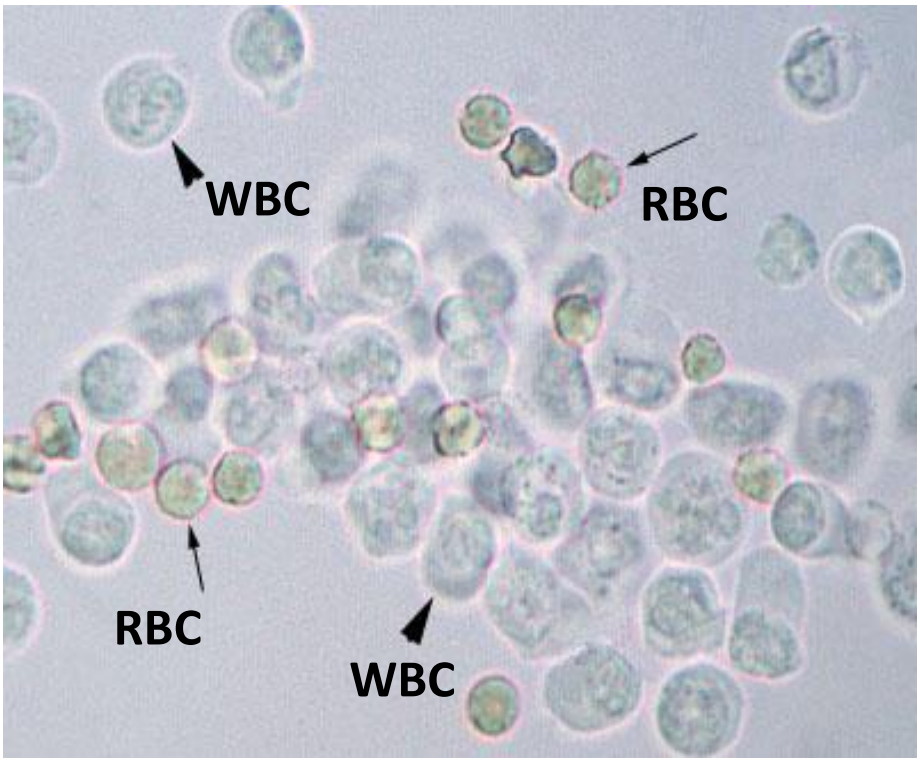
ELEPHANT HEMATOLOGY AND URINALYSIS LAB MANUAL



Elephant Care Asia
Healthcare and Welfare Workshop
Green Hill Valley Myanmar
September 2018

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Preparing and Staining a Blood Smear

Materials:

Blood filled capillary tube or EDTA (purple top) tube

Applicator sticks

Lens cleaner and lens paper

Glass microscope slides (clean in 70% alcohol and air dry)

Dip-quick stains

Pencil to mark slides

Immersion oil

Blood smears should be prepared as soon as possible after collection. Delay can result in changes in parasite morphology and staining characteristics. Thick smears are used to search for blood parasites; thin smears are used for differential white blood cell counts. Under field conditions, if slides are scarce, you can prepare both a thick and a thin smear on the same slide just make sure that only the thin smear is fixed.

Thin smears

1. In thin smears the blood is spread in a layer such that the thickness decreases progressively toward the feathered edge. In the feathered edge, the cells should be a monolayer, not touching one another.
2. Clean the slide and label with the elephant's ID and the date
3. Gently tap the end of the capillary tube or invert the EDTA tube several times and use an applicator stick to place a drop of blood (2-4 mm in diameter) on the slide near its frosted end.
4. Spread the drop by using another slide (called here the "spreader"), placing the spreader at a 30 - 45° angle and BACKING into the drop of blood.
5. As soon as the blood has spread along the edge of the spreader slide quickly push the spreader slide forward. This action pulls the blood.
6. Make sure that the smears have a good feathered edge. This is achieved by using the correct amount of blood and spreading technique.
7. Allow the smears to air dry.
8. Fix the smears by dipping them in absolute (100%) methanol being careful not to dip the frosted end with the ID information.
9. If you are preparing one smear per slide, the spreader then becomes the next slide to receive a smear. Each slide thus serves two duties, as a spreader, then as a slide to receive a smear. If two smears are made per slide, be sure to flip over the spreader to use the other edge for the second smear produced. The spreader then is used to receive the next two smears. If there is surplus

blood on the spreader, wipe it off carefully before flipping it over to make the second smear on the slide.

Common causes of a poor blood smear

1. Drop of blood too large or too small.
2. Spreader slide pushed across the slide in a jerky manner.
3. Failure to keep the entire edge of the spreader slide against the slide while making the smear.
4. Failure to keep the spreader slide at a 30° angle with the slide.
5. Failure to push the spreader slide completely across the slide.
6. Waiting too long after placing the drop of blood on the slide.

Staining the Slide

The Jorvet Dip Quick Stain is a quick and easy stain that gives comparable results to the Wright-Giesma method. These polychromic stains will color acid groups blue (DNA/RNA), basic groups orange (protein eosinophil granules), and metachromic substances violet (mast cell and basophil granules). It is a valuable stain for blood cell differential count and evaluations. It also works well for general diagnostic cytology.

1. The slide is allowed to air dry.
2. After air drying, dip the slide repeatedly in the wide mouth bottle marked #1 fixative for a total of 5 seconds. Hold the slide up over the bottle and allow the excess fixative to drain off.
3. Dip the slide repeatedly in the bottle with component #2 for a total of 5 seconds. Hold the slide up over the bottle and allow the excess stain to drain off.
4. Dip the slide repeatedly in the bottle with component #3 for a total of 5 seconds. Hold the slide up over the bottle and allow the excess stain to drain off.
5. Gently rinse with distilled water and gently blot dry with a paper towel.
6. Always close the lid tightly in the bottles to avoid evaporation loss.

Examining the smear

Use a systematic approach:

1. Scan the smear at low power (10 X objective) to find the optimal area for examination at higher power and to evaluate the distribution of WBCs
2. Perform the differential (see below)
3. Estimate platelet numbers (100 X)
4. Assess morphology (100 X) See appendix C. White Blood Cell Morphology

Thick smears for blood parasites

Thick smears consist of a thick layer of lysed red blood cells (RBCs). The blood elements (including parasites, if any) are ~ 30 times more concentrated than in an equal area of a thin smear. Thick smears are better for detecting some parasites. However, they do not permit an optimal review of parasite morphology. A thin smear may be needed for species identification.

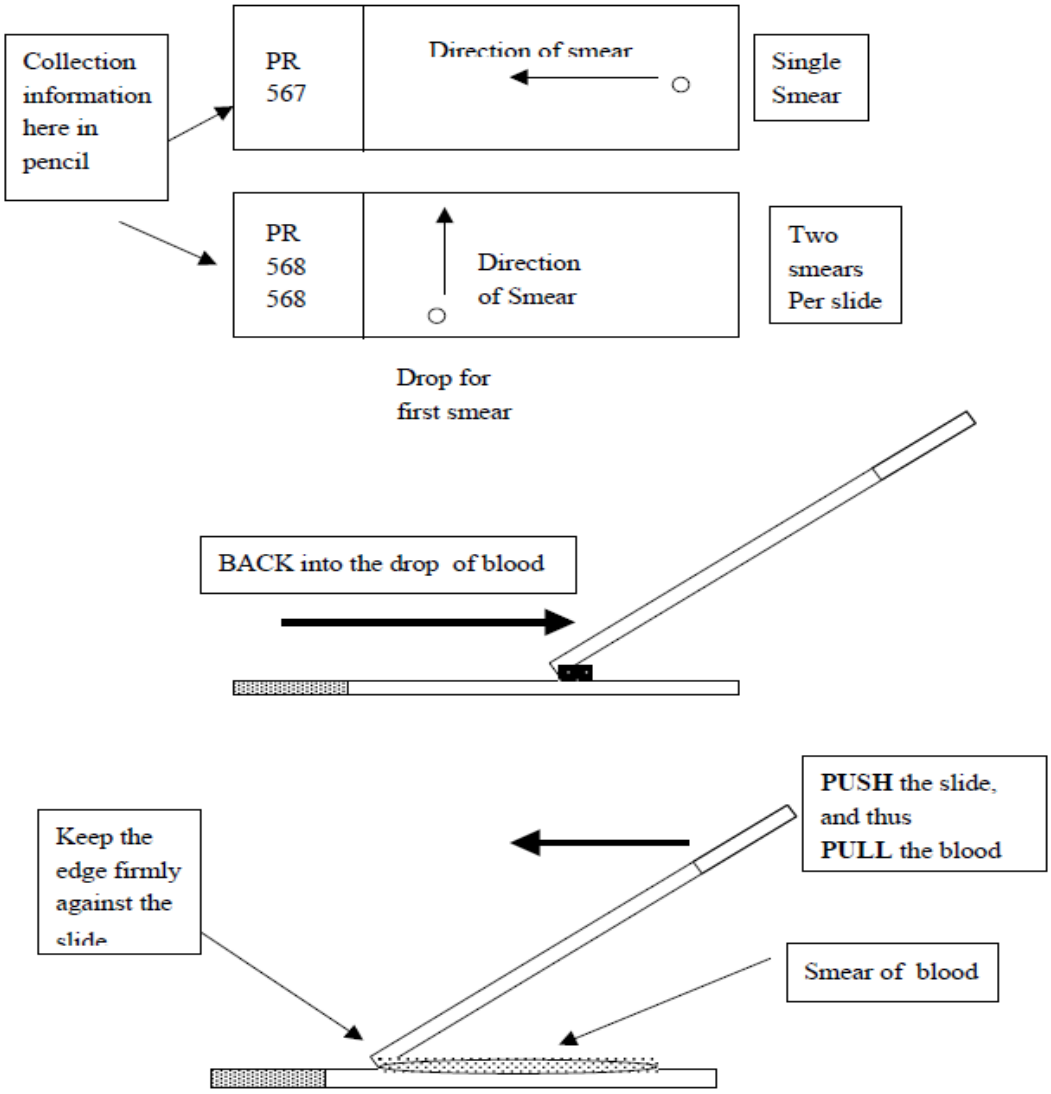
1. Place a small drop of blood in the center of the pre-cleaned, labeled slide.
2. Using the corner of another slide or an applicator stick, spread the drop in a circular pattern until it forms a small circle.
3. A thick smear of proper density is one which, if placed (wet) over newsprint, allows you to barely read the words.
4. Lay the slides flat and allow the smears to dry thoroughly; protect from dust and insects. Insufficiently dried smears (and/or smears that are too thick) can detach from the slides during staining. The risk is increased in smears made with anticoagulated blood. At room temperature, drying can take several hours; 30 minutes is the minimum; in the latter case, handle the smear very delicately during staining. Protect thick smears from hot environments to prevent heat-fixing the smear.
5. Do not fix thick smears with methanol or heat. If there will be a delay in staining smears, dip the thick smear briefly in water to hemolyze the RBCs.

