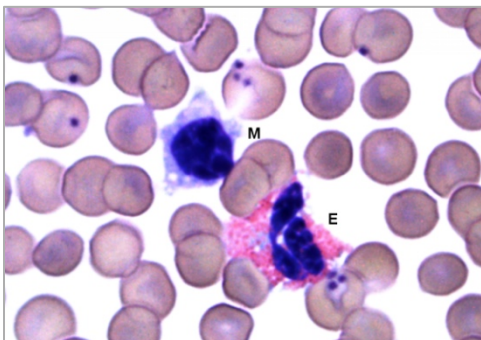
Heterophil (H), basophil (B) and bilobed monocyte (M)²



Unsegmented monocyte (M) and two lymphocytes (L)²



Unsegmented monocyte (M), lymphocyte (L), and scattered platelets²



1 Courtesy of Teri Hermann, Woodland Park Zoo. 2 Images from Fowler, M.E. and Mikota, S.K.(eds). 2006. Biology, Medicine, and Surgery of the Elephant. Blackwell Publishing, Ames, IA. 565p.

Eosinophil (E). The cell M presents an identification dilemma. It could be identified as an unsegmented monocyte based on the following features: 1) it has more cytoplasm than the other lymphocytes and 2) it has two small cytoplasmic granules at about 7 o'clock. Alternatively, it could be identified as a lymphocyte based on these features: 1) it has a heavily condensed nucleus and 2) it has less cytoplasm than the other monocytes. Cells of this type are rare and unlikely to result in significant error on the differential count.²

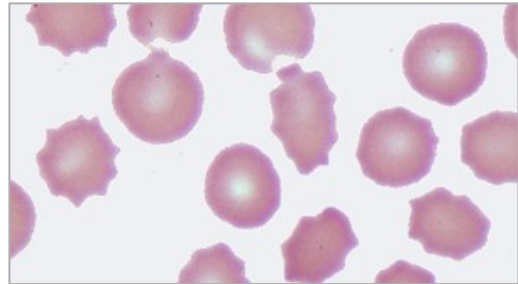
E. Artifacts in Blood Smears

The following information was downloaded from an earlier version at eClinPath.com, Cornell University. The current version with additional images and information can be found here:

https://eclinpath.com/hematology/sample-collection-heme/#Stain_artifacts

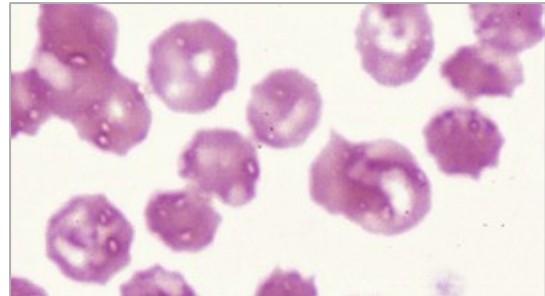
Red cell crenation

Crenated red cells have changed from a disc shape to spheres covered with short, sharply pointed projections. They are also known as echinocytes or burr cells. Slow drying of the smear and aging of blood in the tube are the most common causes.



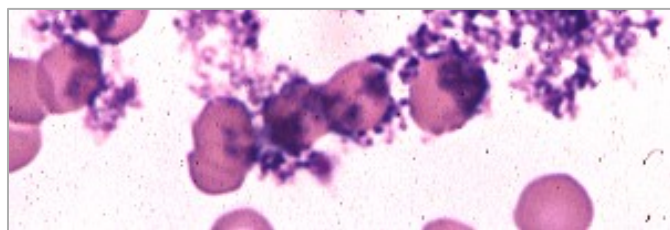
Water Artifact

The red cells shown here are crenated and many have multiple refractile "bubbles" across their surfaces. The refractility is often called water artifact because it results from exposure of the smear to water, either as high humidity in the air or as contamination of the fixative with water. If a smear is affected by water artifact, the fixative solution in the staining jar should be discarded and replaced with fresh fixative. Notice that some of the spicules on the red cell are pointing toward the viewer; this orientation of the spicules can mislead a novice into thinking there are inclusions in the red cell.



Stain Precipitate

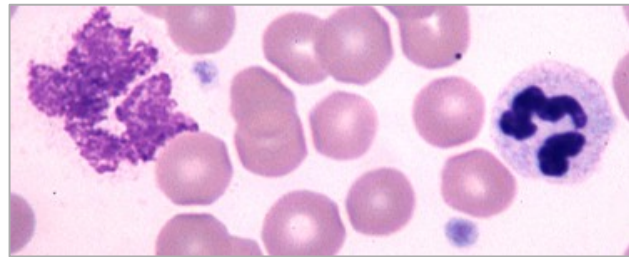
This microscopic field is sprinkled with dark purple granules of precipitated stain. Stain precipitate has many forms and can appear as granules smaller or larger than these. It can be distributed as



patches or dispersed across large areas. It is important to identify this material correctly, so that it is not mistaken for bacteria or for red cell parasites. When precipitate on smears becomes a problem discard the stain, thoroughly clean and dry the staining jars, and refill them with fresh stain. Proper care of staining solutions is important for obtaining the best results.

Smudged Cells

This photo shows an intact neutrophil and a smudged cell. A few partly or completely disrupted leukocytes are found in any blood smear but may be numerous in a poor smear. Aging of blood in the tube



results in degeneration and increased fragility of leukocytes resulting in excessive numbers of smudged cells. The nucleus in smudged cells is more magenta than the deep purple of intact nuclei. Minimal disruption can leave some cytoplasm around the nucleus but the cell border is indistinct. More complete smudging can strip the cytoplasm away from the nucleus, leaving only a blob of nuclear material. Most smudged cells are not included in the differential count unless enough remains for unmistakable identification, e.g. a nucleus surrounded by eosinophil granules can be safely counted as an eosinophil.

F. Causes of Increased and Decreased Total Protein

A variety of problems can cause changes in total protein. Dehydration may be one of the most common in working or tourism elephants followed by inflammation and perhaps tissue necrosis secondary to wounds. Low total protein can be due to malnutrition, malabsorption, parasites, or the other causes listed below.

↑ Total Protein	↓ Total Protein
Dehydration	↓ Production <ul style="list-style-type: none">• Malnutrition• Malabsorption• Liver disease
↑ Acute phase proteins <ul style="list-style-type: none">• Inflammation• Neoplasia	↑ Loss <ul style="list-style-type: none">• Hemorrhage• Burns• Parasites
↑ Gammaglobulins <ul style="list-style-type: none">• Inflammation• Necrosis• Surgery• Tumors	
False ↑ if glucose, Na ⁺ or Cl ⁻ ↑; or if sample is lipemic	

G. Interpreting the Total Protein and PCV Together

Increased PCV and TP <ul style="list-style-type: none"> • Dehydration 	Increased PCV and normal TP <ul style="list-style-type: none"> • Polycythemia (rare) • Dehydration with protein loss
Normal PCV and increased TP <ul style="list-style-type: none"> • Dehydration (common) • Dehydration and anemia • Increased globulin production • Lipemic serum or severely hemolyzed serum can give an increased TP reading on a refractometer 	Decreased PCV and decreased TP <ul style="list-style-type: none"> • Blood loss (most common) • Nonregenerative anemia with protein loss • Nonregenerative anemia and liver dysfunction • RBC destruction and protein loss

H. Urinalysis Reference Values (Wiedner 2009)

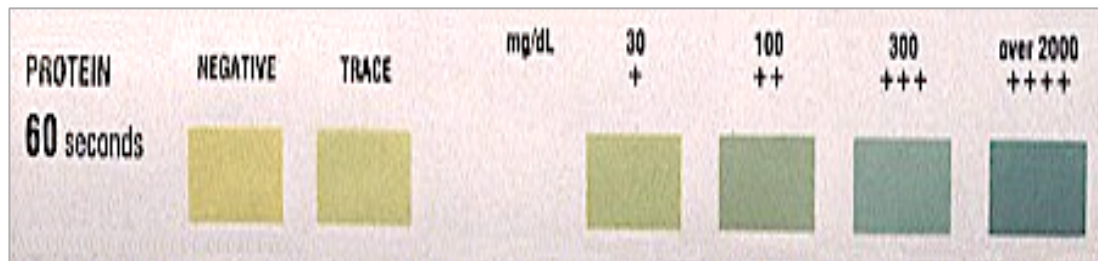
Parameter	Typical finding
Color	pale to dark yellow
Transparency	clear to cloudy
Specific gravity (dip stick)	1.007-1.025 (refractometer more accurate)
pH	6.0 – 8.5 (most are alkaline)
Protein	Neg to trace
Glucose	Neg
Ketones	Neg
Bilirubin	Neg
Blood	Neg to trace
RBCs	Neg
WBCs	Neg
Sediment	Calcium oxalate crystals common

I. Urine Dipstick References

The following information was downloaded from an earlier version at eClinPath.com, Cornell University. The current version can be found at <https://eclinpath.com/urinalysis/> and includes greater detail.

