Laboratory Procedures for Wildlife Rehabilitators

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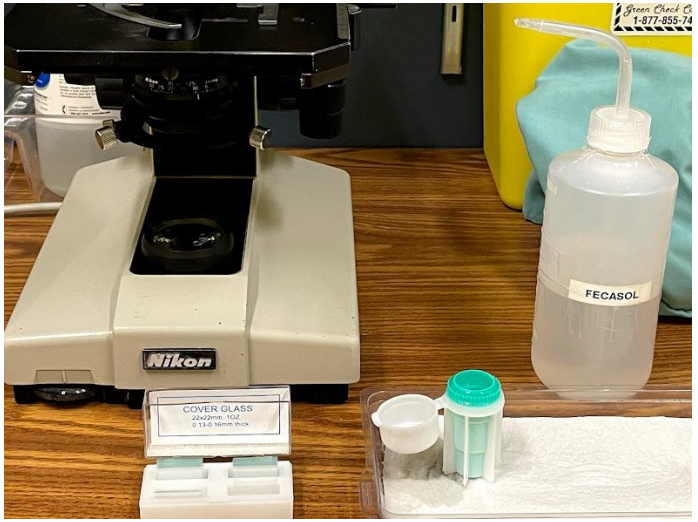
Overview

- When and why
- Equipment
- Common endoparasites
- Fecal procedures
- Viewing slides
- Swabs
- Common ectoparasites
- Hematology
- Urinalysis
- Microbiology
- Consider sending lab samples



Why perform laboratory procedures?

- To detect parasites and disease processes, including zoonotic and infectious ones
- Give further indication to the general condition and prognosis of the patient
- Guide treatment plans and increase chance of healing, recovery and release

When would you perform lab procedures?

- Upon intake if animal is sufficiently stable
- Oiled animal
- Thin body condition
- Eating but not gaining weight
- Crop not emptying
- Cough
- Suspect poisoning or toxicity

- Abnormal colour, consistency, or presence of blood or mucous in feces

Equipment needed

- Compound light microscope
- Fecal kits and flotation solution
- Blood collection supplies
- Centrifuge
- Refractometer
- PPE
- Good reference manual

Equipment needed…

If possible…

- Glucometer (essential for deer fawns)
- Lactate reader
- Stains (Dip Quick, Lugol's, Gram stain, NMB, etc…)
- Lead analyzer

Lab Samples

- Blood
- Feces
- Discharges
- Urine
- Tissue scrapings

Fresh samples best

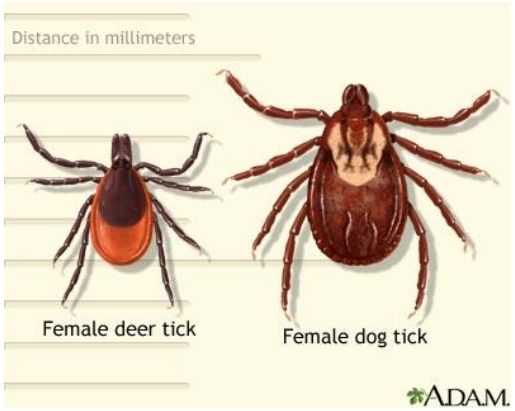
Wildlife and Parasites

- Low load can be normal
- Higher numbers may indicate a disease process or immunocompromised
- Increase in care – stress, other animals, enclosures
- Some parasites are host-specific
- Many are zoonotic – **PPE**
- Know how to diagnose and treat them, and prevent re-infection
- Keep stress down and provide supportive care

Parasite families

- Parasite families
  - **Protozoa** (single-celled organisms): Coccidia, Trichomonas, Giardia
  - **Helminths**: Nematodes (roundworms), Trematodes (flukes), Cestodes (tapeworms)
  - **Arthropods**: hard segmented bodies (ticks, mites, lice, insects)

Treatingdiarrhea.com



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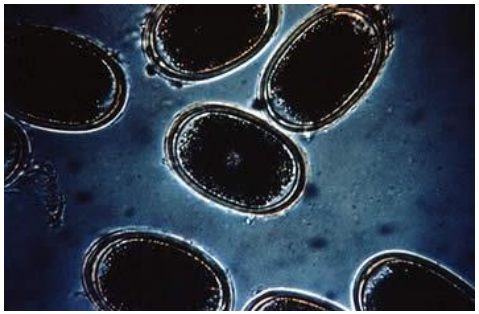
Common Avian Endoparasites

- Capillaria sp. (Threadworm)
- Syngamus sp. (Gapeworm)
- Taenia sp. (Tapeworm)
- Ascaridia/ Porrocaecum(Roundworm)
- Coccidia (protozoa of genera Cystoisospora or Eimeria)
- Giardia (protozoa)
- Trichomonas sp. (protozoa)

Photo credit Neil Merchant



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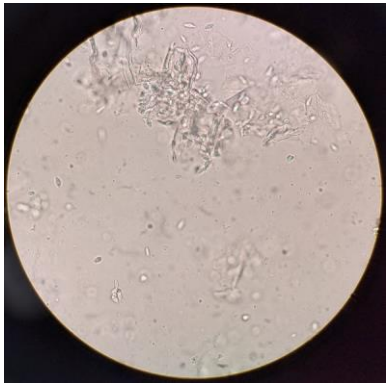


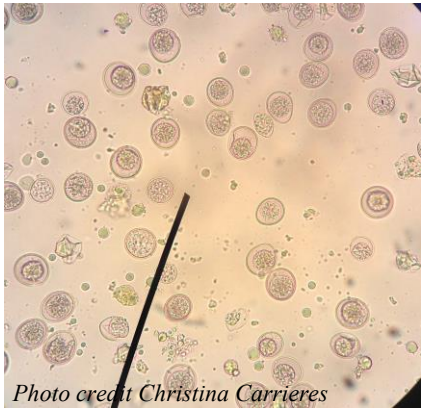
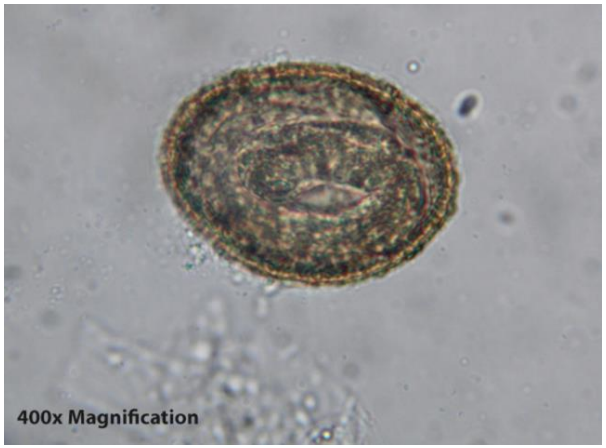