Knott's Techniques for Detecting Microfilariae

1. Prepare 2% formaldehyde (2 ml of 37% formaldehyde + 98 ml H_2O).
2. Mix 9 ml of the 2% formaldehyde with 1 ml of EDTA blood.
3. Centrifuge at 500 × *g* for 10 minutes; discard supernatant. Sediment is composed of WBCs and microfilariae (if present).
4. Examine as temporary wet mounts.
5. Prepare thick and thin smears; allow to dry; stain and examine

References:

http://www.dpd.cdc.gov/dpdx/HTML/Frames/DiagnosticProcedures/body_dp_bloodprocess.htm
www.uvm.edu/~jschall/pdfs/techniques/bloodsmears.pdf
http://www.austincc.edu/mlt/phb/phb_unit9Lab10PreparationOfBloodSmearsFall2010.pdf


Collection information here in pencil
PR 567
Direction of smear
Single Smear
PR 568 568
Direction of Smear
Two smears Per slide
Drop for first smear
BACK into the drop of blood
PUSH the slide, and thus PULL the blood
Keep the edge firmly against the slide
Smear of blood

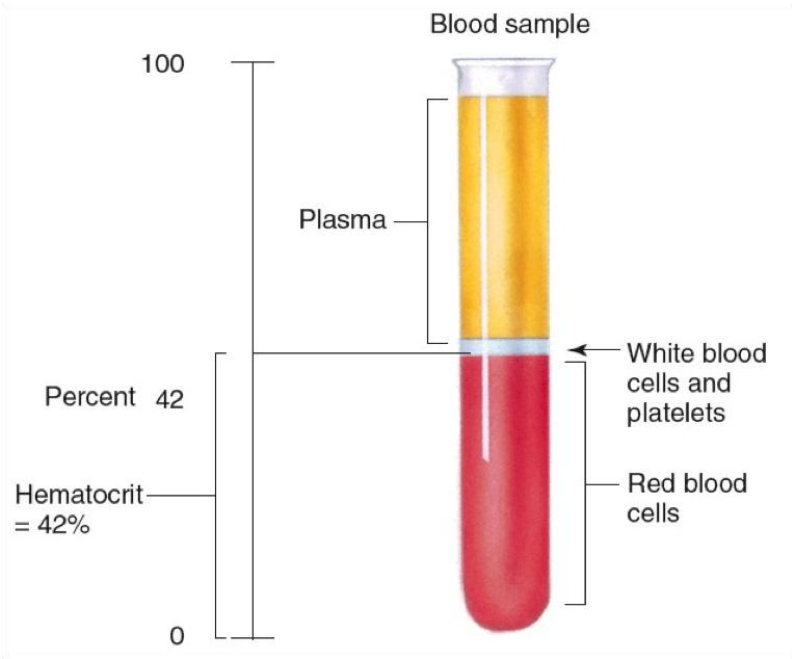
The Complete Blood Count (CBC)

A complete blood count consists of a red blood cell count, hematocrit, hemoglobin, WBC count, and a differential. 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 that this be done manually.

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

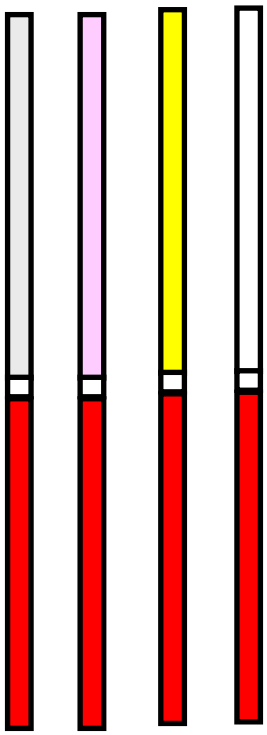
Measuring the Hematocrit

One of the most useful tests that we can run is the hematocrit. The hematocrit is the percent of blood comprised of RBCs. The packed cell volume is the most common method to determine the hematocrit. The PCV is obtained when anti-coagulated blood is centrifuged. The red blood cells become packed at the bottom of the tube, while the plasma is left at the top as a clear liquid. Serum contains all of the plasma proteins except fibrinogen, Factor V, and Factor VIII, which are consumed when the clot forms.



To run a hematocrit you need to use a centrifuge specifically designed for hematocrits or a regular centrifuge that has an adapter that can hold the hematocrit tubes. The hematocrit should be spun at 12,000 rpms. If you don't have access to a lab or a centrifuge there are two Do-It-Yourself versions that you can make.

Plasma Evaluation



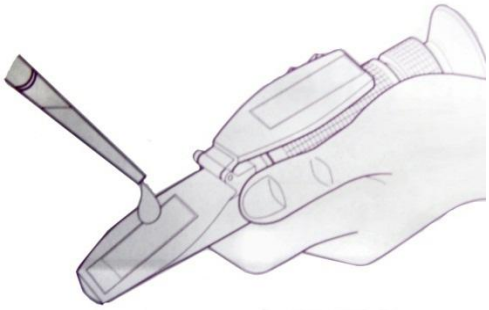
1. Evaluate color and transparency.
    a. Clear and colorless: normal
    b. Pink to red and clear: hemoglobinemia
    c. Yellow: icteric
    d. White to pink and opaque: lipemic
2. Look at the buffy coat the layer between the RBCs and the plasma and estimate the WBC.
3. Examine the plasma above the buffy coat for microfilaria.
4. Measure the total protein using the refractometer (see next section)
5. Record results.

Using a Refractometer to Measure Total Protein or Urine Specific Gravity

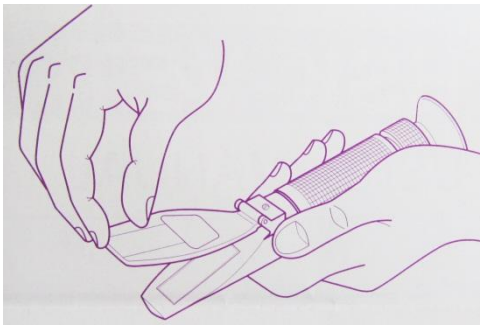
Refractometry can be used to measure plasma, serum, or body cavity protein.

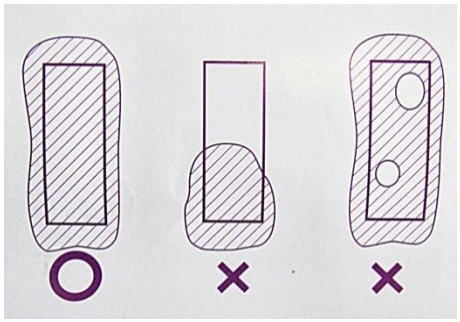
- Temperature-compensated, hand-held refractometers are calibrated to read protein directly in g/dl.
- Most units are temperature-compensated to give accurate readings between 16° C and 38° C (60°F to 100°F).
- Abnormally high concentrations of glucose, urea, sodium, or chloride may result in falsely high protein readings.
- Lipemia alters light transmission through the specimen, producing an indistinct line of demarcation and falsely high protein readings usually result.
- 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).

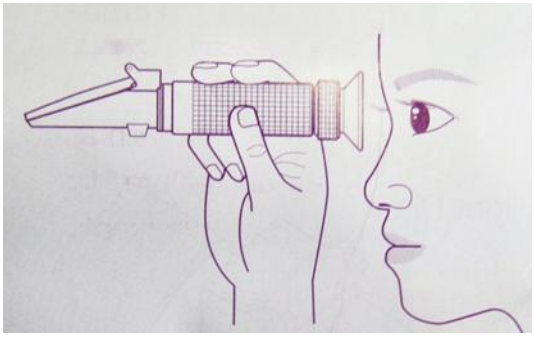


1. Place 1-2 drops of samples on the prism





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 sale

White Blood Cell Count Using the Whi-pette™

Materials:

Pre-filled amber tubes (included in Whi-pette® Kit)

10 microliter pipette and tips (included in Whi-pette® Kit)

Anti-coagulated (EDTA) whole blood sample

Microscope

Hemacytometer

Procedure

1. Draw 10 µl of blood using a fresh pipette tip attached to the microliter pipette. Wipe any excess blood from the outside of the pipette tip with a lint free wipe. Don't forget to depress the pipette half-way down when drawing the sample and all the way down when dispensing the sample.

2. Dispense the sample into an amber tube and flush the pipette several times using the solution in the tube. Be careful not to spill any of the solution.

3. Cap the tube and mix thoroughly.

4. Allow to set 5 minutes to permit the stain to penetrate the cells. During this time set up the hemacytometer and position the cover slip.