Chemical constituents that are a part of a urinalysis and included on a dipstick measurement: pH, protein, glucose, ketones, bilirubin, and heme. The leukocyte, nitrite, and urobilinogen pads on the dipstick are not generally used as they are considered inaccurate (leukocytes) or not diagnostically useful (urobilinogen, nitrite).

1. Urine Protein



The urine protein results 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 can 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. But, 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 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 including renal ischemia and nephrotoxins (such as aminoglycosides).

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

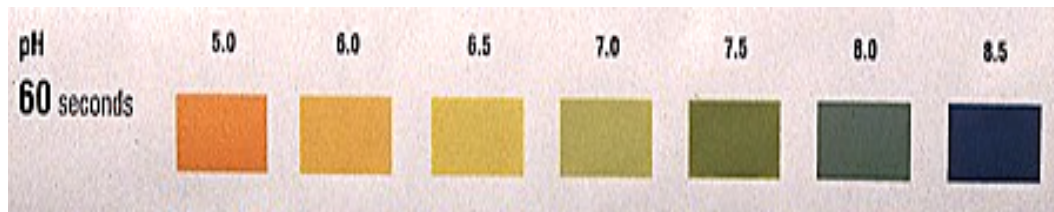
Post-renal proteinuria is due to hemorrhage or inflammation in the urinary tract (ureter, bladder, urethra) or in the reproductive tract that causes protein (in the serum that accompanies inflammation or hemorrhage) to enter the urine once it has been formed and entered the renal pelvis.

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.

A more accurate assessment of proteinuria is obtained with measurement of the urine protein to creatinine ratio which standardizes the protein in the urine to creatinine, which remains constant in the tubules once filtered (not resorbed by the tubules or secreted by the tubules in most species). The dipstick measurement is also altered by the pH and you may see a false increase in protein in an alkaline urine (pH>8).

2. Urine pH



The pH of a urine sample is affected by a variety of factors including:

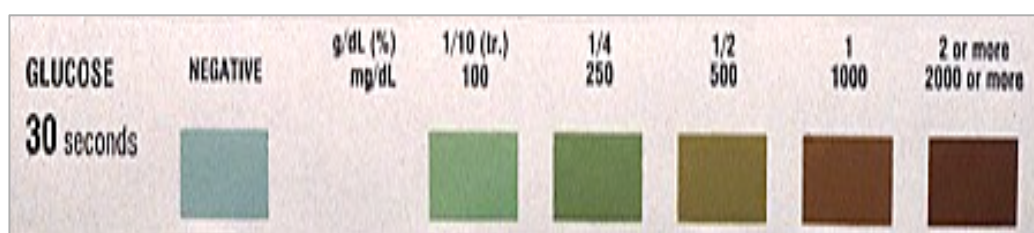
1. renal H^+ excretion and HCO_3^- resorption

- pathologic abnormalities of systemic acid/base balance
- pathologic abnormalities of tubular function: with failure to excrete an acid load or failure to absorb bicarbonate
- dietary factors - herbivores usually have alkaline urine
- age of specimen (loss of CO_2 from the sample to the air raises the pH)
- presence of contaminant or pathogenic bacteria (some convert urea to ammonia, raising pH)

Knowledge of the urine pH is important in interpreting urine sediment findings. Erythrocytes, leukocytes, and casts tend to disintegrate in alkaline urine ($pH > 8.0$). In addition, precipitation of urine crystals in supersaturated urine is highly dependent on urine pH (e.g. struvite will precipitate in alkaline not acidic urine).

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. In addition, studies have shown that the dipstick measurement of urine pH can be quite inaccurate (e.g. a pH value of 7.5 can be anywhere from 7 to 8). In some conditions, impaired renal tubular function in fact causes or perpetuates the underlying acid/base derangement. Meaningful evaluation of acid-base status generally requires blood gas analysis and consideration of clinical signs.

3. Urine Glucose



Glucose is an abnormal finding in urine. The chart on this slide shows the threshold value for different species. 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 and chlorine will produce false positive reactions.

- Formaldehyde
- Outdated reagents
- False negative reactions can result from:
 - High concentrations of ascorbic acid which inhibit the reaction
 - Drugs such as salicylates, tetracyclines

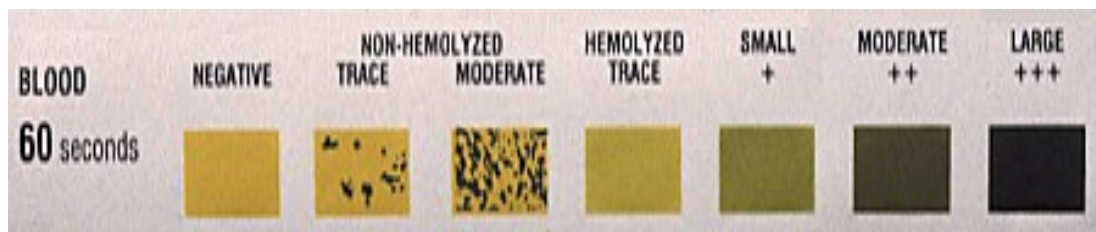
Pathologic glucosuria due to hyperglycemia: In nearly all cases, glucosuria is a result of prior (often, continuing) hyperglycemia to a level in excess of the renal threshold for reabsorption.

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.

Species Threshold

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

4. Urine Blood (heme)



Heme is found within hemoglobin (free in the urine or within erythrocytes) or myoglobin. Thus, the reaction is very sensitive and will detect hematuria, hemoglobinuria and myoglobinuria as indicated in the table.

Causes of a positive urine blood test and their differentiation:

Hematuria

- Red, cloudy urine that usually clears with centrifugation.
- RBCs in the urine sediment
- Absence of clinical or laboratory evidence of hemolytic anemia or muscle disease.
- 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.

Hemoglobinuria

- Red to brown urine that does not clear on centrifugation.
- No RBCs in the urine sediment (RBCs in urine with a very low S.G. or in aged urine may lyse and mask hematuria).
- Concomitant red discoloration of the plasma (hemoglobinemia).
- Evidence of anemia.
- Absence of clinical or laboratory evidence of muscle disease.

Myoglobinuria

- Red to brown urine that does not clear on centrifugation
- No RBCs in urine sediment.
- Clear, normal color plasma. Myoglobin does not bind significantly to serum proteins and is excreted in the urine before reaching concentrations that discolor plasma.
- No clinical or laboratory evidence of anemia.
- Clinical or laboratory evidence ($\uparrow$ CK, $\uparrow$ AST) of muscle disease.

