TARONGA
CONSERVATION SOCIETY AUSTRALIA

Australian Registry of Wildlife Health

Wildlife Pathology Short Course 2019

Practical Laboratory Skills Workshop

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Introduction

Whether your background is wildlife rehabilitation, or you work as part of veterinary clinic or academic centre, a basic knowledge of some choice pathology laboratory techniques can greatly enhance your facilities productivity, but more importantly can aid in the health and wellbeing of an animal or group of animals. As Australian fauna is so unique, diagnostic pathology is considered a specialised field as each species has its own inherent differences when it comes to differential cell types, etc. However, that does not change how the laboratory processes the samples submitted and, if you can master the lab techniques, you can utilise established resources (e.g. texts and publications) to interpret your results.

Goals of workshop

1. Review of appropriate sample collection, storage and shipment

2. Hands on practice of native mammalian, avian, reptilian and amphibian haematological techniques

3. Hands on practice of native mammalian, avian, reptilian and amphibian faecal parasitological identification techniques

Contents

GLOSSARY

AFB	Acid Fast Bacilli
Buffy Coat	The white layer in a centrifuged haematocrit tube between the plasma and the red cells, which contains most of the white blood cells and platelets.
CBC	Complete Blood Count (aka FBC)
CF	Correction Factor
DF	Dilution Factor
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
FBC	Full Blood Count (aka CBC)
HOIF	High Oil Immersion Field
Lith-Hep	Lithium heparin
PCR	Polymerase chain reaction
PCV	Packed Cell Volume (aka haematocrit)
Plasma	Yellowish liquid component of blood that contains salts, enzymes, antibodies and other proteins. Result of using a blood tube with anticoagulant.
Quick Dip	Differential cell count staining method (aka Diff Quik)
RBC	Red Blood Cell
RNA	Ribonucleic acid
SAF	Sodium Acetate Formalin
SDS	Safety Data Sheet
Serum	Amber-coloured, protein-rich liquid which separates out when blood coagulates or is centrifuged. Result of using a blood tube with no anticoagulant.
TPP	Total Plasma Protein
WBC	White Blood Cell

LABORATORY SAFETY

Biosafety

No eating or drinking is permitted in the laboratory.

- All laboratory personnel must wear protective laboratory coats or gowns, and disposable gloves, while performing tests in the laboratory.
- Gowns should not be worn outside the laboratory.
- Gowns should be replaced daily.
- Protective, covered, non-slip footwear should be worn at all times.
- Protective eyewear should be worn when biological sample or chemical splatter is a risk.
- N95 face masks should be worn when handling high infection risk samples i.e. Tuberculosis or Psittacosis.
- Hand washing, with a disinfectant hand wash, should be performed after each sample handling.
- Samples for microbiology should be processed in a biological safety cabinet (minimum Class I). Cabinet should be serviced and tested annually.
- All biological waste should be discarded into dedicated waste bags and sterilized on site or removed by an accredited waste company.
- Benches should be wiped down daily with 70% alcohol or any laboratory grade neutral detergent.

Biological Spill Clean-up

- If lab gown and gloves become contaminated, remove gloves into contaminated waste and place gown in a dedicated tub for disinfection.
- Scrub any bare skin thoroughly with warm water and an appropriate disinfecting soap.
- If eye was splashed, use eyewash (as per manufacturer recommendations).
- Report incident to supervisor (as per institutions incident reporting system) as soon as possible.
- To disinfect benches, Biological Safety Cabinet or floor, soak up any liquid with vermiculite and place in biological waste. Then spray with Cavicide (or other appropriate disinfectant) following manufacturer's instructions.
- If broken glass is involved, disinfect glass first then sweep and place in sharps container.

Disinfectants

Cavicide

Can be used for decontaminating surfaces where a small biological spill has occurred. Can be used to disinfect liquid biological samples prior to disposal (less corrosive on stainless steel surfaces if used long term).

Sodium hypochlorite (10%)

Can be used for decontaminating surfaces where a small biological spill has occurred. Can be used to disinfect liquid biological samples prior to disposal (may be corrosive on stainless steel surfaces if used long term).

Virkon S (1.0%)

Used for disinfecting instruments or decontaminating surfaces (may be corrosive on stainless steel surfaces if used long term).

70% Alcohol

Used for disinfecting laboratory surfaces.

Chemical Hygiene

- All chemicals should be stored appropriately as per their Safety Data Sheets (SDS). This may include a Chemical Hazard Cupboard.
- SDS should be updated every 5 years.
- Appropriate chemical personal protective equipment should be made available as per SDS recommendations.
- Suitable spills kits need to be made available for the types of chemicals used.

SAMPLE COLLECTION

Processing times

It is always optimal to process blood soon after collection but delays can occur. As a general rule, never freeze the sample if delayed unless you have checked to see if this a suitable storage option. Keeping the sample in the fridge or on ice may be sufficient.

Biochemistry: If blood cannot be tested soon after collection, whole blood should be separated, and the plasma or serum stored at 4^{o}C overnight or at -18^{o}C for an extended period (depending on the test).

Complete Blood Count (CBC) (aka Full Blood Count, FBC): Blood film should be made and left to dry as soon as possible after blood collection. The slide can then be fixed and stained the next day.

The Packed Cell Volume (PCV) and Total Plasma Protein (TPP) should be done as soon as possible after blood collection. The sample can be then stored at 4^{o}C overnight.

Samples for cytology and culture collected in these containers should be processed soon after collection or stored for a short period at 4^{o}C.

Blood Collection Tubes[1]

Most blood collection tubes are universally colour coded with certain tube types, or colours, being suitable for specific tests. Always read the packaging first to ensure you are using the correct tube type. Always check with your reference laboratory to determine their collection requirements prior to collecting the sample. Tube size will vary depending on patient size and/or required blood yield.

Cap Colour	Additive	General use
Purple Top	Ethylenediaminetetraacetic acid (EDTA)	Haematology, genetics (blood DNA)
Green Top	Lithium heparin	Biochemistry (plasma), haematology in small birds, reptiles and amphibians
Yellow Top	SST II, Clot activator & serum gel separator, No anticoagulant	Biochemistry (serum), serology
Red Top	Clot Activator, No anticoagulant	Biochemistry (serum), serology
Blue Top	Sodium Citrate	Coagulation studies
Grey Top	Sodium Fluoride/Potassium Oxalate	Glucose tests
Dark Blue Top	No anticoagulant / K2 EDTA	Toxicology and trace elements

*may vary depending on manufacturer

Other Requests:

Blood cultures are collected aseptically into the yellow-topped Isolator tubes (only one per animal required).

Numerous other blood tests are available such as serology, coagulation studies, plasma transfusion, and heavy metals. It is always wise to check with your reference laboratory prior to collection to determine their collection requirements.

Specimen Containers and Swabs

Universal sterile container (70 ml yellow top) are used for:

- Urine (urinalysis (process within 4 hours) and culture)
- Faeces (generally parasitology and culture)
- Pus (cytology and culture)
- Other Body Fluids i.e. ascites, pleural (cytology and culture)

Needle and Syringe: For fine needle aspirate or pus aspiration. Must have needle guard attached. Should be processed immediately for culture and cytology.

Transport Swabs: Like blood collection tubes, there are many types of sample swabs, with a variety of uses. Some examples include:

[1]*There has been a lot written about which anticoagulant tube to use for CBC in birds and reptiles. EDTA is the anticoagulant of choice but can haemolyse the blood of a variety of birds (i.e. crows and magpies) and reptile species (chelonians). Lithium Heparin has the added advantage that in small animals you can use it to do both CBC and Biochemistry. However, it can cause clumping of platelets and leukocytes and interfere with how well the leukocytes stain with Diff Quick. Some biochemistry analysers can now accommodate whole blood in addition to serum or plasma which means blood volume can be smaller but it would be best to check with your reference laboratory, or analyser manufacturers, before assuming whole blood can be used in this manner.*

- Large dacron or cotton tip - with Amies transport medium and plastic shaft (either clear or charcoal). Appropriate for bacterial culture when sample can't be cultured immediately. May also grow some yeast and filamentous fungi.
- Small dacron or cotton tip - with Amies transport medium and thin wire shaft used for collection from small animals, conjunctiva, and choana. Appropriate for bacterial culture when sample can't be cultured immediately. May also grow some yeast and filamentous fungi.
- Dry swabs with no transport medium. Appropriate for some antigen and PCR tests. Culture should be done soon after collection if swab inoculated soon after collection.
- Viral transport swab. Usually a Dacron swab with virus preservation media. Can be used to support select viruses prior to culture but can also be used to do PCR testing.
- eSwab – newer universal swab on the market. Utilizes Floq swab technology and a liquid transport media. Ideal for aerobic and anaerobic bacteria culture, fungal culture, as well as antigen, and PCR.

Microbiology Sample Collection

Bacteria are most often found at the leading edge of the wound, on the margin of normal and abnormal tissue. Thus, the wound margin, or subcapsular region of an abscess, is the best site to collect samples for bacterial culture. The central region of many wounds often contains only necrotic debris.

Hairs or skin scrapings submitted for fungal culture should be placed into a ventilated Petri dish or envelope so that excess moisture does not promote bacterial growth.

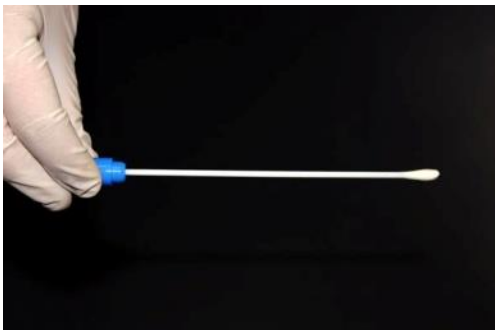
Isolation of bacteria from faeces often poorly represents the intestinal flora. Microbial culture is far more successful when samples are collected by direct cloacal or rectal swabbing.

Bacterial isolation from wedges of tissue collected via biopsy or during post mortem examination is much more successful than from tissue swabs. Isolation of anaerobic bacteria from swabs is particularly difficult. If biopsy is not feasible, aspirates collected into a syringe may be more useful than a simple swab.

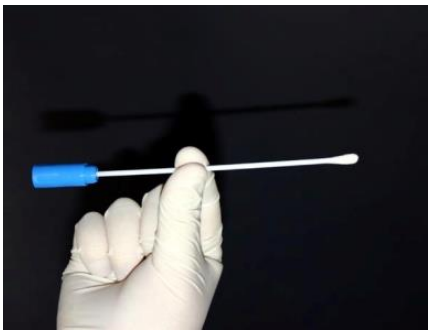
Population health studies in birds often involve the collection of separate swabs from the conjunctiva, choanal slit (the slit in the palate), and cloaca. Two sets of swabs can be collected from each location and placed into bacterial transport medium, and viral culture medium. An additional dry swab may be collected from the conjunctiva and choanal slit combined, to be used for chlamydial antigen capture testing. PCR and serological testing may also be used to identify infection and exposure to organisms such as *Salmonella* spp. and *Chlamydia* spp.

Health studies to characterise the microbial flora of mammals may involve collecting separate swabs from the rectum/cloaca, mouth, conjunctiva, nasal passages, and pouch.

Peel back the plastic wrapping from the swab and transport tube. Grasp the swab firmly by the plastic tip. Ensure that the swab only contacts the intended site[2]. Remove the plug from the transport tube, but do not touch the rim or inside of the tube. Slide the swab into the transport tube and secure the system with gentle pressure. Label the tube.



CORRECT



WRONG – START AGAIN!

Wet mount preparations, impression smears or scrapings should be prepared from any lesion. Smears may then be stained using Gram's technique and Quick Dip (or Wright's stain) to identify microbial agents and observe cell structure. A KOH preparation may be used to digest tissue to delineate fungi or parasites.

Samples collected from anatomic sites that should be sterile i.e. urine collected by cystocentesis, cerebrospinal fluid collected by lumbar puncture, blood culture by venepuncture, should have the skin adequately prepared with antiseptics. These include 75% ethanol, betadine and chlorhexidine suspensions. In the case where blood culture isolator tubes are used, it is necessary to disinfect the top of the tube as well to minimize contamination and false positives.

[2] *It is very easy to identify the bacteria growing on your fingertips, instead of those growing in the animal. Be very careful during the swabbing procedure not to touch any portion of the swab other than the plastic top. If you touch the shaft or tip of the swab, throw it out and start again.*

HAEMATOLOGY TECHNIQUES

Haematology is a broad science involving the study of blood, the organs that make the blood and the diseases that cause blood disorders. Students spend semesters studying the theory and practical aspects of haematology but there are a few rudimentary tests that can be used to gather information about an animal's health.

In our laboratory, on receiving a blood for CBC, the sample is placed on a rotator for a minimum 10 minutes to ensure an even mixing of the blood components and anticoagulant[3]. Once this has occurred, the sample is prepared in the following fashion:

- Depending on the species, a relevant dilution is made for total cell count and the diluted sample placed back on rotator for another 10 minutes. The sample is then added to a haemocytometer, allowed to sit for a few minutes and then a total cell count performed.
- All animals have a PCV and TPP performed.
- If a mammal, haemoglobin level is also performed.
- A blood smear is prepared, stained and read (white cell differential, cell morphology and parasite examination).
- Any pertinent calculations are made.

Preparation of a Blood Film

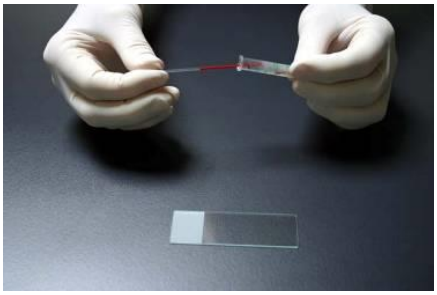
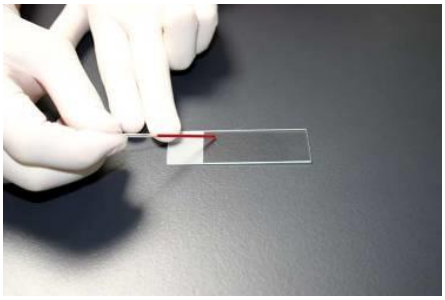
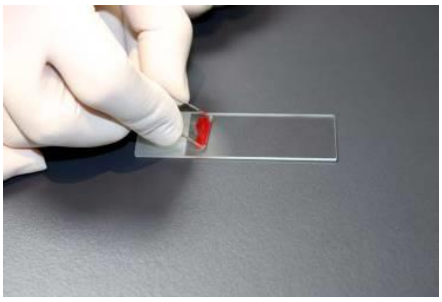
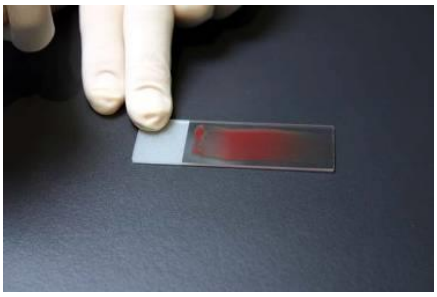
Blood films are used to examine the blood cell morphology under a microscope. Examining blood films can also be useful in identify a variety of blood parasites.

A blood film is prepared from blood collected either directly from an animal or from blood with an anti-coagulant. The goal of making a blood smear is to prepare a thin and even film, with an iridescent, monolayer of cells along the feathered edge at the end of the film.

One recommended method to create a blood film is using the slide-on-slide technique:

- Only touch glass microscope slides by their edges. The oils in fingerprints will repel the blood film.
- Place a glass microscope slide, frosted side up, on the bench top. This slide stays stationary and is supported by the left hand (for right-handed people).
- Label the frosted edge using a pencil.
- Draw up a small amount of blood approximately 1.5 cm up into a capillary tube.
- Deposit a drop of blood onto the microscope slide, approximately 1 cm from the frosted end of the slide.

[3] *Often, especially with paediatric or 'mini' blood collection tubes, there is a tendency for a bubble to form at the top of the sample preventing it from mixing effectively on a rotor or rocker. It is best to check that there is movement of blood in the tube by tipping it back and forth a few times manually, before placing it on any mixing device.*

- Hold a second microscope slide at a 45-degree angle to the slide on the bench top. Back the edge of this second slide into the blood drop until you see the droplet spread evenly along the spreader edge and then slide it out towards the end of the stationary slide.
- Allow the film to air dry and then assess the quality of the smear. If the film has an iridescent quality along the feathered edge at the end of the slide, it should be satisfactory for examination. We recommend making multiple blood smears from every animal.
- Fix the slide for 5 – 20 seconds in 70% methanol. Unfixed smears degrade with exposure to light and air.
- Store blood films in a slide box to prevent the films from being scratched or eaten by flies.
- Ensure slides are dry prior to storage to prevent fungal growth. Especially important in the field.

Quick Dip Stain

The Quick Dip (Thermo Fisher, Vic), also known commonly as Diff-Quik® staining method is a rapid and reliable means of preparing blood films for microscopic examination. The stain set is packaged as three solutions: a fixative and two staining solutions. The outcome using this staining set is very similar to that provided by Wright or Wright-Giemsa staining techniques, except that mast cell and basophil granules appear very pale. Very similar stain sets, with different brand names, are available from pathology supply companies.

- Keep the staining solutions in tightly sealed jars when not in use.
- Use forceps to hold the slide (as prepared above, dried).
- Dip the slide slowly, five times (one second per dip) in the fixative and then allow the excess fixative to drain.
- Dip the slide slowly, five times in staining solution #1 and then allow the excess to drain. Dipping more than five times can increase the eosinophilic (pink) characteristics in the cells.

- Dip the slide slowly, five times in staining solution #2 and then allow the excess to drain. Dipping more than five times can increase the basophilic (blue) characteristics in the cells.
- Rinse the slide with water. It is better to immerse the slide in a jar of water, rather than run water directly over the smear, or run the water gently over the back slide of the slide. Do not blot the slide dry.
- Place the slide vertically in a slide rack and allow it to air dry before microscopic examination.
- If you don't like the staining characteristics of the cells on the slide, you can dip the slide back into one or both of the stain solutions. Alternatively, place the slide back into a small jar of fixative (methanol) to dissolve the stains and then you can start again.
- Depending on workload, stains should be changed at least weekly.

Differential White Cell Count (Diff)

- Prepare a blood film, air dry, fix and stain with Quick Dip method.
- Count 100 white cells and record each type as a percentage of the total count (%). A differential counter can be used for this. 200 cells may be counted if abnormal and results calculated accordingly.
- Calculate absolute values for each group of WBCs present.

Example: Absolute value of Neutrophils = Total WBC x (% Neutrophils / 100)

Also:

- Comment on the red blood cell (RBC) and white blood cell (WBC) morphology.
- For mammals, report the average number of platelets per field under oil (per/HOIF). Count at least 5 fields and take average.
- Look for the presence of blood parasites such as Microfilaria, Haemogregarines, Malaria spp, Babesia spp, etc.

Blood Smear Evaluation

Blood smear examinations are best approached systematically and a consistent technique will ensure that the results obtained from the smear are as reliable and accurate as possible. We would recommend the following approach:

Before beginning any differential count, *we recommend* you take some time to scan around the slide to assess the general appearance of the slide and the cells. This will ensure that you have identified each type of cell (often they can appear morphologically similar depending on stain quality, etc.), assess the overall number of WBCs present, assess RBC morphology and determine if there are clumps of platelets present, etc. This type of assessment can be done at a low power.

The cells are best assessed at the tail end of the blood smear, where cells are separated into a thin monolayer (rather than further up the slide where they are layered atop each other) but not too far into the 'tail' where cells may be fractured and misrepresented in quantity.

If stain quality is inferior, it may be very difficult to assess the slide. RBCs should appear pink (with a pink to purple/blue nucleus in the case of birds, reptiles and amphibians). WBCs should have a definite darkly staining nucleus with light purple to pink cytoplasm (see differentiating between white cells below).

A higher power may be used to perform the actual count of WBCs. Birds, reptiles and amphibian samples are often performed at 100x oil immersion. Confusion can occur between lymphocytes and thrombocytes in these groups so this should be ascertained prior to starting the differential. Mammal smears can be evaluated using oil, a coverslip and a slightly lower power. The slide is scanned in a consistent pattern to ensure the same cells are not counted twice.

It is important to comment on the morphological appearance of the RBCs. Different morphological states are often reported as low (+), moderate (++) or high (+++).

Some of the terms used are defined below:

Anisocytosis: variations in RBC size within the blood film. Small RBCs are known as microcytes and large RBCs are known as macrocytes.

Polychromasia: the occurrence of slightly immature RBCs, which are larger than normal and have a blue-grey colouration. It is normal for there to be small numbers of these cells present but presence in large numbers may indicate anaemia, etc.

Target cells (mammals): Thin, pale (hypochromatic) cells with a round central area of deep pigmentation.

Howell-Jolly bodies (mammals): small, dense, perfectly round basophilic red cell inclusions. Result of remnant DNA after RBC maturation.

Hypochromasia: Occurs with a decrease in the amount of, and concentration of, haemoglobin in RBCs causing a very pale appearance to the central region of the red cells.

Hyperchromasia: Occurs with an increased haemoglobin concentration.

Nucleated red blood cells (NRBCs) (mammals): Immature RBCs. In an adult animal the presence of NRBCs indicates a possible disease process but maybe normal in younger animals or certain adult species such as Koala.

NRBCs (birds/reptiles/amphibians/fish): normal RBCs in these species.

Poikilocytosis: Variations in RBC shape within the same blood smear.

Rouleaux formation (mammals): When RBCs are arranged in a stacked, or linear, arrangement.

Erythroplastids (birds / reptiles): anucleated RBCs; occasionally observed in healthy animals with little clinical significance.

Rubricytes: Immature RBCs that may be seen in the peripheral blood.