5. Use the flushed pipette tip to instill just enough sample to fill both sides of the counting chamber. Take care that the counting area is completely filled and not allowed to overflow into the moat. Should this occur the hemacytometer should be cleaned and recharged before proceeding.

6. Let the sample to sit in the counting chamber for 5-10 minutes before counting (allow cells to settle).

7. Using 100X magnification, count the cells in the 9 large squares on both sides of the chamber. The count should proceed in an orderly fashion, starting at one end of the square, going across to the other side, then down one microscope field and back across until all cells within the square are counted.

Cells that are touching the line between two squares are counted with that square if they are touching either the top or the left line. Do not count cells touching either the bottom or right lines.

8. The totals from each side should be within 10% of each other.

9. Take the total of both sides of your counting chamber and enter the numbers into the equation below:

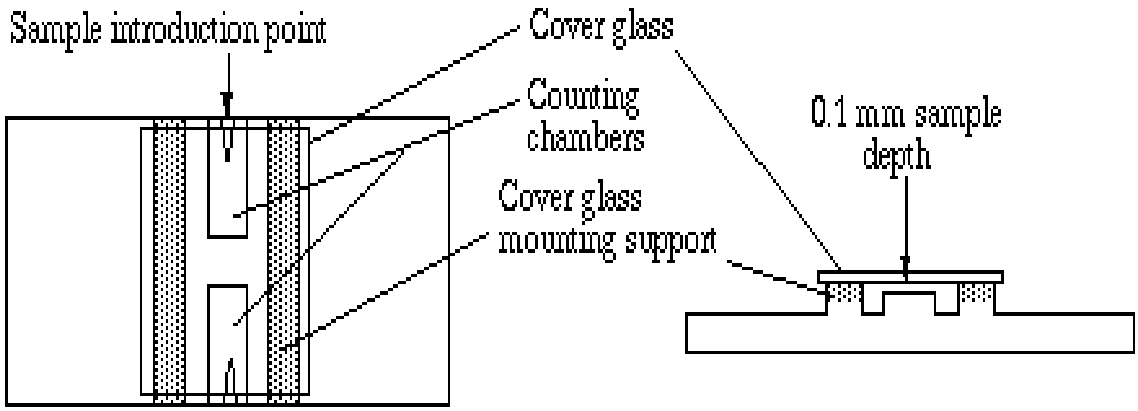
(Side A + B/2) x 110

Example: (78 + 82/2) x 110 = 8,800 µl

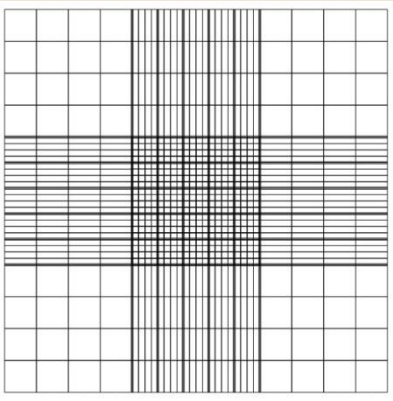
How to Use a Hemacytometer Counting Chamber

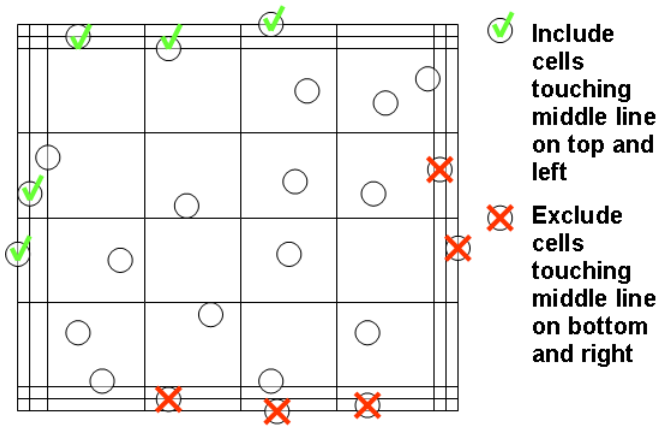
A hemacytometer is a counting chamber used to determine the number of blood cells per unit volume of a suspension. It can be used to count RBCs, WBCs or platelets. Staining of cells facilitates visualization and counting. Cell counts require a properly collected and anti-coagulated blood sample that is fresh and well mixed. If the sample has been refrigerated, it should be warmed to room temperature and remixed. The blood is then diluted using either glass blood dilution pipettes or the Whi-pette™ system. We will use the Whipette™ system in this lab.

The hemacytometer contains two raised bridges which hold the coverslip and two counting areas that are completely surrounded by a moat. Coverslips for counting chambers are specially made and are thicker than those for conventional microscopy. They should be kept with the hemocytometer and not used for other purposes.



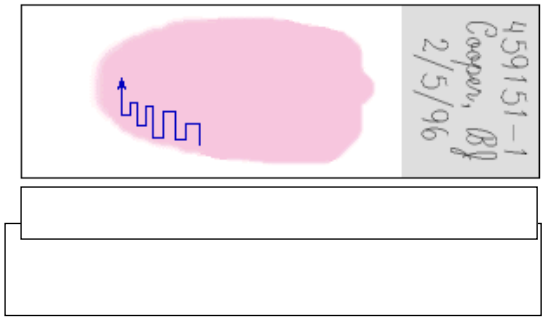
The sample counting area contains a ruled grid, most commonly Neubauer ruling. Neubauer ruling consists of 9 large squares, each measuring 1 mm^2. The depth of the chamber (distance between the grid area and the coverslip) is 0.1mm. The squares are further subdivided into smaller squares. The center square is divided into 400 small squares arranges as 25 groups of 16 each. One entire grid Neubauer rulings can be seen at 40x (4x objective).





Differential White Blood Cell Count

1. The accuracy of the differential count depends on a well prepared blood smear. If too thick, the cells are difficult to differentiate; if too thin the distribution may not be even.
2. After staining, scan the smear at low magnification (10X) to find the best area to examine at higher magnification and check the feathered edge.
3. Select a thin area of the smear where the cells are evenly distributed. Examine at high dry magnification (40X) or oil (100X); oil may be necessary to adequately see all the cell features.



4. Begin the differential count by moving back and forth across the smear in a pattern that avoids covering the same territory. Identify each leukocyte 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.
5. The % of each cell type is the relative differential count.
6. The % of each WBC type multiplied by the WBC count gives the absolute number of that WBC type/µl of blood. The cumulative total of all the absolute cell counts should equal the total WBC.

How to Use the Cell Counter

1. Make sure all the counter units are set at 000. To do this rotate end knobs one time towards the operator. Mark the cell types to be counted below each window.
2. Press down only 1 key 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. On the ring the number shown on each individual counter directly corresponds to the % of that cell type counted.
4. 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. Counts can be continued to a total of 300 or 40-0 when more precise counts are needed

Care of the Cell Counter

1. 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.
2. Cover the cell counter when not in use to protect form dust.
3. Never lubricate the counter.

Urinalysis

The routine urinalysis is a quick and relatively inexpensive test which can be readily performed in a field laboratory. Ideally urine should always be collected at the same time as blood for hematology and clinical chemistry (if possible) 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.

Equipment and Materials

Microscope

Refractometer

Slides

Urinalysis dipsticks

Urine sediment stain

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.

Some examples of various urine colors and corresponding common possible causes:

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 small 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 (↓ 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 ↑ urine S.G.

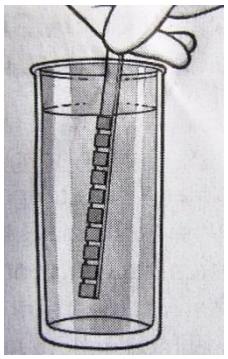
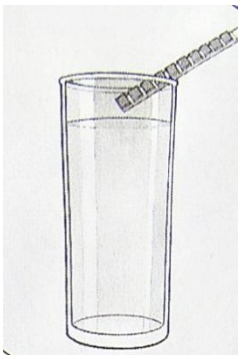
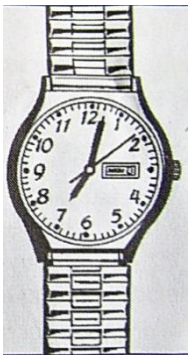
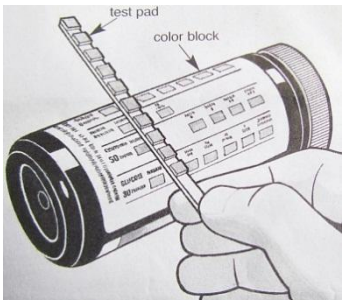
Knowledge of the hydration status is necessary to interpret the S.G. Urine S.G. can vary from 1.001 to 1.065 in most healthy animals but this range also includes values associated with renal abnormalities. S.G. has been inadequately studied in elephants and obtaining a baseline during health and evaluating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

- Mix the urine
- Dip the dipstick ; remove extra urine
- Start timing
- Compare to chart on bottle in good light

7. Urine sediment examination

1. Centrifuge a fresh urine sample for 5 minutes at about 1500 RPM
2. Remove supernatant
3. Add 1-2 drops of stain to sediment
4. Tap the bottom of the tube to mix
5. Transfer one drop to a slide and place a coverslip
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

Banking Serum

The value of banking serum is immense – enabling retrospective disease investigations, applied research, and testing to facilitate animal movements. The following are a few basic tips in setting up a serum bank:

- If -20 freezers are used, make sure they don't have an automatic defrost cycle.
- If -70 freezers are used, make sure there is a plan posted on the freezer for how to respond to an alarm.
- Have back-up generators or a CO2 tank back up for -70 freezers if possible.
- Have an alternate plan if the freezer and backups fail.
- Make sure nothing goes in the freezer that has not been properly logged.
- Make sure a copy of the logbook always stays with the freezer.
- Make sure cryovial labels are permanent, legible, and standardized.
- Use the same sized vials in grid boxes and racks for efficient space use and to facilitate retrieval and backfilling of spaces that become empty through use.
- If infrastructure problems (like frequent power outages) are a concern, consider spots of blood on Whatman FTA cards as your archive backup, if not your primary archive.