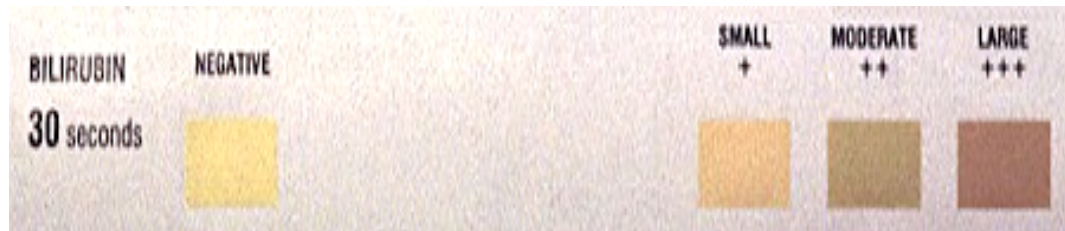
5. Urine Ketones



Ketonuria indicates abnormal energy metabolism such that fat is used instead of glucose. Fat metabolism can result in production of the ketone bodies in

amounts greater than can be metabolized by peripheral tissue; filtration into urine in excess of tubular reabsorption then results in ketonuria. 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.

6. Urine Bilirubin



The bilirubin pad on the multireagent dipstick detects conjugated bilirubin and is sensitive to 0.2-0.4 mg/dl of conjugated bilirubin.

Detection of bilirubin in urine is an abnormal finding. 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 if 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).

7. Urobilinogen

Urobilinogen is formed in the intestine by anaerobic 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. In humans, increased urobilinogen may occur in hemolytic diseases and with reduced functional hepatic mass. The correlation between urine urobilinogen and hepatobiliary disease is poor in animals and the test is not used diagnostically very often in animals.

8. Nitrite

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.

9. Leukocytes

The leukocyte test detects WBCs which may indicate infection is present. If positive on the dip stick, check the urine sediment to see if WBCs are present.

10. Specific Gravity

Specific gravity measurement can be done by dipstick, however measurement of specific gravity using a refractometer is more accurate.

J. Urine Sediment

The following information was downloaded from an earlier version at eClinPath.com, Cornell University. The current version can be found at <https://eclinpath.com/urinalysis/cellular-constituents/>.

A complete urinalysis should include an examination of urine sediment if possible. Centrifuge a standard volume of urine at low speed. The standard volume is the volume that you use every time. Decant the supernatant and gently re-suspend the pellet in a standard volume of urine supernatant (i.e. 0.5 ml). Place a drop of the re-suspended urine on a slide, add a coverslip and examine at 10x and 40x power. To examine unstained sediment, maximum contrast is needed and may be obtained by dimming the microscope light, closing the iris diaphragm and/or lowering the condenser. Using a sediment stain may also enhance contrast.

Under low magnification (10 X) examine the entire coverslip. At this magnification casts, large crystals, debris, and parasitic ova can be seen

Use high magnification (40 X) to examine several random fields. At this magnification WBCs, RBCs, epithelial cells, fat droplets, small crystals, sperm, debris, and bacteria are seen and can be semi-quantified.

RBCs in Urine

Red blood cells (RBC) are reported semi-quantitatively as the number seen per high power field (HPF): none seen; <5, 5-20, 20-100, or >100/HPF. Up to 5 RBC/HPF generally are considered acceptable for "normal" urine.

Increased red cells in urine (hematuria) may be due to hemorrhage, inflammation, necrosis, trauma, or neoplasia somewhere along the urogenital tract in voided specimens.

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). RBCs often appear shrunken or "crenated" in concentrated urine (high S.G.) RBCs may lyse or become ballooned ghost cells in dilute urine (S.G. > 1.006).

RBCs may be confused with fat droplets or yeast. Fat droplets vary in size, are highly refractile, less dense, and float right beneath the coverslip (out of the plane of focus of the cells). Yeast are oval, vary in size, and can often exhibit budding.

WBCs in Urine

WBC are reported semi-quantitatively as the number seen per high power field (HPF) using the high dry objective (40x): none seen; <5, 5-20, 20-100, or >100/HPF.

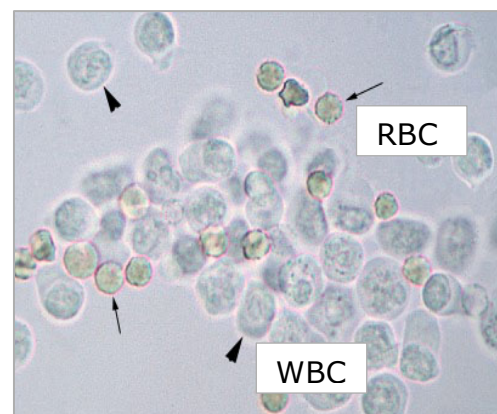
Interpretation: Less than 5 WBC/HPF is normal. More than 5 WBCs/HPF indicates urogenital tract inflammation (pyuria). WBCs are often associated with bacteria in the urine but significant bacteriuria can occur without pyuria.

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 for culture of urine even if no bacteria are seen. Non-septic causes of inflammation, such as uroliths and tumors, also must be considered.

Identification: In regular unstained urine sediments, WBC are small cells, usually 1.5-2x larger than a RBC (see image below), are regularly round, colorless and have a slightly grainy appearance. Like erythrocytes, WBC may lyse in very dilute or highly alkaline urine. The type of WBC (neutrophil, lymphocyte, monocyte etc.) cannot be determined from a regular urine wet preparation, but they are usually neutrophils. A cytologic examination would be required to determine which WBC types are present in urine, however this is rarely indicated for this purpose. WBCs must be distinguished from RBCs and small epithelial cells.

WBCs vs RBCs

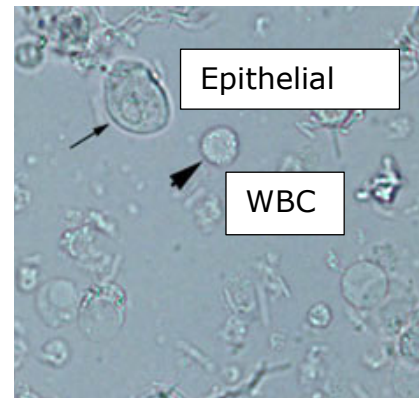
The grainy nature of WBCs helps to distinguish WBCs from RBCs which are smoother. WBC are also colorless whereas RBC are slightly red-tinged from hemoglobin. Identification of the segmented nuclear shape of a neutrophil is helpful, but this feature is often inapparent, particularly if the



specimen is not fresh (the nucleus swells and rounds up with storage). WBC (arrowheads) are larger, colorless and more granular than the redder smaller RBC (arrows), which have no internal texture (although appear slightly biconcave).

WBCs vs Epithelial Cells

Epithelial cells can originate from the kidneys, ureters, bladder, urethra, or reproductive tract. WBCs are generally smaller than epithelial cells and are more round. WBCs can swell with storage and appear larger than normal. Epithelial cells have more angular borders or polygonal shapes and round to oval nuclei.



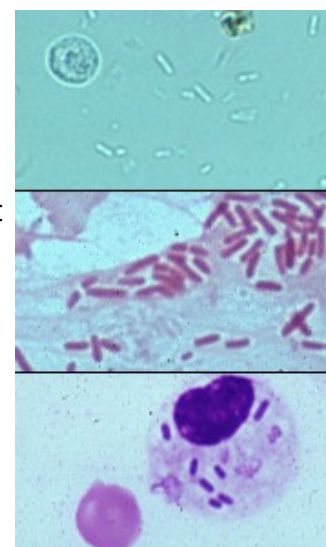
Debris in Urine

Pollens, mold spores, glove powder, and fibers of various types are among the common contaminants referred to as "debris." Generally, such material is significant only as an indication of the conditions of collection and/or handling of the specimen prior to analysis. Debris is more frequently seen in voided urine samples or those collected off the floor or ground. On occasion, confusion may arise if these are mistaken for findings of significance (casts, parasites, etc.).



