Basic Laboratory Techniques in Wildlife Rehabilitation

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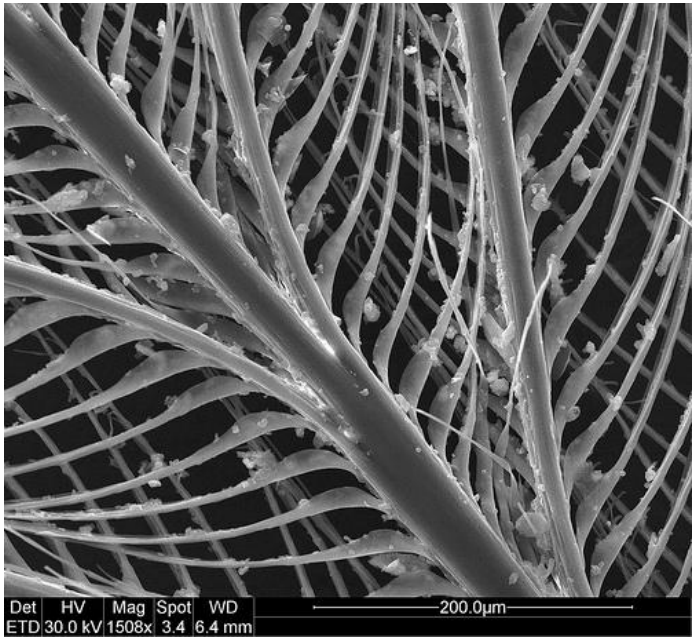
IWRC Symposium 2013

Overview

- Why and when you would perform lab techniques
- Equipment
- Parasites and wildlife
- Parasite families
- Microscope and how to read a slide
- Fecal procedures
- Ectoparasite methods
- Common parasites of avian and mammals
- Microbiology
- Mycology
- Cytology
- Hematology- WBCs, RBCs, platelets
- Sending samples away
- Urinalysis
- Necropsy
- What you can do in your rehab facility lab
- Conclusion



www.flickriver.com

What a birds feather looks like under the microscope

Why perform laboratory procedures?

- To help detect any underlying disease processes- wildlife hide signs
- Provides information to assess current condition
- Provides information to design a treatment plan
- Helps evaluate prognosis
- Tool to monitor progress

http://www.zutrition.com/avian-toxicities/

Bald Eagle with Lead Poisoning

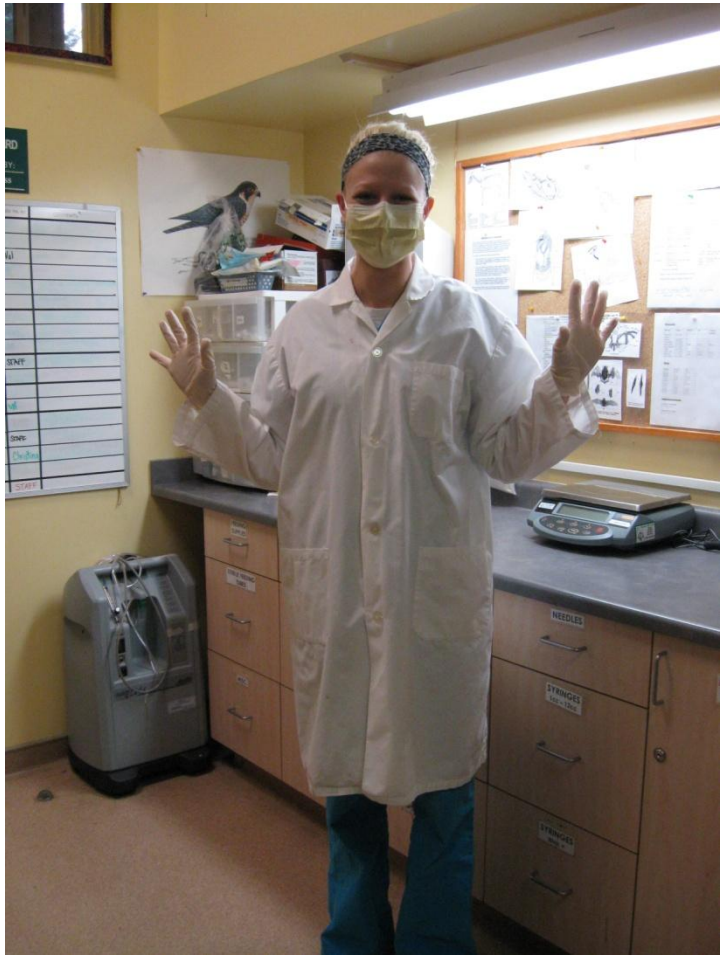
When would you perform laboratory procedures?

- Upon intake if animal is stable enough to handle it
- Oiled animal
- Seabird
- Thin body condition
- Crop not emptying
- Eating but not gaining weight
- Suspect poisoning, toxicity
- Abnormal color, consistency, or blood in feces
- Anorexia

Basic Laboratory Equipment

- Microscope
- Slides and Cover Slips
- Lighter
- Stains (Gram stain and Diff Quik)
- Fecal kits and floatation solution
- Blood collection supplies- vacutainer, hematocrit tubes, alcohol, syringe/needles
- Centrifuge
- Refractometer
- Glucometer
- Good reference manuals

Parasites and Wildlife



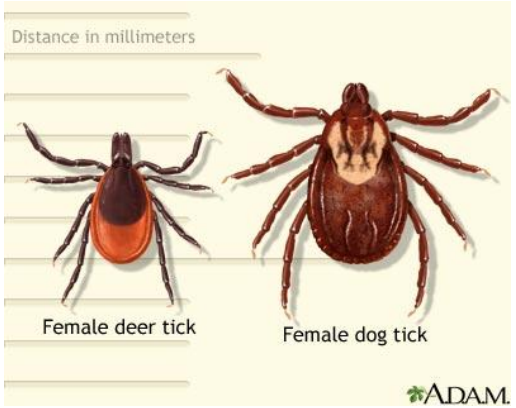
- Parasites in **low** numbers are **normal** in wildlife
- **Higher** numbers may indicate a **disease** process or animal is immunocompromised
- Some parasites are host-specific
- Many of the parasites encountered are zoonotic so wear PPE
- Know how to diagnose, treat, and prevent re-infection
- Decrease stress, provide supportive care
- Isolate if necessary

Parasite families

- Parasite families
  - **Protozoa** (single-celled organisms): Coccidia, Trichomonas, Giardia, avian blood parasites
  - **Helminths**: Nematodes (roundworms), Trematodes (flukes), Cestodes (tapeworms), Gapeworms, Hookworms, Whipworms, Threadworms.
  - **Arthropods**: hard segmented bodies (ticks, mites, lice, insects)