Identification of White Blood Cells (WBCs)

For the beginner, WBC differentiation of a peripheral blood smear can be daunting but with practice can be mastered. It is important to be readily able to assess the WBCs in healthy specimens because it will help in the classification of WBCs produced during various disease processes such as infection or leukaemia.

Some terms used in the reporting of WBC morphology:

Toxic granulation: Increased granulation in neutrophils and heterophils that can be a response to infection or inflammation.

Left shift: Is used in reference to immature myeloid cells (granulocyte cell line) being present in the peripheral blood. This is usually a response of the bone marrow pushing out precursor cells in response to a disease process.

Right Shift: Mature neutrophils usually have 2 to 3 segmented nuclei. In a right shift, the neutrophils may have 6 or more nuclei known as neutrophil hyper segmentation.

Atypical Lymphocytes: differ from the regular description by having more diffuse chromatin pattern, lower nuclei to cytoplasmic ratio, and have a dark blue staining cytoplasm. Quite often these are B-lymphocytes or plasma cells.

Haematology techniques for Mammals

Packed Cell Volume (PCV/Haematocrit)

PCV is the proportion of Red Blood Cells (RBC) to plasma in a sample of whole blood. Amongst other things, a high PCV can indicate dehydration or polycythaemia and a low PCV can indicate blood loss or anaemia.

- Fill microhaematocrit tube to about 2/3 to 3/4 the length of tube.
- Wipe blood from outside with a tissue.
- Seal one end with Plasti-seal.
- Place in Haematocrit centrifuge and balance with an opposite tube. Screw on top and press "start" button. Speed is fixed at 12,000 rpm/ 3 minutes.
- Record PCV (%) using PCV scale reader (see Appendix I).

At this point you should also take the opportunity to describe the colour of the plasma i.e. is it of normal appearance (clear, pale yellow colour), is it haemolysed (clear, red wine colour), lipaemic (cloudy) or icteric (excessive yellow pigmentation).

Total Plasma Protein (manual TPP)[4]

Most biochemistry panels will run a total protein. If a full panel is not required, a manual total plasma protein can be done using a refractometer. Most times a TPP is done in conjunction with the PCV.

If needed, zero the refractometer for room temperature using distilled water.

Break haematocrit tube (used to measure the PCV) just above the "buffy coat" and place a large drop of plasma on the refractometer.

Take reading from "serum" scale (g/L).

Total White Cell Count (WCC)

Using a modified unopette method for mammals, only the white blood cells (WBCs) will remain in solution (with RBCs having lysed upon mixing). 20µl of EDTA or Lithium Heparinised blood is added to 1.98 ml of lysing diluent (1% acetic acid with a few drops of Quick Dip II added). Place on the rotator for a minimum of 10 minutes then add to a haemocytometer (see Appendix II). Count all 9 large squares and calculate total WBC count (see Appendix III).

Haemoglobin

Our lab utilises the Reflotron system (Roche Diagnostic) and also an I-Stat instrument (REM Systems). Larger labs will utilise an automated system to calculate haemoglobin (Hb) in mammals.

White Blood Cell Descriptions for Mammals

See Appendix V for WBC descriptions for mammals. Mammalian WBCs are quite similar amongst the different species with minimal variation between the different cell lines. As always, exceptions to the rule do apply.

Reticulocyte Count

Reticulocytes are immature RBCs that contain residual cytoplasmic RNA. Reticulocytes can be graded depending on how the RNA appears after staining. A large clump of RNA maybe referred as being reticulated whereas a few granules of RNA maybe referred as being punctuate.

A reticulocyte count may help with the determination as to whether an anaemia is classified as being regenerative or non-regenerative. An over simplified definition of a regenerative anaemia is where the anaemia is due to either blood loss or haemolysis. In most cases, a reticulocytosis would be noted. A non-regenerative anaemia is where the anaemia is due to an ineffective erythropoiesis. In this case reticulocytosis would not be noted.

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[4] *The principle of the refractometer is that the degree of light refraction in an aqueous solution is proportional to the quantity of solids in solution. Most of the solids in plasma are proteins, so the degree of light refraction is highly dependent on protein concentration. Margins of error can be introduced when other solids in a sample are elevated i.e. lipid, glucose etc. It is considered however to be a quick, easy and inexpensive way to determine hyperproteinaemia and hypoproteinaemia.*

In a small tube place 2 drops of mixed whole blood with 2 drops of Reticulocyte counting fluid (new methylene blue). Mix and leave for 15 to 20 minutes. Prepare a film from this mixture and air dry. Count 500 red cells and express Reticulocytes as a percentage (%).

Useful Haematological Indices

Mean corpuscular volume (MCV) (FL) = PCV% x 10 / RBC count

Mean corpuscular haemoglobin (MCH) (Pg) = Hb (g/L) / RBC count

Mean corpuscular haemoglobin concentration (MCHC) (g/L) = Hb (g/dL) x 100 / PCV%

Haematology techniques for Birds and Reptiles

In birds and reptiles, a total cell count of RBCs, WBCs and thrombocytes are obtained. Parameters such as haemoglobin concentration is not performed in our laboratory due to the method being different to that designed for mammals. Indices such as PCV are relied upon to determine conditions such as anaemia.

PCV and TPP

Determined as per for a mammal.

Total Red Blood Cell Count

The modified red cell unopette method is used. 10ul EDTA or Lithium Heparinised blood is added to 1.99 ml of normal saline, mixed for about 10 minutes on a rotator and added to an Improved Neubauer Haemocytometer. The RBCs are counted in the centre small and four corner squares within the large central square of the chamber. For calculation see Appendix IV.

Total White Blood Cell Count

Using the same sample as for the total RBC count, load the Improved Neubauer Haemocytometer, count the total number of white cells (WBC) present in the 9 large squares = N. For calculation see Appendix III.

Estimated White Blood Cell Count

Considered a 'down and dirty' method, a WBC estimate is useful when a counting chamber is not available. Unfortunately, this method can be subjective and inaccurate.

One documented WBC estimation method from stained blood smears (Campbell and Ellis, 2007): Using x100 oil immersion, determine the average number of WBCs in 5 fields divide by 1000 and multiply by 3,500,000. This will give you an estimate of the total WBC count per µL. Most Australian reference ranges are 'per L' so a further conversion would need to be made.

If the sample is anaemic (based on PCV), then the following adjustment needs to be applied:

(estimated WBC count x actual PCV) / normal PCV (for that species)

Thrombocyte Count

Using the Improved Neubauer Haemocytometer, count the total number of thrombocytes present in the 9 large squares = N. Calculate total thrombocytes in the same way you calculate total WBC (see Appendix IV).

Blood Film Preparation, Examination and Evaluation

Performed as for a mammal.

White Blood Cell Descriptions for Birds and Reptiles

See Appendix VI and VIII for WBC descriptions for birds and reptiles. Avian WBCs are very similar amongst the different avian species. There are some subtle differences i.e. most species of bird eosinophils have orange cytoplasmic granules, however in birds such as cockatoos and curlews the granules are a bluish colour. Reptile WBCs have many distinct differences between species. An Eastern Water Dragon heterophil may have a segmented nucleus with very small pinkish coloured cytoplasmic granules where as a heterophil in a Green Turtle will have an ovoid nucleus with large orange cytoplasmic granules. As a rule, it is always important to scan the slide first and gauge the WBC population that is present. Particularly in birds and reptiles, it is critical to establish a difference between a lymphocyte and thrombocytes along with determining the other cell lines.

FAECAL PARASITOLOGY

We can ascertain a lot of information about an animal's health by examining its faecal sample. A quick macroscopic observation can give an insight into a potential underlying disease. Is the sample formed, unformed, loose or liquid, is there any blood or mucus, can adult helminths be seen? Amongst other things, parasites can cause substantial morbidity and mortality so at, Taronga animal population and health assessments routinely include faecal analysis. Last year we examined over 1300 samples. If indicated, we would also culture for bacterial pathogens and yeast.

General Rules

As a rule, '*fresh is best*' for faecal parasite examination. Any samples collected for full work up (including culture), protozoal check or any other testing must be tested on the same day they are collected. Faeces collected for helminth examination only, provided the sample is moist, may be placed in the refrigerator for up to 24 hours before being tested. If longer storage is required, samples can be preserved in Sodium Acetate Formalin (SAF) fixative.

When interpreting a faecal floatation, you should keep in mind what makes up the animal's diet. Scales, feathers, insect exoskeletons, flower parts, plant fibres and pollen will all be present in the faecal sample and may often look like a potential parasite. It is also worth keeping in mind that the parasites of your patient's prey may also be present in the faecal sample. We should also be mindful that not all parasites found will be the cause of clinical disease or debilitation and some host/parasite relationships should be maintained (e.g. wild animals for release, translocation, etc.).

Diagnostic method for the detection of parasites in faeces

Our laboratory has adopted the OvaTector®1 system. Many other methods can be employed.

When collecting the sample, fill the centre cupule with fresh faecal matter (hold approx. 2 grams) and label the sample with the specimen identification. Replace the lid for clean and transportable sample.

Faecal Wet preparation

For detection of moving parasites such as flagellates, ciliates, larvae and amoeba.

Remove the lid of the faecal container and place the cylinder, lip end up, over the centre receptacle – this will form the flotation system.

Place a few drops of normal saline into the system and mix the suspension (add enough saline to form a thick suspension) with the wooden applicator stick.

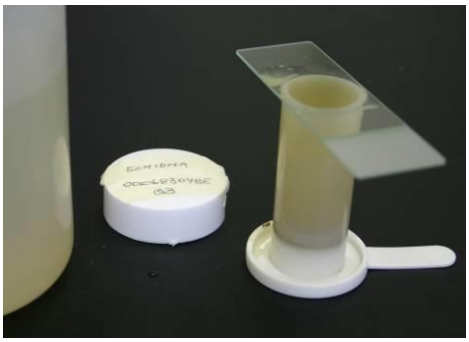
Using the applicator, drop some of the suspension onto a glass slide and cover with a glass cover slip (do not press down too firmly on the cover slip as the aim of this procedure is to observe live and moving parasites). Examine under low power and 40x for larvae, flagellates and ciliates, amoebic trophozoites, cysts, yeast[5], etc. Lugol's Iodine can also be added to enhance amoeba detection.

Cryptosporidia: Using the applicator stick and a labelled fresh slide, smear some of the faecal suspension and allow slide to dry. Stain with modified acid fast stain (as per protocol below) and examine under 100x.

Faecal flotation

For detection of parasite ova and protozoa.

Remember: this procedure will use the remainder of the specimen, thus always do this procedure last!



- - Half fill the cylinder with saturated sodium nitrate (66.7 % (w/v)) - specific gravity 1.20[6]. We have also used sugar-sodium chloride with some success.
- - Mix the suspension thoroughly with the applicator stick.
- - Push the sieve down into the cylinder gently until the handle rests below the top of the cylinder.
- - Add more saturated sodium nitrate until a convex meniscus forms at the top of the cylinder.
- - Place a glass slide, or cover slip, over the meniscus and let stand for 15 minutes – the ova will float through the sieve and adhere to the slide.
- - After 15 minutes, lift and turn the slide in a smooth motion and cover with a cover slip (alternately lift the cover slip and place on a slide).
- - Examine under low power and 40x.

Faecal Concentration Method

Alternative method using the Evergreen Faecal Parasite Concentrator (FPC) ®2 to detect amoebic cysts, ova, larvae and protozoa.

- Label the tubes and samples appropriately.
- Add 9mL formal saline to the graduated, flat bottomed tube.

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[5] *Keep a close eye out for yeast also. Koalas normally have large numbers of grain shaped yeast, Saccharomyces spp. Young hand-raised marsupials, echidnas, finches, and some parrots are highly susceptible to gastrointestinal yeast infections. Large numbers of yeast or many budding yeast cells within the faeces of these species should be viewed with concern. Whenever fungal hyphae are evident amongst the yeast, in a fresh sample, there should be a high degree of suspicion that the fungi have begun to invade tissue.*

6 If specifically interested in trematode ova such as Fasciola spp, a saturated salt solution with a specific gravity of 1.30 maybe required. Trematode ova are usually quite large and dense so requiring a higher specific gravity.

- Add 3 spoons of faeces and mix the sample thoroughly using the applicator/spoon provided.
- Add 3 drops of Triton X-100 to the mixed samples.
- Add 3mL ethyl acetate.
- Screw the FPC strainer (tapered centrifuge tube with strainer attachment) tightly onto the flat-bottomed tube and shake vigorously for 30 seconds.
- Tip the tube upwards and flick the tube gently so that the liquid drains into the centrifuge tube.
- Unscrew the sieve and flat-bottomed tube and discard, place a cap on the centrifuge tube.
- Gently centrifuge the sample at 500 x g for 10 minutes.
- Carefully pour off the supernatant to leave a spun deposit in the bottom of the centrifuge tube.
- Add 2-3 drops of saline to the deposit to suspend the sediment.
- Prepare slides with a plastic pipette, add a cover slip and examine under low power and 40x for parasites. Can use a small drop of Lugol's iodine to help detect protozoa.
- Smears can be made and dried for Modified ZN or other parasitology stains.

1) OvaTector – disposable faecal diagnostic system: BGS Medical Products Inc., 2000 South Tamiami Trail, Venice, Florida 34293

2) Faecal Parasite Concentrator: Evergreen Scientific, 2254 East 49th Street, PO Box 58248, Los Angeles, CA 90058-0248

ECTOPARASITE COLLECTION

A small drop of alcohol placed onto an embedded tick or stickfast-flea will often encourage the parasite to release its hold so that it can be removed with gentle traction. Many parasitologists prefer to examine live ticks. These ticks can be transported in a plastic vial containing a moist cotton ball, and small ventilation holes. If immediate transport is not possible, ticks and fleas can be preserved in 70% ethanol.

Mites and lice can move quickly and be challenging to collect. Close examination of the animal with a dissecting microscope will improve the likelihood of finding these parasites. Check the whiskers, ears, pouch, underarms, and groin of all mammals, and the wings of bats. Reptiles will often harbor mites between the scales around the eyes, and between the lower jaws. Mites and lice attached to bird feathers can be difficult to collect and often it is easier to snip an affected portion of the feather into the collection vial.

Mites and lice can be collected by dragging a cotton ball or cotton bud moistened with ethanol along the infested region. The cotton ball or cotton bud can then be cut off into a small cryovial containing 70% ethanol and 5% glycerol, as described above. It will be much easier for the parasitologist to find these small organisms in a small vial. A vacuum cleaner can be used to collect parasites from fur or feathers, if filter paper is placed at some point along the tube with suction. A human lice comb is another alternative for fur. Fixed specimens can be stored at room temperature for prolonged periods as long as the seal does not allow the ethanol to evaporate.

Alcohol will destroy most labels, except those written in pencil. The label should contain the name of the host, location that the host was found, date of collection, site of collection, and your name. This information can be written in pencil on a small piece of manila card and included in the specimen vial.

The identification of burrowing mites, such as sarcoptic mites in wombats, and scaly-leg mites in birds are best diagnosed by chemically digesting (with potassium hydroxide) portions of the skin taken by scraping the affected region with a scalpel blade or collecting a small skin biopsy.

MICROBIOLOGICAL STAINING METHODS

All reagents can be easily purchased commercially.

Gram Stain

Over 100 years old but still one of the front-line stains used in bacteriology today.

- Make a thin smear of the sample onto a glass slide.
- Fix the smear to the slide with gentle heating and allow to cool.
- Place the slide on a staining rack and flood with Crystal Violet. Let sit for 30 seconds.
- Wash the slide with Lugol's iodine and leave on for 30 seconds.
- Rinse the slide quickly with acetone and wash with water. Repeat if the smear is a too thick.
- Flood the smear with dilute carbol fuchsin for 30 seconds.
- Gently wash the slide in a container of tap water and leave it to thoroughly dry or gently blot dry with paper towel.
- Examine the smear using oil immersion microscopy. Gram-positive bacteria are bluish / purple, whilst gram-negative bacteria are pinkish/red.

Ziehl Neelsen (ZN) Stain

Used to detect Acid Fast Bacilli (AFB), which include *Mycobacteria* species. AFBs will appear as beaded, red bacilli when examined under oil immersion microscopy against a green background.

- Flood slide with strong carbol fuchsin and heat until steam rises. Leave stain for 10 mins.
- Rinse with water.
- Decolourise with 3% acid-alcohol for exactly 5 mins.
- Rinse with water.
- Counter stain with 1% malachite green for 2 mins.

To make: **Acid-Alcohol** (3% acid in 95% ETOH)

- 20 mls distilled water, then add
- 30 mls conc. Hydrochloric Acid
- Make up to 1 Litre with Absolute (100%) Ethanol

Modified Ziehl Neelsen

Used to stain for Cryptosporidium, Myxosporidia and Microsporidia, which will stain red on a green background.

- Using an applicator stick, smear a thin sample of the thick suspension from the wet preparation onto a glass slide and heat fix
- Flood the slide with carbol fuchsin (cold) for 5 minutes, wash with tap water
- Decolourise with 3% acid alcohol for approximately 15 seconds, wash with tap water
- Counter stain with 1% malachite green for 1 minute, wash with tap water
- Examine under low power and 100x

HELPFUL REFERENCES

Texts:

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Clark, P. (2004). *Haematology of Australian Mammals.* (CSIRO Publishing)

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Claver, J. A., Quaglia, A, E. (2009). Comparative Morphology, Development and Function of Blood Cells in Nonmammalian Vertebrates. *Journal of Exotic Pet Medicine* 18, 87-97.

Heatley, J. J. and Johnson, M. (2009) Clinical Technique: Amphibian Hematology: A Practitioner's Guide. *Journal of Exotic Pet Medicine* 18, 14-19.

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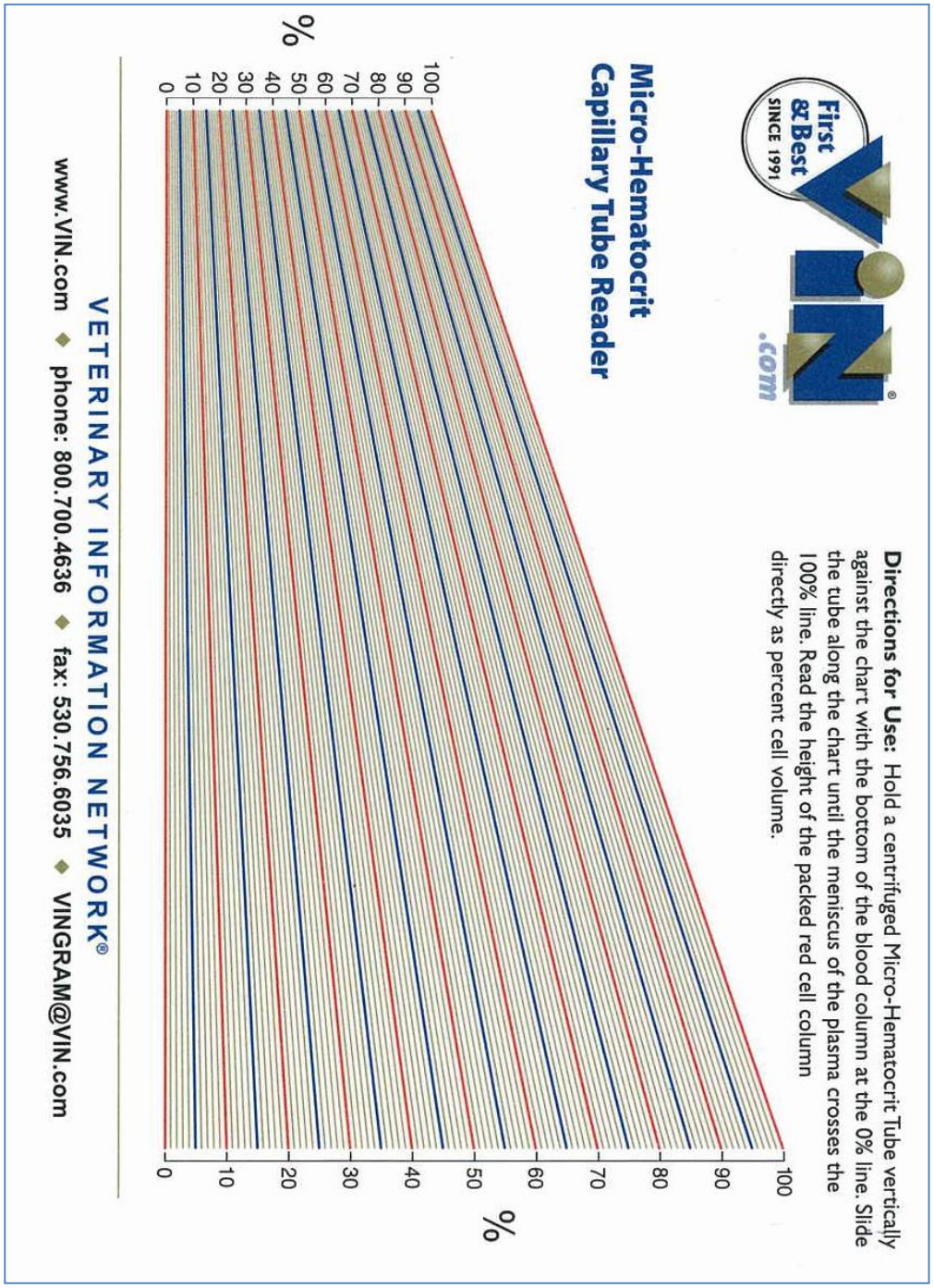
Scullion, F. T. and Scullion, M. G. (2009) Gastrointestinal Protozoal Diseases in Reptiles. *Journal of Exotic Pet Medicine* 18, 266-278.