The following information should be included in the serum logbook:

- Date sample collected
- Elephant name
- ID #
- Sex Age
- Owner
- Volume of serum
- Quality of serum (clear, hemolyzed, slightly hemolyzed, very hemolyzed)
- Grid #
- Date out
- Recipient
- Purpose
- Comments

Appendix A. Causes of Anemia

Blood Loss

Acute	Chronic
Trauma / surgery	Parasites (strongyles, liver flukes)
GI ulcer / hemorrhage	Hematuria (blood in urine)
Toxin (e.g. rat poison)	Neoplasia
D.I.C.	Thrombocytopenia
	Vitamin K deficiency

Blood Destruction

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

Decreased Blood Production

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

Appendix B. Causes of Increased and Decreased WBCs

↑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

↑ Eosiniphils	↑ Monocytes
Parasite infections	Chronic infections (TB)
Hypersensitivity reactions	Chronic inflammatory bowel disease
Leukemia (not reported in elephants)	Other granulomatous diseases

Appendix C. Elephant White Blood Cell Identification

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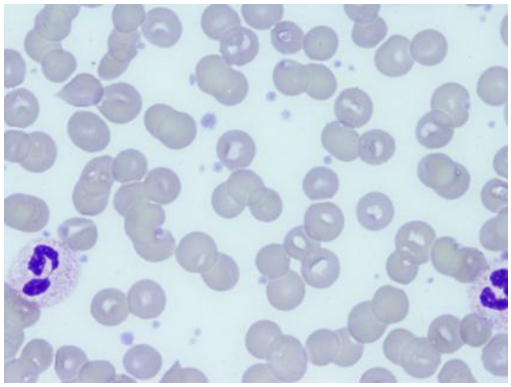