Bacteria in Urine

Identification: Rod-shaped bacteria and chains of cocci are often readily identifiable. The top panel of the image at right shows E.coli bacilli. Small amorphous crystals, cellular debris, and small fat droplets can either mask or mimic cocci. If there is any doubt about the presence of bacteria, a Gram-stained smear of urine sediment (middle panel) should be examined. This gram stain confirms the presence of gram negative bacilli. Note that only extracellular bacteria can be visualized on an unstained urine sediment. Intracellular bacteria can only be identified by cytologic examination of a Wright's-stained smear of the urine sediment (the bottom panel demonstrates phagocytized bacteria within a neutrophil. The neutrophil's nucleus is swollen as a storage-related artifact).



Interpretation: Bacteria may be insignificant contaminants or important pathogens. Distinguishing between these possibilities relies on clinical signs, the method of urine collection, the number and types of bacteria, the presence of leukocytes in urine, the length of urine storage, and any underlying disease in the animal.

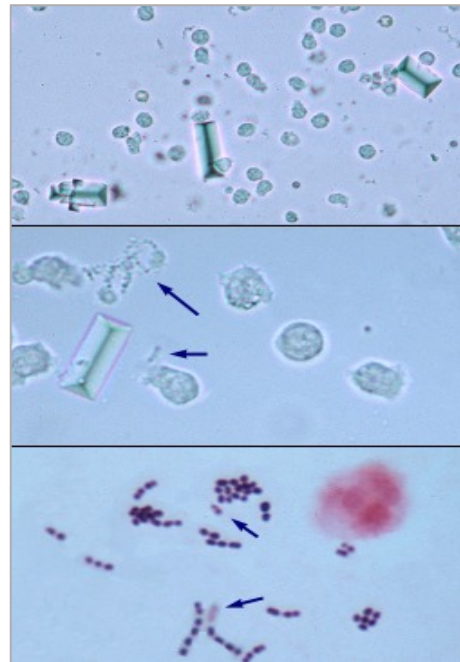
Since urine in the bladder of normal animals is sterile, bacteria are not normally seen in urine, however this does depend on the method of collection. In most other species, a few bacteria from the distal urethra and/or genital tract) may be seen in voided urine, however usually numbers are low if a good mid-stream collection was obtained. Due to the long urethra in the male elephant and the long vestibule in the female, bacteria are more common and more difficult to interpret.

Bacteriuria of clinical significance, e.g., bacterial cystitis, is usually accompanied by increased WBCs (pyuria). However, some animals with pyelonephritis or underlying immunosuppression (e.g. diabetes mellitus) may have clinically relevant bacteriuria without pyuria.

Cocci in Urine

Upper panel: Low magnification view showing increased numbers of leukocytes and several struvite crystals (unstained wet prep). The leukocytes provide clear evidence of an inflammatory process; the background appears "busy", but bacteria are not reliably identifiable at this magnification.

Middle panel: High magnification view of unstained wet prep showing leukocytes and clumps and chains of bacteria (arrows). Amorphous crystals or debris, however, can have a virtually identical appearance. Use of phase contrast microscopy can help in distinguishing between the two, but examination of a gram-stained drop of the urine sediment is most reliable.



Lower panel: High magnification view of a gram-stained slide. A neutrophil and gram positive cocci arranged in clusters and short chains are shown (arrows: some organisms have partially or completely decolorized). This inflammatory process is caused or complicated by bacterial infection.

Urinary crystals

The following information was downloaded from an earlier version at eClinPath.com, Cornell University. The current version can be found at <https://eclinpath.com/urinalysis/crystals/>. See Wiedner 2009 for images of crystals observed in elephant urine.

Crystalluria indicates that the urine is supersaturated with the compounds that comprise the crystals, e.g. ammonium, magnesium and phosphate for struvite. Crystals can be seen in the urine of clinically healthy animals or in animals with no evidence of urinary disease (such as obstruction and/or urolithiasis). However, some crystals can be pathologically relevant in certain circumstances. Note that crystals may not form in all urines supersaturated with these compounds. A number of *in vivo* and *in vitro* factors influence the types and numbers of urinary crystals in a given sample as indicated in the table below.

<i>In vivo</i> factors	<i>In vitro</i> factors
Concentration and solubility of crystallogenic substances contained in the specimen	Temperature (solubility decreases with temperature)
Urine pH	Evaporation (increases solute concentration)
Diet	Urine pH (changes with standing and bacterial overgrowth)
Excretion of diagnostic imaging and therapeutic agents	

Crystals are subjectively quantified in urine as few, moderate and many. For crystals that are large, e.g. calcium carbonate and struvite, this assessment is made from low power (10 X objective). For smaller crystals (e.g. amorphous, calcium oxalate dihydrate), the high power (40 X objective) is used.

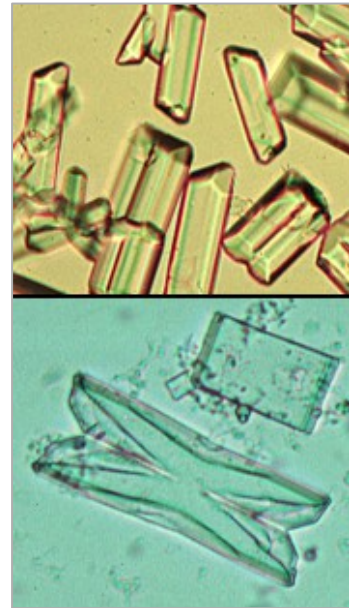
Crystals that have been observed in elephant urine include calcium carbonate, magnesium ammonium phosphate (struvite), calcium oxalate, and amorphous phosphate (Wiedner 2009).

Struvite

Struvite crystals (magnesium ammonium phosphate, triple phosphate) usually appear as colorless, 3-dimensional, prism-like crystals ("coffin lids"). Occasionally, they instead resemble (vaguely) an old-fashioned double-edged razor blade (lower frame).

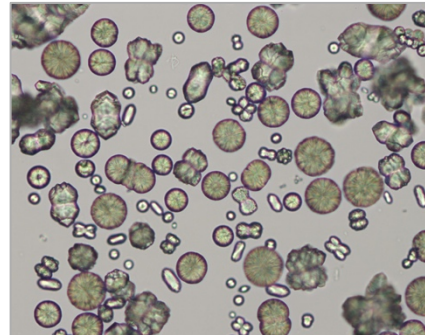
Struvite crystals are the most common type in urine from dogs and cats. They are often seen in urine from clinically normal individuals. Though they can be found in urine of any pH, their formation is favored in neutral to alkaline urine so they may be seen in elephant urine which is alkaline.

Urinary tract infection with urease-positive bacteria can promote struvite crystalluria (and urolithiasis) by raising urine pH and increasing free ammonia.



Calcium carbonate

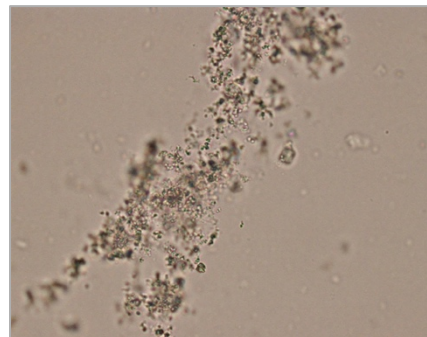
Calcium carbonate crystals are variably sized crystals that frequently appear as large spheroids with radial striations. They can also be seen as smaller crystals with round, ovoid, or dumbbell shapes. They are colorless to yellow-brown and can impart a brownish tinge to the urine, when they occur in high numbers.



Amorphous crystals

Amorphous crystals appear as aggregates of finely granular material without any defining shape at the light microscopic level. They can be comprised of urates, phosphates or xanthine. Amorphous urates (Na, K, Mg, or Ca salts) tend to form in acidic urine and may have a yellow or yellow-brown color.