Stacey, N. I., Alleman, A. R., Saylor, K. A. (2011). Diagnostic Hematology of Reptiles. *Clinical Laboratory Medicine* 31, 87-108.

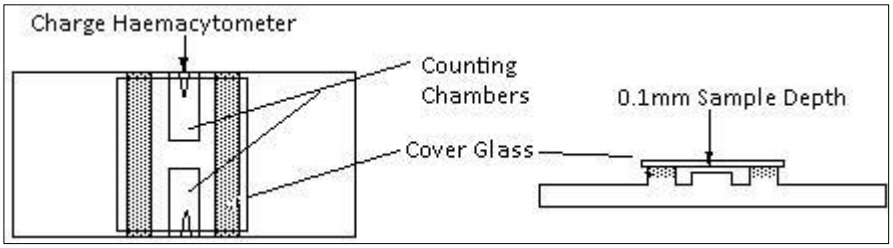
Work, T. M., Raskan, R. E., Balazs, G. H., and Whittaker, S. D. (1998) Morphologic and Cytochemical Characteristics of Blood Cells from Hawaiian Green Turtles. *American Journal Veterinary Research* 59: 1252-1257.

APPENDIX I: Micro-haematocrit Capillary Tube Reader



APPENDIX II: The Improved Neubauer Haemocytometer

A Haemocytometer is comprised of two parts, a glass cover glass and two counting chambers surrounded by a moat. The cover glass is thicker than a regular coverslip and is a better quality so as to have no surface aberrations.



There are a few different Haemocytometers on the market and the one we use is the Improved Neubauer Haemocytometer. The depth between the cover glass and the ruled area is 0.1 mm. There are two ruled areas on the Haemocytometer each being 9 mm^2, divided into nine large squares. Each of the four large corner squares is divided into 16 smaller squares. The central large square is divided into 400 tiny squares arranged in 25 groups of 16 by triple boundary lines.

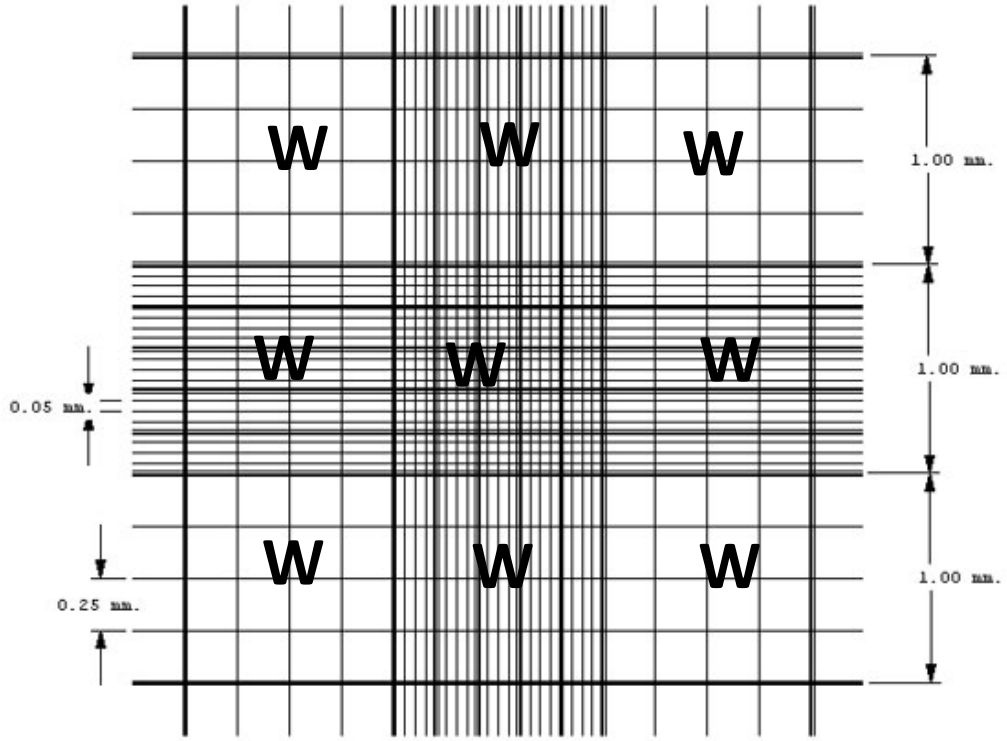
Prior to use the cover glass and Haemocytometer should be thoroughly cleaned and free from any dirt or residue and be completely dry. The cover glass can then be placed on top of the Haemocytometer and the sample added. Pipettes or capillary tubes can be used to do this. It is important to charge the Haemocytometer in one uninterrupted flow, careful not to overflow the sample into the moat.

Haemocytometer can then be placed in a moistened chamber for 5 – 10 minutes to allow the cells to settle.

The Improved Neubauer has triple ruling surrounding all the large squares (1mm x 1mm) as well as around the small squares (0.05mm x 0.05mm). When counting, as a rule only count the cells in or touching the left and upper middle grid line, ignoring any cells touching the right and bottom middle grid line.

The total volume of the ruled area is 0.9 µl (mm^3) with each large square 0.1 µl and each small square 0.004 µl. From these values and the dilution factor, it is a matter of calculating the cells per litre of blood.

APPENDIX III: Total White Blood Cell Count Method



** In some texts, the description of the manual count using the counting chamber may have the raw WBC count in mm^3. Keep in mind this is equivalent to 1µl. Then you can convert to litres.

For Mammals:

1. Count the total number of WBCs (**N**) present in the 9 large squares (W)
2. Multiply N by the correction factor, CF (**10%)** for volume of sample under coverslip (*0.9µl volume to 1.0µl*).
3. Multiply by the dilution factor (**DF**). *For mammals, this will be* ***100***
4. Multiply by 10^6. *Brings the total volume to one litre*

Example:

N = 50 cells
CF = 50 cells x 10%
(N + CF) = 55 cells

Total WBC (per L) = ((N + (N x CF)) x DF x 10^6

Then Total count (WBC) = 55 x 100 / µl
= 5.5 x 103/ µl

Then to convert to WBC/L = 5.5 x 103 x 106 µl
= 5.5 x 109 / L

For Birds and Reptiles (also for Thrombocyte Count)

Similar to Mammals, however a different dilution factor

1. Count the total number of WBCs (N) present in the 9 large squares (W)
2. Multiply N by the correction factor, CF (10%) for volume of sample under coverslip (0.9 µl volume to 1.0 µl)
3. Multiply by the dilution factor (DF). For birds and reptiles, this will be 200
4. Multiply by 106. Brings the total volume to one litre

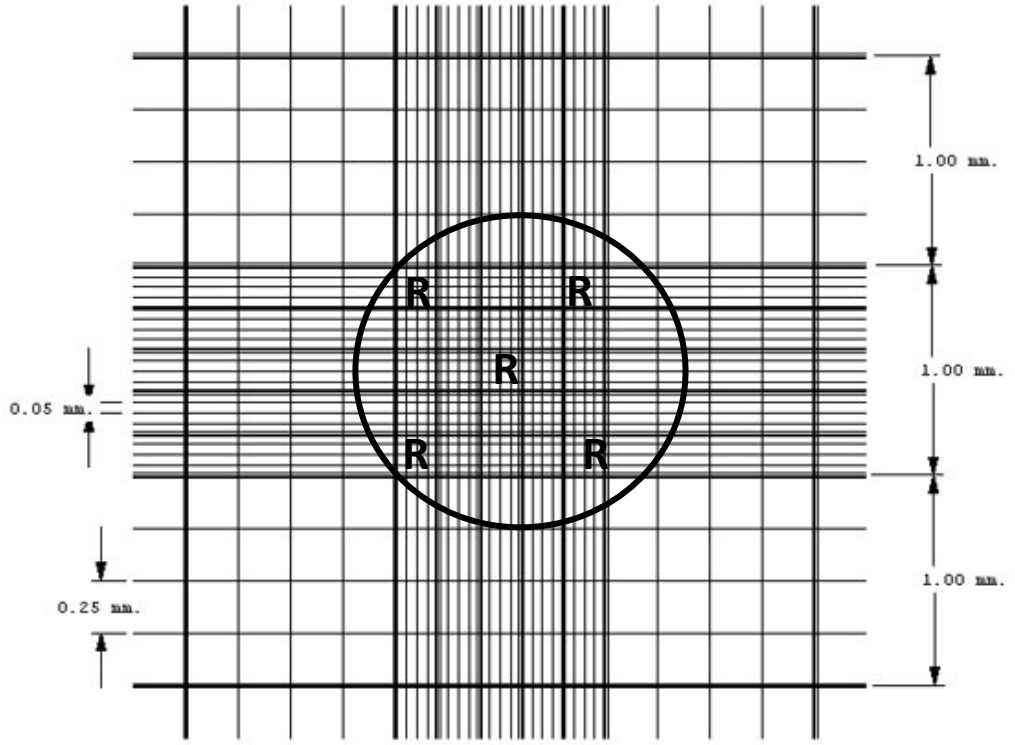
Example:

N	= 50 cells
CF	= 50 cells x 10%
(N + CF)	= 55 cells

Total WBC (per L) = ((N + (N x CF)) x DF x 106

Then	Total count (WBC)	= 55 x 200 / µl
		= 11.0 x 103/ µl
Then convert to WBC/L		= 11.0 x 103 x 106/ µl
		= 11.0 x 109 / L

APPENDIX IV: Total Red Blood Cell Count Method (Birds and Reptile)



Because there are so many more RBCs in the blood compared to WBCs, the count is focused around the central square of the chamber.

1. Count the number of RBCs (N) present in 5 of the smaller squares (R). Good distribution represented in the diagram
2. Multiply N by the correction factor, CF (50) for volume of sample under coverslip within the large central square (1 µl ÷ (5/25 x 0.1 µl) = 50)
3. Multiple by the dilution factor (DF). For birds and reptiles, this will be 200
4. Multiple by 106. Brings the total volume to one litre

Example:

N	= 600 cells
CF	= 600 cells x 50
(N + CF)	= 30,000 cells

Total RBC (per L) = (N + (N x CF)) x DF x 106

Then, Total count (RBC)	= 30,000 x 200 / µl
	= 6.0 x 106 / µl
Then to convert to RBC/L	= 6.0 x 106 x 106 / µl
	= 6.0 x 1012 / L

APPENDIX V: Mammalian White Blood Cell Characteristics

CELL		NUCLEUS				CYTOPLASM			
	Size (µm) / Shape	**Shape**	**Staining Quality**	**Chromatin**	**Position**	**Relative Amount**	**Colour**	**Granules**	**Vacuoles**
1. Granulocytes (Segmented)									
Neutrophil	10-15; Round	Band to segmented	Purplish blue	Coarse	Slightly eccentric	Large	Faint pink	Faint pink	Absent
Eosinophil	10-15; Round	2-3 lobes	Purplish blue	Coarse	Slightly eccentric	Large	Bluish pink	Bright orange to bluish-pink	Absent
Basophil	10-15; Round	Outline covered with granules	Pale blue	Indistinct	Slightly eccentric	Moderate	Bluish pink	Bluish to dark purple	Absent
2. Lymphocyte	6-18; Round but may have irregular outline	Round, oval or slightly. indented	Deep purplish blue	Large coarse clumps	Central	Small to moderate	Pale blue	Few azurophilic	Absent
3. Monocyte	12-18; Round to oval	Round, indented, band or lobed	Purple	Fine strands	Central, Slightly eccentric	Moderate	Grey or grey/blue	Gary or grey/blue	May contain in activation
4. Thrombocytes	2-4; Round	None	None	None	None	Absolute	Pale blue	Pale blue	Absent

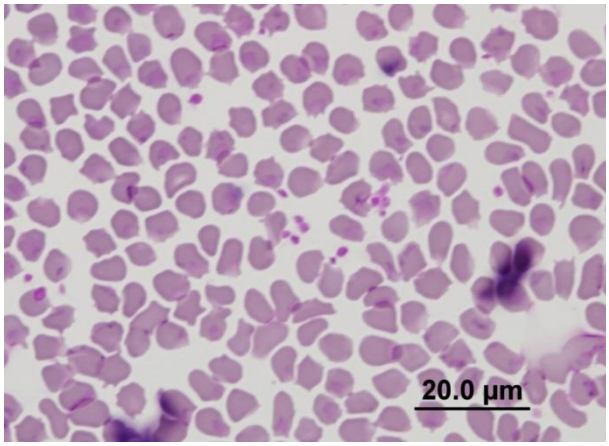
APPENDIX VI: Reptilian White Blood Cell Characteristics

CELL		**NUCLEUS**				**CYTOPLASM**			
	Size (µm), Shape	**Shape**	**Staining Quality**	**Chromatin**	**Position**	**Relative Amount**	**Colour**	**Granules**	**Vacuoles**
1. Granulocytes (Segmented)									
Heterophil	10-23; Round	Round, oval or lobular	Purplish blue	Densely clumped	Eccentric	Large	Colourless	Bright orange, refractile, rod to spindle shape	Absent
Eosinophil	11-17; Round	Round, oval or bi-lobed	Purplish blue	Densely clumped	Slightly eccentric	Large	Clear to light blue	Round and strongly eosinophilic (some species blue)	Absent
Basophil	8-15; Round	Round	Light purple	Densely clumped	Slightly eccentric	Moderate	Bluish pink	Round; dark blue to purple	Absent
2. Lymphocyte	5-15; Round to polygonal. May have bleb-like protrusions	Round or slightly indented	Pale to deep purplish blue	Densely clumped	Central to slightly eccentric	Small	Pale blue	Occasional azurophilic	Absent
3. Monocytes	8-25; Round to Oval	Round, oval, indented, band or lobed	Pale purple	Slightly clumped	Slightly eccentric	Moderate	Blue-grey	Fine dust-like orange and pale blue	May contain in activation
Azurophils	8-25; Round to Oval	Round, oval, indented, band	Purple	Slightly clumped	Eccentric	Moderate	Blue-grey	Fine dust-like and azurophilic to purple	May contain in activation
4. Thrombocytes	8-16 x 5-9; Oval to fusiform; Irregular in activation	Oval, indented	Pale to deep purple	Densely clumped	Central	Small	Colourless to pale blue	Some species may contain a few azurophilic granules	May contain in activation

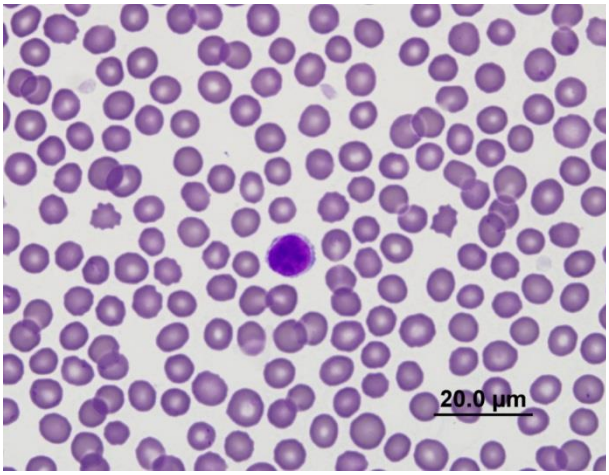
APPENDIX VII: Avian White Blood Cell Characteristics

CELL		NUCLEUS				CYTOPLASM			
	Size (µm) / Shape	**Shape**	**Staining Quality**	**Chromatin**	**Position**	**Relative Amount**	**Colour**	**Granules**	**Vacuoles**
1. Granulocytes (Segmented)									
Heterophil	Round	Lobed (2 to 3)	Purple	Coarse and clumped	Eccentric	Large	Colourless	Rod to oval or spindle-like. May also be refractive. Dark orange to brown-red	Absent
Eosinophil	Round	Lobed	Deeper purple	Clumped	Slightly eccentric	Large	Clear blue	Round to rod shape; strongly eosinophilic (some species blue)	Absent
Basophil	Round	Can be non-lobed	Light blue	Indistinct	Slightly eccentric	Moderate	Bluish pink	Round and very basophilic	Absent
2. Lymphocyte	Round or elongated	Round, oval or slightly indented	Pale purple	Large coarse clumps	Central to Slightly eccentric	Small to moderate	Pale blue	May have few azurophilic to basophilic	Absent
3. Monocyte	Round to amoeboid	Round, indented, band or lobed	Pale purple	Fine clumping	Slightly eccentric	Moderate	Blue grey	Fine dust-like eosinophilic granules	May contain in activation
4. Thrombocytes	Round to oval	Round to oval	Deep purple	Densely clumped	Central	Small	Colourless to pale grey	Some species may contain fine eosinophilic granules	May contain in activation

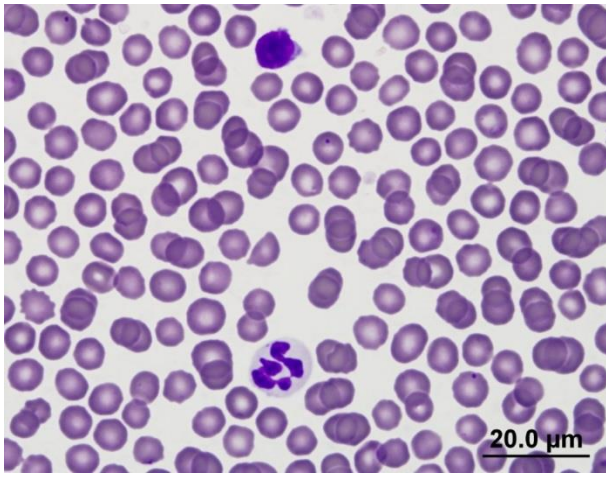
IMAGE GUIDE 1: Typical Blood Morphology of Mammals



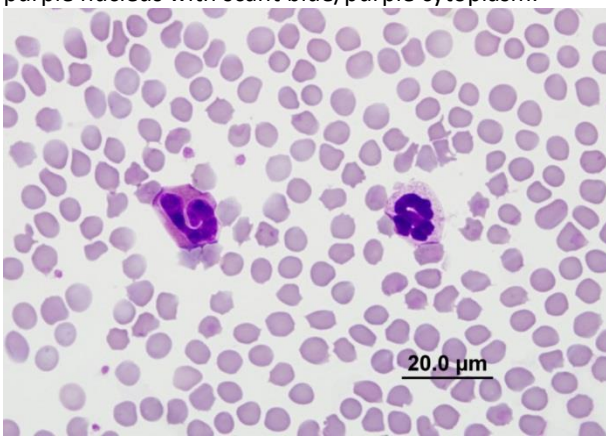
Bettong, Thrombocytes. May vary in size and shape but notably much smaller than birds, reptiles and amphibians with no nucleus.



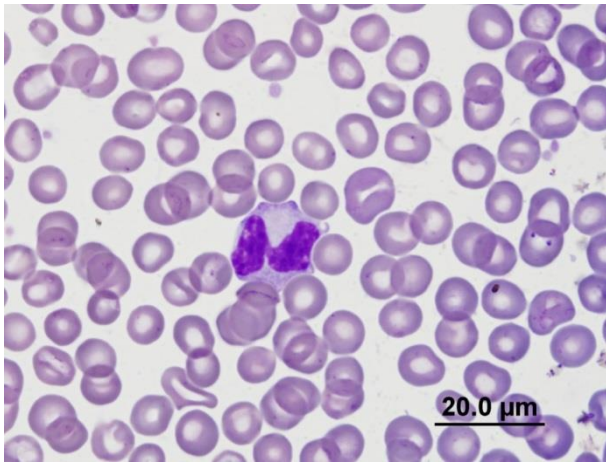
Short-beaked Echidna, Lymphocyte. Note that mammalian red cells are not nucleated and are much smaller than the bird, reptile and amphibian RBCs. Lymphocytes, as with other species, consist of a large purple nucleus with scant blue/purple cytoplasm.



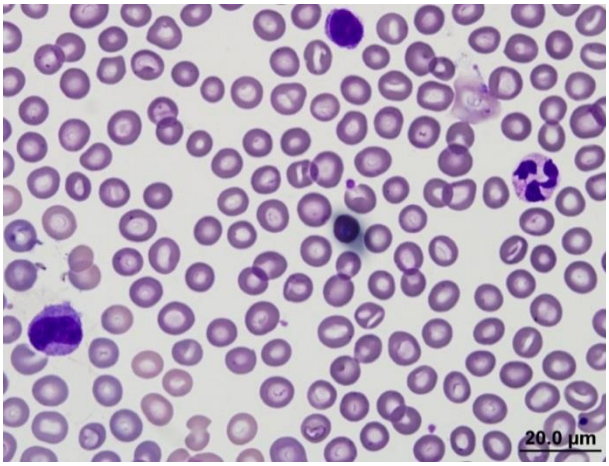
Short-beaked Echidna, Neutrophil (bottom), Lymphocyte (top). The neutrophil is a large cell with a large, multilobulated dark purple nucleus and faintly pink cytoplasm. The lymphocyte, by comparison is smaller with a dense purple nucleus and scant blue/purple cytoplasm.



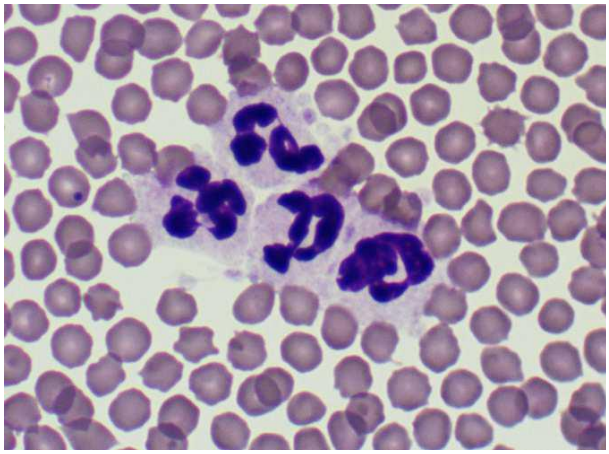
Bettong, Neutrophil (right) and Eosinophil (left). Neutrophil has a multilobulated, dark purple nucleus with coarse pink cytoplasm. Eosinophil is of a similar size but has a bright orange/pink cytoplasm. Appearance of both cell types differs from species to species.