Photo credit Christina Carrieres

*Most identified in fecal floats except Trichomonas and Giardia are seen on a direct smear

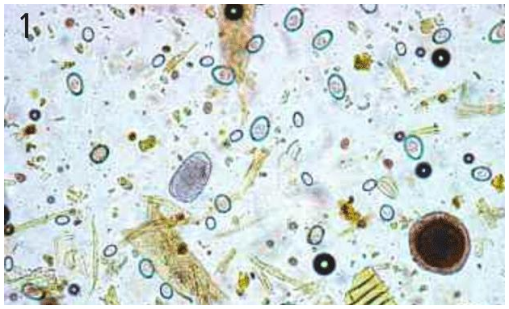
Mammal Endoparasites

- Taenia sp., Dipilydium sp. (Tapeworm)
- Toxocara sp., Toxascaris sp., Baylisascaris sp. (Roundworm)
- Ancylostoma (Hookworm)
- Trichuris (whipworm)
- Coccidia
- Giardia

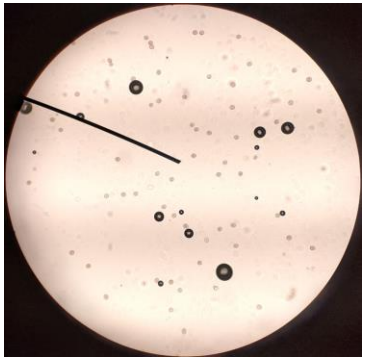


Artifacts (pseudoparasites & spurious parasites)

- Bubbles
- Pollen
- Fat droplets
- Urate crystals
- Plant fibers
- Plant cells
- Yeast
- Feather fragments
- Fur
- Host specific parasites from prey species (spurious)
- Free living organisms



https://www.cliniciansbrief.com/columns/37/fecal-sample-analysis



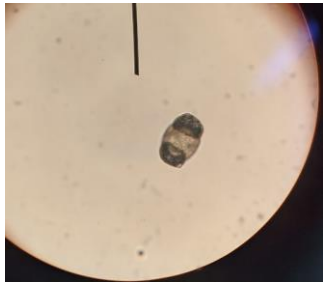


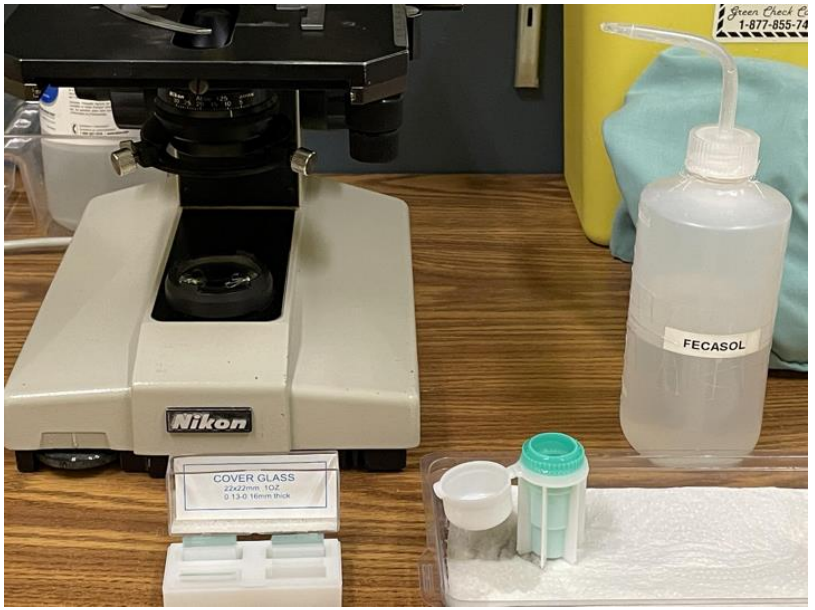
Photo credits Neil Merchant

Fecal Collection

- Fresh is best (<2 hours)
- Label with species, case number, date
- Note fecal colour, consistency, and presence of (gross) parasites, blood
- Avians- collect fecal part only, not the urates or urine
- Can keep in the fridge up to 4 days

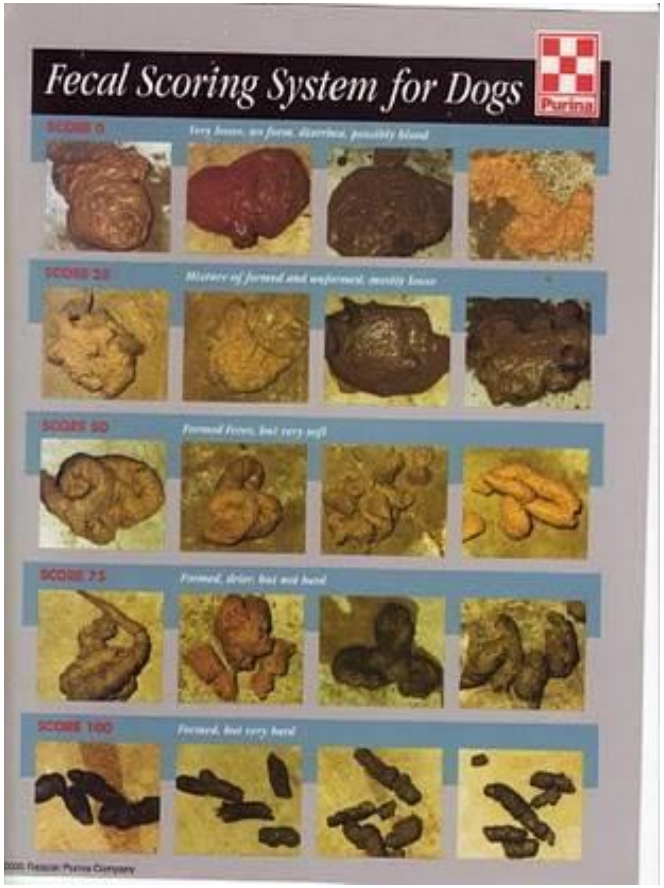
Fecal exam

- 1. Gross examination of feces
- 2. Fecal flotation (and Centrifugal flotation)
- 3. Direct smear
- *4. Fecal sedimentation*
- *5. Gram stain*