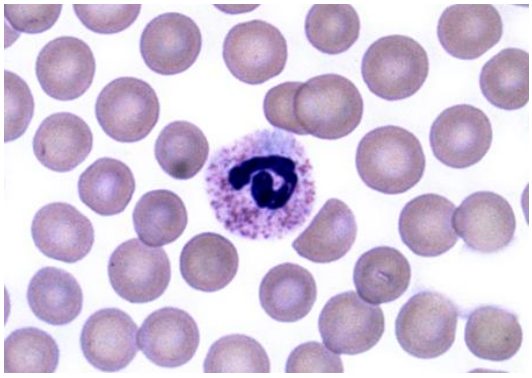

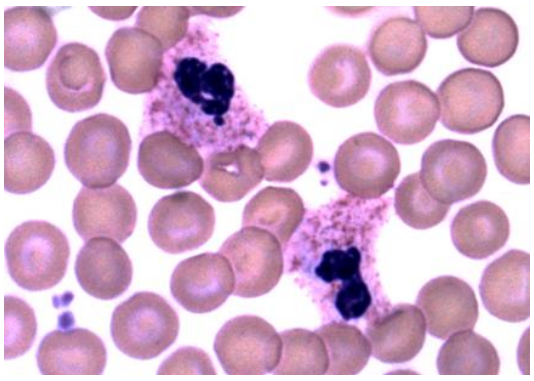

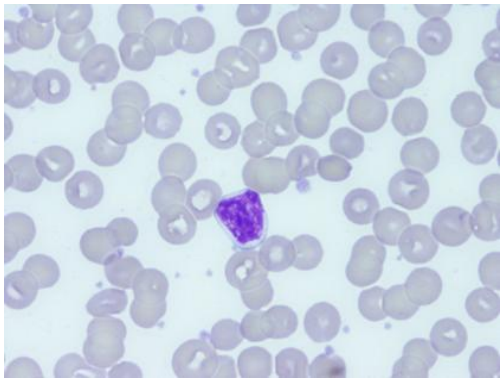

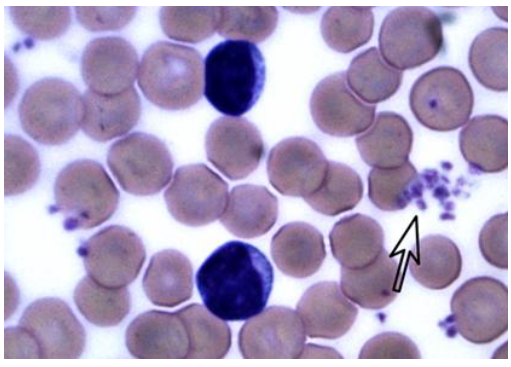

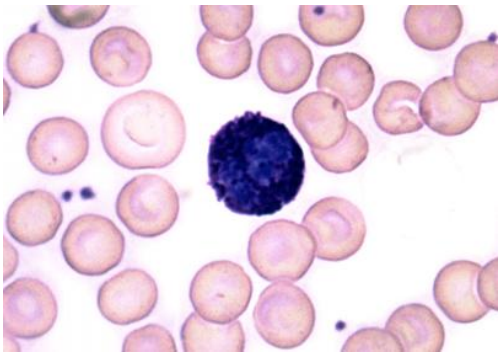

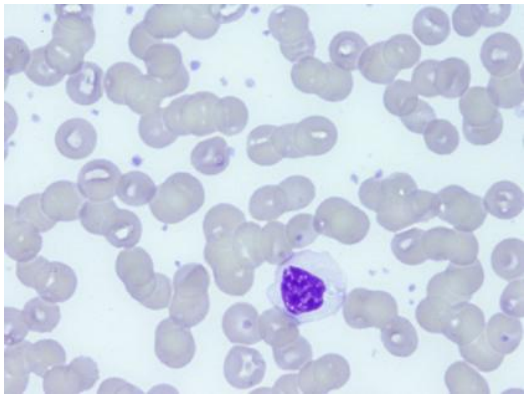

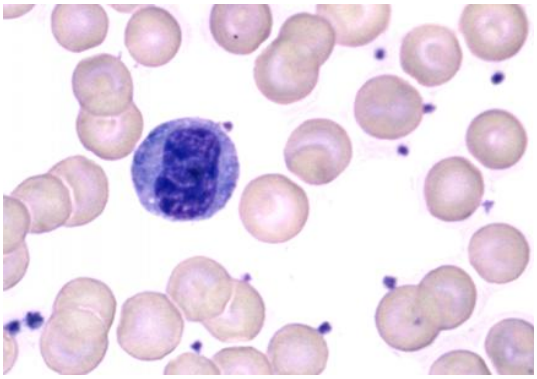

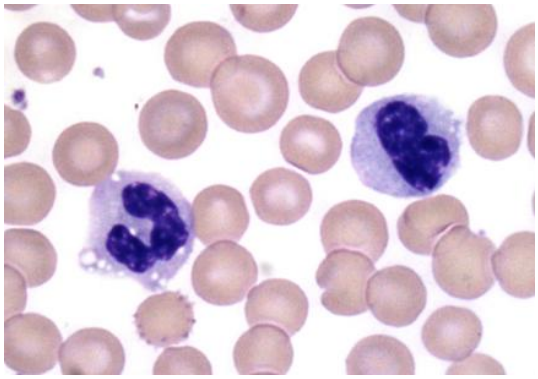

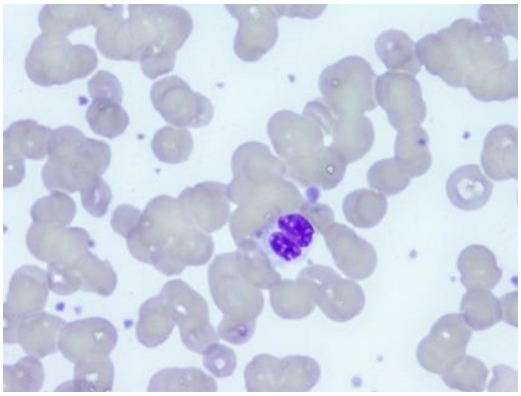

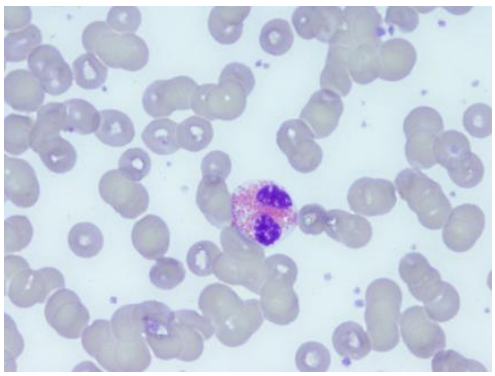

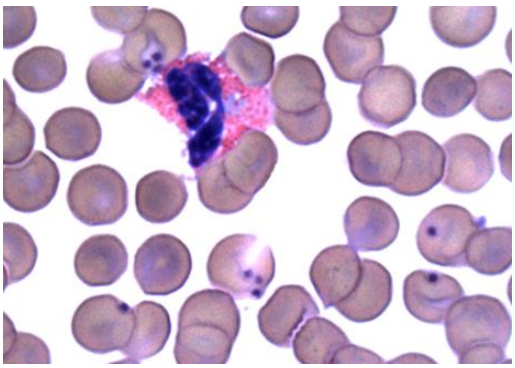

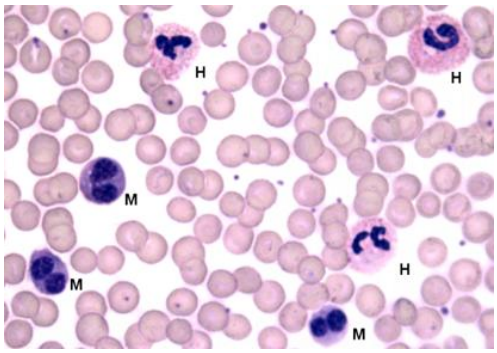
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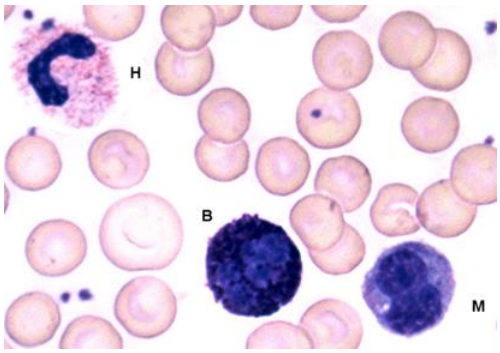
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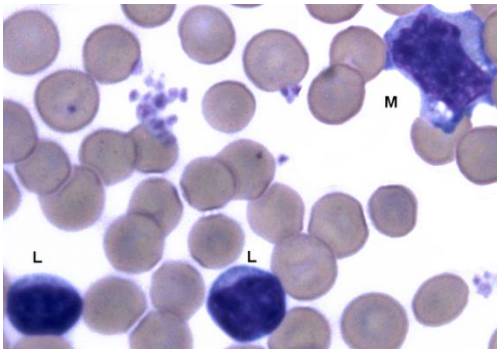
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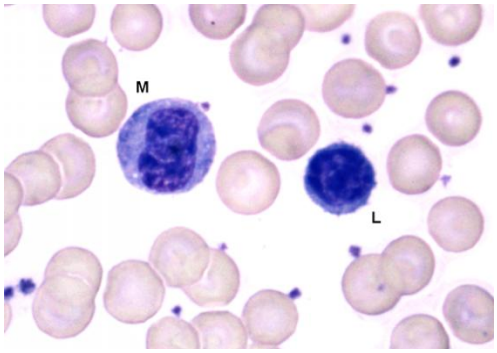
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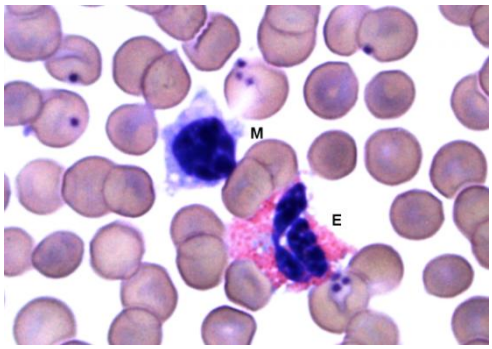
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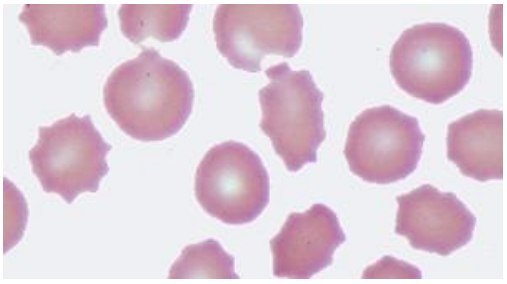
Appendix D. Artifacts in Blood Smears

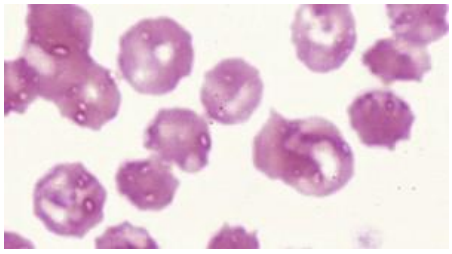
Source: http://ahdc.vet.cornell.edu/clinpath/modules/heme1/morph.htm

Red cell crenation



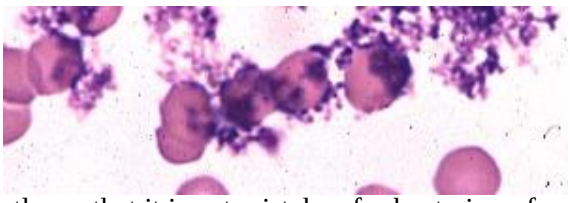
Crenated red cells have changed from a disc shape to spheres covered with short, sharply pointed projections. They are also known as echinocytes or burr cells. Slow drying of the smear and aging of blood in the tube are the most common causes.

Water Artifact



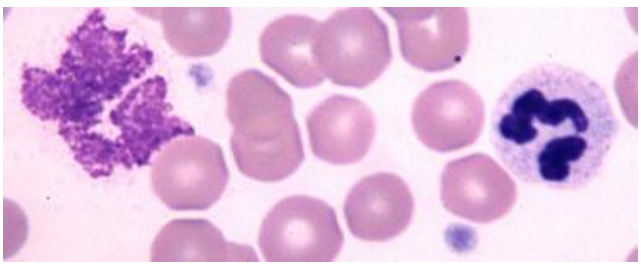
The red cells shown here are crenated and many have multiple refractile "bubbles" across their surfaces. The refractility is often called water artifact because it results from exposure of the smear to water, either as high humidity in the air or as contamination of the fixative with water. If a smear 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.

Appendix E. CBC and WBC Reference Ranges

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	References
PCV	%	28-40	[a] References: Silva 1993a,
Hb	g/dl	8-15	Silva 1993
RBC	x 10^6/µl	2.5-5.0	
MCV	fl	80-160	b, Niemuller 1990,
MCH	pg	35-50	Ratnasooriya 1990,
MCHC	g/dl	25-40	Sreekumar 1990,
Platelets	10^3µl	200-600	Jainudeen 1971, Nirmalin
Reticulocytes	%	0.00	1971 ISIS 2002.
RDW	%	28-32	
ESR	mm/hr	65-150	

White Blood Cell (WBC) Reference Values

Parameter	Absolute Range (x 10^3/µl)	Relative Range(%)	References
WBC	10-18	-	Silva 1993a, Silva 1993b, Nirmalin
Heterophils	2-4	25-30	1967, Jainudeen
Lymphocytes	5-8	30-40	1971, Ratnasooriya
Monocytes	2-4	25-30	1990, Allen, 1985
Eosinophils	0.1-1	< 5	
Basophils	0.00-.03	< 1	

Coagulation parameters in elephants and horses.

Test	Horses[a]	Elephants	References
Platelet count (x 10^3/ul)	100-600	200-600 [b]	[a] Morris 1999 [b]
Prothrombin time (sec)	9.5-13.5	9.6 ± 0.7 [c] (8.2-10.4)	Lewis 1974,
Activated partial thromboplastin time (sec)	39-64	65.6 ± 9.2 [c] (52.1-83.5)	duPleiss 2002 [c] Gentry 1996
Fibrinogen (g/dl)	200-400	461 ± 49 [c] (401-546)	

Appendix F. Causes of Increased and Decreased Total Protein

↑ **Total Protein**	↓ **Total Protein**
Dehydration	↓ Production - Malnutrition - Malabsorption - Liver disease
↑ Acute phase proteins - Inflammation - Neoplasia	↑ Loss - Hemorrhage - Burns - Parasites
↑ Gammglobulins - Inflammation - Necrosis - Surgery - Tumors	
False ↑if glucose, Na^+ or Cl^- ↑; or if sample is lipemic	

Appendix G. References Ranges for Elephant Urinalysis*

Parameter	Typical finding
Color	pale to dark yellow
Transparency	clear to cloudy
Specific gravity	1.007-1.033
Protein	6.8 – 8.0 (most are alkaline)
Glucose	Neg
Ketones	Neg
Bilirubin	Neg
Blood	Neg to trace
RBCs	Neg
WBCs	Neg
Sediment	Calcium oxalate crystals common

* Weidner, E. B., Alleman, R., and Isaza, R. (2009). Urinalysis in Asian elephants. Journal of Zoo and Wildlife Medicine 40, 659-666.

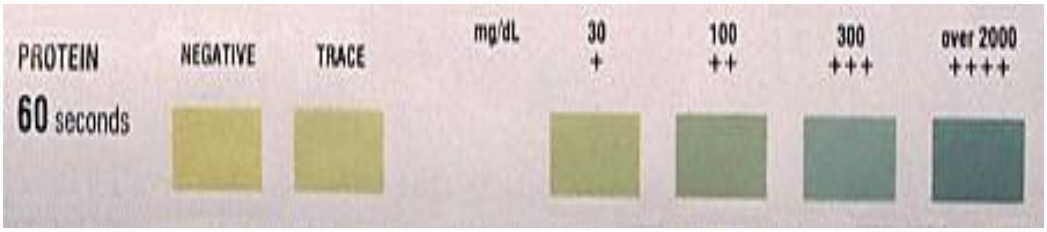
Resources for Further Study

E-ClinPath Online textbook, Cornell University College of Veterinary Medicine: www.eclinpath.com

Appendix H. Urine Dipstick References

E-ClinPath Online textbook, Cornell University College of Veterinary Medicine: www.eclinpath.com

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 occurs 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 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 including renal ischemia and nephrotoxins (such as aminoglycosides).

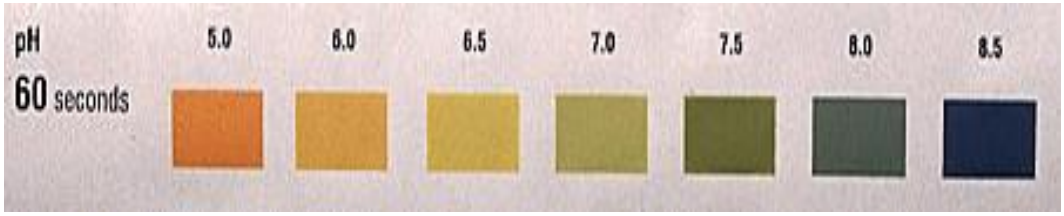
Interstitial proteinuria is due to hemorrhage or inflammation within the kidney.

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.

2. Urine pH



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

1. renal H+ excretion and HCO3- 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 CO2 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.

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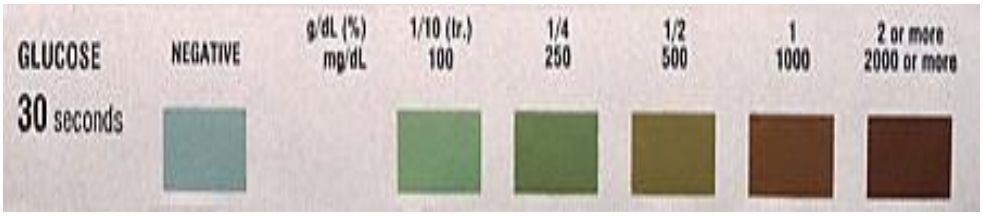
3. Urine Glucose



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

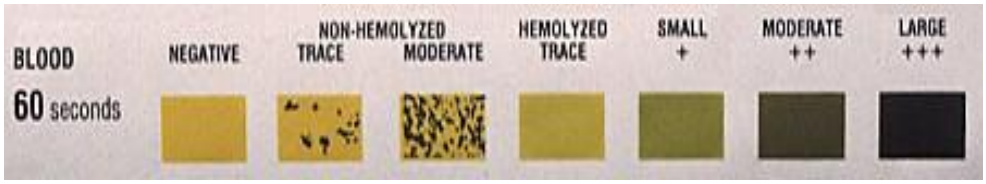
Pathologic glucosuria due to hyperglycemia: In nearly all cases, glucosuria is a result of prior (often, continuing) hyperglycemia to a level in excess of the renal threshold for reabsorption.

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 below.

Causes of a positive urine blood test and their differentiation:

Hematuria
1) Red, cloudy urine that usually clears with centrifugation.
2) RBCs in the urine sediment
3) Absence of clinical or laboratory evidence of hemolytic anemia or muscle disease.
4) 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
1) Red to brown urine that does not clear on centrifugation.
2) No RBCs in the urine sediment (RBCs in urine with a very low S.G. or in aged urine may lyse and mask hematuria).
3) Concomitant red discoloration of the plasma (hemoglobinemia).
4) Evidence of anemia.
5) Absence of clinical or laboratory evidence of muscle disease.

Myoglobinuria
1) Red to brown urine that does not clear on centrifugation
2) No RBCs in urine sediment.
3) Clear, normal color plasma. Myoglobin does not bind significantly to serum proteins and is excreted in the urine before reaching concentrations that discolor plasma.
4) No clinical or laboratory evidence of anemia.
5) Clinical or laboratory evidence (↑ CK, ↑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 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).

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 is to detect WBCs which may indicate infection is present. If positive on the dip stick it is advisable to check a urine sediment and see if WBCs are also present there.

10. Specific Gravity

Specific gravity measurement can be done by dipstick, however measurement by specific gravity is more accurate.

Appendix I. Urine Sediment

Source: E-ClinPath Online textbook, Cornell University College of Veterinary Medicine: www.eclinpath.com

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-quantifies.

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 becomes 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. WBC must be distinguished from RBCs and small epithelial cells.

WBCs vs RBCs

The grainy nature of WBC 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).

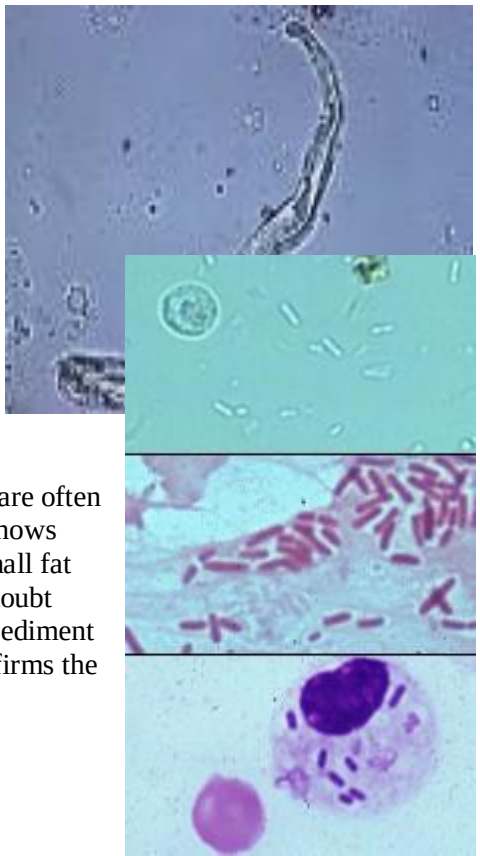
WBCs vs Epithelial Cells

Epithelial cells can originate from the kidneys, ureters, bladder, urethra, reproductive tract. WBCs are generally smaller than epithelial cells and 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 or are nuclei.

Epithelial cell

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 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.).



urine

Bacteria in Urine

Identification: Rod-shaped bacteria and chains of cocci are often readily identifiable. The top panel of the image at right shows bacilli. Small amorphous crystals, cellular debris, and small fat droplets can either mask or mimic cocci. If there is any doubt the presence of bacteria, a Gram-stained smear of urine sediment (middle panel) should be examined. This gram stain confirms the

E.coli

about

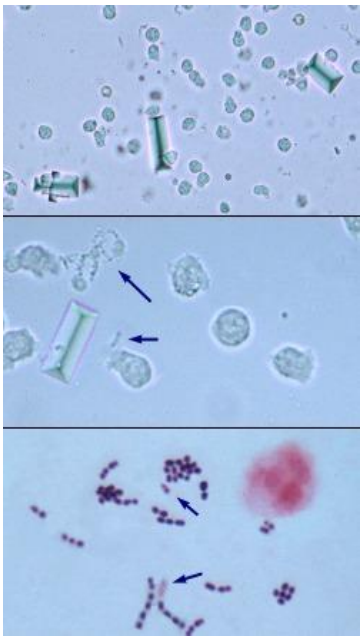
presence of gram negative bacilli. Note that only extracellular bacteria can be visualized on an unstained urine sediment. Intracellular bacteria can only be identified by cytologic examination of a Wright's-stained smear of the urine sediment (the bottom panel demonstrates phagocytized bacteria within 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 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. 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 complicated by bacterial infection.

Urinary crystals

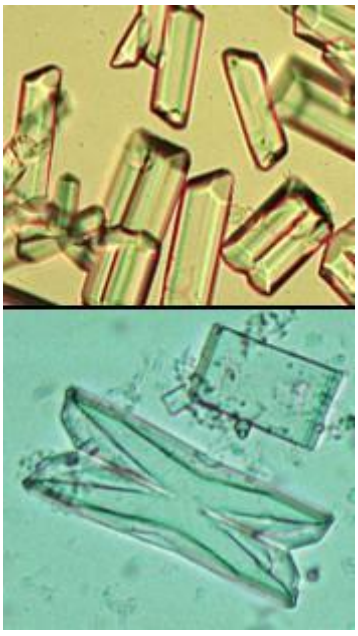
Source: http://ahdc.vet.cornell.edu/clinpath/modules/UA-ROUT/crystsed.htm

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 (see example of ammonium biurate below). 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.

In vivo factors	*In vitro* 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.

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.

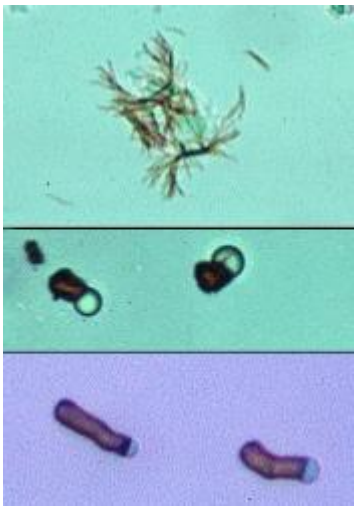
Urinary tract infection with urease-positive bacteria can promote struvite crystalluria (and urolithiasis) by raising urine pH and increasing free ammonia.

Bilirubin

Bilirubin crystals form from conjugated bilirubin (water soluble) and are needle-like to granular crystals that are yellow in color. They tend to precipitate onto other formed elements in the urine. In the top picture, fine needle-like crystals have formed on an underlying cell. This is the most common appearance of bilirubin crystals. In the lower two pictures, cylindrical bilirubin crystals have formed in association with droplets of fat, resulting in a "flashlight" appearance. This form is less commonly seen.

Bilirubin crystals are seen most commonly in canine urine, especially in highly concentrated

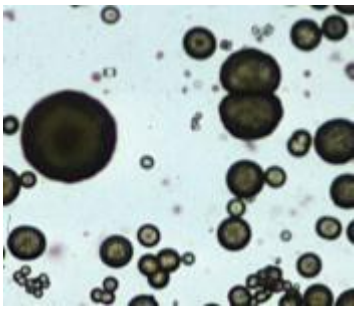
specimens. They are less common in urine of other species. In dogs, they often are of no clinical significance (healthy dogs can have low, but detectable, bilirubin levels in urine). Bilirubin crystals (or a positive chemical reaction on the urine dipstick) in feline, equine, bovine, or camelid urine is an abnormal finding and the animal should be investigated for an underlying cholestatic process.



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. These crystals are common in the urine of normal horses, rabbits, guinea pigs and goats.