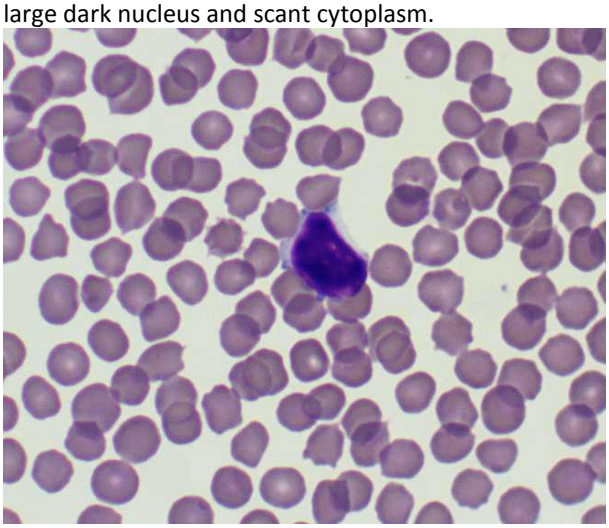
Koala, Monocyte. The monocyte is a large cell with a dark purple, lobulated (in this instance) nucleus and a large amount of purple cytoplasm.



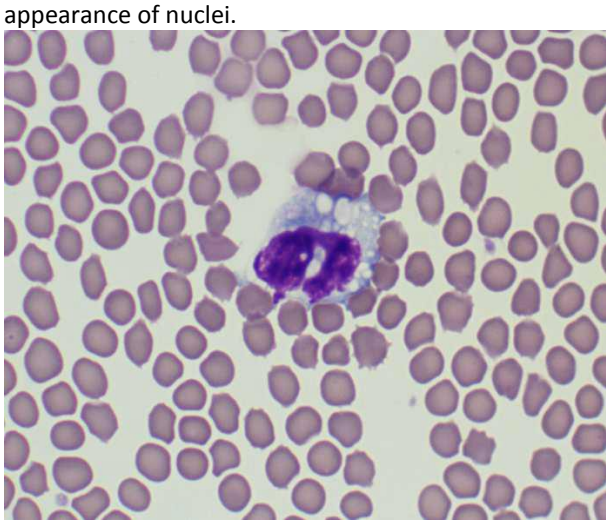
Koala, Monocyte (left), Lymphocyte (top) and Neutrophil (right). In this example, the monocyte has a band-like nucleus with darkly staining cytoplasm and is of a similar size to the neutrophils. The neutrophil has a multilobulated nucleus and pale pink staining cytoplasm. The lymphocyte is small with a large dark nucleus and scant cytoplasm.



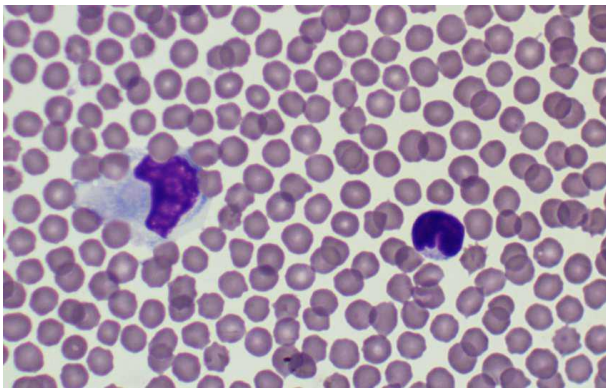
Brush-tailed Bettong, Neutrophils. Note the different appearance of nuclei.



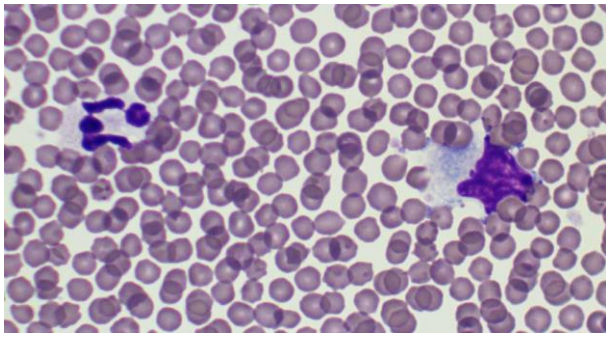
Brush-tailed Bettong, lymphocyte



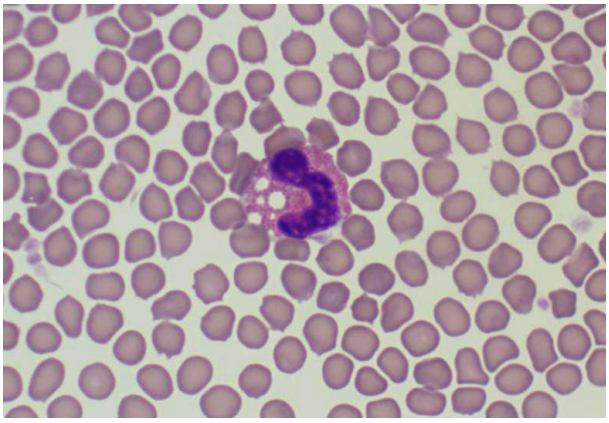
Brush-tailed Bettong, Monocyte



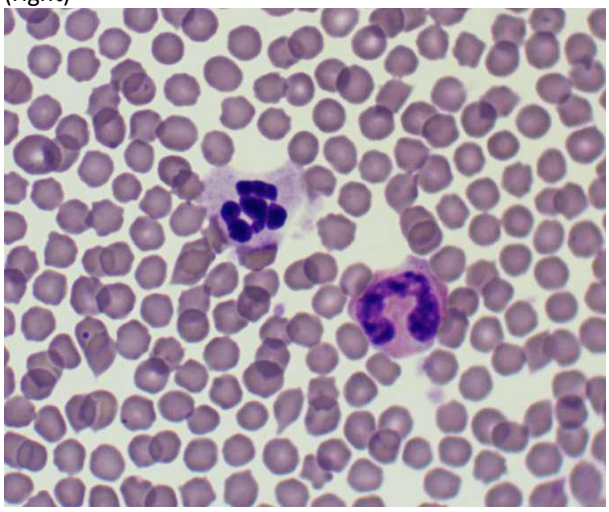
Brush-tailed Bettong. Monocyte (left) and lymphocyte (right)



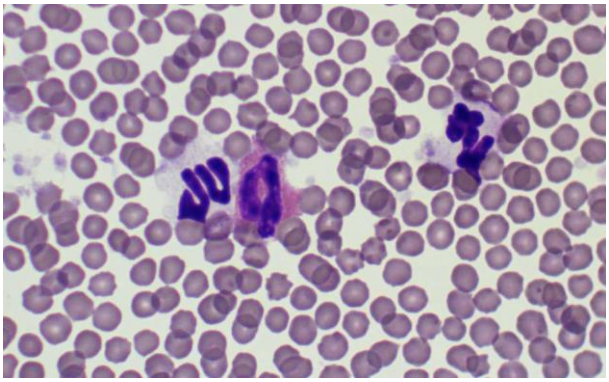
Brush-tailed bettong. Neutrophil (left), and monocyte (right)



Brush-tailed Bettong. Eosinophil.

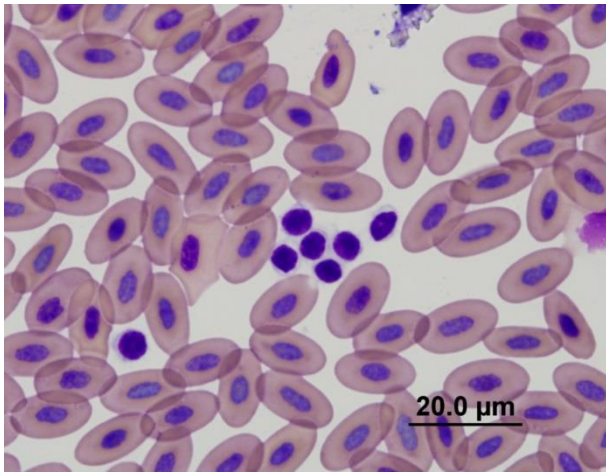


Brush-tailed Bettong. Neutrophil (left), and eosinophil (right)

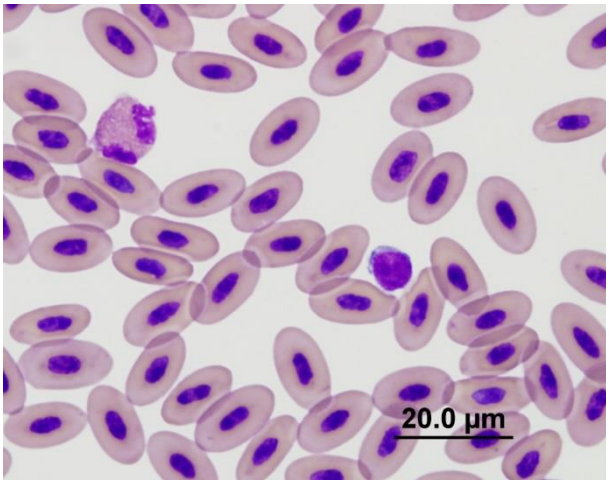


Brush-tailed Bettong. Neutrophils (2) and Eosinophil.

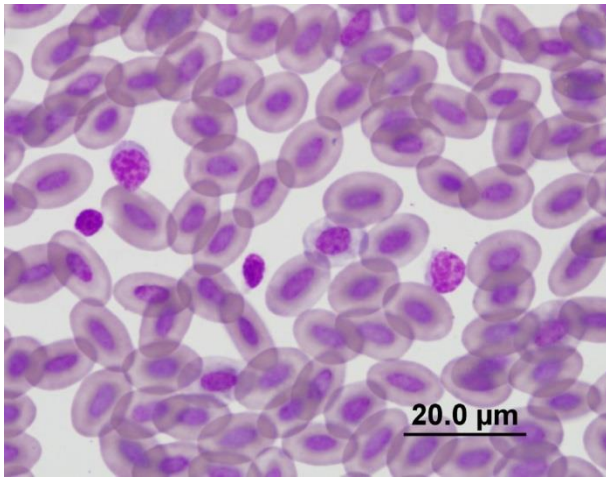
IMAGE GUIDE 2: Typical Blood Morphology of Birds



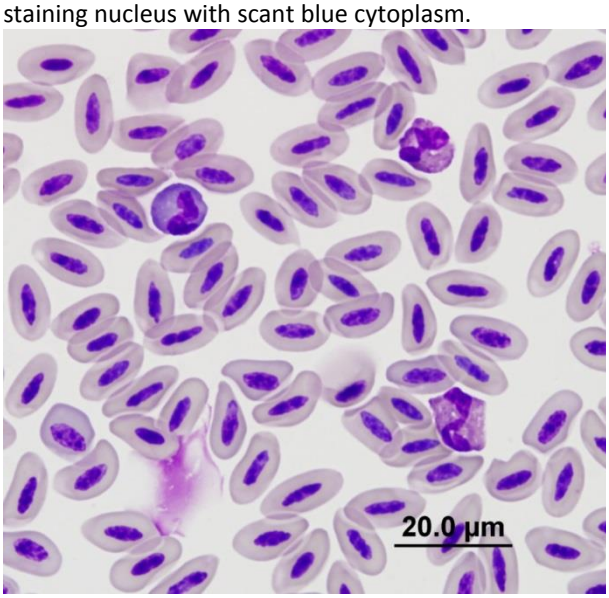
Australian Pelican, Thrombocytes. The thrombocytes appear as dark round nuclei with pale grey, almost colourless, cytoplasm.



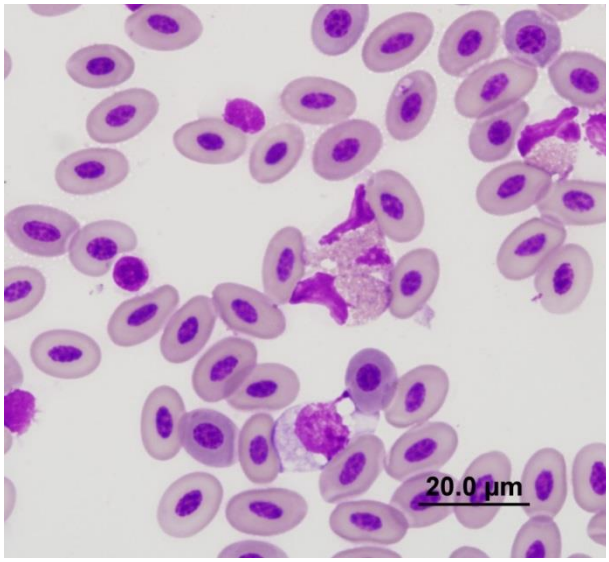
Australian Pelican, Heterophil (left), Lymphocyte (right). Heterophil contains a multilobulated, purple, eccentric nuclei and pink, granular cytoplasm. Lymphocyte is much smaller and has a large, purple staining nucleus with scant blue cytoplasm.



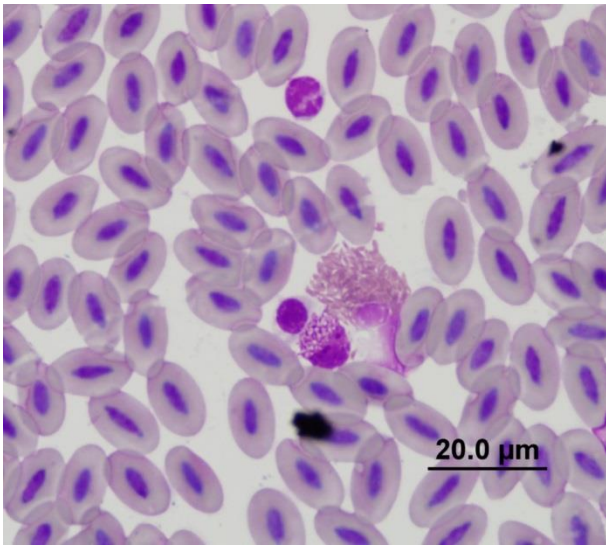
Little Penguin, Thrombocytes and Lymphocytes. Although they often appear quite similar, the defining difference between lymphocytes and thrombocytes is that lymphocytes have scant, blue/pale purple staining cytoplasm while thrombocytes have a clear or unappreciable cytoplasm.



Red-tailed Black Cockatoo, Heterophils (right), Eosinophil (left). Heterophils typically have pale pink staining cytoplasm with a lobulated purple nucleus. In this sample, the eosinophils appear blue/grey with a dark purple, lobed nucleus. This is typical of many cockatoo species.

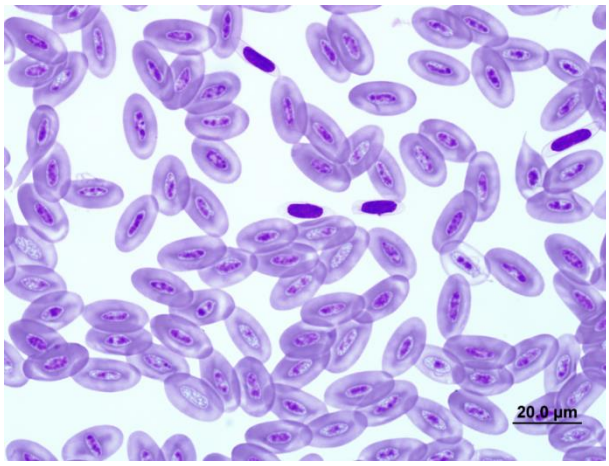


Little Penguin, Heterophils (central, right), Monocyte (bottom). Heterophils typically have pale pink staining cytoplasm with a lobulated purple nucleus. The monocytes are large with a purple nucleus and a lot of purple/blue staining cytoplasm. They may also contain clear cytoplasmic vacuoles. Note the RBC on upper right has cytoplasmic parasite.

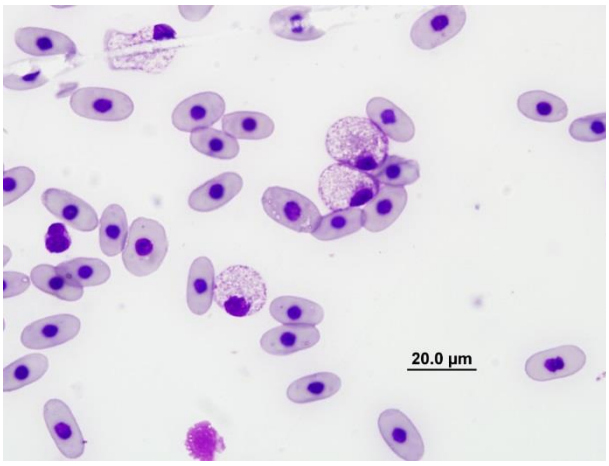


Brolga, Lymphocytes, heterophil (broken) and probable eosinophil (adjacent). This odd cell in the centre of field is likely an eosinophil due to the dark pink granules within the cytoplasm and the number of these cells encountered within the differential. It would be very uncommon to encounter multiple basophils in a single diff. Note the oval to lobular shaped granules of the broken heterophil. Lymphocytes are typical with large, round, purple nucleus and scant blue/pale purple cytoplasm.

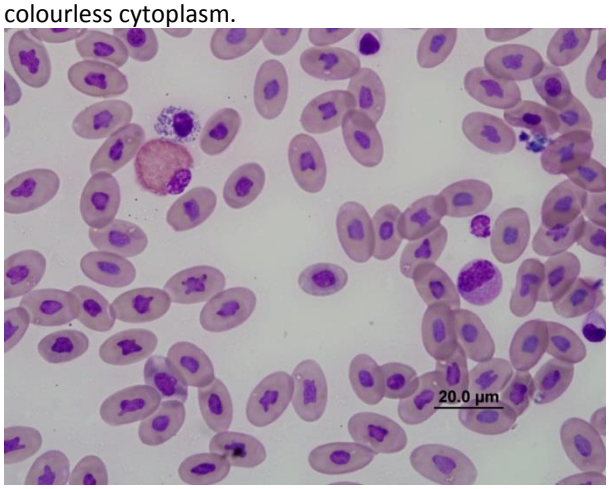
IMAGE GUIDE 3: Typical Blood Morphology of Reptiles



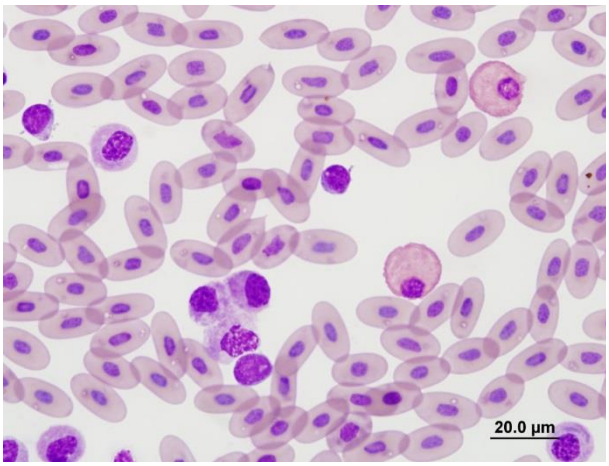
Flowerpot Blind Snake, Thrombocytes. Note that the red cells of reptiles are nucleated. The shape of thrombocytes in reptiles may vary but here they appear as an elongated, cigar-like shape with almost colourless cytoplasm.



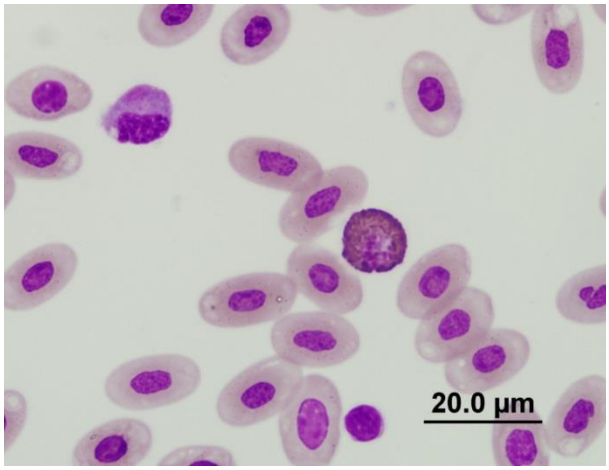
Saltwater Crocodile, Neutrophils. Neutrophils seen here have a dense, non-lobulated nucleus with pink cytoplasm. The appearance of cells can differ depending on species.



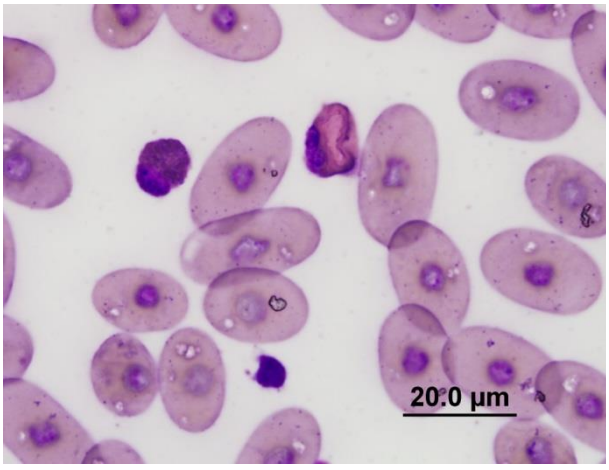
Diamond Python, Heterophil (left), Azurophil (right), Basophil (left). Heterophils have large pink granules and a dense, round, purple eccentric nucleus. The basophil is a smaller cell with a darkly staining, round, slightly eccentric nucleus surrounded by dark, round, cytoplasmic granules. Azurophil is a dark purple cell with a dark band like nucleus.