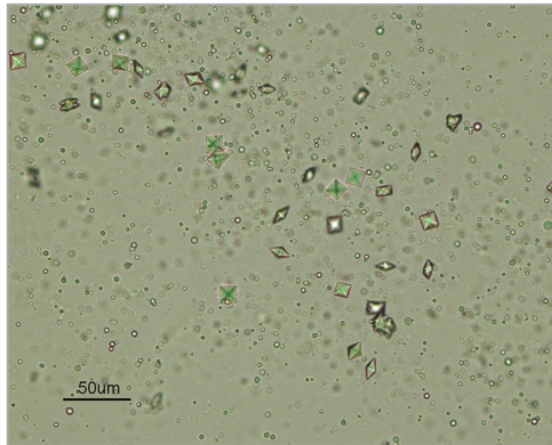
Amorphous phosphates are similar in general appearance, but tend to form in alkaline urine and lack color. Xanthine crystals are usually in the form of "amorphous" crystals. Generally, no specific clinical interpretation can be made based on the finding of amorphous crystals. Small amorphous crystals can be confused with bacterial cocci in some cases, but



can be distinguished by Gram-staining. Degenerating crystals or cells can also resemble "amorphous" crystals.

Calcium oxalate dihydrate crystals

Calcium oxalate dihydrate crystals typically are colorless squares whose corners are connected by intersecting lines (resembling an envelope). They can occur in urine of any pH. The crystals vary in size from quite large to very small. In some cases, large numbers of tiny oxalates may appear as amorphous unless examined at high magnification. These crystals are often seen in normal urine from domestic animals and can also be an artifact of storage (they can develop in stored urine), emphasizing the need to perform a urinalysis on fresh urine samples.



Drug crystals

Many drugs excreted in the urine have the potential to form crystals. Hence, a review of the patient's drug history is prudent when faced with unidentified urine crystals. Most common among these are the sulfa drugs.

Contaminants

Sperm in Urine

They may be seen in urine from males. 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

Casts were not observed in elephant urine in the study by Wiedner 2009. For a general discussion of casts see <https://eclinpath.com/urinalysis/casts/>.

K. Hematology Report Form

ELEPHANT HEMATOLOGY REPORT FORM

Elephant name: _____

Elephant ID: _____ Sex: ____ F ____ M ____

Health status: Normal ____ Abnormal ____

Date of collection: Day: ____ Month: ____ Year: ____ Time of collection: ____

Name of person collecting sample: _____

Activity during collection: Calm ____ Active ____ Excited ____

Collection site: Ear ____ Saphenous ____ Cephalic ____

Type of sample collected: EDTA ____ Serum ____ Na citrate ____ Other ____

Storage conditions: Room temperature ____ Refrigerated ____ Other ____

Sample deteriorated: Yes ____ No ____

Date of analysis: Day: ____ Month: ____ Year: ____ Time: ____

Person performing analysis: _____

Appearance of serum: Clear ____ Icteric ____ Lipemic ____

Hemolyzed 1+ ____ 2+ ____ 3+ ____ 4+ ____

Hematology Results

Hct: ____

Total Protein (refractometer): ____

Total WBC (Manual): ____ Automated: ____

Differential: Lymphs Neutrophils Monocytes Eos Basophils

%

/mm³

Estimated platelets/hpf: ____

Blood parasites seen: yes ____ no ____ Describe: _____

Estimated WBC from buffy coat: Normal ____ High ____ Low ____

Estimated WBC from slide (average # WBCs / 45-50 X field) x (1500): ____

Comments:

L. Urinalysis Report Form

ELEPHANT URINALYSIS REPORT

Elephant name: _____

Elephant ID: _____ Sex: __ F __ M __

Health status: Normal ____ Abnormal ____

Date of collection: _____ Time of collection: _____

Name of person collecting sample: _____

Activity during collection: Calm ____ Active ____ Excited ____

Sample collection: Beginning ____ Mid-stream ____ End ____

Storage conditions: Room temperature ____
Refrigerated ____
Other ____

Sample appearance: Clear ____ Slightly cloudy ____ Cloudy ____
Opaque ____ Flocculent ____

Color: colorless ____ light to medium yellow ____ dark yellow ____
red ____ reddish brown to brown ____
greenish tint ____ other (describe) _____

Specific gravity (refractometer): _____

Dip Stick Evaluation

Leukocytes cells / μ L _____
Nitrite _____
Urobilinogen μ mol/L _____
Protein g/L _____
pH _____
Blood cells/ μ L _____
Specific gravity _____
Ketones mmol/L _____
Bilirubin _____
Glucose mmol/L _____

Microscopic Sediment Examination

WBC / hpf _____
RBC / hpf _____
Cocci / hpf _____
Rods / hpf _____
Crystals _____
Epithelial cells _____
Other _____

Comments:

M. How to Make a Hand-Powered Paper Centrifuge

The hematocrit (HCT) is the percentage of red blood cells and is an indicator for anemia and dehydration. If a conventional HCT centrifuge is not available, an unconventional, hand-made substitute can be made easily. This device is based on a whirligig toy that most people may remember from their own childhood: a string loop with a small object in the middle, which spins around by pulling the string ends in the right rhythm.

The Paperfuge described here can hold HCT capillary tubes with a length of 40 mm, preferably heparinized (e.g., HemoCue America StatSpin™ Glass Microhematocrit Tubes). If larger tubes are used (74 mm), the size of the paper disks needs to be enlarged, which will reduce the accuracy.

How to make the Paperfuge

The Paperfuge is made of the materials displayed below.

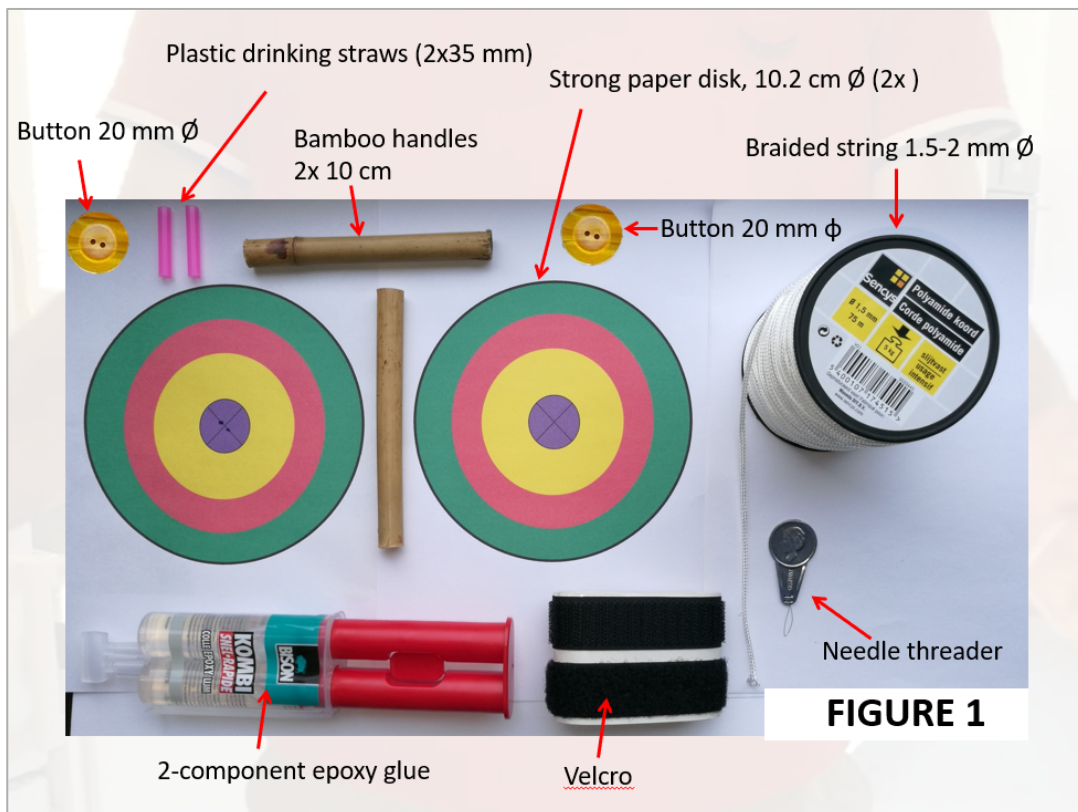


Figure 2. Printable page for making one Paperfuge. If printed on A4-sized paper, the disk diameter will be 10.2 mm.