1. Gross examination of feces

- **Consistency**: liquid (diarrhea), soft, hard, grainy
- **Color**: green (can mean parasites, anorexia), dark brown (also means anorexia for 24hrs) red (can be due to food-berries), etc.
- **Blood**: dark black/brown and tar-like (melena=digested blood) or frank blood red (undigested blood, bleeding from lower GI tract), some parasites cause extensive damage to the intestinal lining
- **Mucus**: intestinal inflammation, parasitism
- **Gross parasites**: larvae or portions of parasites are sometimes visible to the naked eye



wideningcircle.blogspot.com/2011/01/poop-char...Remove frame

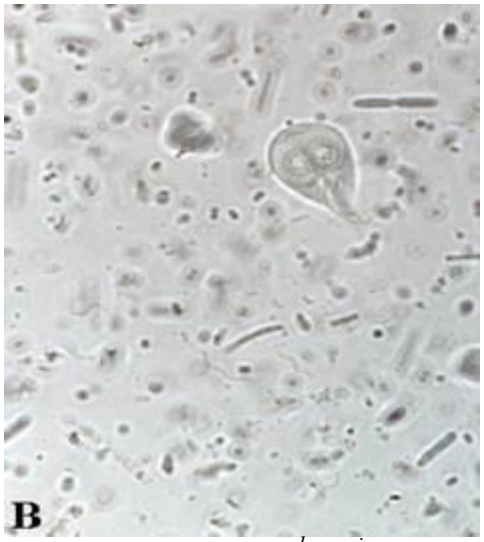
2. Fecal float

atozvetsupply.com

- To detect ova/oocytes
- Passive flotation vs centrifugal
- Ideally should perform 3x
- Commercial fecal flotation kits: Fecalyzer, Ovassay & Ovatector
- Flotation solutions: zinc sulfate, sodium nitrate, sugar
- Solution with SG 1.18-1.3
- Ova float to the surface of the liquid and large particles sink
- Read slide right away, solution can distort parasites
- Flukes too heavy for flotation (fecal sedimentation)

3. Direct Smear

- The simplest method for parasite examination
- Can detect motile protozoan trophozoites: Giardia, Trichomonas, can also see coccidia, larvae and eggs
- Can use with feces, tissue swabs (ex. Crop swab)
- Advantage: small amount of feces
- Disadvantage: Less sensitive, can be inaccurate, also leaves much fecal debris



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Giardia sp.

Fecal analysis – Gull

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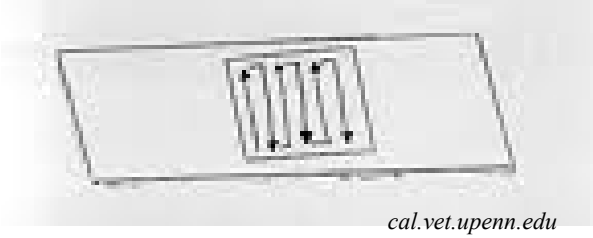
Microscope and reading a slide

- **Low power objective (10x) first**
  - Low power for scanning
  - Wet mounts: Fecals, swabs, urine
  - Lower condenser
- **High dry objective (40x)**
  - Examine object more closely
  - Field of view is decreased, need more light
- **Oil immersion (100x)**
  - Turn up light
  - Raise condenser
  - To view cell detail, bacteria (blood slides and gram stain)



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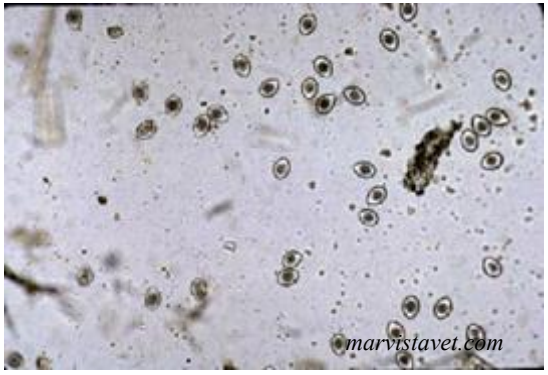
Things to remember

- Wear gloves
- Disinfect area afterwards
- Wash hands after
- Write results in chart and choose treatment
- Results either N.O.O. (no ova observed) or OVA
- Standardize techniques on how to record:
  - 1+ = 1-2 ova per low power field (LPF) (light load)
  - 2+ = 3-5 per LPF (moderate)
  - 3+ = 6-8 per LPF (heavy)
  - 4+ = > 9 per LPF (very heavy)
  - TNTC



Swabs

- Crop swab: Trichomonas sp., yeast, overgrowth of bacteria
- Can also swab choanal slit, nares, and near larynx for respiratory disease
- Ear swab in mammals for mites, yeast, overgrowth of bacteria
- Wounds and GI tract (cloacal) can be swabbed
- Sterile swab with sterile saline, can stain with Gram stain or Dip Quick
- Culture using sterile transfer media swab

ocw.tufts.edu

Photo credit Dr.Jean Sander at https://www.merckvetmanual.com/poultry/helminthiasis/helminthiasis-in-poultry

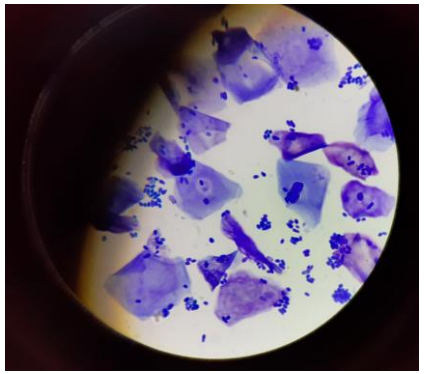
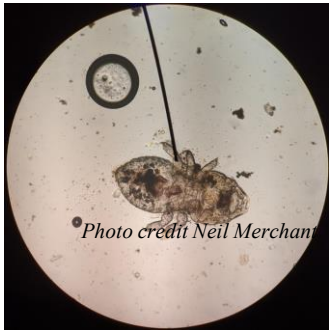


Photo credit Christina Carrieres

Mobile trichamonas trophozoites

Common Avian Ectoparasites



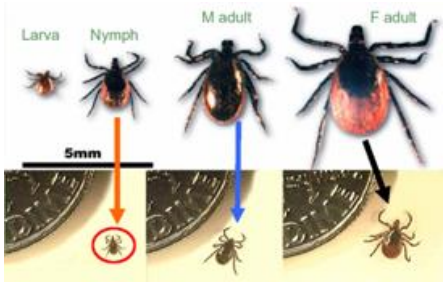
- Lice (Order Mallophaga)
- Scaly Leg Mites (Knemidokoptes)
- Maggots/ Bot fly larvae
- Ticks
- Flat flies (Hippoboscidae)
- Avian skin mites (Ornithonyssus sp.)
- Feather mites
- Avian follicular mites (Harpyhynchus sp.)