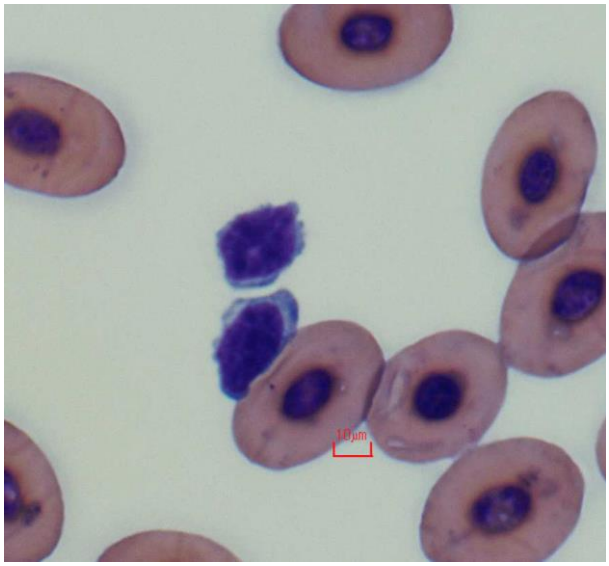
Carpet Python, Heterophil (right), Azurophil (scattered), Lymphocyte (upper left and central). The heterophils have large pink granules and a dense, round, purple eccentric nucleus. The Azurophil is a dark purple cell with a dark round nucleus and are considerably larger, with more cytoplasm, than the lymphocytes present.



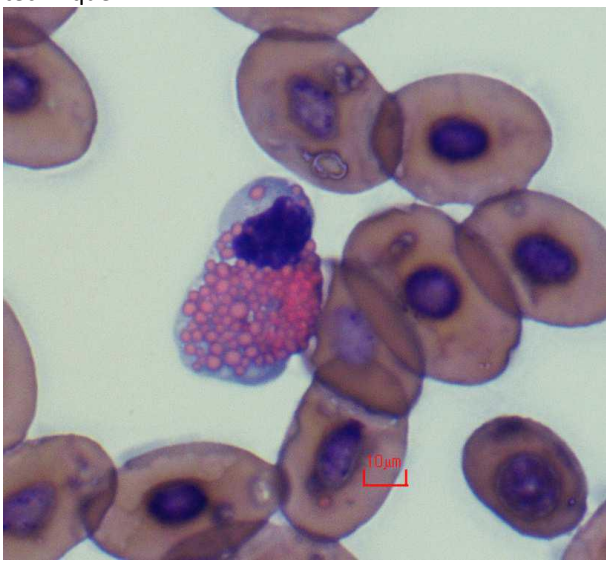
Red-bellied Black Snake, Heterophil (right), Azurophil (left). The heterophils have large pink granules and a dense, round, purple eccentric nucleus. The Azurophil is a dark purple cell with a dark, indented, eccentric nucleus and appears to have a clear cytoplasmic vacuole.



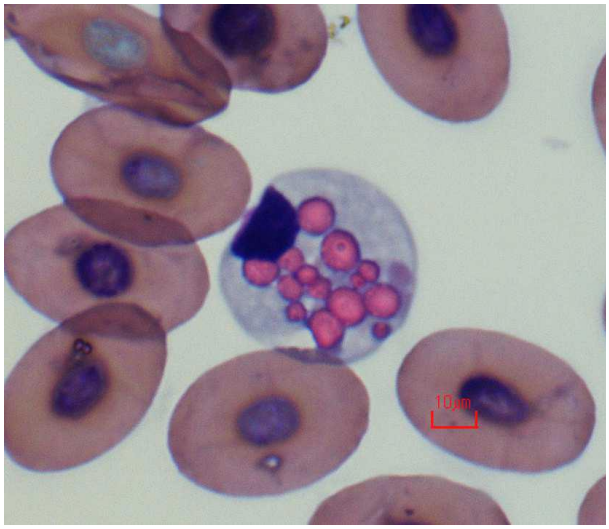
Macquarie Turtle, Heterophil (right), Eosinophil (left), Lymphocyte (bottom). When scanning this slide it is evident that, although similar, the eosinophils appear as slightly smaller, with more densely granular, darker staining cytoplasm. The heterophils in this animal appear slightly squashed due to poor staining technique.



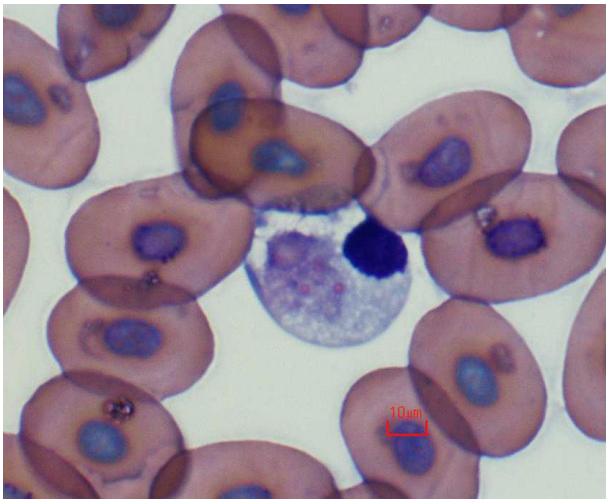
Green Sea turtle, lymphocytes. Similar morphology to other reptile and avian species.



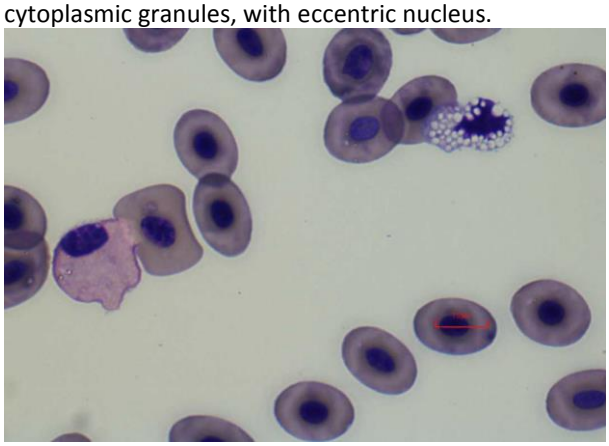
Green Sea turtle, eosinophil. Notice the many forms the Sea Turtle eosinophils can take. Dense, round cytoplasmic granules



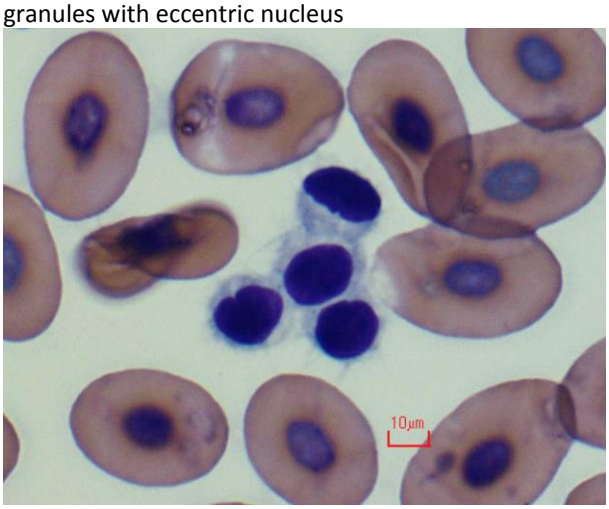
Green Sea turtle, eosinophil. Large, sparse, cytoplasmic granules, with eccentric nucleus.



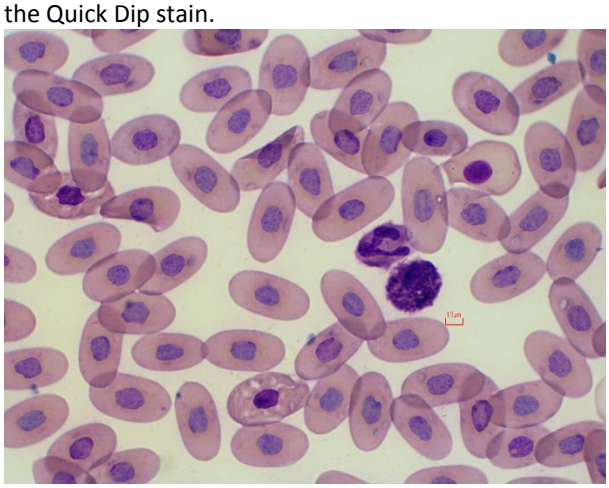
Green Sea turtle, eosinophil. Few cytoplasmic granules with eccentric nucleus



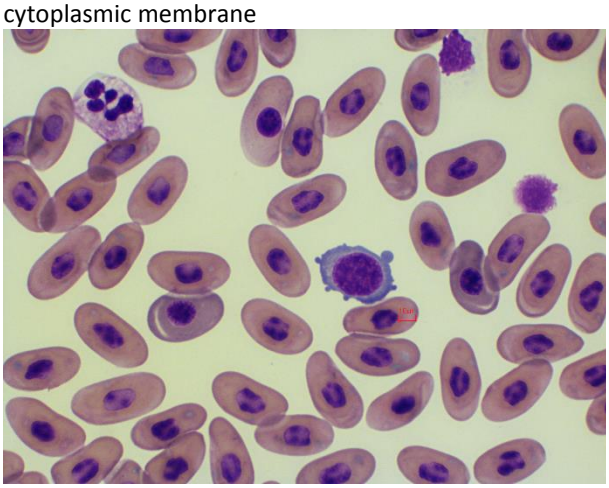
Sea turtle, heterophil (left), basophil (right). Notice the reverse staining in the basophil. Possible effect of the Quick Dip stain.



Green Sea turtle, thrombocytes. Similar size to lymphocytes but more elongated with a subtle blue cytoplasmic membrane

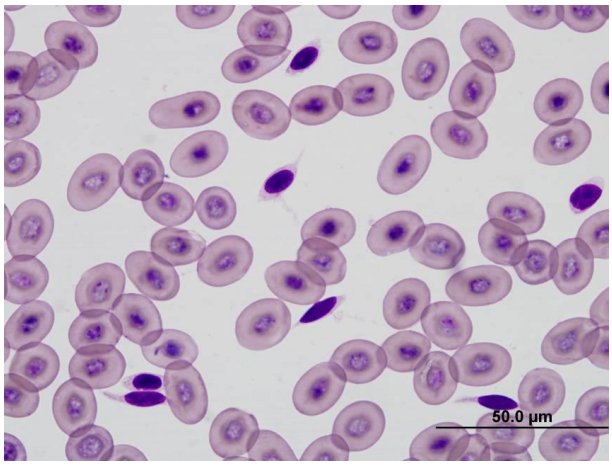


Eastern water dragon, heterophil (top) and basophil (bottom). Heterophil with dark blue, lobulated nucleus and pink cytoplasm. Basophil packed with dark purple granules.

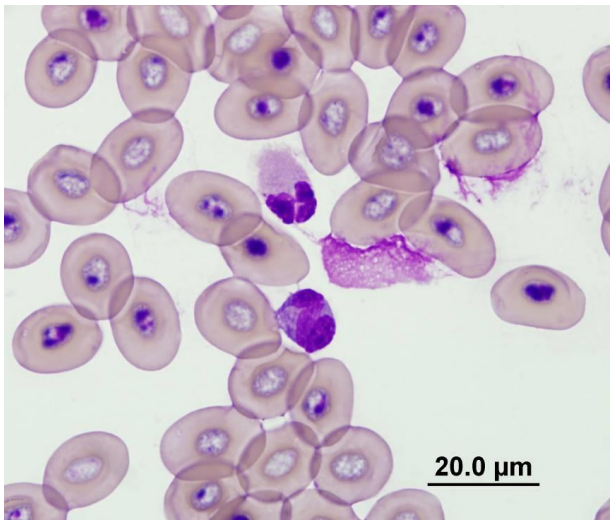


Eastern water dragon, immature red blood cell (centre-right) and heterophil (top left)

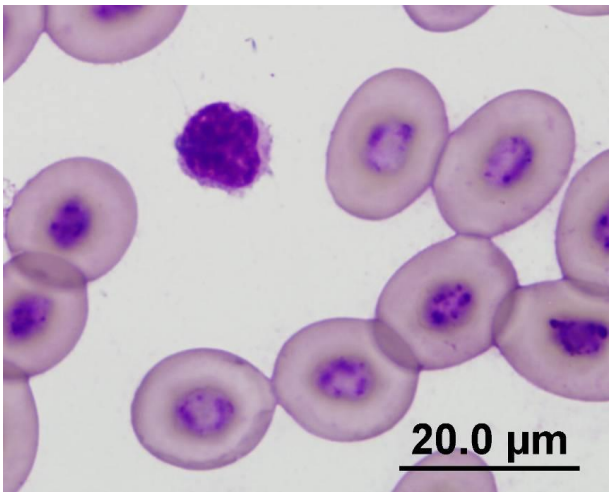
IMAGE GUIDE 4: Typical Blood Morphology of Amphibians



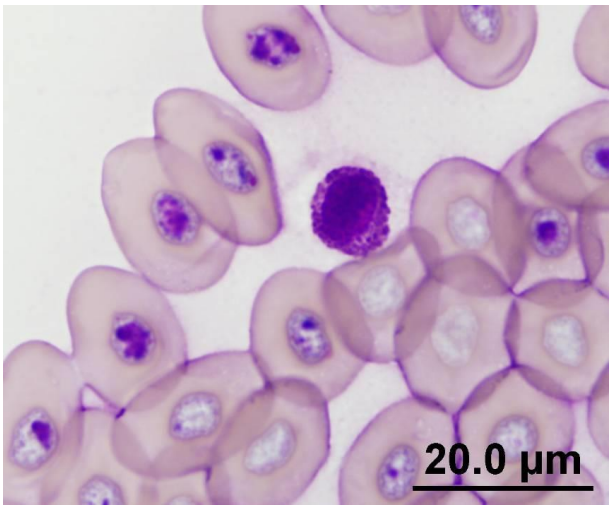
Green Tree Frog, Red Blood Cells and Thrombocytes. Note that the red cells of amphibians are nucleated unlike those of mammals. They typically appear to be larger than those of other vertebrates. The shape of thrombocytes in the amphibian may vary but are typically this elongated, cigar-like shape with almost colourless cytoplasm.



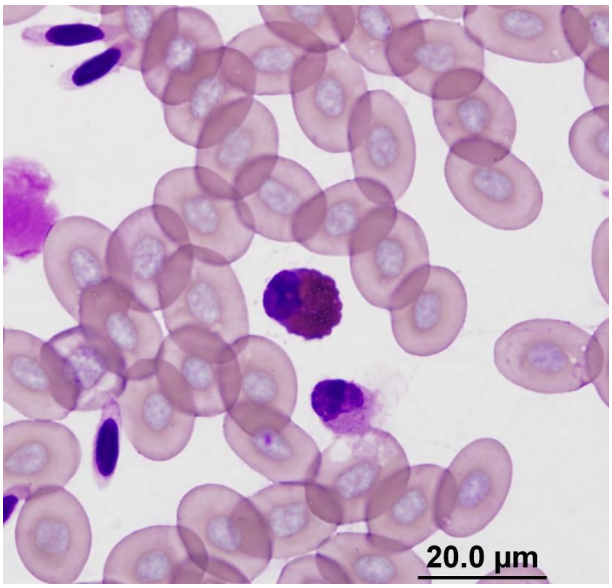
Magnificent Tree Frog, Heterophil and Azurophil. The Heterophils (upper) typically have a multi-lobulated nucleus and a large amount of pale purple to slightly pink staining cytoplasm. The azurophil (lower) is typically darker staining, with a large, often kidney shaped, dark purple nucleus and lots of darkly purple staining cytoplasm.



A Green Tree Frog, Lymphocyte. Lymphocytes are typically smaller than heterophils, eosinophils and azurophils. They have a dark purple staining nucleus and scant purple staining cytoplasm. Sometimes it may be difficult to differentiate lymphocytes and thrombocytes therefore we recommend that you take some time to scout around the entire slide to become familiar with the morphology of each cell type before proceeding to the actual differential cell count.

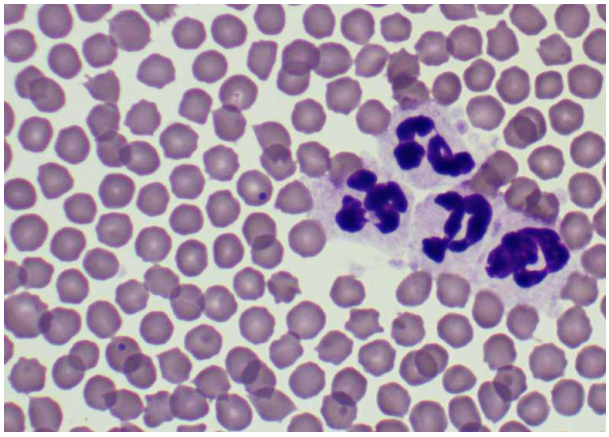


Magnificent Tree Frog, Basophil. The basophils are easily recognisable due to the large dark blue granules present in the cytoplasm of the cell. The nucleus is often obscured by the basophilic granules but when visible, is typically non-lobed. It is not uncommon for a differential to contain no basophils.

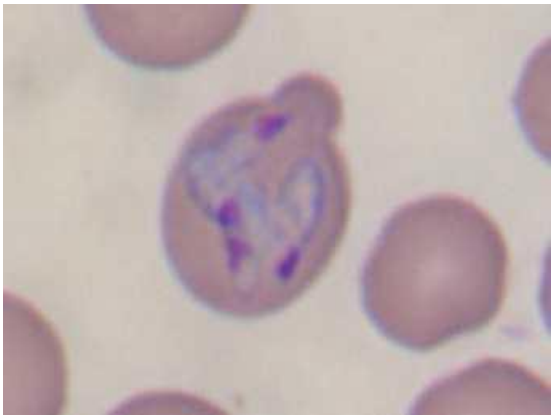


Magnificent Tree Frog, Eosinophil and Heterophil. The eosinophils (upper) have a dark pink, often very granular, cytoplasm and often have a bi-lobed nucleus (not seen here). The deeply dark pink cytoplasm is what differentiates the eosinophil immediately from the heterophil (lower). There are two lobular thrombocytes at the top left of this image, and another on the lower left.

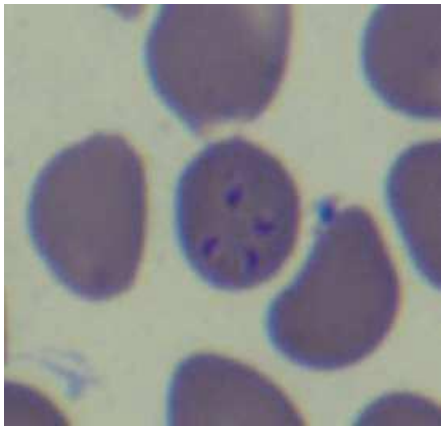
IMAGE GUIDE 5: Typical Blood Parasites and Inclusions



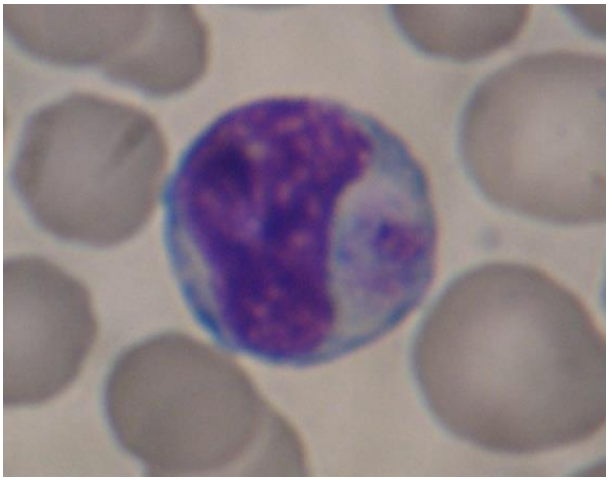
Howell Jolly Bodies. Dark, dense, small round red cell inclusions – normal artefact that occurs during red cell maturation.



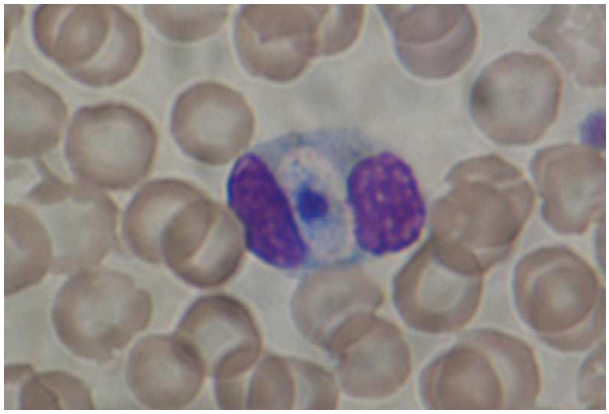
Eastern Grey Kangaroo, Babesia.



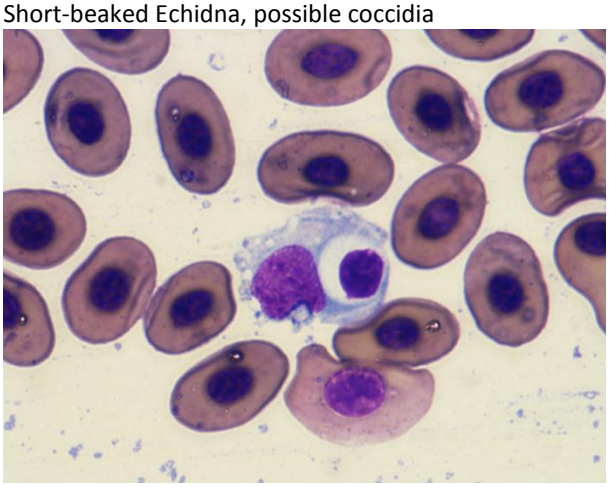
Short-beaked echidna. Theileria.