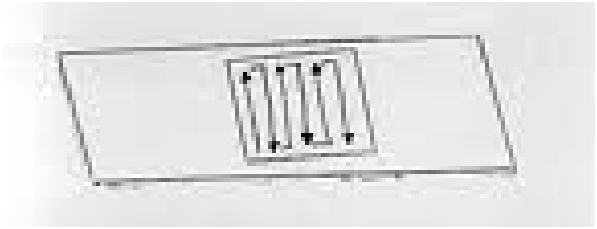
Treatingdiarrhea.com

www.findavet.com



nvsd44.bc.ca

Microscope and Reading a Slide



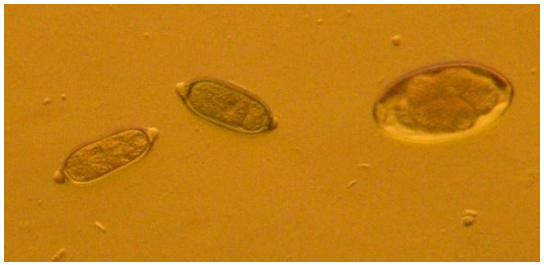
cal.vet.upenn.edu

- **Low power objective (10x) first**
  - faster to read, less detail and light needed
  - scan for platelet clumps, staining, large abnormal cells
- **High dry objective (40x)**
  - to examine object more closely
  - field of view is decreased, need more light
- **Oil immersion (100x)**
  - need lots of light,
  - small field of view
  - to see bacteria, yeasts, spores, and cell details

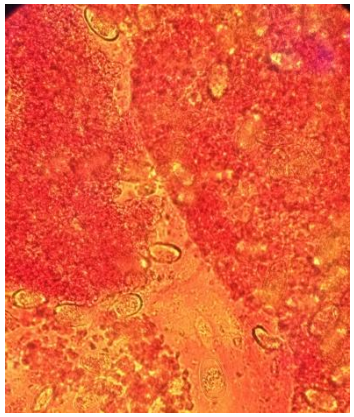
Laboratory – How to…

treebeard31.wordpress.com

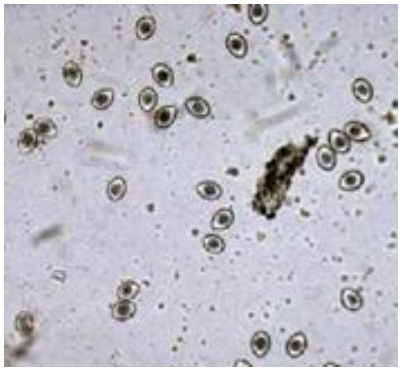
- Wear gloves
- Gather supplies and perform tests
- Sanitize surfaces in contact with sample
- Dispose of used supplies and samples appropriately (hazard vs. regular waste)
- Wash hands after procedure
- Write results in chart, select appropriate treatment



Gapeworm, Capillaria



Hepatic Coccidiosis



Coccidia

marvistavet.com

Fecal Analysis - Procedures

- 1. Gross examination of feces
- 2. Fecal floatation, centrifugation, sedimentation
- 3. Direct smear
- 4. Gram stain

Fecal Analysis - Collection

- Need a fresh sample- rapid development and changes can occur
- Label with species, case number, date
- Note feces color, consistency, and presence of (gross) parasites and/or blood
- Avian- collect fecal part (dark colour) only, not the urates or urine (white or light green)
- Can keep in the fridge up to 4 days

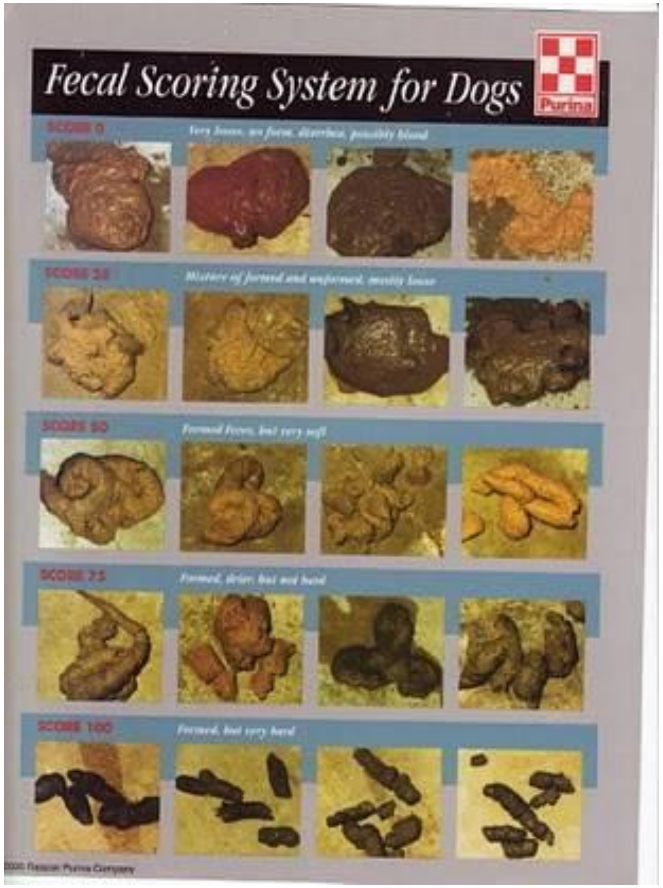
www.pet-informed-veterinary-advice-online.com...Remove frame

GWGU dropping

1. Gross examination of feces

- **Consistency**: liquid, soft, hard, granular, gelatinous
- **Odor:** normal vs abnormal for the species
- **Color**: green, dark brown, black, red, etc.
- **Blood**: dark black/brown and tarry stools (bleeding from upper GI)

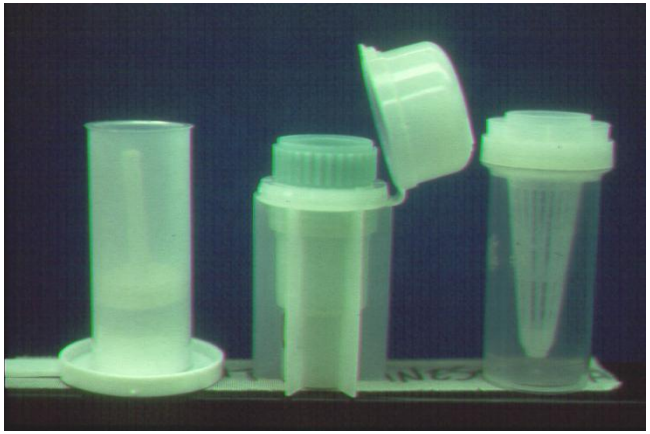
  red or maroon-coloured (bleeding from lower GI)
- **Mucus**: intestinal inflammation, parasitism, or infection
- **Gross parasites**: larvae or portions of parasites are sometimes visible to the naked eye (e.g. Tapeworm)



widening circle.blogspot.com/2011/01/poop-char...Remove frame

2. Fecal float

atozvetsupply.com



Ovatector, Fecalyzer and Ovassay kits

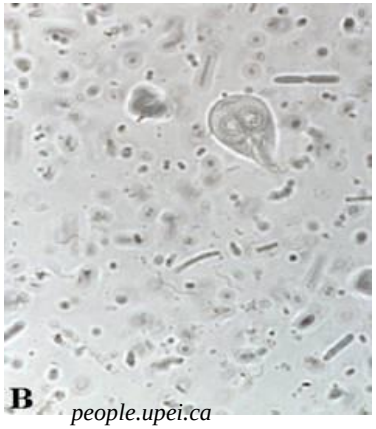
Wildlife under the microscope

- Detects ova/oocytes from internal parasites
- If Negative: Perform fecal analysis for 3 consecutive days
- If Positive: Perform fecal analysis 2-3 days **after the last treatment**
- Uses a solution with a higher specific gravity than that of the eggs
- Many floatation solutions: zinc sulfate, sodium nitrate, sugar, etc.
- Allow 10-15 mins. for eggs to float
- Read slide ideally within 30 mins.
- Floatation not used for Trematodes (flukes) as too heavy for solution **or** Giardia as solution will lyse organism

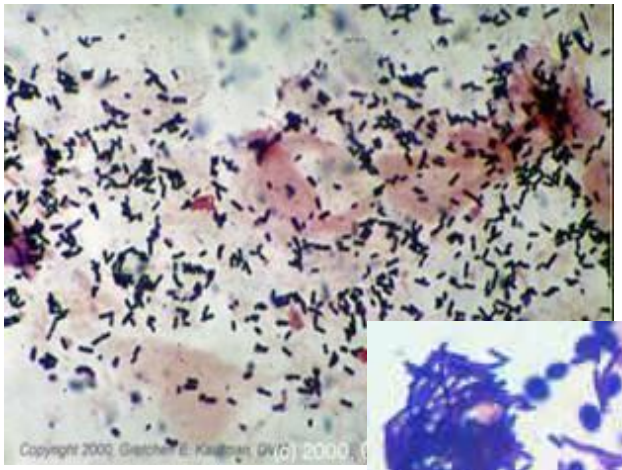
3. Direct Smear – Wet Mount

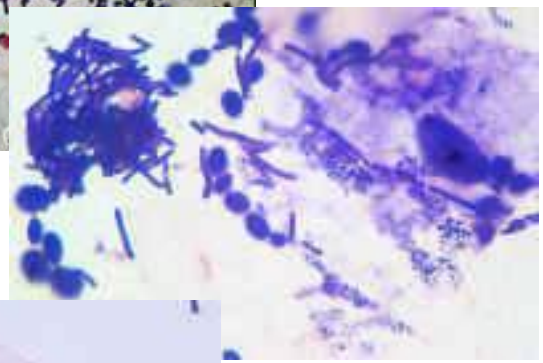
- The simplest method for parasite examination
- Detects motile protozoan trophozoites: Giardia, Trichomonas, but also coccidia, larvae and eggs
- Use Q-tip to collect a very small amount of fecal matter and add one drop of saline.
- **Advantage**: uses a very small amount of feces and can be analysed immediately.
- **Disadvantage**: small sample not a good representative for parasite load,or presence of parasites, large amount of fecal debris.

Giardia sp.



3. Direct Smear - Gram Stain





masaav.org

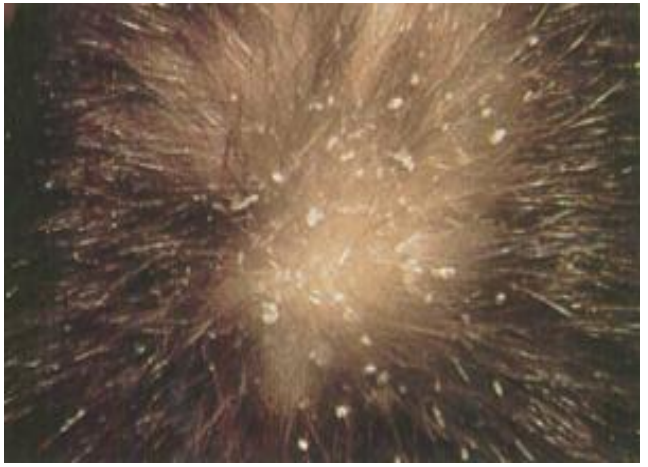
- Can detect bacteria, yeast (purple colored), spores, Clostridium sp., Campylobacter sp. etc.
- Differentiate between two types of bacteria: gram positive and gram negative
- Evaluate the balance of bacterial flora (cocci vs. bacilli)

Fecal Analysis – Recording Results

- Results either No Ova Observed (N.O.O) or Ova Observed (OVA).
- **Standardize techniques on how to record**:
  - 1+ = 1-2 ova per low power field (LPF) (light load)
  - 2+ = 3-5 (moderate)
  - 3+ = 6-8 (heavy)
  - 4+ = > 9 (very heavy)
  - TNTC
- Wet Mount interpretation
- Gram stain interpretation

Ectoparasites Analysis - Procedures

- Three methods used:
  - **1. Squash Smear**: to see tapeworm segments and ectoparasites (saline + cover slip)
  - **2. Scotch tape method**: to visualize lice, mites, and others
  - **3. Skin scrape:** to detect mites such as Mange (Sarcoptes, Demodex), Scaley leg mite
- Preserve them in 70% ethanol or formalin
- Find through physical examination except for mites (will see lesions)



Scotch tape method for Cheyletiella

Cheyletiella under the microscope

Common Avian **Ecto**parasites

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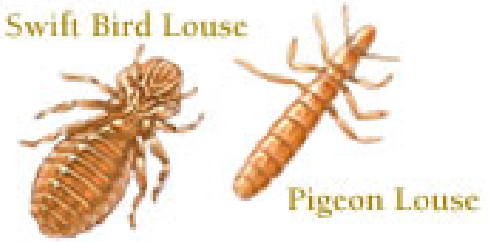


bugguide.net

- Lice (Order Mallophaga)
- Fleas
- Fly larva (maggots)
- Ticks
- Flat flies (Hippoboscidae)
- Scaley Leg Mites (Knemidokoptes)- thickening of skin
- Flower Mites
- Avian skin mites (Ornithonyssus sp.)- black specks on bedding, hand
- Feather mites
- Avian follicular mites (Harpyhynchus sp.)- nodules on skin

*****Diagnosed by microscopic exam (scraping) of feathers and skin or gross observation

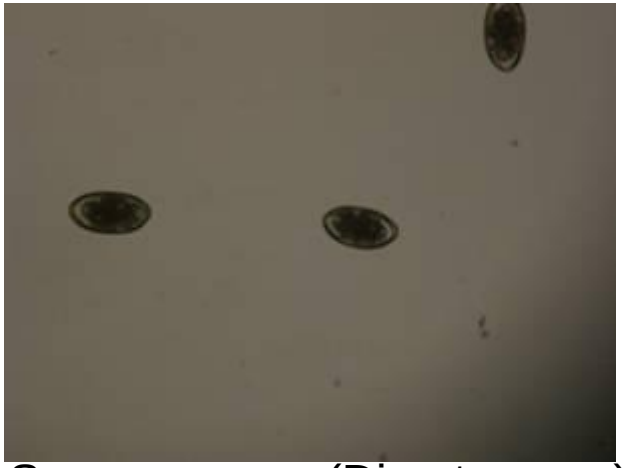
Common Avian **Endo**parasites

Fecal Analysis:

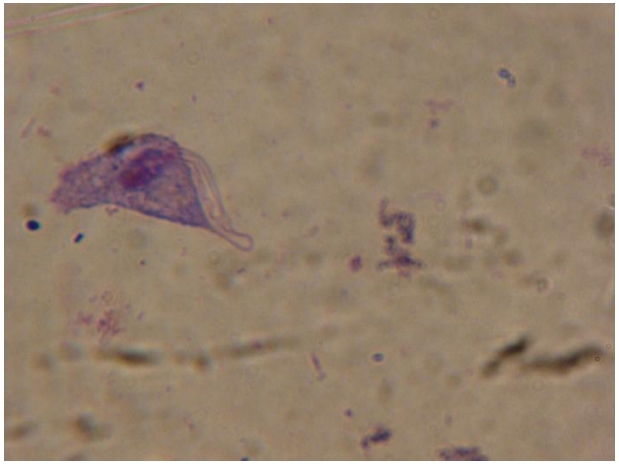
- Capillaria sp. (Threadworm)
- Syngamus sp. (Gapeworm)
- Coccidia (Protozoa of genera Isospora or Eimeria)
- Taenia sp. (Tapeworm)
- Ascaridia (Roundworm)
- Giardia (Protozoa) (direct smear)

Crop Swab:

- Trichomonas sp. (Protozoa)
- Yeasts (fungal infection)

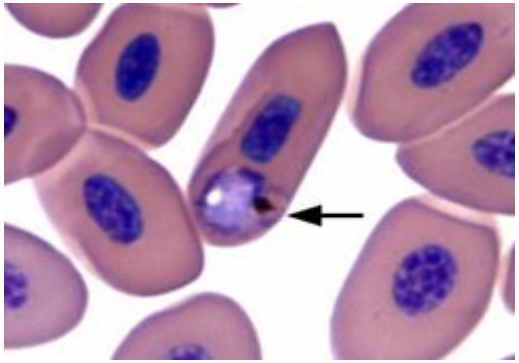


Syngamus sp. (Direct smear)



Trichomonas sp. (Diff Quik)

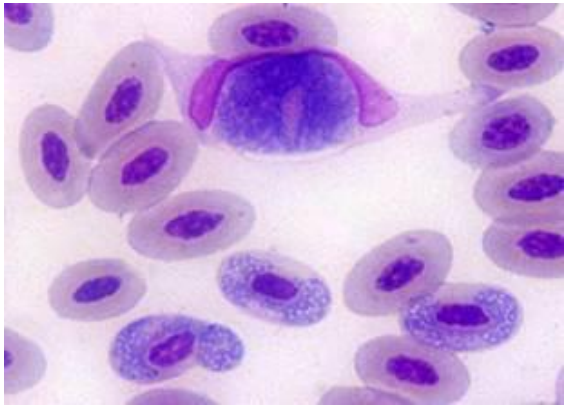
Avian Blood Parasites

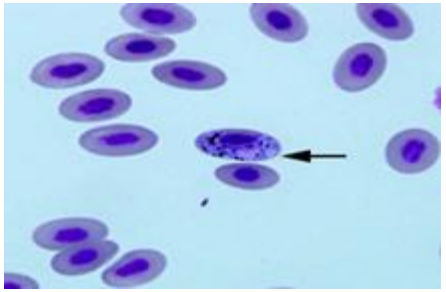


Plasmodium sp.

- All protozoans
- Plasmodium (Malaria)
- Leukocytozoon
- Hemoproteus: considered non-pathogenic in most avian species.
- Transmission by biting arthropods
- Anyone's experience?

*Diagnosed by blood smear stained with Diff-Quik (not smaller than 25G needle for blood collection)





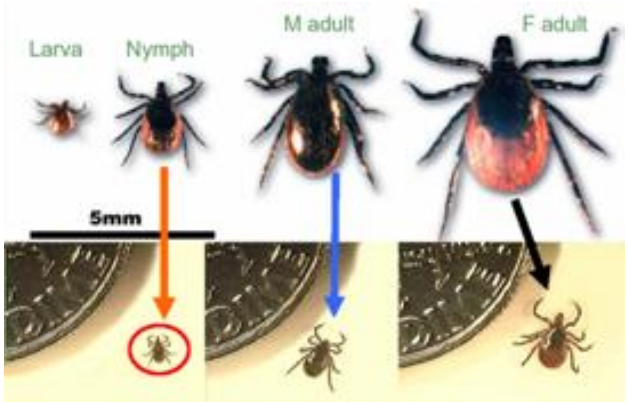
Leukocytozoon sp. and Hemoproteus sp.

Hemoproteus sp. *vet.uga.edu*

Mammal Endoparasites

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

Mammal Ectoparasites



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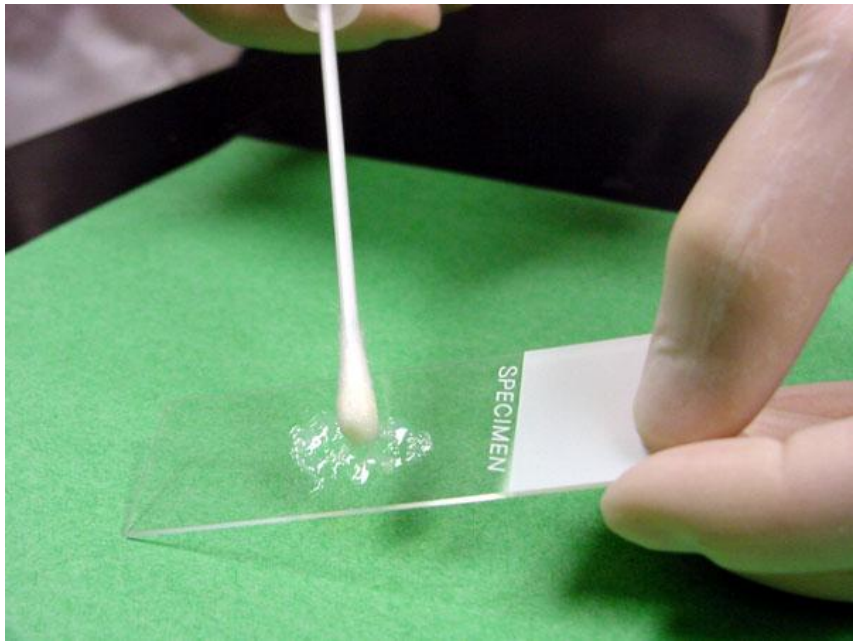
flea-control.org

- Mange (Sarcoptes, Demodex)
- Fleas (Ctenocephalides)
- Lice (Pediculus)
- Ear mites (Notoedres)
- Cuterebra
- Cheyletiella
- Maggots
- Ticks

heartspring.net

Microbiology

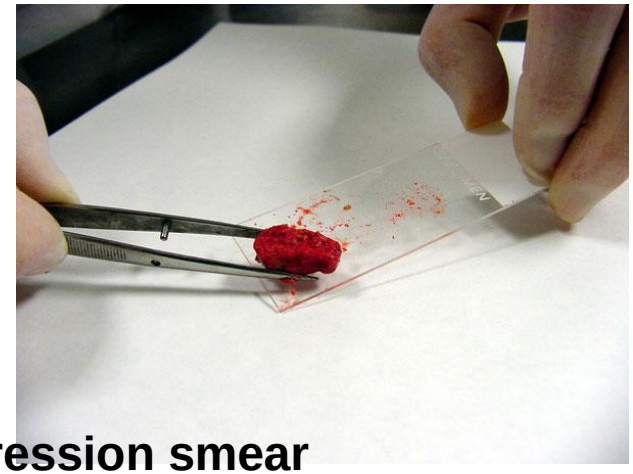
- Gram stain
- Remember normal flora: areas normal to have bacteria (digestive tract, mouth) vs. sterile areas
- Sterile swab used to culture bacteria (ID and antibiotic sensitivity)
- Fungassay (fungal culture)



http://loudoun.nvcc.edu/vetonline/vet132/micro/unit2/swabsmear.jpg

Cytology

Skin scrape



Impression smear

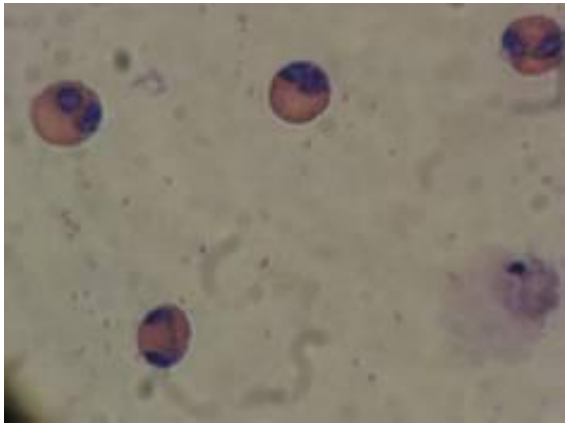
Cytology: **Removing cells from tissue and examining them under the microscope**

- Evaluate cells in body fluids, purulent discharge, skin masses, and internal organs
- Look for abnormal cells (neoplastic) or presence of inflammatory cells (WBC)
- Can be tricky to identify cells- send to pathologist or consult with veterinarian
- **Techniques**: fine needle aspirate of a lump, impression smear, swab, scraping
- Stain with Diff Quik

Swabs

- **Crop swab**: to detect 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
- Other areas such as **wounds and GI tract (cloacal)** can be swabbed
- Skin lesions
- Use a sterile swab with sterile saline, can stain with Gram stain or Diff Quik
- Can also do a culture this way using a sterile transfer media swab

ocw.tufts.edu

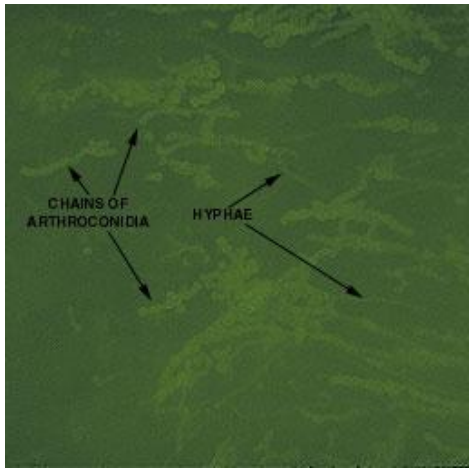


WBCs and bacteria from swollen joint of GWGU, Diff Quik 100x

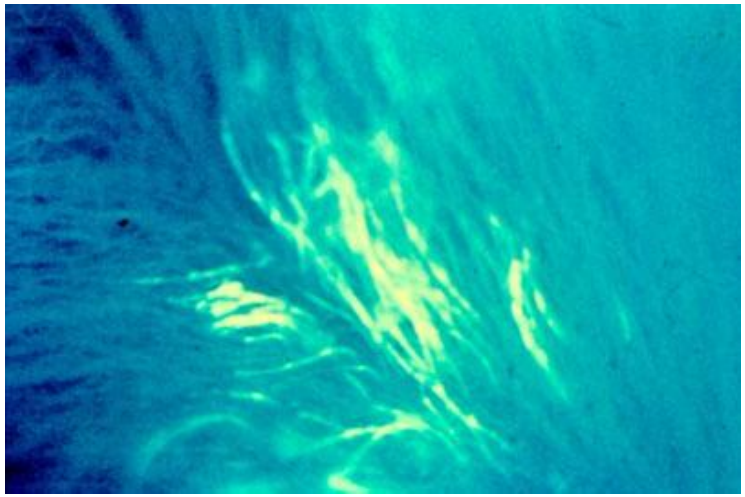
Mycology

- When to consider investigating: visual observation of skin lesion, foul smell, aural or oral accumulation of abnormal material
- Yeast (Candida)
- Ringworm (Dermatophyte): culture with fungassay, 50% of cases light up with a woods lamp if the fungus is *Microsporum canis* species
- Sometimes fungi can be seen by stained direct smear or KOH smear (hair and skin scraping)

Positive fungassay



KOH direct smear



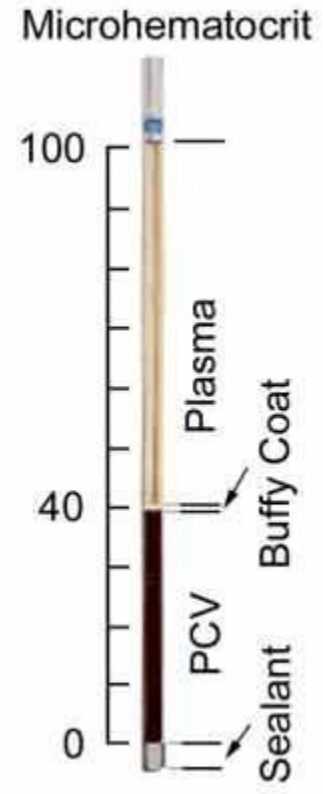
Positive flourecence

http://loudoun.nvcc.edu/vetonline/vet132/micro/unit2/microbiology_unit2_lesson3.htm

Basic Hematology

- **1. Packed Cell Volume (PCV)**
- **2. Buffy Coat and plasma color**
- **3. Total Protein (TP)**
- **4. Blood glucose**
- **5. WBC differential and estimate, platelet estimate, RBC morphology**
- **6. Note any blood parasites, and abnormal toxic cells**
- 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)



- Determines the percentage of whole blood composed of red blood cells (RBCs)
- Also known as the hematocrit (Hct)
- Very accurate within 1%
- Reference ranges:
  - Avian 35-55%
  - Mammals 30-55%

PCV Values

Low PCV < 30% indicates anemia caused by:

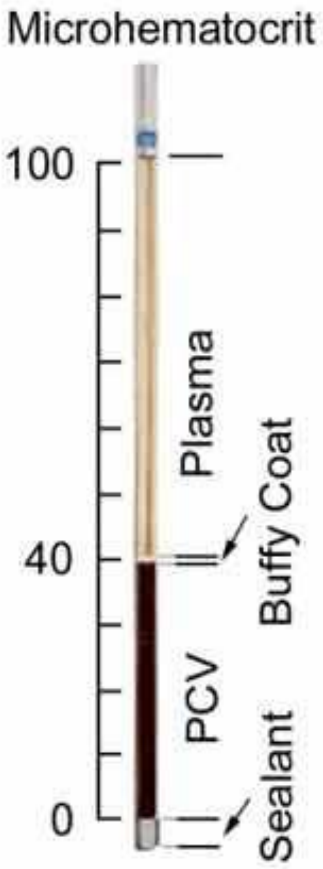
- Hemorrhage
- Parasites
- Destruction of RBCs
- Decreased production of RBCs
- Treatment:
  - Iron dextran injection
  - Pentaspan or Hetastarch
  - Consider transfusion or euthanasia at < 15%

High PCV > 55 % can indicate:

- Dehydration
  - Treatment: fluid therapy

2. Buffy Coat

- Estimates the WBC and platelet level in mammals
- Thicker buffy coat = increased number of WBCs
- Whitish-gray layer just above the RBC layer
- Healthy patient: <1%
- Over 2% consider antibiotics, **NO** Iron administration



Plasma Layer

- Clear - yellow fluid above the buffy coat layer
- Proteins (hormones, antibodies, enzymes, etc.), water, salts, glucose, fats
- **Colors**:
  - **Red**: hemolysis, possible poor handling technique
  - **Yellow**: icterus, liver issue (mammals)
  - **White**: lipemic, recent ingestion of fatty meal
  - **Normal** is clear in mammals, slightly yellow in birds

3. Total Protein (TP)

- The total protein measures the <u>total amount of protein in the liquid portion of the blood</u>
- Indicates general health status of the patient
- Healthy avian patient should be between **3.0-6.0 g/dL**
- **TP > 6 g/dL**: indicates dehydration, chronic disease, infection
- **TP < 6 g/dL**: hypoalbuminemia due to malnutrition, malabsorption, chronic liver disease, starvation
- **TP < 2.0 g/dL**: poor prognosis, consider euthanasia

Blood Glucose

- Using a glucometer and a drop of blood
- Most useful in mammals
- **Normal values: species specific**
- **Hypoglycemia: Below normal range**
  - Starvation, malnutrition
  - Treatment: dextrose
- **Hyperglycemia: Above normal range**
  - Diabetes
  - Pancreatitis
  - Stress can elevate blood glucose levels

articlesbase.com

ikigai-de-crabahuteuse.over-blog.com

"STRESS"

Blood Collection Sites

Mammals:

- Jugular vein
- Cephalic vein (forearm)
- Saphenous (lateral side of back leg)
- Femoral (medial side of back leg)
- Marginal ear vein (rabbits, felines)

Avian:

- Ulnar vein (ventral aspect of elbow)
- Medial metatarsal vein (along the metatarsus)
- Jugular vein (R one is two-thirds larger than the L)
- Superficial digital veins through webbing (large species)
- **No** toe nail clips

Avian blood collection sites

Medial metatarsal vein

Jugular vein

Needle sizes: bigger than 25G but are exceptions

Ulnar vein

ltryhelp.com

Mammal blood collection sites

Cephalic vein

Ear vein in rabbit

Saphenous vein

Femoral vein

Jugular vein

Pictures taken from “wildlife under the microscope”

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 100 ml, safe maximum amount of blood taken is 10 ml
  - Effects of blood loss (hypovolemic shock)

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Making Blood Films

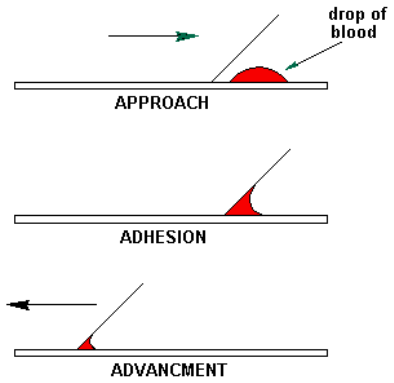
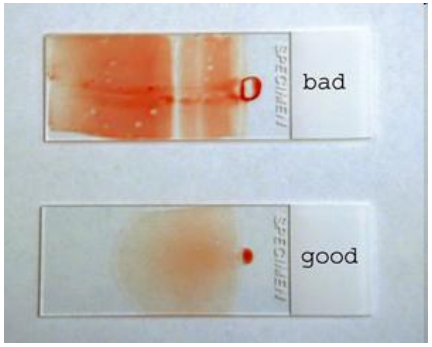


Fig. 13 - How to prepare a blood smear.



cartage.org.lb

- To perform the differential WBC count, estimate platelet numbers and evaluate morphology of WBCs, RBCs, and platelets
- Make the blood film as soon as possible after collection
- Use blood from needle or vaccutainer using a hematocrit tube
- Mix vaccutainer tube gently first (blood with anticoagulant)
- Use one small drop of blood
- Make sure slide is clean

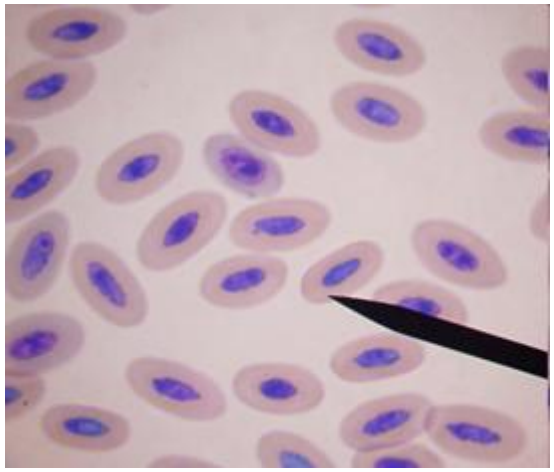
Erythrocytes/Red Blood Cells (RBCs)

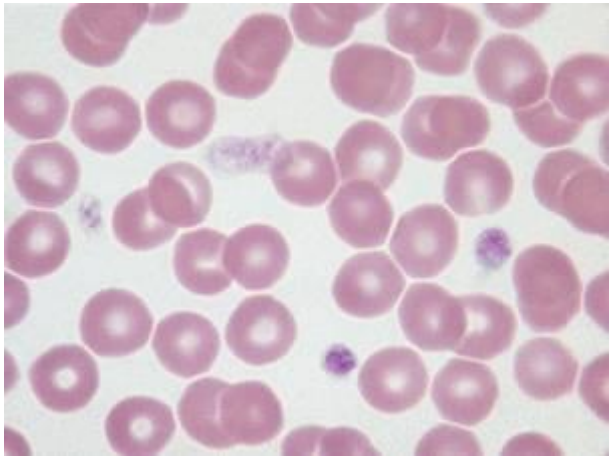
Function:

- RBCs carry hemoglobin, which transports oxygen throughout the body
- Made in the bone marrow in mammals, in liver and spleen in birds
- Lifespan: 28-45 days in birds, 90-120 days in mammals

Avian vs. Mammal:

- Avian: Elliptical and nucleated; larger than mammal
- Mammals: Round and not nucleated, have an area of central pallor; smaller than avian
- Look under oil immersion for abnormalities (100x)



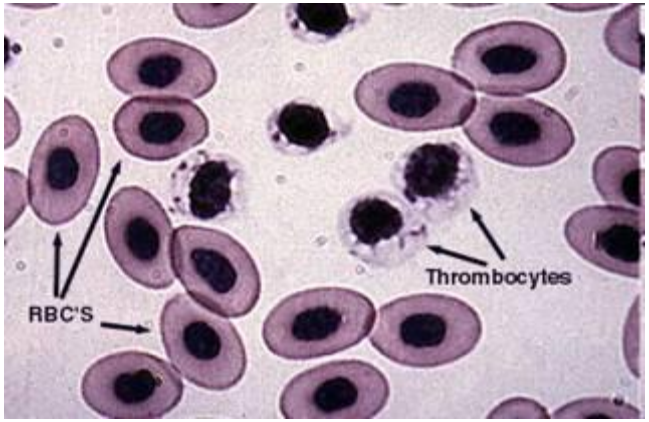


ahdc.vet.cornell.edu

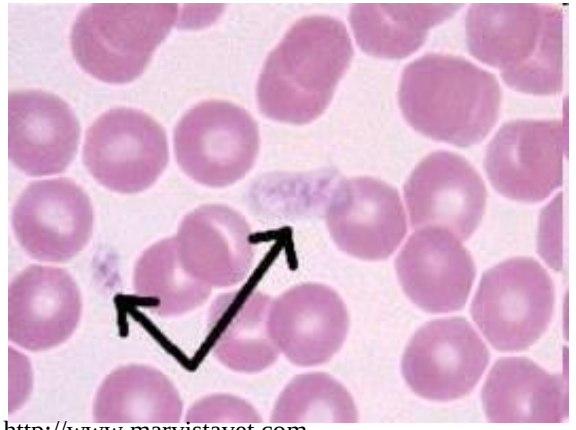
Thrombocytes (Platelets)

- **Function:**
  - Hemostasis, wound healing, phagocytosis, clotting
  - Can clump on a blood film

- **Avian vs. Mammal**:
  - Avian: **nucleated**, small rim of gray cytoplasm
  - Mammals: not nucleated, much smaller than avian
- Determine if they are normal, increased or decreased:
  - Normal: 1-5 platelets/ oil immersion field (100x)
  - Consider amount of clumping



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

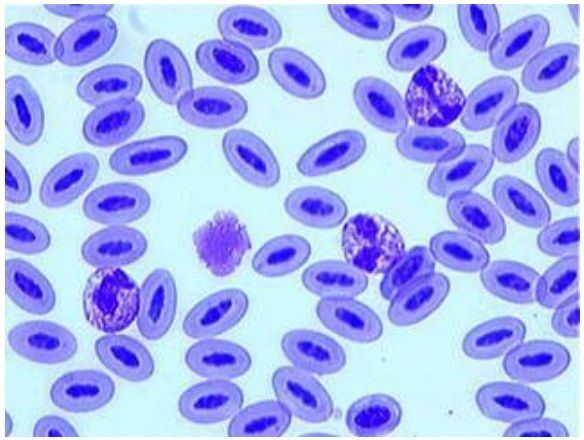


http://www.marvistavet.com

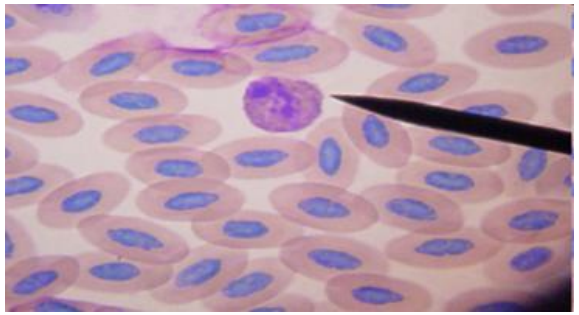
White Blood Cells (WBCs)

- Avian and mammals function similar: to defend the body against foreign invaders (immune system)
- **Neutrophil** (mammals), **Heterophil** (avian, rabbits, some rodents): phagocytosis
- **Eosinophil**: allergic reactions, parasites, phagocytosis
- **Basophil**: initiation of immune and allergic reactions
- **Monocyte**: phagocytosis and antigenic processing
- **Lymphocyte**: antibody production and immunity
- **Leukocytosis**: increase, due to inflammation, infection, tissue damage
- **Leukopenia**: decrease due to virus, septicemia

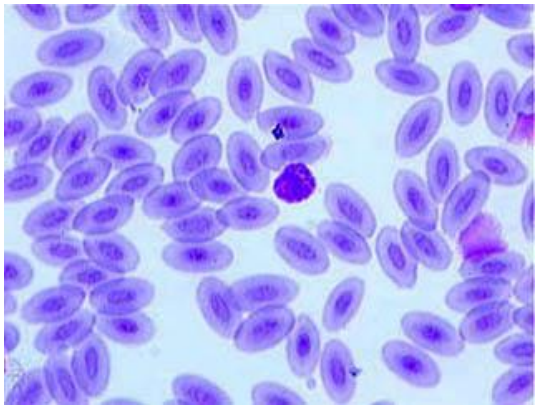
Avian WBCs



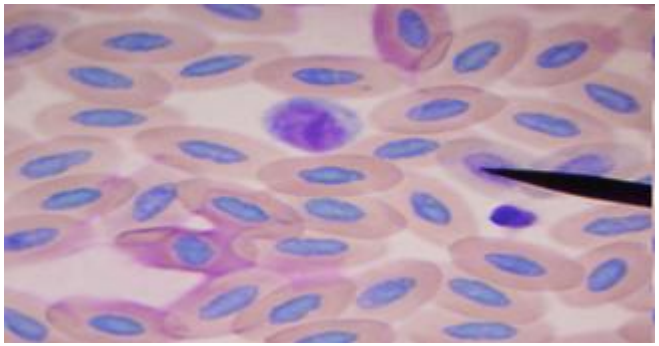
Heterophil and smudge cells



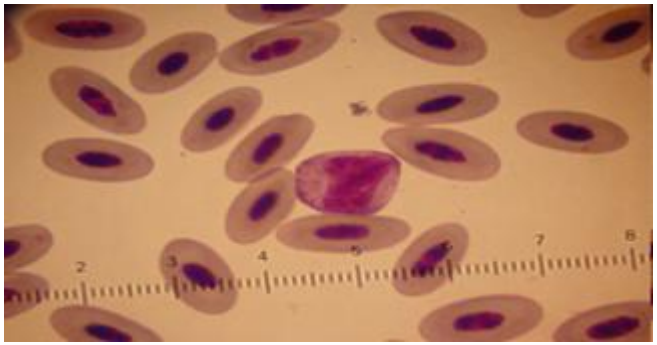
Eosinophil



Basophil



Lymphocyte



Monocyte

Mammal WBCs

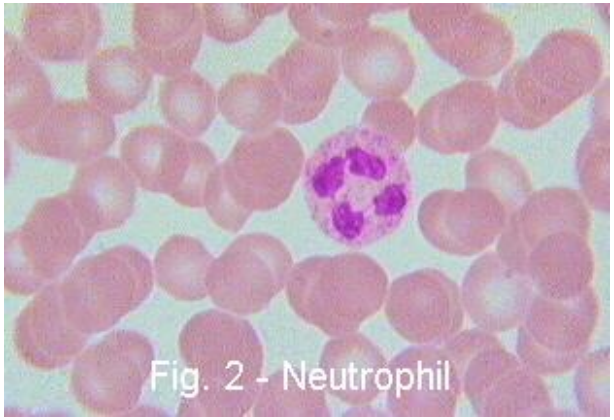


Fig. 2 - Neutrophil

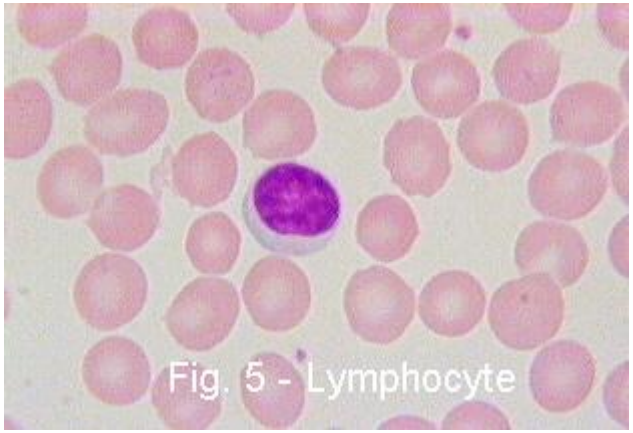


Fig. 5 - Lymphocyte

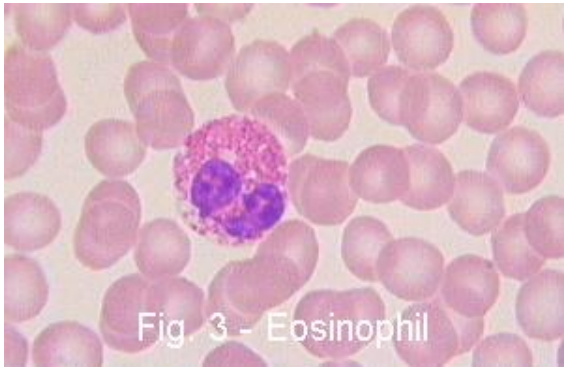


Fig. 3 - Eosinophil

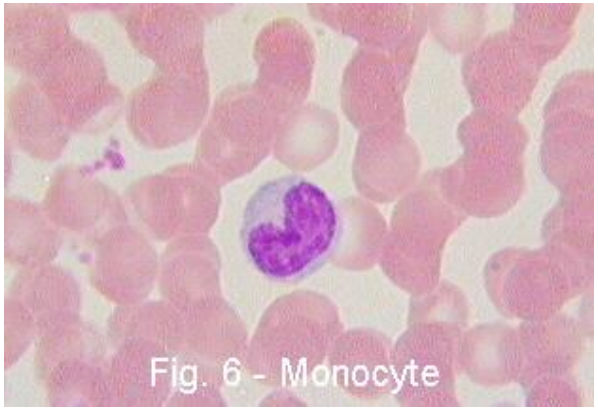


Fig. 6 - Monocyte

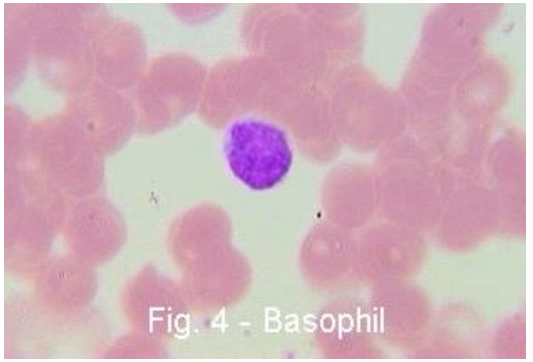


Fig. 4 - Basophil

http://www.funsci.com/fun3_en/blood/blood.htm

Sending samples away

- Include as much history as possible
- Blood chemistries, virology, histopathology, cytology, parasitology, toxicology, culture and sensitivity
- Call lab prior to collecting samples to know what test requires what and how to ship
- Tissue samples- send in 10% buffered formalin, no thicker than 2-3cm
- Don’t freeze histo samples, package so there is no leakage

alwaslvetclinic.com

What do you suspect?

Poultryhub.org

Necropsy samples

- Important to send body, tissue, or fluid samples to lab after unexplained death
  - Can give you information on cause of death
  - Can be linked to symptoms
  - Helps you learn from the case and change protocols for future cases
  - Population health monitoring

http://www.unbc.ca/nlui/wildlife_diseases_bc/parvovirus.htm

Inflamed bowels indicative of which mammal viral disease?

Urinalysis

Three parts:

- **1. Gross exam**: odor, color, clarity, specific gravity
- **2. Chemical exam**: Dipstick
- **3. Sediment exam**: Centrifuge and microscope (bacteria, crystals, cells, blood, WBC)
- Analyze **within 1 hr** or put in fridge for up to 6hrs, AM sample is best
- Urinalysis useful in mammals but not as much in birds
  - Birds- collect urine on wax paper

carnegyanimalhospital.com

peteducation.com

Things you can do in your center

- Laboratory Standard Operating Procedures (standardize lab work)
- Create a chart with blood values for each species to learn species normals (intake vs. pre-release)
- Start a ‘library’ of interesting slides/photos to learn from, and use as a reference
- Quality control: for refractometer, glucometer, any lab machines
- **Teach others!**

Conclusion

- OBSERVATION: notice abnormalities to know when to test
- Consult with your veterinarian, send lab work to the lab for further answers
- Most useful basic procedures: Fecal analysis, PCV, TP, crop swabs, blood glucose in young mammals
- Can use a few simple lab tests to help in patient diagnosis, treatment, and prognosis
- Standardize techniques for consistent values

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www.nationalgeographic.com

Plankton sample under the microscope

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