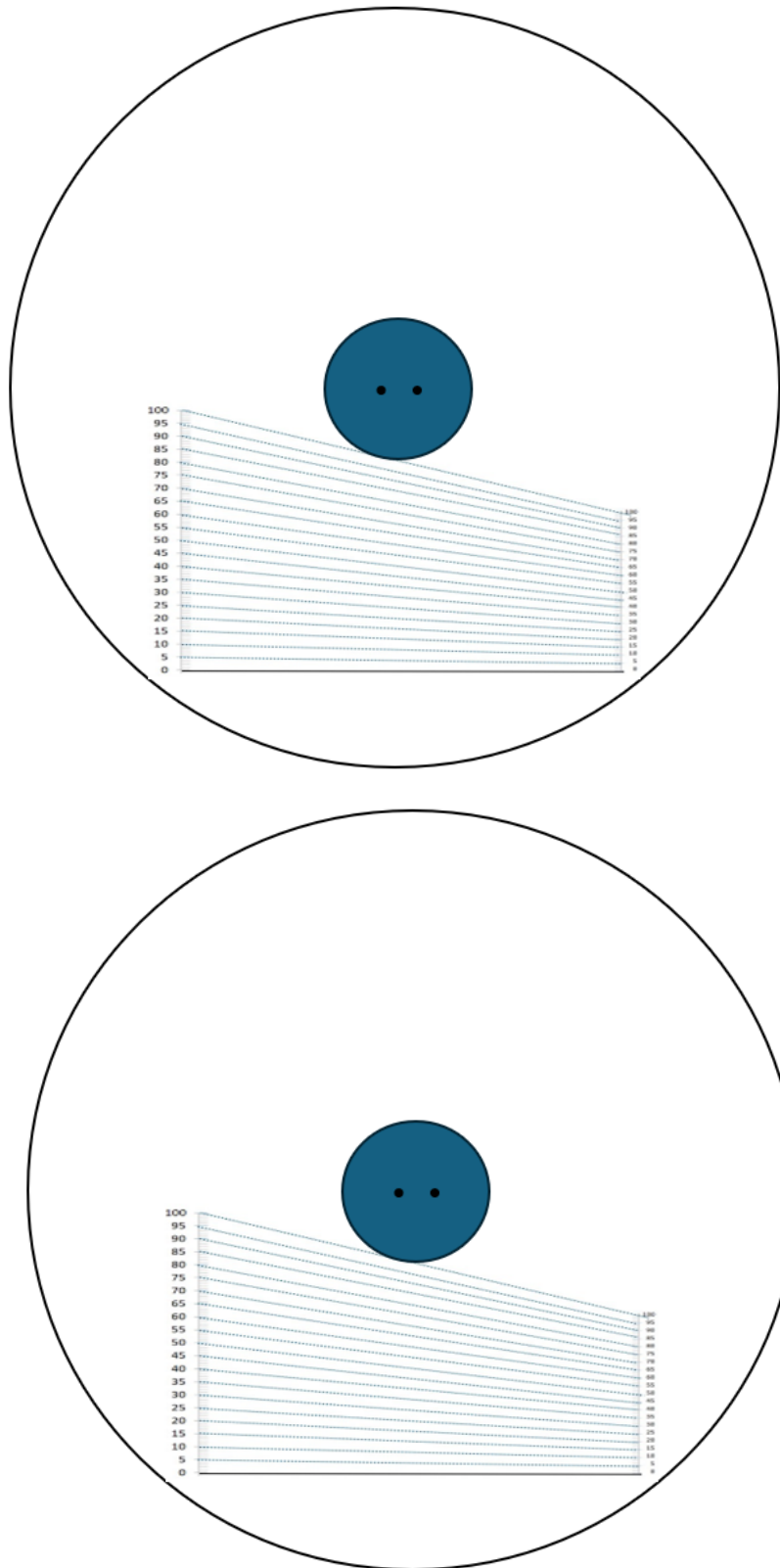


Figure 3 (below). Closed "PAPERFUGE", showing the paper disk, the button with 2 holes, the braided string and the cross mark; another cross mark is on the underlaying disk at the corresponding location.

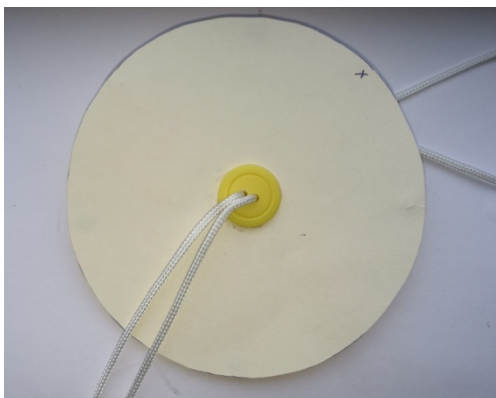


Figure 4 (below). Opened "PAPERFUGE", showing the cross-marks, 2 drinking straws, 4 pieces of Velcro and the braided string.

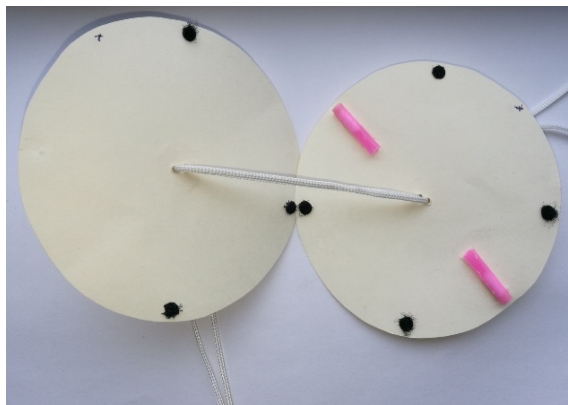
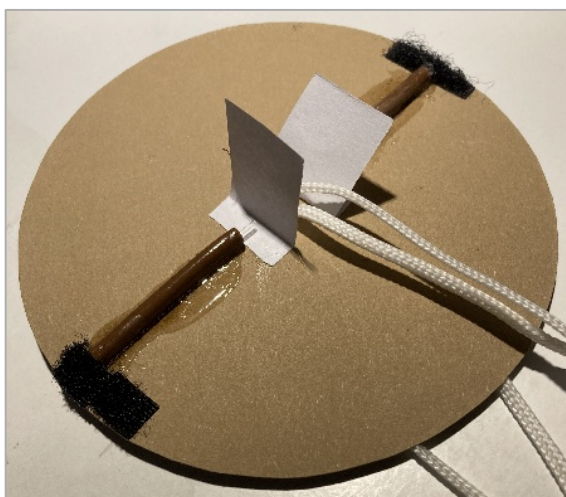
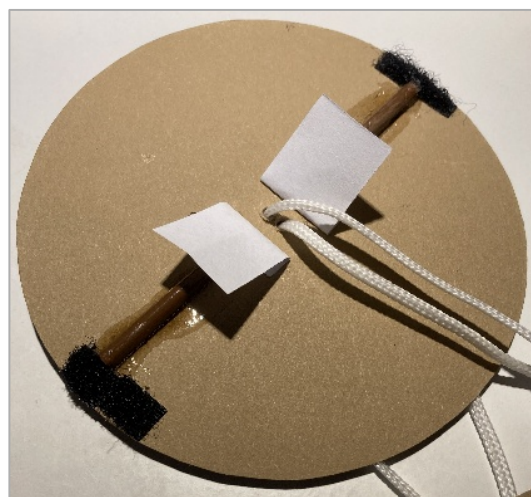


Figure 5 (below). To prevent the microtubes from falling out of the Paperfuge at the start and end of spinning, a small piece of paper is glued to the paper disk. Once the tube is in place, the paper is folded over it and kept in place by the opposite paper disk.



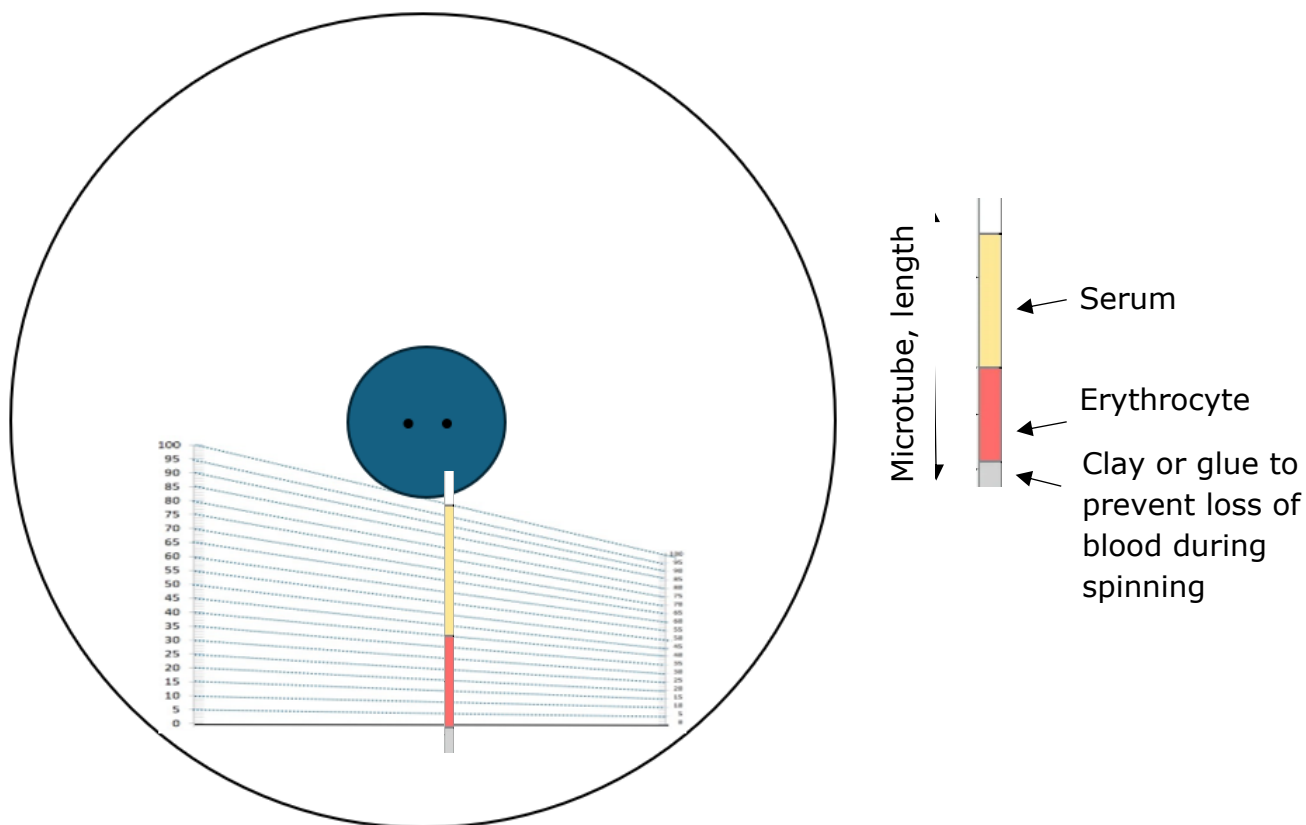
See detailed instruction for creating this Paperfuge on the next page.

Step-by-step instructions for creating a Paperfuge:

1. Draw 2 circles on a strong paper (10.2 cm diameter) and mark 2 holes (figure 1).
2. Cut out the paper disks and prick the 2 holes in each of them as indicated in figure 2.
3. Cut two 35 mm cm cane ends.
4. Cut 2 or 4 pieces of Velcro from the 2 different strips.
5. Mark the places where you will glue the cane ends and the Velcro on the inner side of the paper disks and put a cross-mark (figure 3) on the inner and outer side of each paper disk exactly opposite to each other. Make sure that the 2 holes in the buttons are in line with each other.
6. Glue the buttons on the outside in the middle of each disk.
7. Close one end of each cane by filling it with a droplet of 2- component epoxy glue.
8. Glue 2 cane ends on the inner side of one paper circle as indicated in figure 4.
9. Glue the Velcro (2 or 4) on the inner side of each paper disk exactly opposite to each other. If Velcro is not available, paper tape can be used instead. However, this will reduce the lifespan of the device.
10. Glue 2 pieces of paper near the open end of the canes (figure 5).
11. Cut a 150 cm long string.
12. Cut 2 pieces of bamboo (10 cm long).
13. Lead the string through the holes in the buttons carefully using the needle threader (or with the help of a very thin thread).
14. Hold the string ends in a flame for a few seconds.
15. Tie the string ends together.
16. Secure the end of each string loop to a bamboo stick.

Reading the hematocrit

In the example shown on the next page, the bottom of the erythrocytes must be aligned with the 0%-line, while the top of the serum must be aligned with the 100%-line. The result is: HCT =41-42%.



Reference

Bhamla, M. Saad and Benson, Brandon and Chai, Chew and Katsikis, Georgios and Johri, Aanchal and Prakash, Manu. 2016. An ultra-low cost, hand-powered centrifuge inspired by the mechanics of a whirligig toy. *Nature Biomedical Engineering*

<https://www.biorxiv.org/content/early/2016/08/30/072207.full.pdf>

N. Proper Use and Care of Microscopes

CLINICAL HOW-TO / PEER REVIEWED



Proper Use & Care of Microscopes

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Fort Valley, Georgia

Microscopes are used daily in veterinary practice to view various biologic products (eg, blood, urine, fecal material, fluid aspirates, skin scrapings, ear cerumen). Team members should be familiar with proper techniques for viewing different specimens and the use of each lens objective (eg, 4x, 10x, 40x, 100x). The microscope ocular lenses, objective lenses, stage, and stage clips should be cleaned after each use, and the body of the microscope should be wiped down and inspected at the end of each shift after its last use of the day.

See related article, **The Surprising Cost of Skipping Practice Equipment Maintenance**, page 34

Microscope Supplies

The following are suggested cleaning supplies for daily microscope care.

- Cotton tip applicator
- Distilled water
- Suction bulb or ear balloon
- Kimwipes or lens tissue
- Microfiber or other soft cloth
- Optical cleaning solutions
- Soft brush

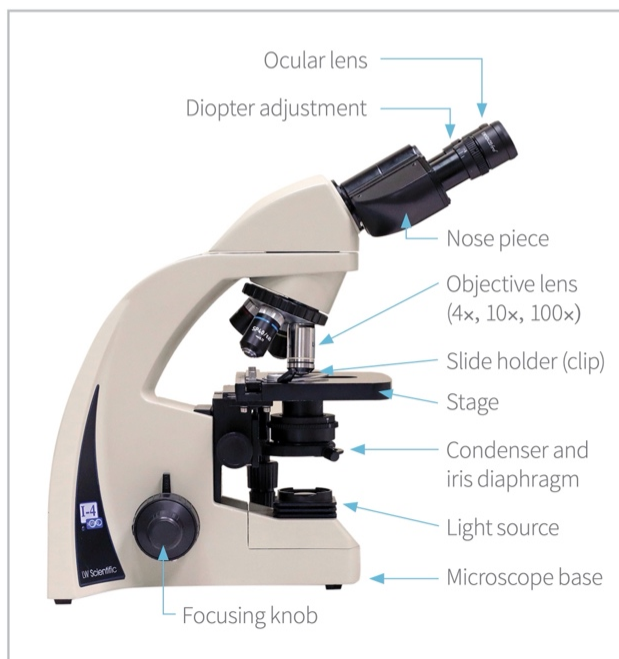
Samples viewed on an improperly cleaned microscope are at risk for contamination, which can lead to erroneous results and misdiagnosis. Because samples may contain transmissible disease organisms, veterinary team members should wear gloves when handling slides, and slides should be disposed of according to practice protocol.

Microscope Basics

Microscopes in veterinary practice typically use 10× ocular lenses with 4×, 10×, 40×, and 100× (oil immersion) objective lenses. Microscopes may have multiple eyepieces to allow more than one team member to view the same slide simultaneously. Objective lenses are housed in a nosepiece that can be rotated to select each lens. Slides are placed on the stage below the objective lens and held in place with metal clips. (See **Figure 1.**)

Examination of Samples

Blood, urine, and fecal material are some of the most common substances viewed under the microscope at a veterinary practice. Blood prepared as a smear is stained to view cell morphology. Stained blood smears must be completely dry before examination under oil immersion. Begin by examining cells at 10× and graduate through the objectives, stopping at each to focus using the fine focus dial and light adjustment for sharp results. The iris should be open to allow good lighting of the specimen but may need to be adjusted periodically during scanning. Identifying certain cells at 10× or 40× may be possible, but the 100× oil immersion lens likely will be used to evaluate individual cells. When using a hemocytometer, blood is most often diluted to visualize and count cells manually using 10× or 40×, and light should be adjusted to a lower standard to show contrast between cells and background.



▲ **FIGURE 1** Parts of a microscope

A damaged microscope should be removed from service immediately to prevent injury to team members.

Fecal material is prepared as a coverslipped wet mount and examined for intestinal parasites and ova using a direct smear or concentration (ie, flotation or sedimentation) technique. For any preparation, begin at 10×, focus, and then move to 40×. Using the oil objective lens is not necessary.

Urine sediment is prepared as a coverslipped wet mount and examined to identify cells, crystals, casts, parasitic ova, or blood in the same manner as fecal evaluations (10×, 40×). Staining is discouraged because many of the available stains readily grow bacteria and form crystals that can be misleading when evaluating the sediment.^{1,2}

Solid materials (eg, skin scrapings, cerumen) are suspended in a small drop of mineral oil on a slide with a coverslip. The 4× or 10× objectives are used to identify skin or ear mites. Skin and ear cytology preparations to evaluate for bacteria, fungi, and cell morphology should be reviewed using the 10× and 40× objectives.²

Cytologic and microbial samples (eg, vaginal cytology, tissue and bone marrow aspirates, fluid aspirates) can be visualized microscopically for diagnostic purposes similarly to blood smears, beginning at 10× and working toward 100× oil immersion. (See **Table 1**.)

Cleaning the Microscope

The ocular lens, objective lenses, stage, and stage clips should be cleaned after every slide, and the entire microscope should be thoroughly examined and cleaned at the end of the day or at the beginning or end of each shift. Storing all the necessary cleaning supplies in one central location near the microscope helps ensure

that cleaning is completed as scheduled. (See **Microscope Supplies**.)

Start by inspecting the cord for frayed wires or damage. A damaged microscope should be removed from service immediately to prevent injury to team members. To gain access to the objectives for cleaning, lower the stage or raise the arm, depending on the type of microscope being used. Moisten cotton tip applicators with the appropriate cleaning solution and gently clean each objective lens and wipe dry with new lens paper.^{1,3}

TABLE 1 Sample Requirements for Common Microscopy Samples

Examination	Objective Lens	Coverslip (Y/N)	Staining (Y/N)
Blood smear	10×, 40× 100× (oil)	N N	Y Y
Blood, diluted	10×, 40×	N (use a hemocytometer)	N
Urine sediment	10×, 40×	Y	N
Fecal material	10×, 40×	Y	N
Skin scraping	10×, 40×	Y	N
Fluid, bone marrow, and tissue aspirates; ear, skin, and other cytology samples	10×, 40× 100× (oil)	N N	Y Y
Vaginal cytology	10×, 40×	Y or N ^a	Y or N ^a

^aVaginal cytology slides can be viewed after drying/fixation, after air-drying/staining and nonfixed, or examined as a stained and coverslipped wet mount.²

TABLE 2 | **Cleaners Recommended for Microscopes**

Cleaning Product	Microscope Component	Indication/Notes
Cotton applicators or Kimwipes/lens tissue moistened with distilled water	Objective and ocular lenses	Water-soluble dirt
Compressed air	Ocular lens	Loose dirt and debris
Suction bulb/ear balloon/soft brush	Ocular lens	Loose dirt and debris
Xylol, acetone, 70% alcohol, or blue Windex may be used sparingly in the presence of nonwater-soluble dirt or oil; however, they may damage cemented optical components if used daily	Ocular lens	Mild to extreme nonwater-soluble dirt, oil
Ammonia solution (1 eye dropper of ammonia to ½ cup water). Do not use daily or routinely as lenses and cemented optical components may be damaged. ⁵	Objective lenses	Mild to moderate nonwater-soluble dirt
Xylol (xylene) ^a should be used only when sticky or gummy buildup requires a harsher cleanser. Do not use daily; xylol is carcinogenic and will corrode rubber and cemented optical components.	Objective lenses	Sticky, oily, waxy buildup
Commercial optical cleaning solutions	Objective and ocular lenses	Commercial optical cleaning solutions offered by microscope manufacturers are best; use daily according to manufacturer instructions
Warm, soapy water or a water-dampened cloth, and a cloth or lens paper to dry	Stage and stage clips	Best cleaned with warm water or warm soapy water and a microfiber cloth
Distilled water	Condenser/Diaphragm	Use distilled water on a microfiber cloth to wipe away dust, then dry
Dampened microfiber cloth with water and an airbrush to remove hair or fibers	Light source	Allow to cool before cleaning; do not clean while hot
Warm, soapy water	Metal body/framework	Salt, sugar, and general residue
Microfiber cloth	Body	Moistened with soapy or plain water

^aCarcinogenic—use gloves and avoid breathing fumes.

A well-maintained microscope ensures that samples are free from contamination and results are accurate.

Use distilled water to remove water-soluble debris from the microscope lenses and body, and dry with lens paper.^{1,3} Use a solvent such as blue Windex or xylol for nonwater-soluble material.³ (See **Table 2**.) Wipe the eyepiece(s) with Kimwipes or lens paper moistened, not soaked, in 70% ethanol before drying.^{1,3,4} Use a suction bulb to remove any hair or fibers to prevent scratching.

Look through the eyepiece(s) and rotate each one to ensure that no debris remains on the ocular lens. If it is necessary to remove the eyepiece to clean the inner glass surface, never “probe” into the tube housing; instead, place a lens cap or piece of aluminum foil over the opening while cleaning the eyepiece.⁵

When working with Fecasol, sugar, or other fecal floatation solutions, the microscope stage, clips, and other areas that may be splashed with solution should be cleaned with a water-moistened Kimwipe to prevent the residue from attracting pests and dust. After saturated sodium chloride is used, take care to clean the microscope because this substance will corrode and damage metal.

Wipe the entire microscope body, including the light source, with a warm, moist microfiber cloth. Center the 4× objective over the stage, turn the power off, ensure it is dry, and drape the dust cover over the microscope.

Microscopes should be serviced annually by a professional microscopy service, and lenses and bulbs should be replaced as needed.

Conclusion

All veterinary team members responsible for preparing and viewing microscopy samples should be familiar with the different components of the microscope and the viewing requirements for each biologic substance. A well-maintained microscope ensures that samples are

free from contamination and results are accurate. Daily cleaning and maintenance also protect the practice’s investment in this valuable resource. ■

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TAKE ACTION

- 1 Educate all team members responsible for preparing and viewing microscopy samples about a microscope's components and the viewing requirements for each biologic substance.
- 2 Emphasize the importance of microscopes being well-maintained on a daily basis to ensure samples are contamination-free and results are accurate.
- 3 Service microscopes annually using a professional microscopy service, and replace lenses and bulbs as needed.

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