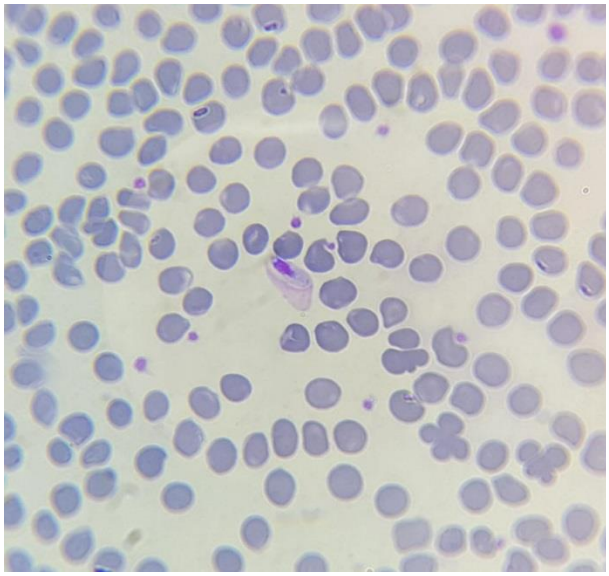
Short-beaked Echidna, possible coccidia



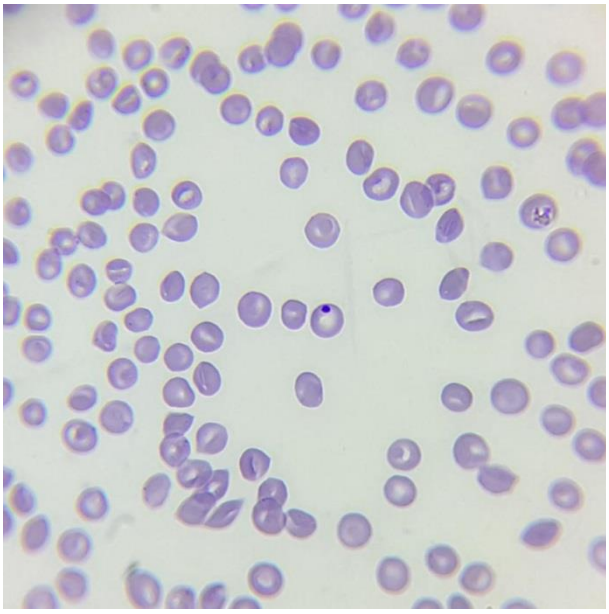
Bush Rat, possible hepatazoon



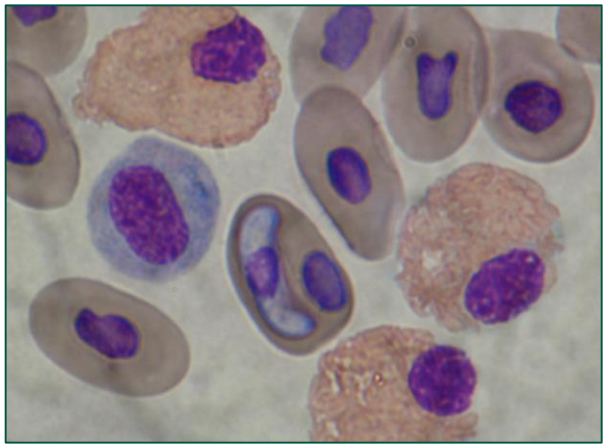
Green Sea turtle, possible coccidian (Caryospora)



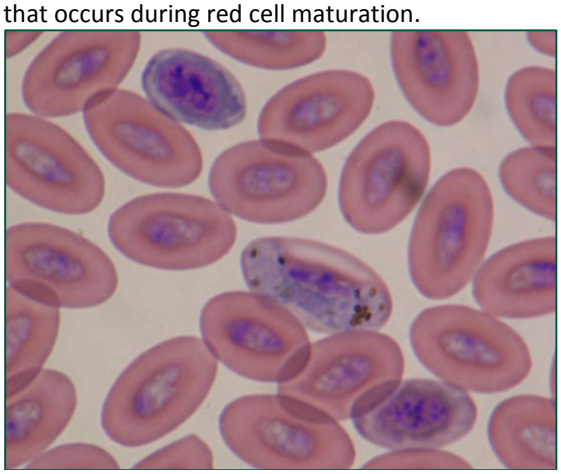
Southern Brown Bandicoot, hepatozoon



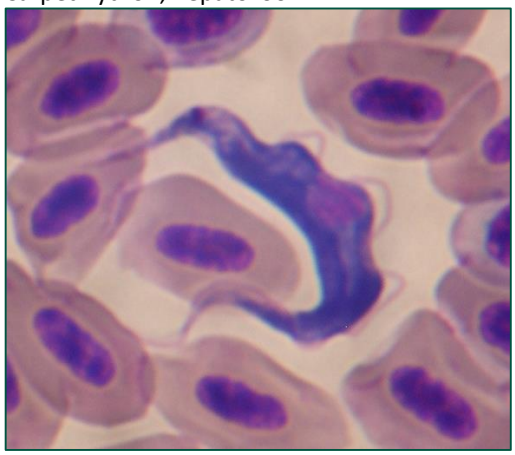
Southern Brown Bandicoot, Howell Jolly Body. Dark, dense, small round red cell inclusions, normal artefact that occurs during red cell maturation.



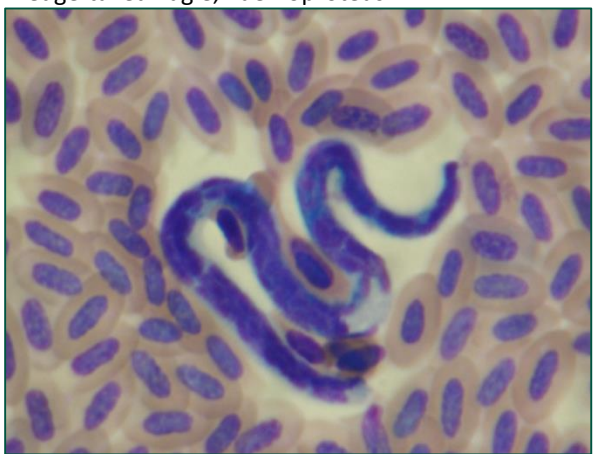
Carpet Python, hepatozoon



Wedge-tailed Eagle, haemoproteus



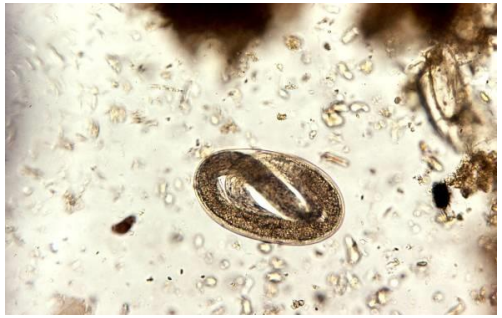
Regent Honeyeater, trypanosome,
notice the undulating flagellum



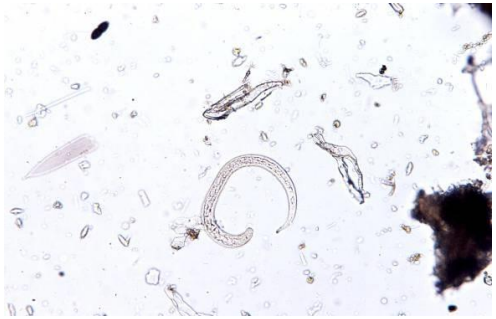
Striped Honeyeater, microfilaria

IMAGE GUIDE 6: Faecal Parasites – Strongyle-type ova

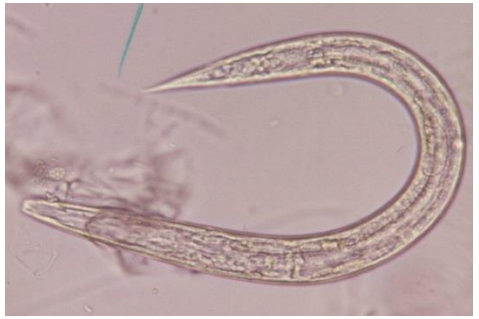
Stongyle-type ova (STO's) can be identified by their large size, thin shells, often broad ellipse shape, sometimes with moving larvae inside. Strongyles and trichostrongylid species are similar in size and appearance and therefore difficult to identify. If identification is necessary, faecal culture yielding L3 larvae is required.



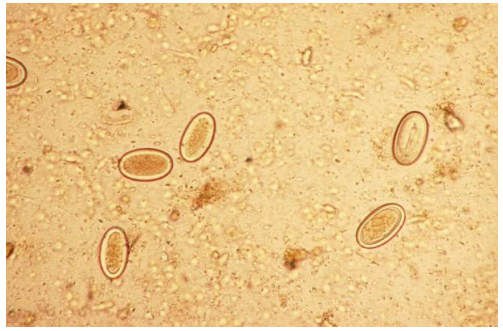
Strongyle-type ova, Eastern Grey Kangaroo, 20x



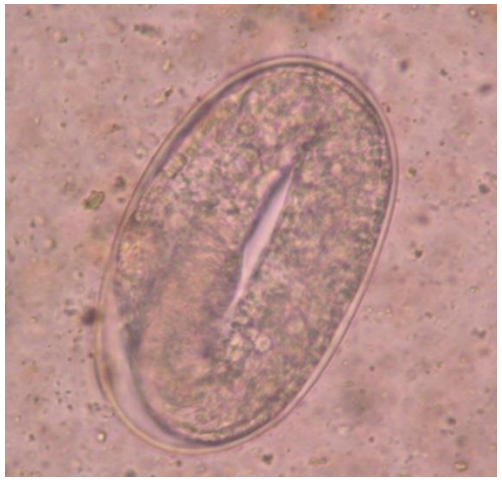
Strongyle larva, Brushtail possum, 20x



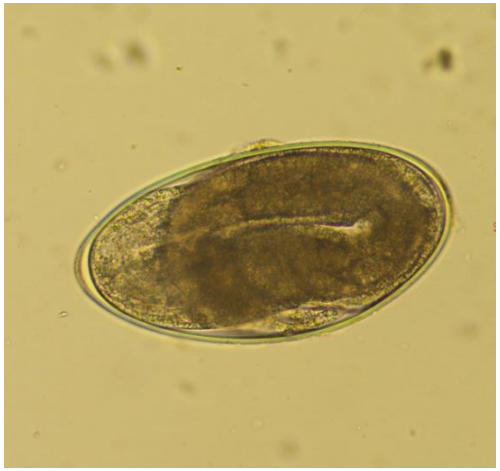
Strongyle larvae, macropod, 40x



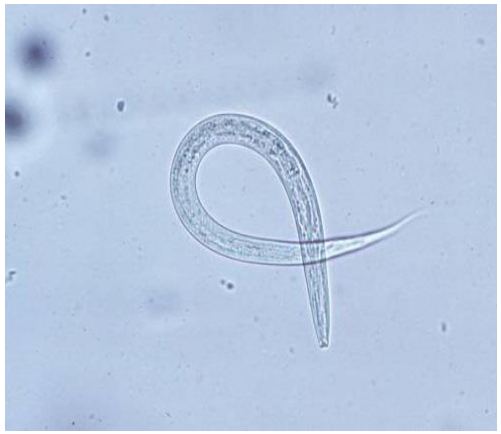
Spiurid, Eastern Water Dragon



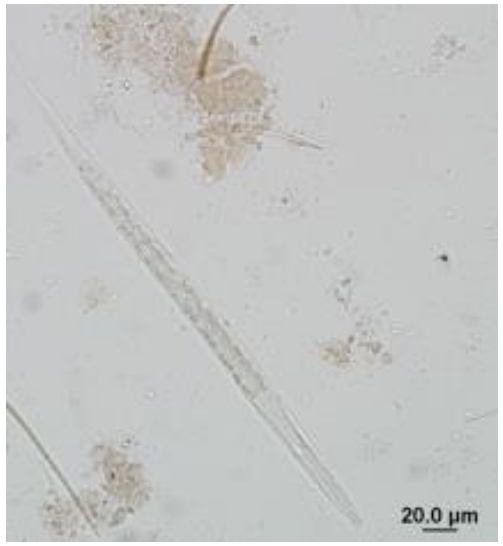
Strongyle-type ova, Quokka, 40x



Strongyle-type ova, macropod, 40x



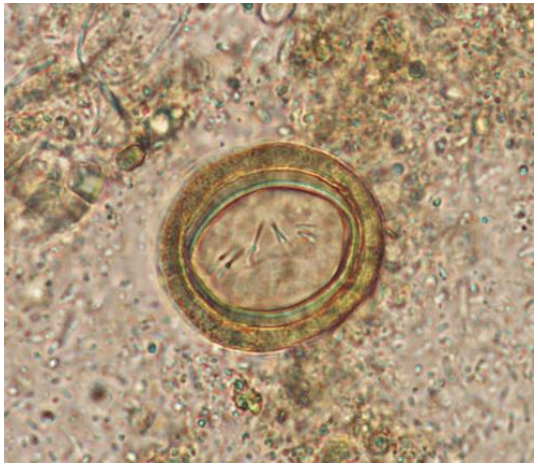
Strongyle larva, Quail, 200x



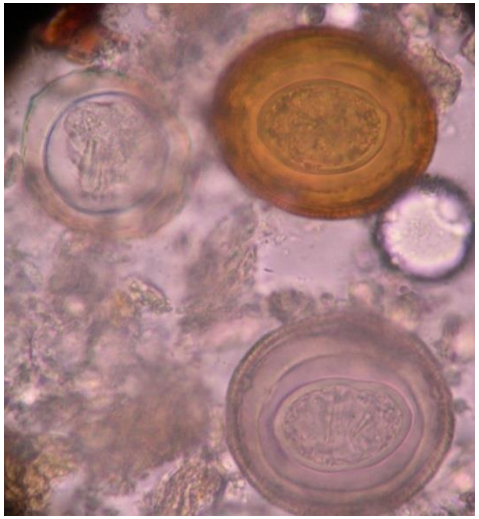
Strongyle larvae, frog, 10x

IMAGE GUIDE 7: Faecal Parasites – Cestodes

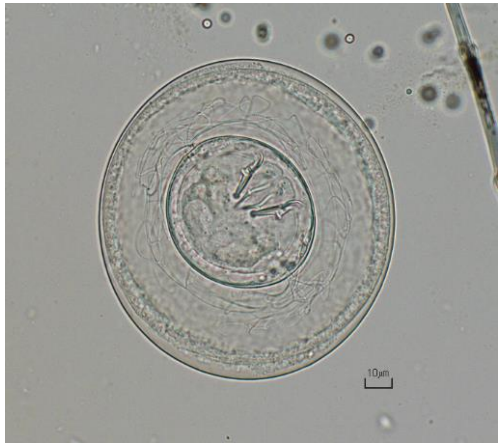
Cestodes can be identified, in most circumstances, by the presence of characteristic hooked econosphere. Ova can be spherical or ovoid and typically have a thick shell that is often striated.



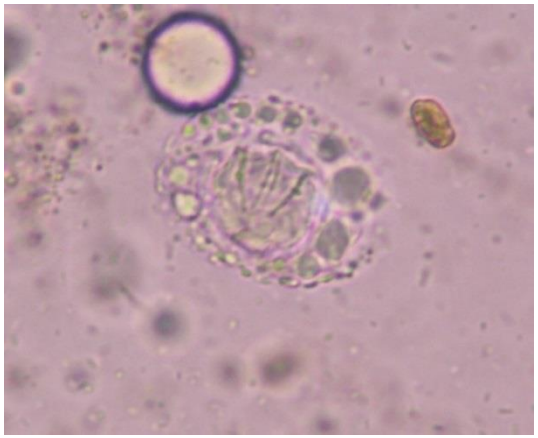
Anoplotaenia dasyure, 40x, Tasmanian Devil



Cestode ova, Long-nosed Bandicoot, 40x

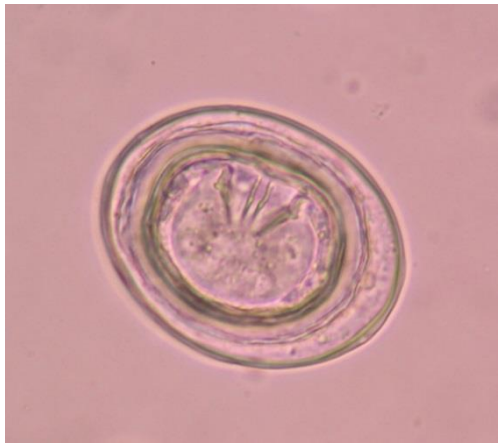


Cestode ova, Australian Raven , 10x

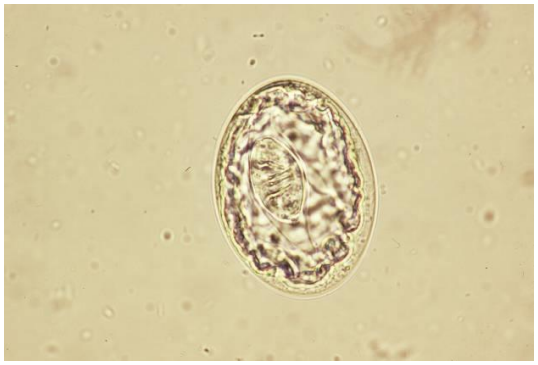


Linstowia echidnae, 40x, Short-beaked Echidna

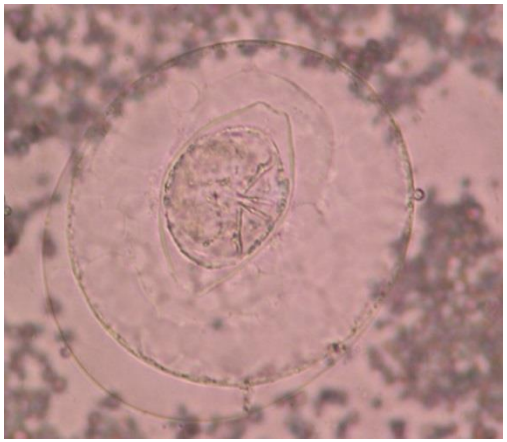
Raillitiella celebensis, 40x, Bush Rat



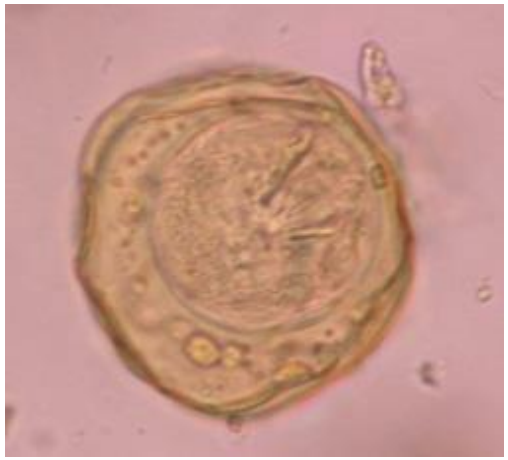
Hymenolepsis nana, 40x, Domestic Mice/Rats



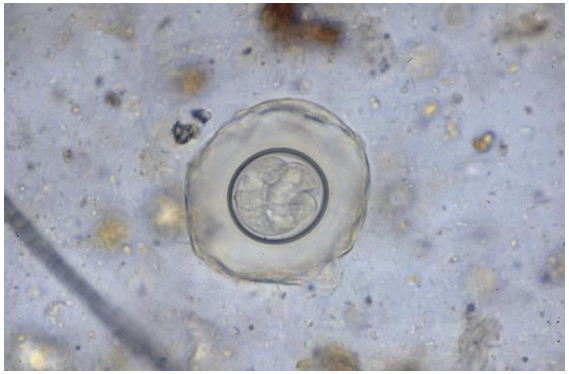
Cestode ova, Dollarbird, 40x



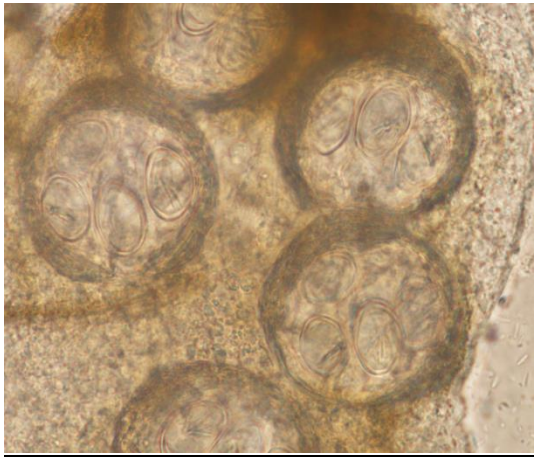
Cestode ova, Regent Honeyeater, 40x



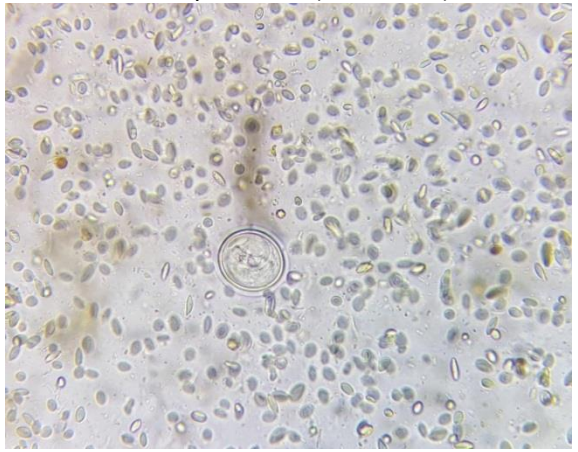
Cestode ova, Noisy Pitta, 40x (not to scale)



Cestode ova, King Brown, 10x



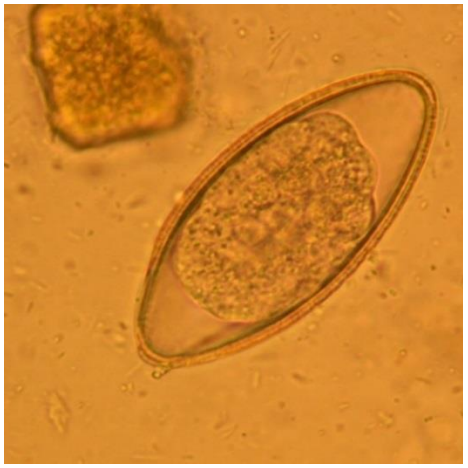
Cestode ova, Blue-tailed Skink, x40



Cestode ova, Southern Brown Bandicoot, 10x

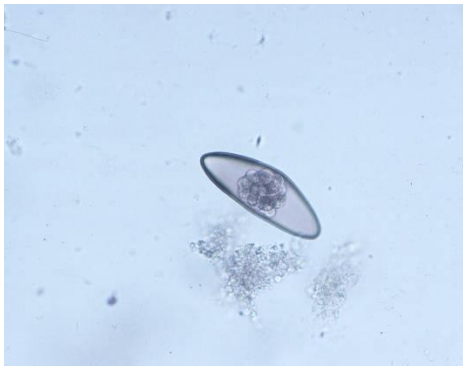
IMAGE GUIDE 8: Faecal Parasites – Oxyurids

Oxyurids can be identified by its asymmetrical walls, one convex and the other flattened.