- *Diagnosed by microscopic exam of feathers and skin or physical exam

Mammal Ectoparasites

flea-control.org

commons.wikimedia.org



heartspring.net

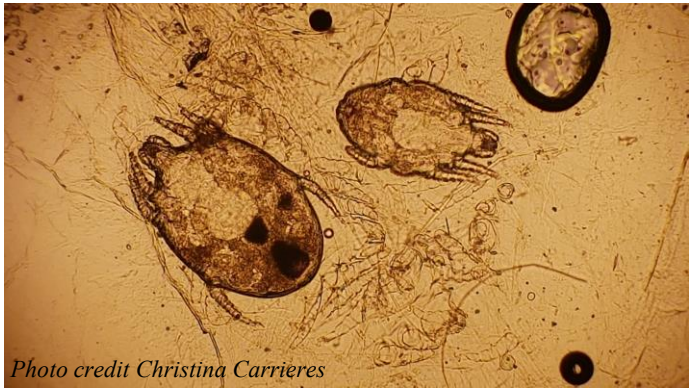


Photo credit Christina Carrieres

- Mange (*Sarcoptes*, *Demodex*)
- Fleas (Ctenocephalides)
- Lice (Pediculus)
- Ear mites (*Psoroptes*, *Otodectes*)
- Cuterebra (bot fly)
- Cheyletiella mites
- Maggots
- Ticks

Ectoparasite methods

https://www.cliniciansbrief.com/article/skin-scraping-external-parasites

- Detection and identification
  - 1. Magnifying lens or dissecting microscope
  - 2. Cellophane tape method: to visualize lice or superficial mites
  - 3. Skin scrape**:** to detect mites such as Mange (Sarcoptes and Demodex), Scaley leg mite (Knemidocoptes), and Cheyletiella
  - 4. Fur: pulled to detect Demodex at follicles
  - 5. Ear swabs: ear mites (also bacteria, yeast)
- Can preserve them in 70% ethanol or 10% formalin

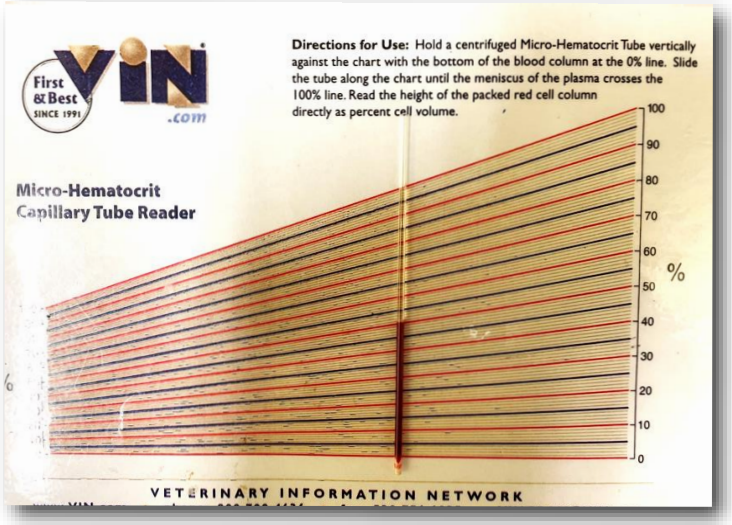
- Find through <u>physical examination</u> except for burrowing mites

Hematology

- 1. Packed Cell Volume (PCV)
- 2. Buffy Coat, plasma color
- 3. Total Protein (TP)
- 4. Blood glucose
- 5. WBC differential and estimate, platelet estimate, RBC morphology
- 6. Note any blood parasites
- Recheck any abnormal bloods after any treatment
- Remember this is a wild animal, handling and captivity will alter blood results

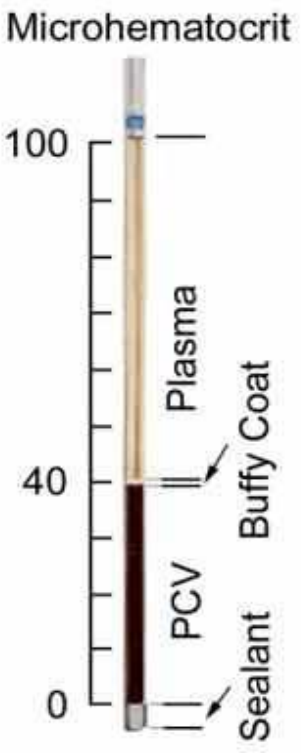
1. Packed cell volume (PCV)

- Percentage of whole blood composed of red blood cells (RBCs)
- Also known as the hematocrit (Hct)
- Very accurate within 1%
- Reference range:
  - Avians 35-55%
  - Mammals 30-55%



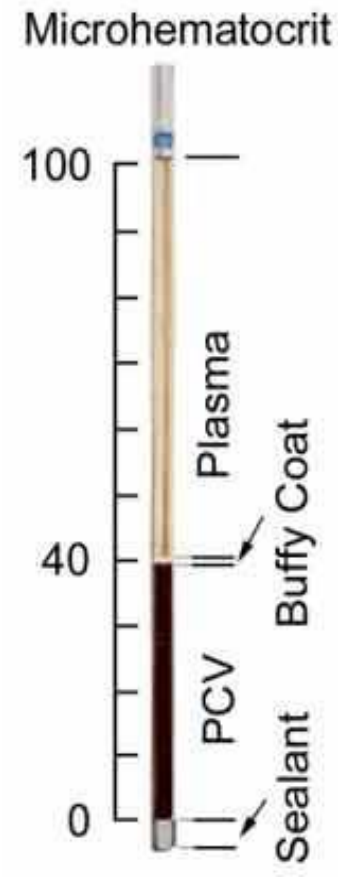
PCV Values

- **Low** PCV < 30% can indicate anemia
- 3 causes:
  - Blood loss: acute vs chronic
  - Low production
  - Destruction of RBCs
- Treat the cause (if known/suspected)
  - Iron dextran injection (caution)
  - Vit K injection if rodenticide
  - Consider transfusion or euthanasia at < 15%
- **High** PCV > 60 % can indicate:
- Dehydration (TP will also be increased)
  - Tx: fluid therapy



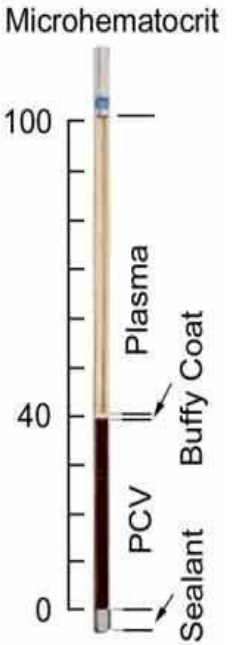
2. Buffy coat

- Whitish-gray layer just above the RBC layer
- Represents WBCs and platelets
- Healthy patient: <1%
- Over 2%, indicates possible infection or inflammation
- BC layer can be transferred to microscope slide to detect microfilariae



Plasma layer

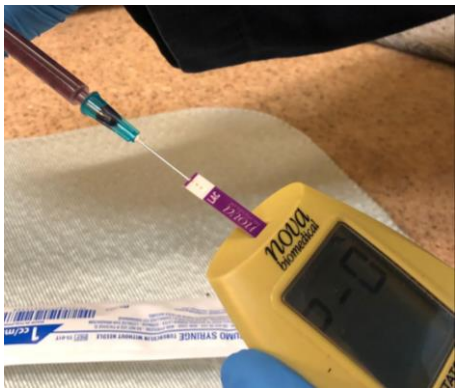
- Clear to yellow fluid above the buffy coat layer
- Consists of proteins (hormones, antibodies, enzymes, etc.), water, salts, glucose, fats
- Color of plasma:
  - Red: hemolysis, due to poor handling technique
  - Yellow: icterus, in mammals due to liver issue (deer can be normally yellow)
  - White: lipemic, due to recent ingestion of fatty meal
  - Normal is clear in mammals, slightly yellow in birds



3. Total Protein (TP) of Plasma

- Indicates general health status of patient
- Mammal 5.5-7.5 g/dl; Avian 3.5-5.5g/dl
- TP > 6 g/dL: can indicate dehydration (has less water in its plasma)
- TP < 2.0 g/dL: do not give solid food
- TP < 1.0 g/dL: poor prognosis, consider euthanasia

4. Blood Glucose



- Using a glucometer and a drop of blood
- Normal values: 5-10 mmol/L
- Hypoglycemia: < 5 mmol/L
  - malnutrition
  - tx: IVF with dextrose
- Hyperglycemia: >38.8 mmol/L
  - Due to diabetes
  - Stress can elevate blood glucose levels

Avian blood collection sites

poultryhelp.com

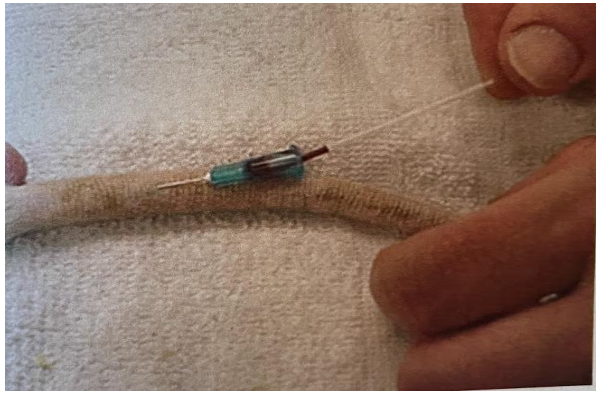
- Medial metatarsal or tibial
- Cutaneous ulnar
- Jugular (right)
- Interdigital web vein

Blood collection – Gull

(1:55)

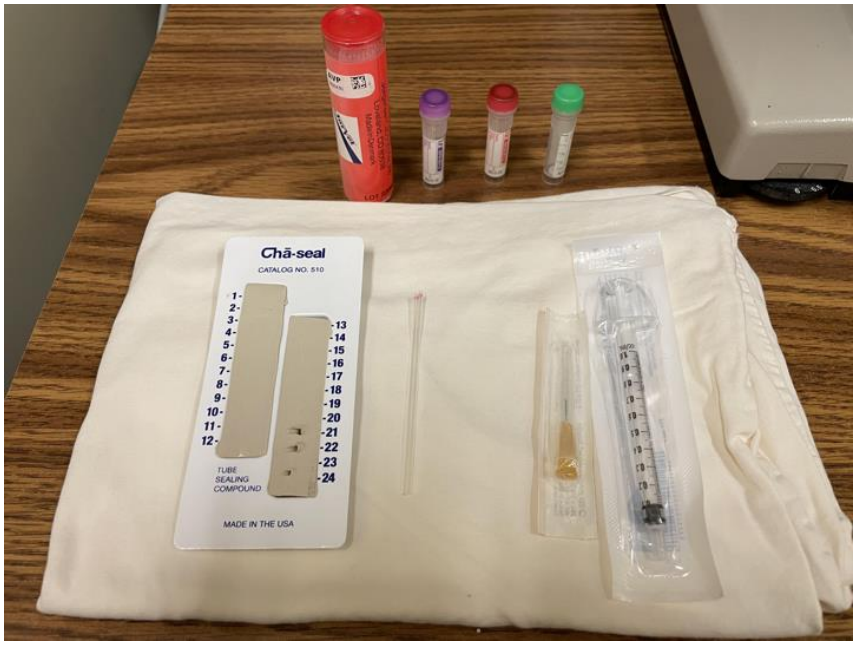
Mammal blood collection sites

- Lateral saphenous
- Femoral
- Cephalic
- Marginal ear vein
- Jugular
- Tail



Tseng et al. (2005) p.27

Blood Tubes



- Avian
  - Avian blood is susceptible to changes when stored in EDTA (purple top tube)
  - Use green top tubes (lithium heparin) for PCV/TP and red top for serum chemistries
  - Avian blood clots very quickly
  - Collect and transfer to the appropriate receptacle as quickly as possible
- Mammal
  - EDTA for blood smears and PCV/TP (purple top)
  - Red top for chemistries (contact lab for specialty tests)

Blood Volume

- **Maximum** safe blood volume for birds and mammals:
  - 1% of patients body weight
  - Equivalent to 10% of blood volume
  - Only applies to **healthy** animals
- General rule of thumb:
  - Birds: 1.0 ml/100 g
  - Example: 1000g bird has a total blood volume of 100ml, safe maximum amount of blood taken is 10ml

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Making Blood Films

- WBC differential and estimates, estimate platelet numbers and evaluate morphology of WBCs, RBCs, and platelets
- Make the blood film as soon as possible after collection
- Don’t use too much blood
- Mix tube gently first
- Make sure slide is clean
- Many techniques used

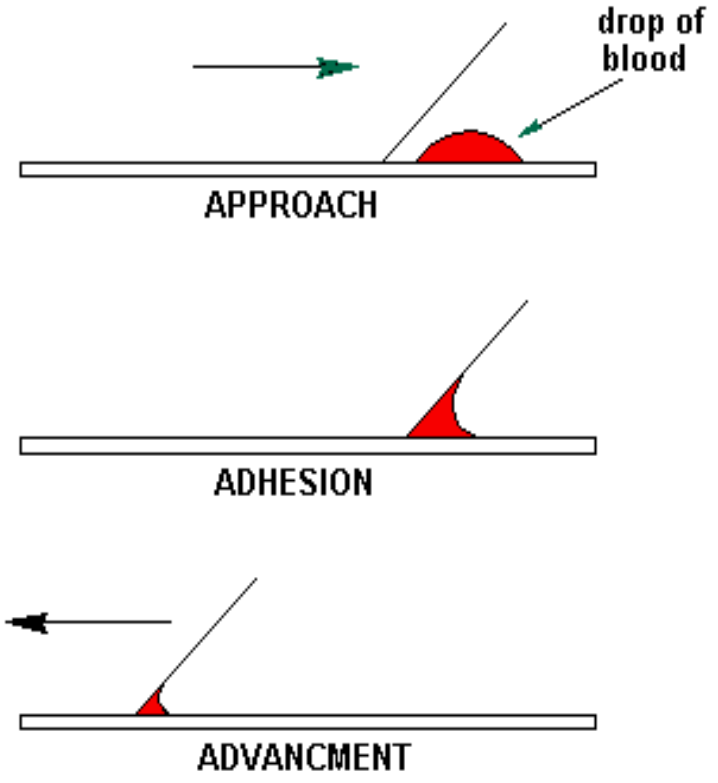


Fig. 13 - How to prepare a blood smear.

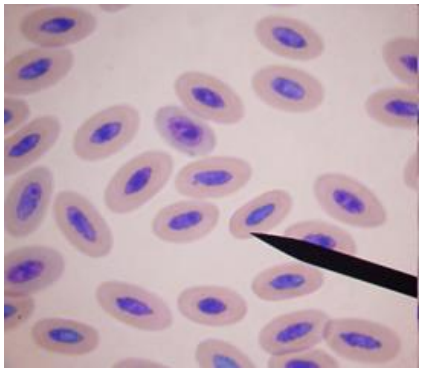
cartage.org.lb

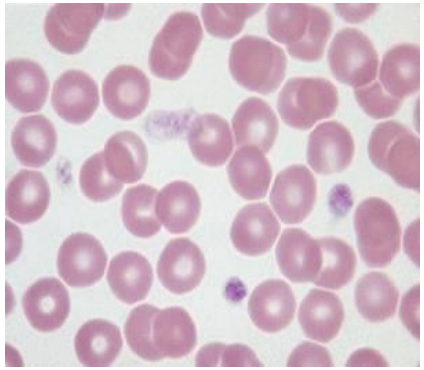
A bit on stains

- Don’t add more stain to the bottle; empty, clean, and dry thoroughly first before refilling
- Change every 4wks or until visible debris is present, or stain on slide not good
- Two commonly used: Gram stain and Diff Quik
- Others used: new methylene blue for reticulocyte count

Erythrocytes/Red Blood Cells (RBCs)

- **Function**:
- RBCs carry hemoglobin, which transports oxygen throughout the body
- Made in the bone marrow in mammals and in liver and spleen in birds
- Lifespan of 28-45 days in birds, 90-120 days in mammals
- **Avian vs. Mammal Appearance**:
- Avian larger in size than mammals
- Avian RBCs are nucleated and elliptical in shape and the cytoplasm is orange-pink
- Mammals are not nucleated, have an area of central pallor
- Look under oil immersion for abnormalities





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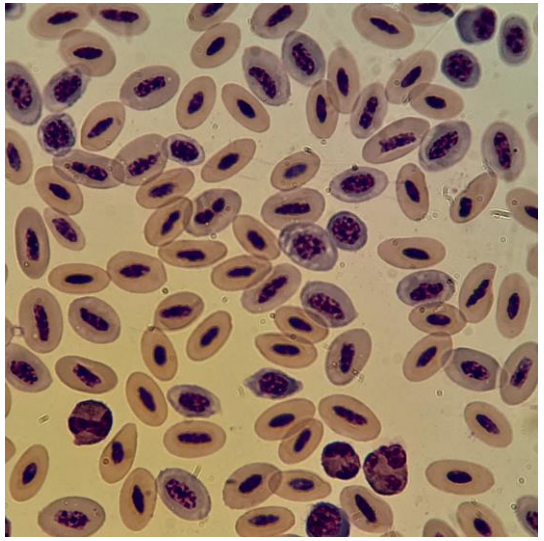
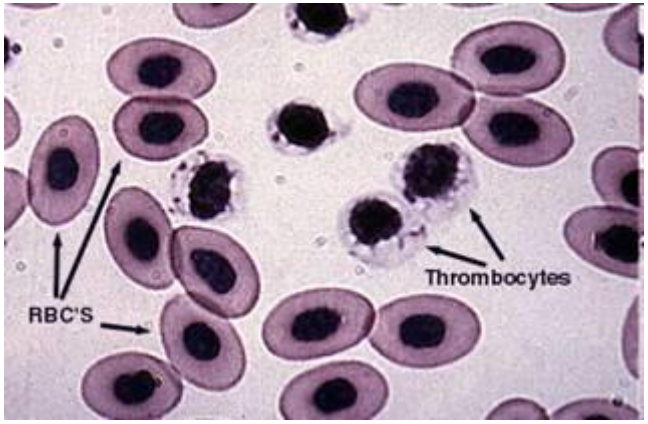


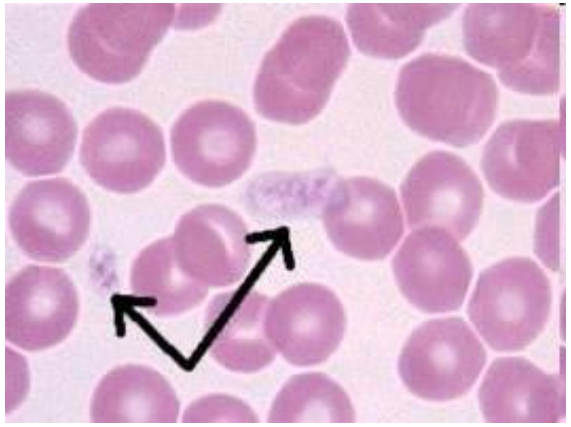
Photo credit Christina Carrieres

Thrombocytes (Platelets)

- **Function:**
  - Hemostasis (blood clotting), clot and wound healing
  - Can clump on a blood film
- **Avian vs. Mammal Appearance**:
  - Avian: large round nucleus and small rim of gray cytoplasm
  - Mammals: not nucleated, much smaller than avian
- Determine if they are normal, increased or decreased:
  - Normal: 1-5 thrombocytes/ oil immersion field (if no clumps)



http://www.spcollege.edu/hec/vt/vtde/avianhemo/avian1/21.jpg

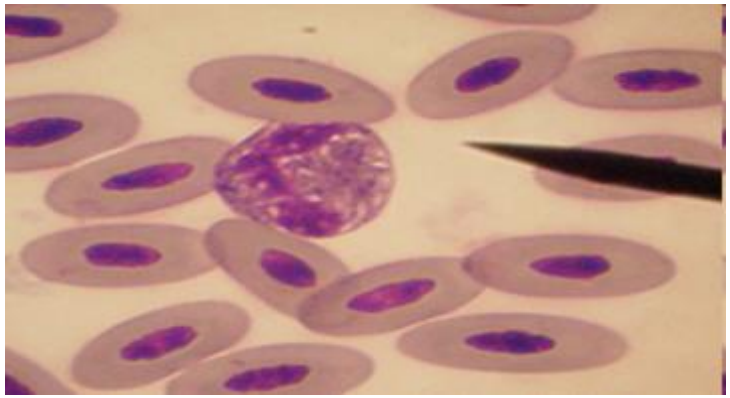


http://www.marvistavet.com

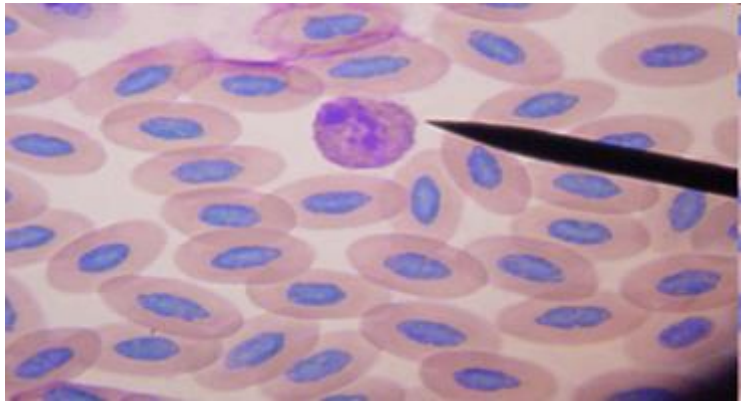
White Blood Cells (WBCs)

- Observe the shape and size of the cell, the nucleus, the presence of vacuoles, and cytoplasmic granules
- Avian leukocytes function similarly to mammals: to defend the body against foreign invaders (immune system)
- Neutrophil (mammals), Heterophil (Avian): phagocytosis
- Eosinophil: allergic reactions, anaphylaxis, phagocytosis
- Basophil: initiation of immune and allergic reactions
- Monocyte: phagocytosis and antigenic processing
- Lymphocyte: antibody production and immunity

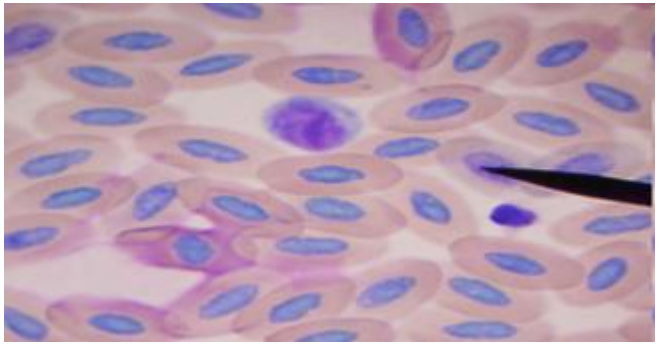
Avian WBCs



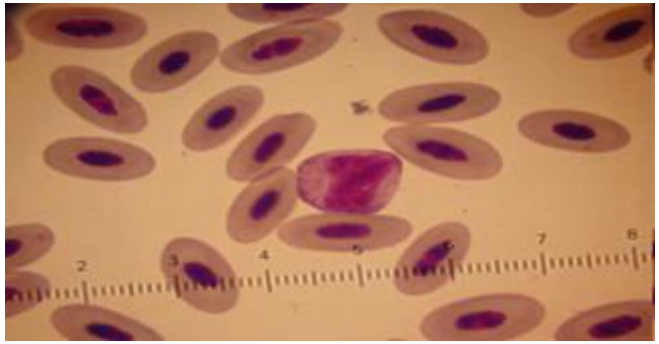
Heterophil



Eosinophil



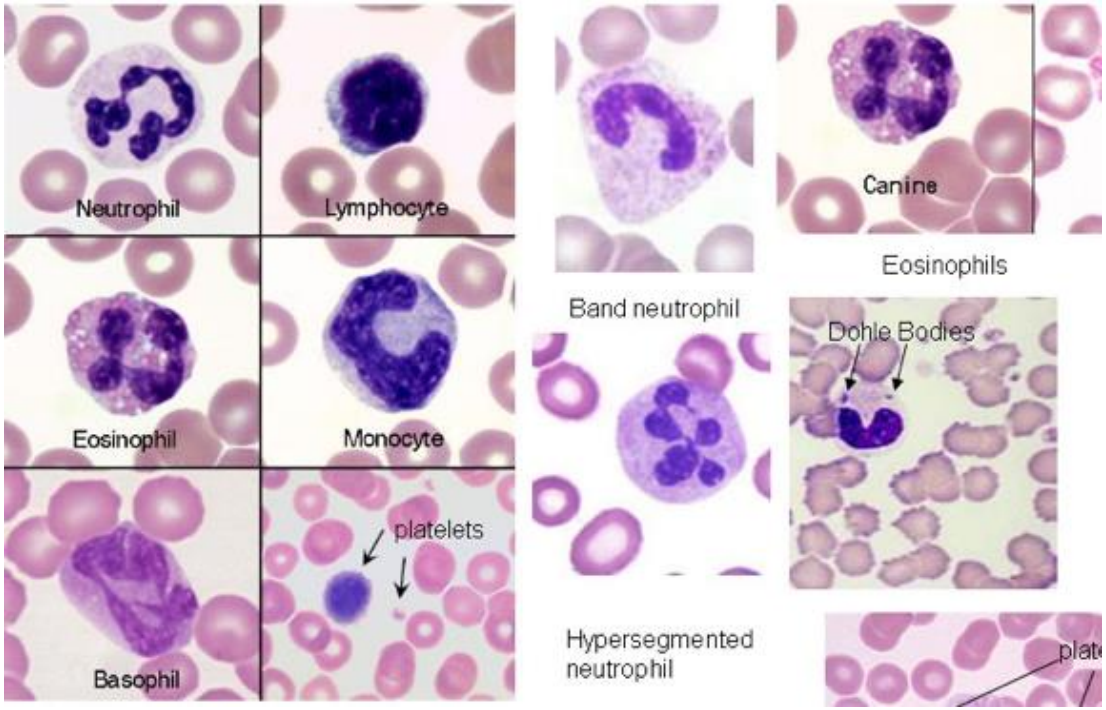
Lymphocyte



Monocyte

http://www.exoticpetvet.com

Mammal WBCs


Neutrophil
Lymphocyte
Eosinophil
Monocyte
platelets
Basophil
Band neutrophil
Canine
Eosinophils
Dohle Bodies
Hypersegmented
neutrophil

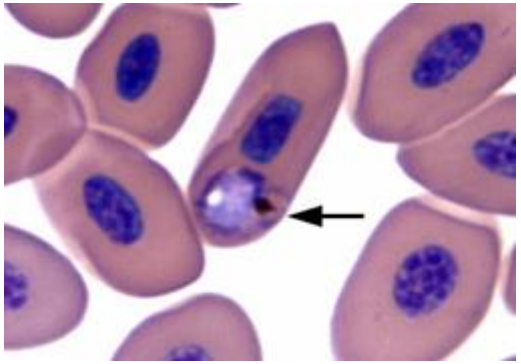
WBC estimate

- Make a well-prepared slide
- Read at feathered edge where cells are evenly distributed, on high dry (40x)
- Can put oil on slide with a coverslip on top
- Count WBCs in 10 fields
- Average and multiply by 2000 (mammals x 1600)
- If PCV is below normal, calculate corrected estimate
- Not as reliable as hematocytometer, need a good slide and experienced person
- Practice, practice, practice

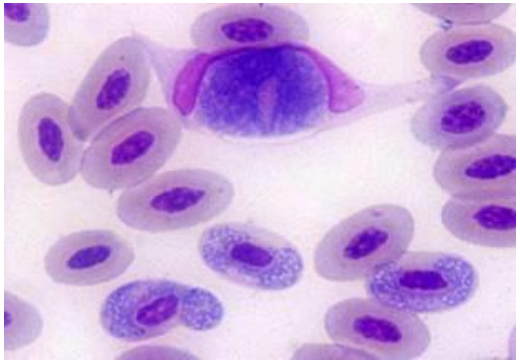
Avian Blood Parasites

- All protozoans
- Plasmodium: Malaria
- Leukocytozoon - pathogenic in young
- Hemoproteus: considered non-pathogenic in most avian species
- Transmission by biting Arthropods

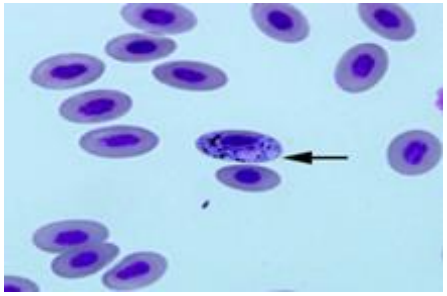
- *Diagnosed by blood smear stained with Diff-Quik



Plasmodium sp.



Leukocytozoon sp. and Hemoproteus sp.



Hemoproteus sp.

Urinalysis

- Three parts:
- 1. Gross exam: odor, color, clarity, specific gravity
- 2. Chemical exam: dipsick
- 3. Sediment exam: microscopic (to see WBCs, bacteria, casts, crystals)
- Analyze within 1 hr or put in fridge for up to 6hrs, AM sample is best
- Urinalysis useful in mammals but not so much in birds

carnegyanimalhospital.com


peteducation.com

pet-informed-veterinar.

Urinalysis

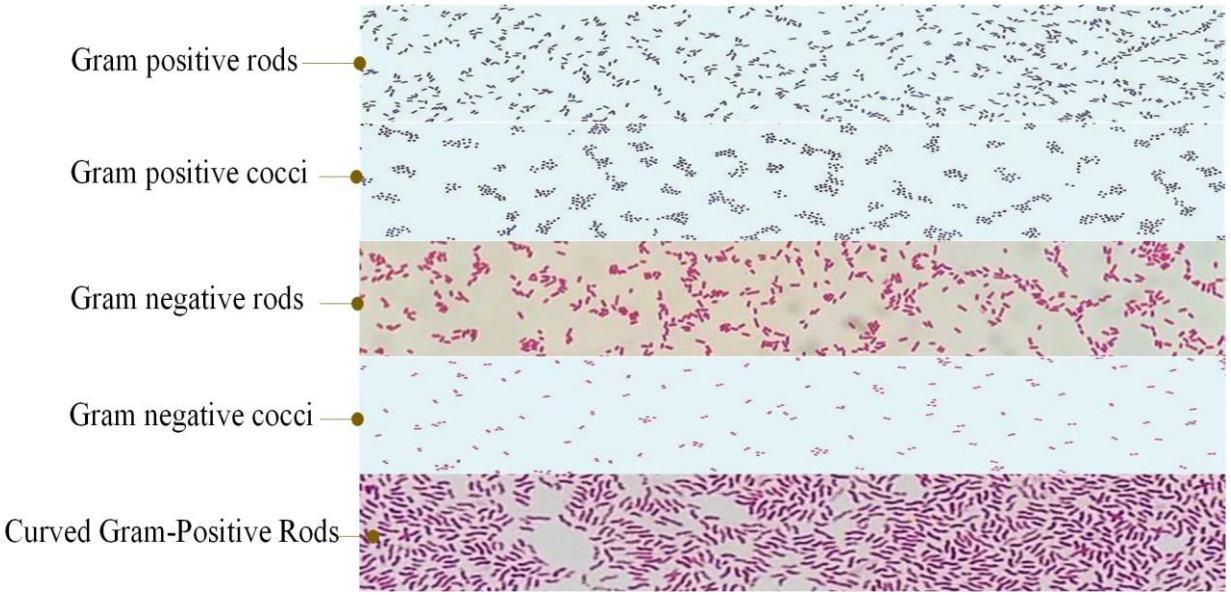
- Perform a urinalysis if gross sample is abnormal, if patient is straining to urinate, or suspect kidney problems
- Normal for rabbits and rodents to have a strong odor and cloudy color
- Red colour normal in rabbits from plant pigments in diet
- Ammonia odor can mean infection

Microbiology

- Gram stain
- Swabs
- Normal flora: areas normal to have bacteria- digestive tract, mouth, etc.
- Areas that are sterile should be free of bacteria- urinary tract, bloodstream, etc.
- Sterile swab used to culture bacteria to identify and for antibiotic sensitivity

Gram stain

- The ability to differentiate between two types of bacteria: gram positive(purple) and gram negative (red)
- Cells stain pink
- Can tell rods from cocci
- Can also detect yeast (show up as purple/blue colored), Clostridium sp., Campylobacter sp. etc.



https://microbiologie-clinique.com/gram-stain-principle-steps-interpretation.html

Sending samples away

- For further diagnostics- CBC, chemistries, electrolytes, histopathology, cytology, toxicology, culture and sensitivity
- Call lab prior to collecting blood to know how to collect and ship
- Tissue samples- send in 10% buffered formalin (usually no thicker than 3cm)
- Necropsies - Important to send body or tissue samples to lab after unexplained death
- Know the reportable disease in your area!

alwaslvetclinic.com

Things you can do in a rehab center

- Laboratory Standard Operating Procedures (standardize lab work)
- Chart with bloodwork for each species to learn species normals
- Start a ‘library’ of interesting cases/slides to learn from, and as a reference
- Have one person dedicated to lab techniques and becoming skilled
- Quality control and Annual maintenance of equipment: microscope, refractometer, glucometer, lab machines

Conclusion

- A few simple lab tests help in patient diagnosis, treatment, and prognosis
- Become familiar with performing fecals, PCV, TP, blood smears and blood glucose
- Learn normals for the species you see
- Recognize your limitations- know when to send lab work to the lab or vet
- Standardize techniques for consistent values

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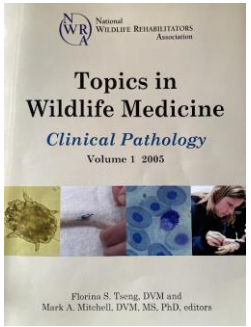

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Thank you for your contributions!

Questions?