Amorphous crystals

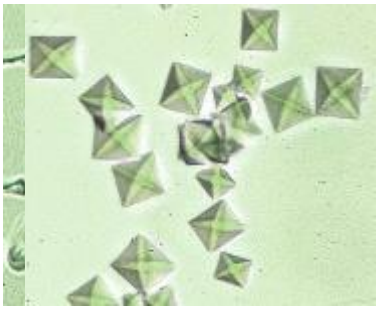


Amorphous crystals appear as aggregates of finely granular material without any defining shape at the light microscopic level. They can be comprised of urates, phosphates or xanthine. Amorphous urates (Na, K, Mg, or Ca salts) tend to form in acidic urine and may have a yellow or yellow-brown color. Amorphous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

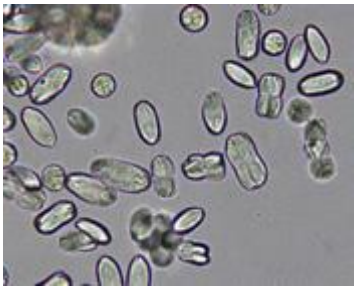
These crystals are often seen in normal urine from domestic animals and can also be an artifact of storage (they can develop in stored urine), emphasizing the need to perform a urinalysis on fresh urine samples.



Calcium oxalate monohydrate

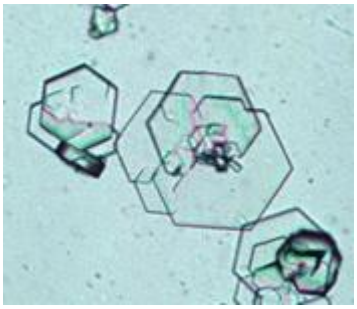
Calcium oxalate monohydrate crystals vary in size and may have a spindle, oval, or dumbbell shape (for examples, see the two unlabeled crystals in the lower left corner of the image to the right). These forms of calcium oxalate monohydrate indicate supersaturation of the urine with calcium and oxalate and, along with calcium oxalate dihydrate crystals ("square envelopes"), can be seen in the urine of animals that have no urologic problems or those suffering from oxalate urolithiasis, hypercalciuric or hyperoxaluric disorders. They are infrequent in the urine of normal dogs and cats but can be seen commonly in the urine from healthy horses.

A particular form of calcium oxalate monohydrate are flat, elongated, six-sided crystals ("picket fences") which are the larger crystals in the image above (which represents urine from a dog with ethylene glycol toxicosis). The arrow in the photo indicates a "daughter" crystal forming on the face of a larger underlying crystal. These "picket fence" forms of calcium oxalate monohydrate are frequently associated with ethylene glycol intoxication in dogs and cats, but are not always observed in the urine of affected animals (i.e. not 100% sensitive). They can also be seen in the urine of animals with hypercalciuria from other causes, e.g. paraneoplastic hypercalcemia with lymphoma.



Another rare form of calcium oxalate monohydrate are the "hempseed" variant.. The image on the right is from the urine of a dog with many of these crystals. The dog did not have ethylene glycol poisoning and the crystals are assumed to be secondary to supersaturation of the urine with calcium and oxalates, which precipitated in the acidic urine.

Cystine



Cystine crystals are flat colorless plates and have a characteristic hexagonal shape with equal or unequal sides. They often aggregate in layers. Their formation is favored in acidic urine.

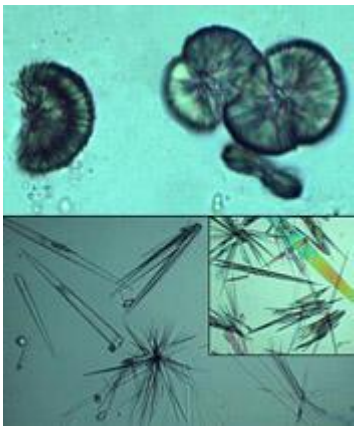
Cystine crystalluria or urolithiasis is an indication of cystinuria, which is an inborn error of metabolism involving defective renal tubular reabsorption of certain amino acids including cystine. Sex-linked inheritance is suspected since male dogs are almost exclusively affected. Many breeds, as well as mongrels, have been reported affected . Renal function otherwise appears to be normal and, aside from a tendency to form uroliths, the defect is without serious consequence.

Drug crystals

Many drugs excreted in the urine have the potential to form crystals. Hence, a review of the patients drug history is prudent when faced with unidentified urine crystals.

Most common among these are the sulfa drugs. Both panels on the right are from patients receiving trimethoprim-sulfadiazine. The differing appearance may relate to variation in drug concentration, urine pH, and other factors. The upper panel is from a feline case, the lower from a horse. The inset in the lower panel shows the crystals as they appeared when polarized.

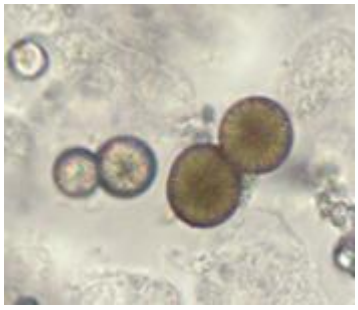
Other examples include radiopaque contrast agents (Hypaque, Renografin) and ampicillin which



may precipitate in acid urine as fine needle-like crystals (not shown).

Other crystals

We frequently see several different types of crystals that are of uncertain origin. Whenever an "unknown" crystal is encountered, we usually perform solubility studies (chemical, i.e. hydrochloric acid, glacial acetic acid and sodium hydroxide, or heat) to help identify the crystals. Some crystals, such as uric acid, calcium phosphate and sulfa drug-related crystals have specific solubility characteristics which, with their shape and pH of the urine (and of course, clinical history), can aid in their identification. Sometimes, despite doing these solubility studies, the identity of the crystal remains a mystery. The clinical relevance of such crystals is questionable, but drug or chemical toxicity should always be considered in an animal presenting with clinical signs of renal failure and unidentified crystals in the urine. The crystals shown in the image above were seen in the urine of a cat with acute renal failure due to melamine toxicosis. These crystals were highly characteristic of melamine and facilitated diagnosis in affected animals.



Contaminants

Sperm in Urine

They may be seen in urine from males collected by voiding, catheterization, or cystocentesis. Rarely, they may be observed voided urine from a recently-bred female. The presence of sperm is reported as part of the complete sediment examination, goal of which is to report all microscopic findings.

in

the

Casts

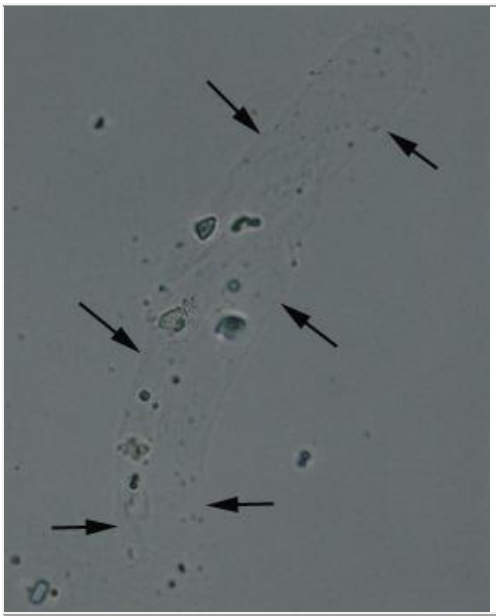
Casts are quantified for reporting as the number seen per low power field (10x objective) and classified as to type (e.g., waxy casts, 5-10/LPF). Casts in urine from normal individuals are few or none (and are usually hyaline or granular in nature).

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. Furthermore, casts are unstable in urine and are prone to dissolution with time, especially in dilute and/or alkaline urine.

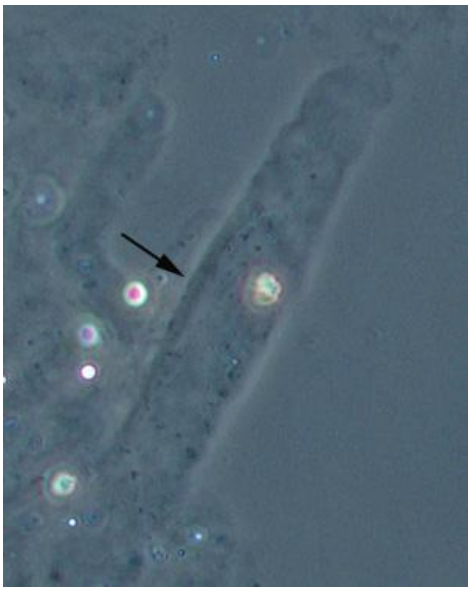
Although the presence of numerous casts is solid evidence of generalized (usually acute) renal disease, it is not a reliable indicator of prognosis. If the underlying cause can be removed or diminished, regeneration of renal tubular epithelium can occur (provided the basement membrane remains intact).

Hyaline casts

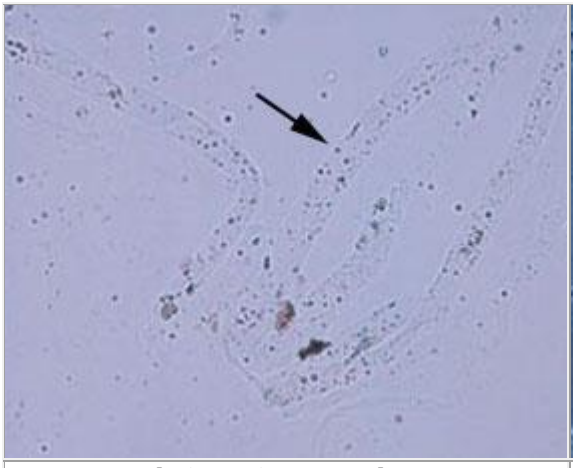
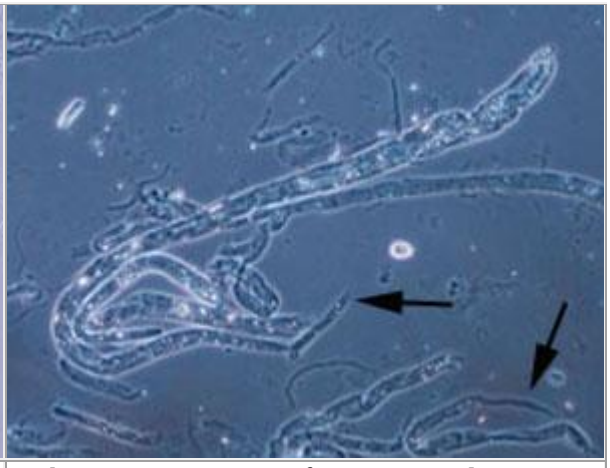
Hyaline casts are formed in the absence of cells in the tubular lumen. They are very difficult to see in wet preparations of urine and must be distinguished from mucus strands. Generally, hyaline casts have parallel sides with clear margins and blunted ends, whereas mucus strands are more variable in size with irregular margins (see below). Reduced lighting is essential to see hyaline casts in urine sediment preparations. Lighting can be reduced by lowering the substage condenser (or close the iris diaphragm). Hyaline casts are also easier to see if other particles (fat, debris) are caught up within them (see image on the left below). Hyaline casts are far easier to visualize using phase contrast microscopy (see image on the right below), but this is not available on standard microscopes. Note that when fat droplets stick to hyaline casts, they are still called hyaline and not fatty casts.



Hyaline casts (black arrows) in a wet preparation of urine sediment as visualized with the condenser racked down or the iris diaphragm closed.

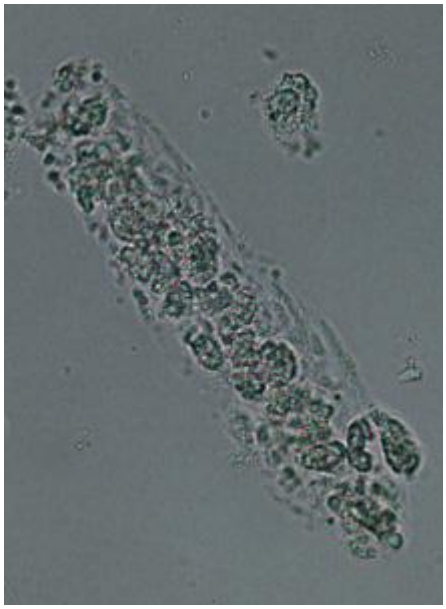


Hyaline casts (indicated by the black arrow) as seen under phase contrast microscopy. The edges of the cast are easier to see with this technique.

	
Mucus strands (arrow) are mostly seen in urine from horses. They mimic casts, however they often have irregular borders, fold over, and taper at one or both ends	A phase contrast image of mucus strands. Tapering ends are evident in several of the strands (arrow), which are also more wavy and fold over compared to hyaline casts

Hyaline casts can be present in low numbers (0-1/LPF) in concentrated urine of otherwise normal patients and are not always associated with renal disease. Greater numbers of hyaline casts may be seen in association with proteinuria of renal (e.g., glomerular disease) or extra-renal (e.g., overflow proteinuria as in myeloma) origin.

Cellular casts



Cellular casts most commonly result when disease processes such as ischemia, infarction, or nephrotoxicity cause degeneration and necrosis of tubular epithelial cells. The presence of these casts indicates acute tubular injury but does not indicate the extent or reversibility of the injury.

A common scenario is the patient with decreased renal perfusion and oliguria secondary to severe dehydration. Ischemic injury results in degeneration and sloughing of the epithelial cells. The resulting casts often are prominent in urine produced following rehydration with fluid therapy. The restoration of urine flow "flushes" numerous casts out of the tubules.

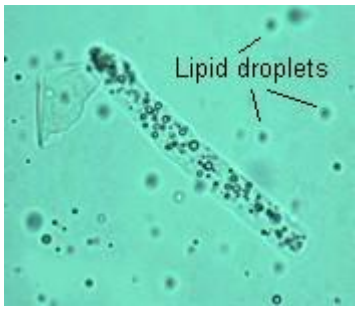
Leukocytes can also be incorporated into casts in cases of tubulo-interstitial inflammation (eg, pyelonephritis). It is rarely possible to distinguish between epithelial casts and leukocyte casts in routine sediment preparations, however, since nuclear detail is obscured by the degenerated state of the cells.

Granular casts

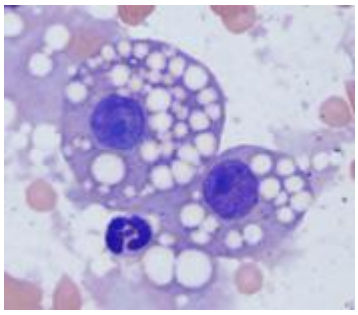
Granular casts, as the name implies, have a textured appearance which ranges from fine to coarse in character. Since they usually form as a stage in the degeneration of cellular casts, the interpretation is the same

as that described for cellular casts. Low numbers of granular casts may be seen in the urine of animals without tubular injury, so the presence of casts should be interpreted along with clinical signs, rest of the urinalysis results, biochemistry results and other pertinent diagnostic information.

"Fatty" casts



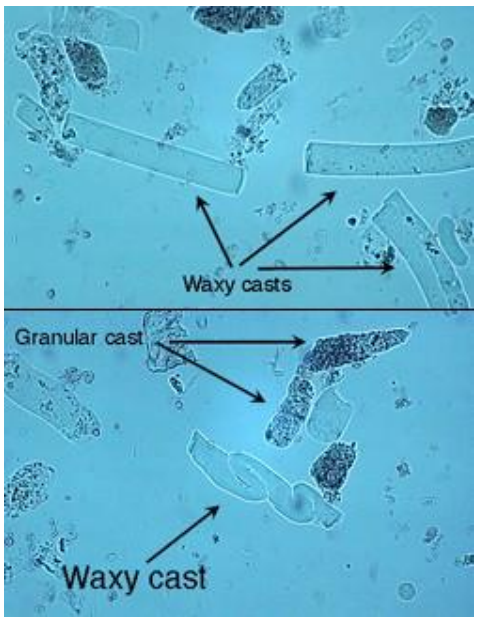
Fatty casts are identified by the presence of refractile lipid droplets within the protein matrix of the cast. The background of the cast may be hyaline or granular in nature. Fatty casts, like granular casts, are thought to represent tubular degeneration. Note that the term fatty cast is not used for hyaline casts with fat droplets adhered to them (which are frequently seen in urines in which free lipid droplets are present as well). Pictured on the right is a fatty cast with a hyaline matrix. Also notice the free lipid droplets in the background.



Feline proximal renal tubular epithelial cells contain numerous fat droplets (100x oil immersion, Wright's stain)

Free lipid can be a normal finding in animal urine (as an isolated finding, lipiduria is seldom of clinical significance) and is likely from degeneration of sloughed renal tubular or transitional epithelial cells. Shedding of these cells in urine can occur under physiologic (low numbers can be shed in healthy animals) or pathophysiologic (renal tubular injury) conditions. Once liberated from degenerating or ruptured cells, fat frequently sticks to hyaline casts. This may happen more in cats, because their renal tubular epithelium can normally contain small to moderate amounts of fat (see image to the left). Differentiation of true fatty casts from hyaline casts with adherent fat can be difficult and is based on the character of the cast matrix, rather than on the lipid content per se.

Waxy casts



Waxy casts have a smooth consistency but are more refractile and therefore easier to see in a regular wet sediment preparation of urine compared to hyaline casts. They commonly have squared off ends, as if brittle and easily broken, and also have smooth parallel-sided borders.

Waxy casts indicate tubular injury of a more chronic nature than granular or cellular casts and are always of pathologic significance.Illustrated to the right is urine from a dog with acute and chronic renal tubular injury as shown by the presence of waxy and coarse granular casts in the urine.

Acknowledgements Sincere thanks to veterinary technician Deborah Maloy VMT, for reviewing this manual, Dr. Bruce Rideout for input on serum banking, and veterinary technician Teri Hermann, Woodland Park Zoo for permission to use blood cell images.

Appendix J: Hematology Report Form

Hematology Report Form
Elephant Care Asia Healthcare and Welfare Workshop
Green Hill Valley Myanmar September 2018

Elephant name: _____

Elephant ID: _____ Sex: ___ F ___ M ___

Health status: Normal ___ Abnormal ___

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

Collected by: _____

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 of analysis: ____
Person performing analysis: _____

Appearance of serum: clear ___ icteric ___ lipemic ___
hemolyzed 1+ ___ 2+ ___ 3+ ___ 4+ ___

Hematology Results

HCT (Automated centrifuge): _____ **HCT (Paprerfuge):** ____

Total Protein (refractometer): _____

WBC Using Whi-pette
Side 1: _____ Side 2: _____

Formula: (Side A + B / 2) x 110 = WBC (µl)

Total WBC: _____

Differential:	Lymphs	Neutrophils	Monocytes	Eos	Basophils
% / mm3					

Estimated platelets / hpf: _____
Blood parasites seen: yes ___ no ___
Estimated WBC from buffy coat: _____
Estimated WBC from slide (average # WBCs / 45-50 X field) x (1500): _____
Comments:

Appendix K. Urinalysis Report Form

Urinalysis Report Form
Elephant Care Asia Healthcare and Welfare Workshop
Green Hill Valley Myanmar September 2018

Elephant name: _____

Elephant ID: _____ **Sex:** ___ F ___ M ___

Health status: Normal ___ Abnormal ___

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

Collected by: _____

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 to brownish red ___
reddish brown to brown ___ greenish tint ___ other (describe) _____

Specific gravity (refractometer): _____

Dip Stick Evaluation	**Microscopic Sediment Examination**
Occult blood _____ RBC/ µL	WBC / hpf _____
Bilirubin _____ mg/dL	RBC / hpf _____
Urobilinogen _____ mg/dL	Cocci / hpf _____
Ketones _____ mg/dL	Rods / hpf _____
Protein_____ mg/dL	Crystals _____
Nitrite Negative _____ Positive _____	Epithelial cells _____
Glucose _____ mg/dL	Other _____
pH _____	
Specific gravity _____	Comments
Leukocytes _____ WBC/ µL	
Ascorbic acid _____ mg/dL	