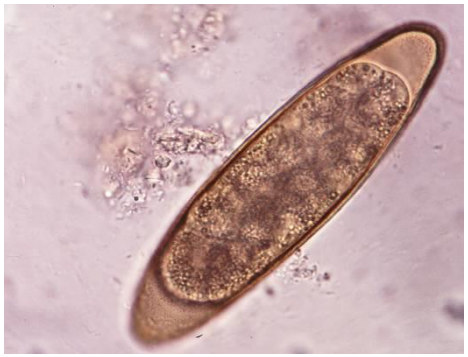


Aspicularis tetraptera, 40x, Rodents

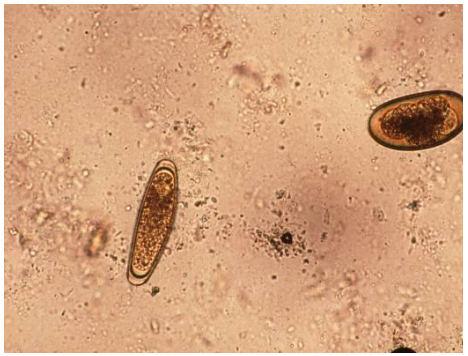
Syphacia muris, 40x, rodents



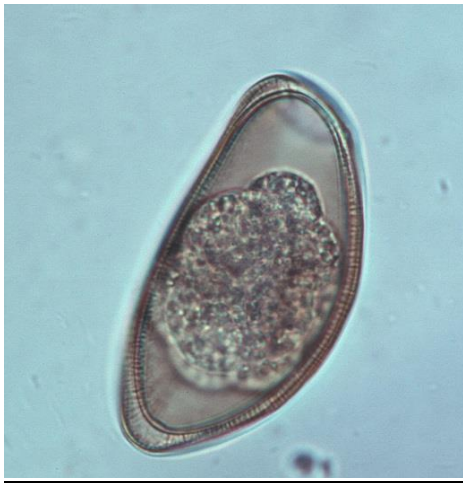
Oxyurid, Rainbow Lorikeet, 10x



Oxyurid, White-lipped green tree frog, 40x



Oxyurid, 2 types, Shingleback



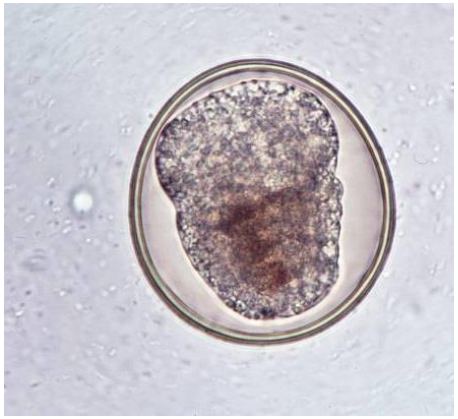
Oxyurid, Inland Bearded Dragon, x40

Oxyurid, Giant Cave Gecko, x40

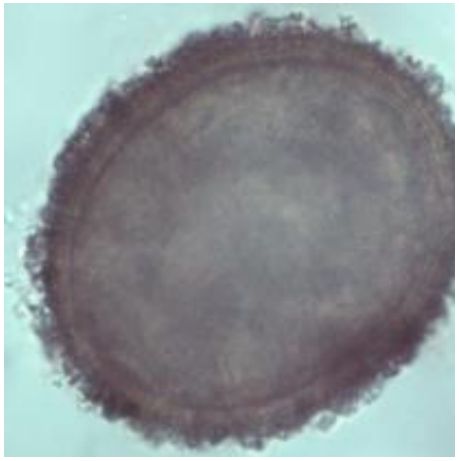
Oxyurid, Spotted-tree Frog, x40

IMAGE GUIDE 9: Faecal Parasites – Ascarids

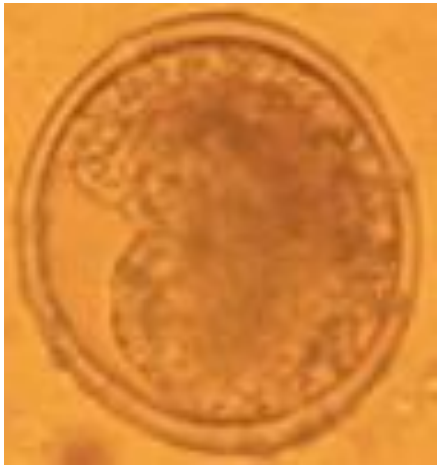
Ascarid ova can be identified by their characteristic shape; round to oval with a thick shell wall which is often mamillated (covered with rounded mounds or lumps)



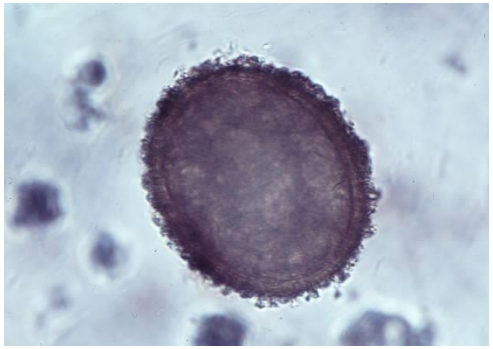
Ascarid ova, New Zealand Fur Seal, 40x



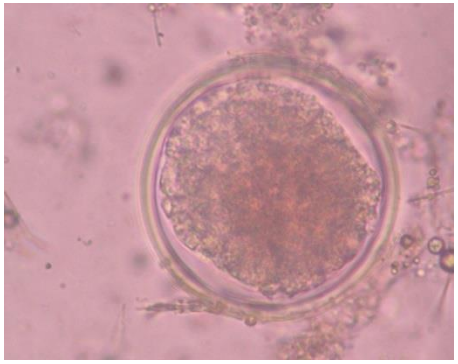
Corticated Ascarid, x40



Anasakis species, marine mammal, 40x

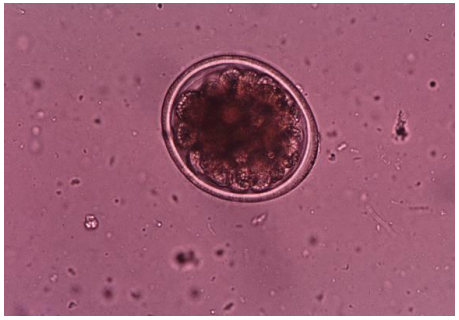


Ascarid, Mitchells Monitor, 40x

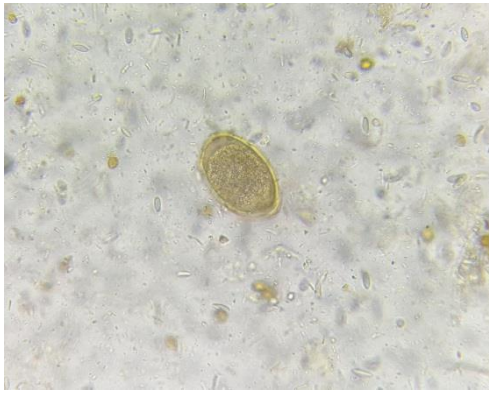


Ascarid, *Anasakis sp.,* Little Penguin, 40x

Ascarid, 40x



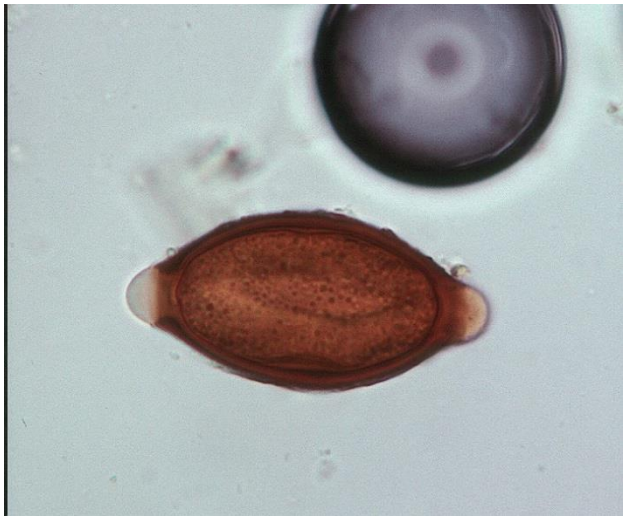
Ascarid, Diamond Python, 40x



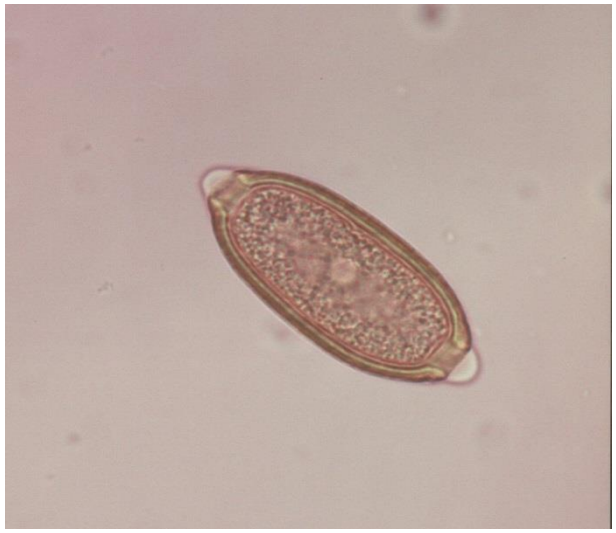
Southern Brown Bandicoot, Ascarid, 10x

IMAGE GUIDE 10: Faecal Parasites – Capillaria

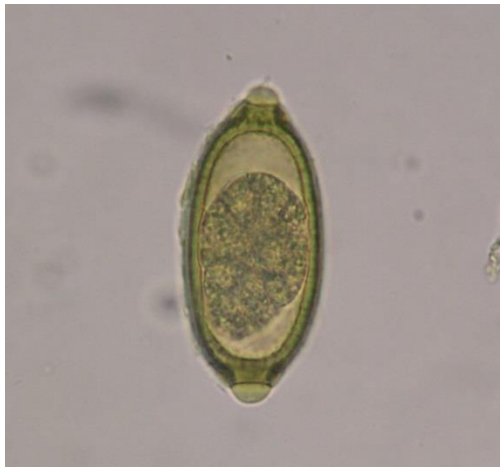
Capillaria ova can be identified by their characteristic shape; elongated, barrel-shaped with a polar “plug” at each end.



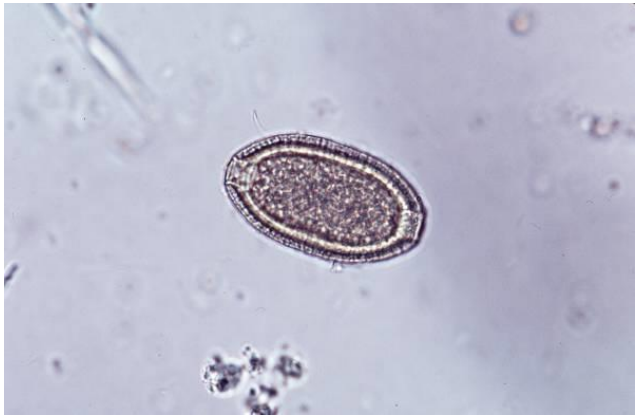
Trichuris trichuria, x40, Primates



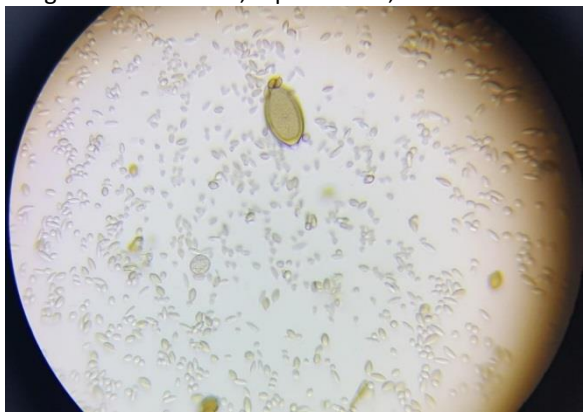
Long-nosed Bandicoot, capillaria ova, 40x



Black swan, Capillaria, 40x



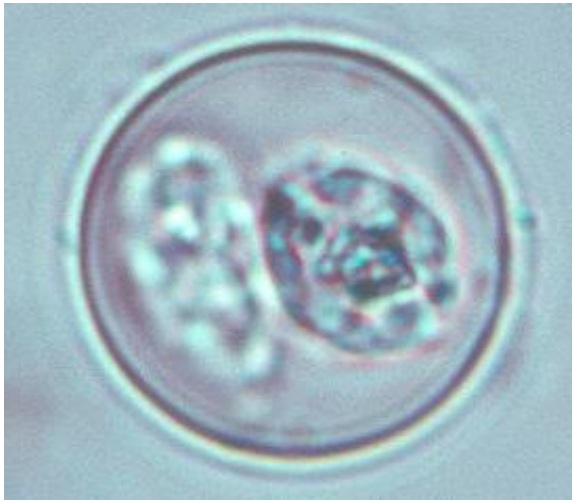
Capillaria, Diamond Python, 40x



Southern Brown Bandicoot, Capillaria (top) and coccidia (lower mid-left)

IMAGE GUIDE 11: Faecal Parasites – Coccidia

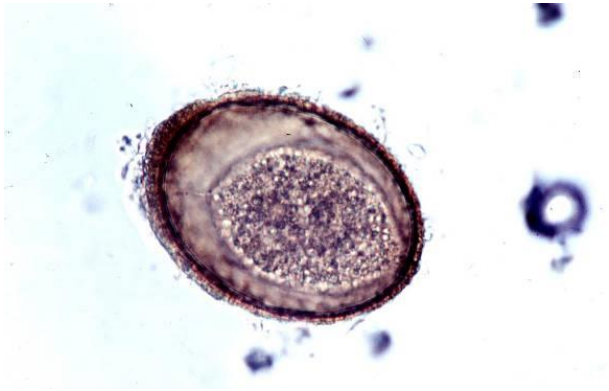
Coccidia are very small compared to other faecal parasites. They can be sporulated or unsporulated and are difficult to speciate based on morphological appearance on faecal float.



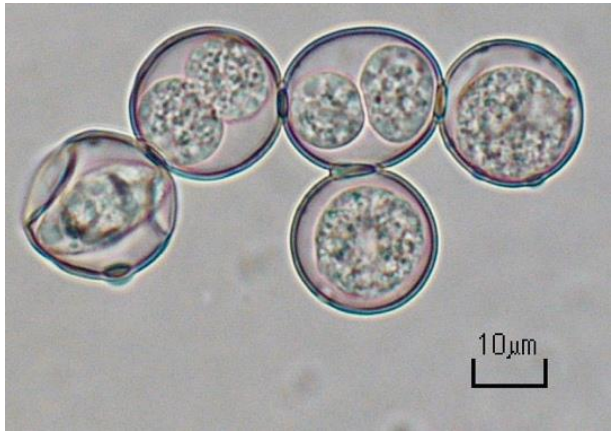
Sporulated coccidian, Isospora, Regent Honeyeater, 40x

Coccidia unsporulated, SB Echidna, 40x

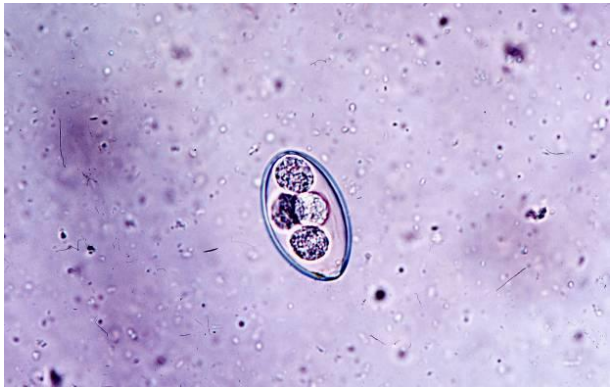
Sporulated Coccidia (Eimeria), SB Echidna, x40



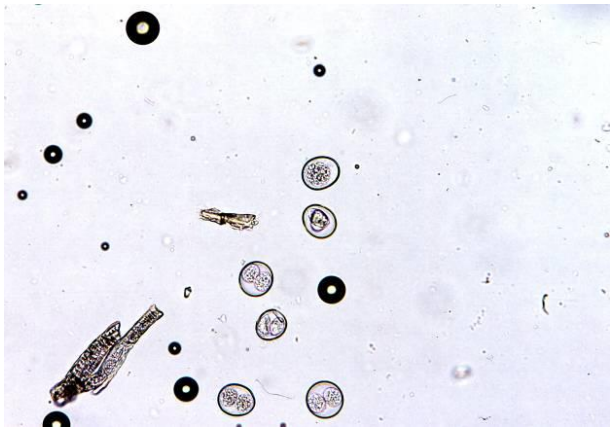
Eimeria, Southern Hairy-Nosed Wombat, 40x



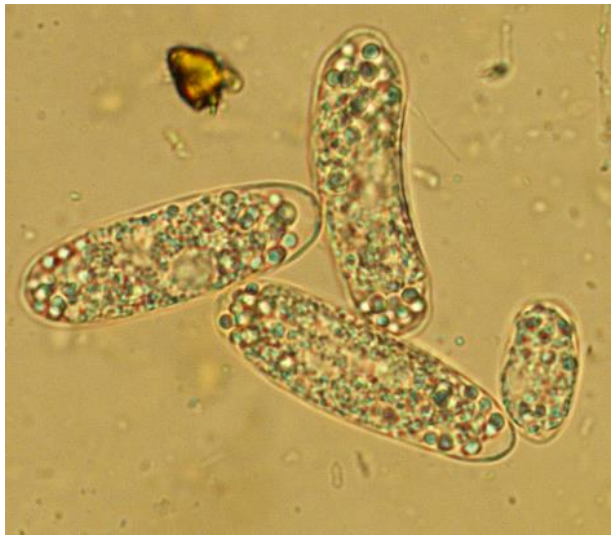
Coccidia (Isospora), Australian raven, 40x



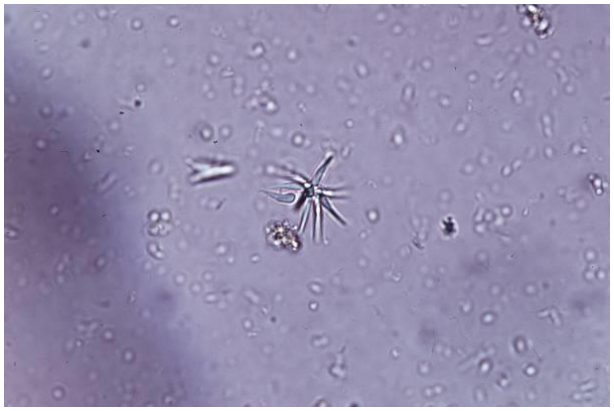
Eimeria, Western Grey Kangaroo, 40x



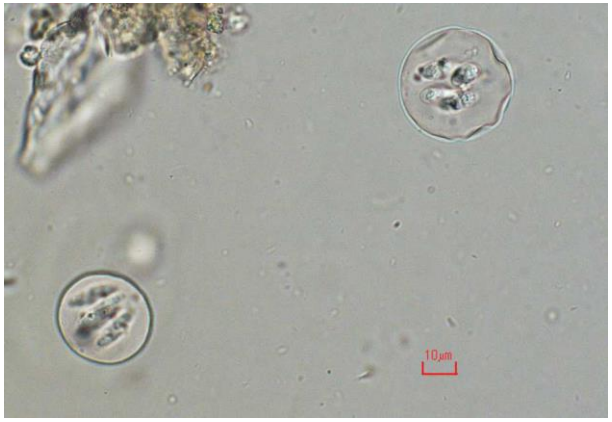
Isospora and air bubbles, Silvereye, 20x



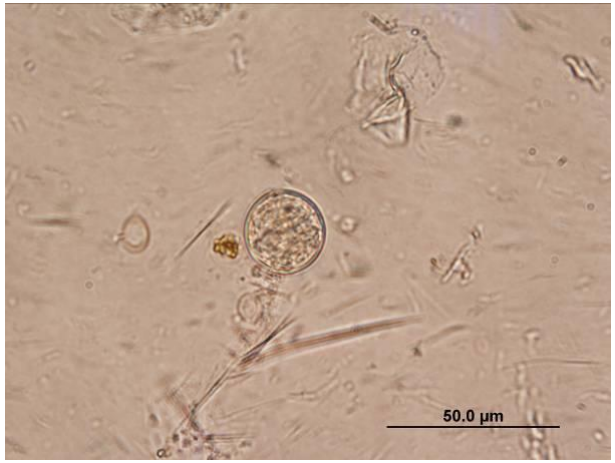
Unsporulated Caryospora, Sea Turtle, x40



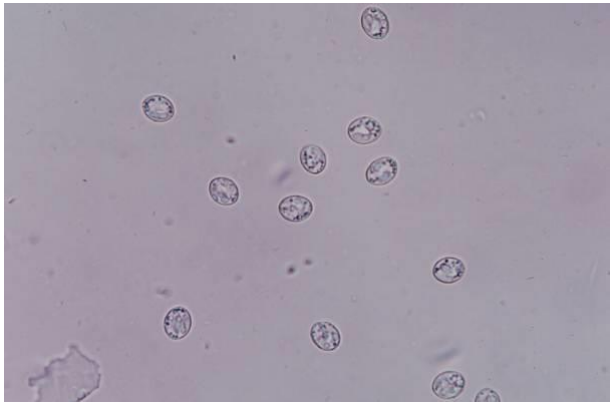
Coccidia sporulated, Caryospora cheloniae, Green Sea Turtle



Eimeria oocysts, Green Sea Turtle, 40x



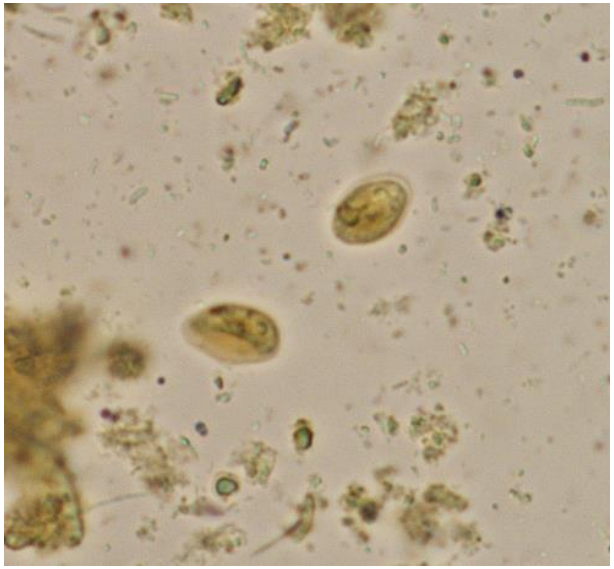
Coccidia, Loggerhead Turtle



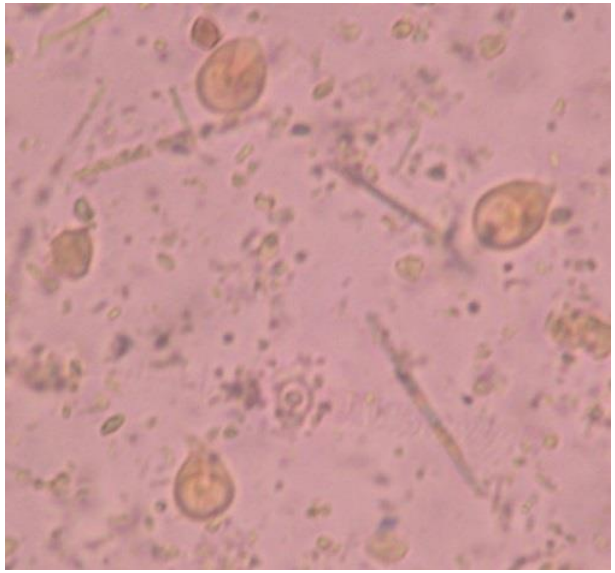
Coccidia, Diamond Python, 40x

IMAGE GUIDE 12: Faecal Parasites – Protozoan: Flagellate

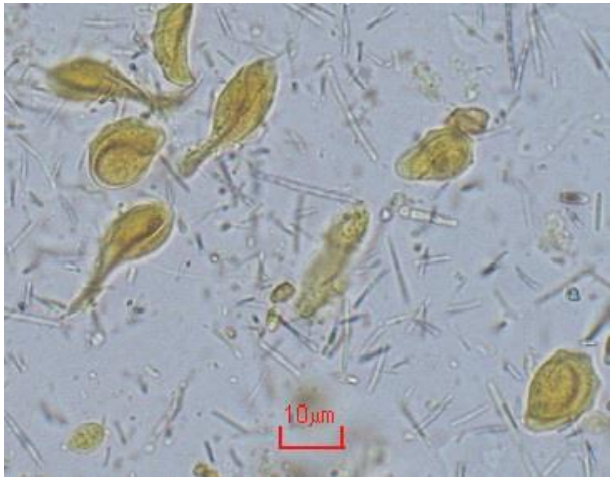
Flagellates can be identified in wet preparation zipping all over the slide. They may move in an undulating or rolling fashion and are identifiable by one or more, long, undulating, whip-like membranes which propel them forward. They come in different shapes and sizes.



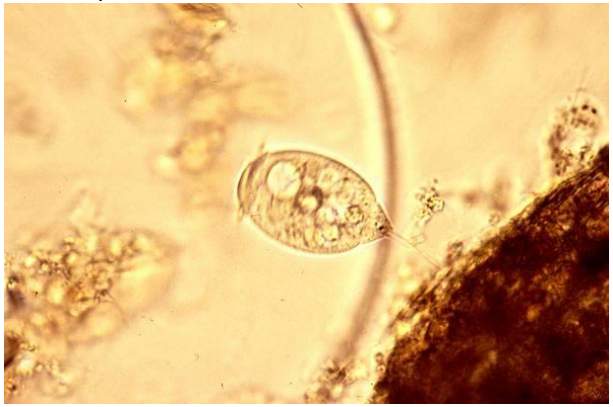
Giardia cysts, 40x



Giardia trophozoites, 40x



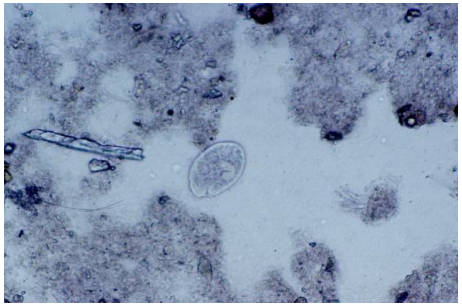
Blue-tongue Skink, Flagellates, Iodine, 40x



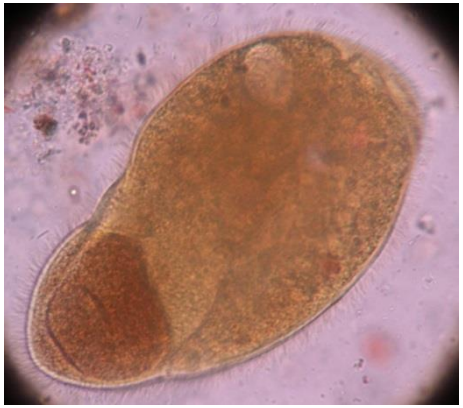
Protozoan parasite, Green Tree Frog, 40x

IMAGE GUIDE 13: Faecal Parasites – Protozoan: Ciliates

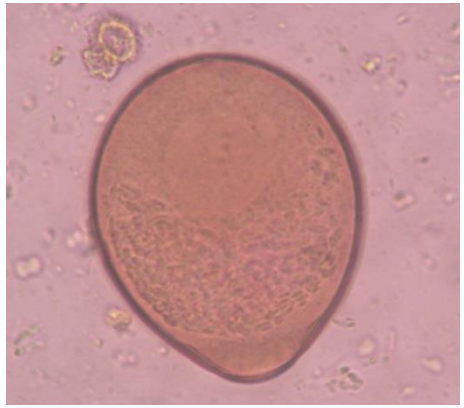
The ciliates are a group of protozoans characterized by the presence of hair-like organelles called cilia, which are identical in structure to eukaryotic flagella, but are in general shorter and present in much larger numbers, with a different undulating pattern than flagella (wiki).



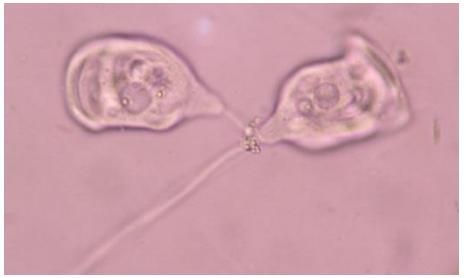
Ciliated Protozoa, Rufous Bettong, 160x



Inland Bearded Dragon, Nyctotherus trophozoite, iodine, x40



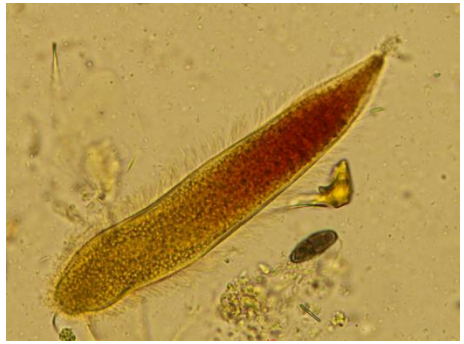
Inland Bearded Dragon, Nyctotherus cyst, iodine x40



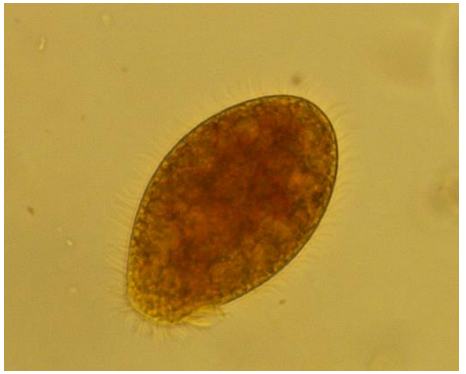
Stalked ciliate (Vorticella), Frog Tank, x40

Sailfin, Nyctotherus trophozoite, iodine, x40

GGB Frog, Ciliate, iodine, x40



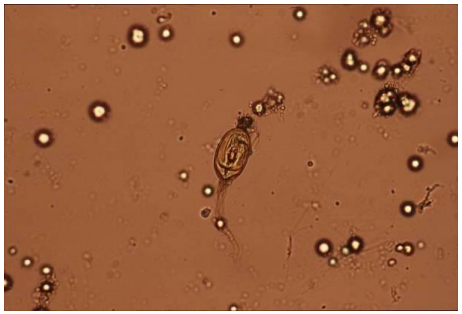
GGB Frog, Ciliate, iodine, x40



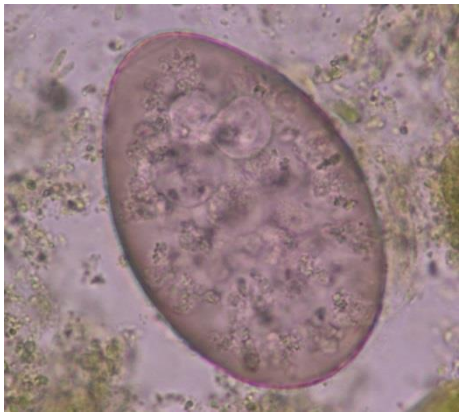
GGB Frog, small ciliate, iodine, x40

IMAGE GUIDE 16: Faecal Parasites - Trematodes (flukes)

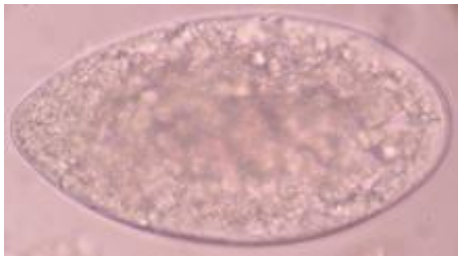
Trematode eggs have a different morphology depending on developmental stage.



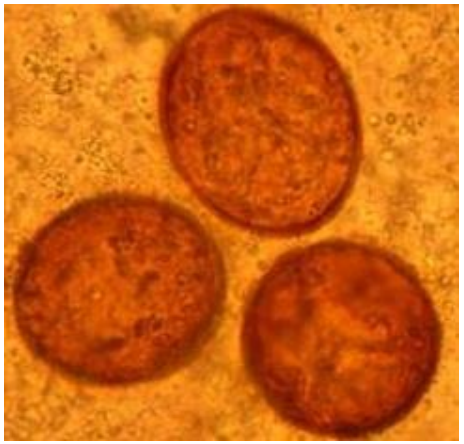
Trematode ova, Avocet, 10x



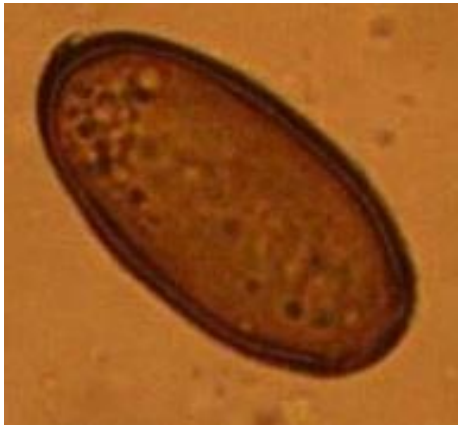
Wedge-tailed Eagle, Trematode, x40



Trematode ova, 40x

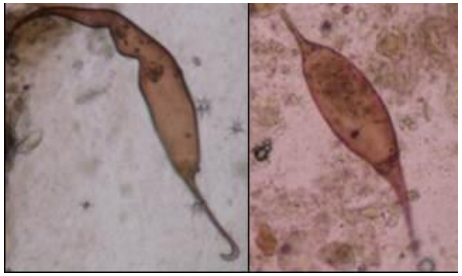


Trematode - Sea Turtles, Spirorchids Type III, x40



Trematode - Sea Turtles, Enodiotrema , x40

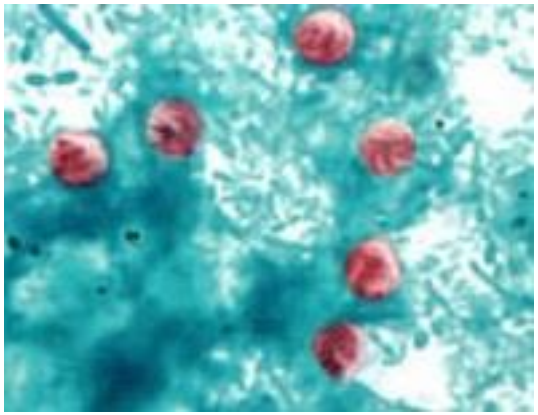
Trematode - Sea Turtles, Pronocephalids, x40

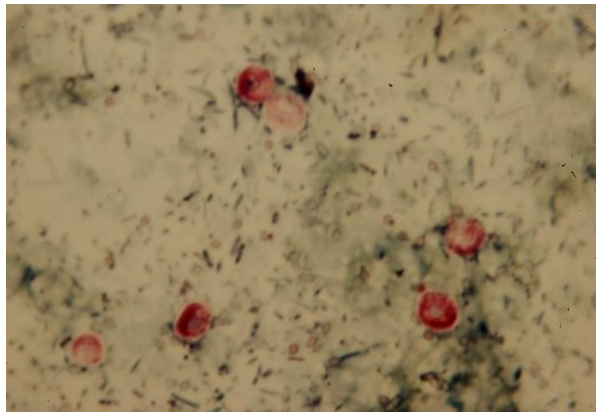


Trematode, Sea Turtles, Spirorchids Type I, x40

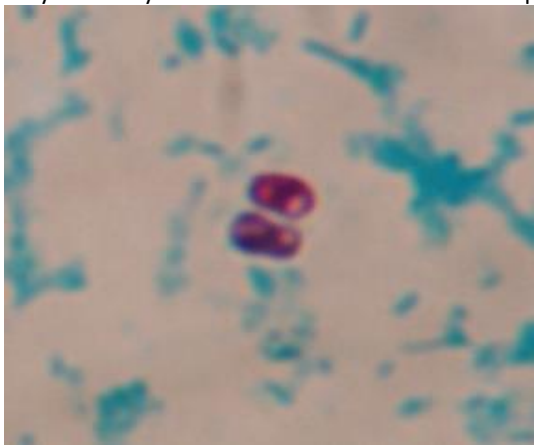
Trematode, Sea Turtles, Spirorchids Type II, x40

IMAGE GUIDE 17: Miscellaneous faecal parasites

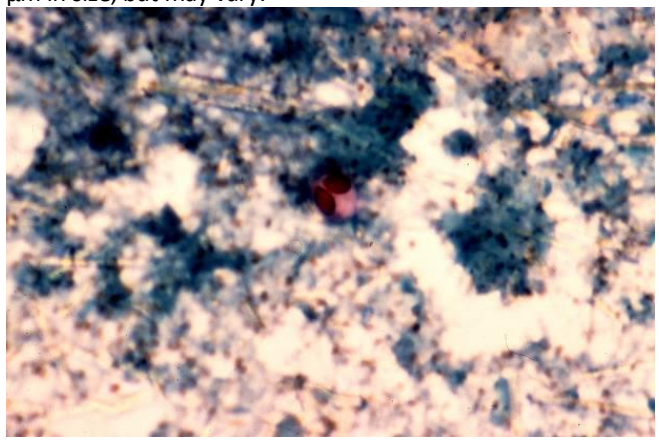




Cryptosporidium, Acid fast stain, Taipan (right): An apicomplexan parasite, oocytes may be seen by microscopic examination of a stool sample stained with acid-fast stain, but they are easily confused with artefacts similar in appearance. Most cryptosporidia are 3–6 µm in size, but may vary.



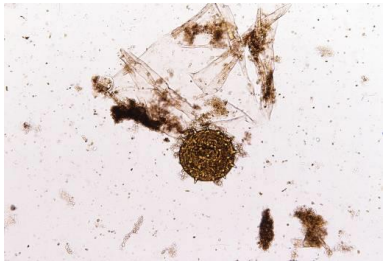
Possible Microsporidia, 100x: Microsporidia are a group of spore-forming unicellular parasites (once considered protozoans, now known to be fungi). Acid-fast stain.



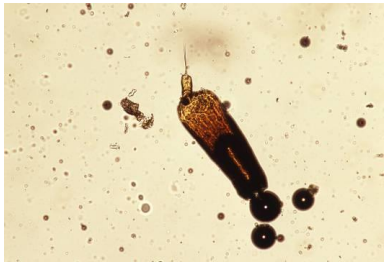
Myxosporidia, Acid Fast Stain, Penguin. Myxosporea is a subclass of microscopic parasites, belonging to the Myxozoa clade within Cnidaria. They have a complex life cycle, which comprises vegetative forms in two hosts, an aquatic invertebrate (generally an annelid) and an ectothermic vertebrate, usually a fish (Wikipedia).

IMAGE GUIDE 18: Common Faecal Pseudoparasites

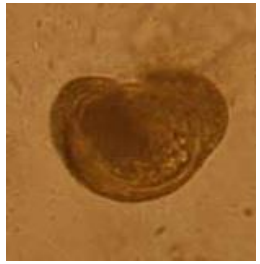
Cyniclomyces yeast, RT Possum, Koala



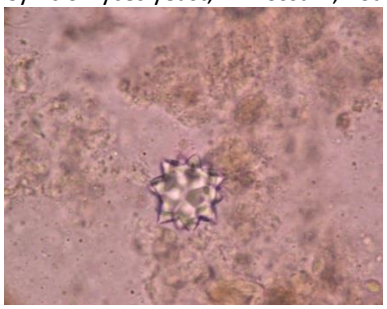
Plant material



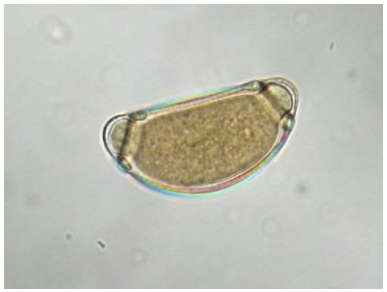
Plant material



Pollen 40x

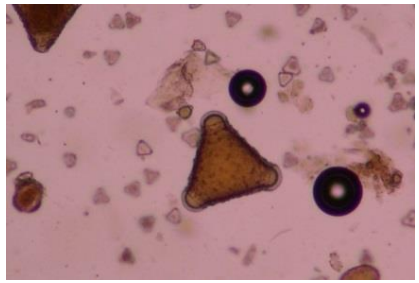


Plant material (commonly seen in sea turtles)



Banksia pollen, x40 - Looks like capillaria.

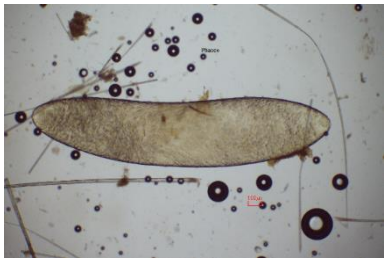
Pollen and plant material



Eucalyptus pollen, x10

Insect Wing scales, x40

Adelina (Octosporella) sp., x40 - coccidia that infects insects. Seen in Echidna faeces



Cricket eggs appear as small grains of rice. Maybe mistaken for a mite egg or STO in a reptile faecal

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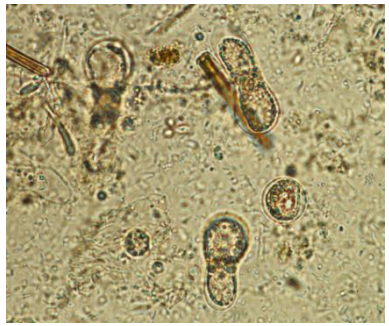

Fungi *Basidiobolus ranarum*, Skink faecal x40

NOTES: