Parasitology, Microbiology, Cytology and Histopathology (and Chemistries)

Saundra E. Willis DVM DACVIM

Parasitology

Fecal testing in health:

- If routine deworming done: double or single centrifugation, or passive floatation
- If routine deworming not done: double centrifugation for optimal accuracy

Fecal testing in disease: consider one or more of the following

- Direct smear in clinic
- Double Centrifugation Fecal
- Giardia/Crytosporidium IFA
- Giardia elisa
- Prophylactic deworming
- Other testing
- (note: for salmon poisoning, trematode eggs are best found on a complete fecal exam including direct smear and double centrifugation)

Direct Mount:

- Wet mount made by mixing drop of fecal sample in drop of saline or water
- Mix gently
- Coverslip and examine for motile parasites, worms and other larvae, 10x and 40x
- Best done immediately, ie. in clinic

Tritrichomonas InPouchTF for identification of *Tritichomonas foetus*

- Fecal sample size should be no larger than the size of a peppercorn
- Incubate pouch in vertical position at room temperature as refrigerator temperature will kill the trichomonads
- Avoid cat litter
- (please read: https://cvm.ncsu.edu/wp-content/uploads/2016/05/ownersguide-to-feline-t-foetus.pdf)

Fecal Cytology:

- Fresh sample (less than 15 minutes old). Technique: Spread thin on slide, air dry, heat fix, stain
- Normal fecal cytology: >90% mixed population of rods, Few epithelial cells, Few/no WBC's, no RBC's
- Scan 10x
- Epithelial cells, abnormal cells
- Intestinal irritation, neoplasia
- Scan 40x
- Increased WBC's: colonic inflammation
- Increased RBC's: intestinal bleeding
- Bacterial numbers and types

Technique	Whipworm (false negatives)	Roundworm (false negatives)	Hookworm (false negatives)
Direct Smear	92.61%	85.38%	72.82%
Ovassay	32.02%	25.88%	4.85%
Centrifugation	4.93%	10.53%	0.97%

Internal Parasites can be difficult to Diagnose

Fecal Solutions:

Sodium Nitrate	1.18-1.20	Good all purpose
Zinc Sulfate	1.18-1.20	Best general all- purpose
Sheathers Sucrose	1.27	Excellent all-purpose
Water	1.00	(example only)

Fecal (O/P) Double Centrifugation:
Fecal Sedimentation: Concentrates feces and therefore ova and larvae, Used to gather heavy ova such as Trematode eggs

Fecal Centrifugation: Centrifugation increases flotation of ova, oocysts, etc to the top of the solution
Creates highest yield of ova, decreases amount of debris to sort through

Double Centrifugation:
Prepare a fecal emulsion using one to six grams feces and 10-12 mls. of water in a paper cup, strain the emulsion through a gauze square into a second paper cup, examine for larvae and worms.

Label one 15ml conical centrifuge tube with the specimen number, Pour the fecal emulsion into the labeled tube to the 15ml mark, Add water to the tubes to balance them (if necessary), Centrifuge for 10 minutes at 1,500 rpm

When the centrifugation is complete, decant supernatent, add sugar solution to the halfway point on the tubes, Mix the fecal sample with the sugar solution with an applicator stick. Complete mixing is important to avoid chunks of material on the slide. Avoid sample contamination by always using new mixing sticks between samples, and not allowing pipettes to touch samples. Add more sugar solution to each tube to reach the 15ml mark. Centrifuge the tubes again for 10 minutes at 1,500 rpm.

Carefully set up tubes in the test tube rack. Use a plastic pipette to gently run additional sugar solution down the side of the tube, create a slight positive meniscus. Disturb the contents as little as possible
Set a 22mm square coverslip on top of each tube. Let stand for 5-10 minutes. Remove the coverslip by lifting straight up and place on a slide. Label the slide
Examine for parasites
10X magnification
40X magnification

Recipe for Sheather's Solution

- Heat 36 ml (1.5 c) water to just boiling
- Add 454g (2.25 c) granulated white sugar
- Mix until completely dissolved
- Pour into plastic container and cover immediately
- Store at 4C for long periods of time. Store at room temp. when container is being used daily

Fecal Quantification: Large Animal, Modified McMaster Test
1 gram feces/15 mls water. Double centrifugation using Sheather's Sugar. Flotation for one hour. Every egg counted. Number given per species per one gram.

Microbiology
Sample Submission:
Culturette
Culturing tissue: Piece of tissue in saline, CTT
Culturing urine: Send urine, cystocentesis preferred
Mycoplasma cultures: Standard c/s best
Provide history, and note anything you are looking for.

Cytology
History:
Size of mass (measure! be clear on units)
Location (site: cutaneous, subcutaneous)
Nature of mass (firm, hard, soft, fluid-filled)
Known duration and any changes in that time
Relevant medical history
Blood work results – hypercalcemia, neutrophilia, etc.
Avoid abbreviations unless common
If multiple sites, please be clear in history and on slides
Large masses – it is helpful to note different aspirate sites so know where to biopsy

Equipment:

- Gather and prepare ALL supplies prior to aspiration

- Needle size: 21-22 gauge, Larger gauge (16-18) – bone
- Syringe: 6 mL (or 3 mL)
- Glass slides – frosted edge
- Tubes – EDTA, serum/clear top

How to Approach the Lesion

- Is it ulcerated: If so, go deep
- Impressions – often of limited diagnostic utility. Exceptions: fungal infections, some well-exfoliating tumors
- Is it large: aspirate multiple sites
- Is it fast growing (and large): avoid the center

Collection Techniques:
Non-aspiration/fenestration: Helps minimize blood contamination and often better preserves cells. Have air-filled syringe ready prior to collection. Have syringe attached when aspirating internal structures. Finger over hub of needle in case of a fluid-filled structure

Aspiration: Good for more sensitive areas or very firm/hard masses. Pull back on plunger and quickly release to minimize blood contamination. Ensure vacuum is released prior to withdrawing needle
Imprint/scrapes for biopsy specimens: Dab on gauze/paper towel until surface is dry/tacky, press slide onto surface – do NOT drag/smear along the slide surface

Can also use backside of scalpel blade for firm tissues (similar to skin scrape) and then smear those cells onto a slide

A note when using ultrasound…Use as little gel as possible!

Application to the slides:
Use the air-filled syringe to push the aspirated material onto the slide. Aim for the "butt" of the slide (near the frosted edge0 such that there are more cells to look at (less "wasted" cells). This will make sure slides are correctly stained, given the variation in type of automated stainer between labs and that high power can be used to view the slide.

In-House Evaluation:
Stain 1 slide prior to submission. Assess cellularity, cell intactness: have I sampled the tissue I thought I did? SUBMIT THIS SLIDE WITH THE OTHERS

Dif-Quick Stain:
Do not heat fix. Have chemical fixative. Have 2 sets – "dirty" and "clean". Change and/or filter regularly. Dye will precipitate out with time and/or will become exhausted. Abundant stain precipitate on slide: Quick dip in the solvent (blue stain/1st step). It is easy to understain – appropriate times are crucial. Thick smears require longer time.

Packaging
Ensure slides are COMPLETELY air-dried. Exception: lipomas/fat-rich samples will NEVER appear dried. Do NOT refrigerate any slides. Ensure slides are all labeled: Name, Site. Use pencil or markers specific for slide labeling (sharpies and other "permanent" markers can be dissolved).

Specific Tissues and Samples:

Cystic Fluid-Filled Masses
Fluid often of limited diagnostic usefulness on its own. Always try to aspirate the wall of the mass. Both the wall and the fluid count as one site, so still submit both! Put sample into BOTH an EDTA and serum/plastic-topped tube . EDTA: cytology, Serum: chemistry testing, culture. If may want both cytology and culture, recommend two separate tubes

Always prepare at least 1-2 slides at time of collection and submit with fluid.

Urine:
Split sample into 2 tubes. Centrifuge 1 and prepare 1-2 smears of the pellet as soon as possible following collection. Submit unspun urine in tube, spun pellet in tube, and air-dry slides on a cytology form

Mammary Gland:
Often CANNOT differentiate benign from malignant mammary tumors. REQUIRE HISTOPATHOLOGY.
Good to diagnose mast cell tumors, lipomas, and other non-mammary origin tumors

Skin (non-mass lesions):
Cytology very limited utility in many chronic skin diseases, Require histopathology for assessment of architecture
Mites, pyoderma should be able to be identified in-clinic.

Histopathology
Sample Submission: Right proportion of formalin to sample, 1 to 10. Formalin only penetrates 1cm into tissue. Make cuts into larger masses to facilitate penetration of formulin. Larger tissues can be wrapped in bags and placed on ice. Call for help in sending in tissues. From our technicians: Tightly close jar, and tape shut.

Chemistry Submission
1. 1-2 mils of serum required (always send a bit more for additional testing)
2. Allow blood to clot for 30 minutes to begin clot retraction, decreases hemolysis
3. Centrifuge in SST or RTT
4. Avoid hemolysis: undertake a clean venipuncture, allow vacuum in tube to draw sample into tube
5. Avoid lipemia: 8-12 hour fast is ideal. Ultracentrifugation can be done at the laboratory to remove lipemia by creating a "creme layer" on top of the cleared serum. Serum is then aspirated from below the "creme" layer with a pipette.

Blood-Chemistry Panel
A blood-chemistry panel measures electrolytes, enzymes and chemical elements of the blood to assess several different organ systems in the body. This will help detect endocrine disease, renal and kidney disease, hepatic or liver disease, gastrointestinal disease (including the pancreas); disorders of acid-base, chemicals such as calcium, electrolytes such as potassium, protein disorders and blood lipids such as cholesterol.

Blood Chemistry Analytes include:
- Albumin- one of the major proteins in the blood. With globulins, comprise total protein. Low levels can be seen with hepatic (liver) disease, certain types of kidney disease, and gastrointestinal disease. Blood loss can also lower albumin and globulin levels.
- ALP (alkaline phosphatase) – enzyme that increases in liver disease and with elevations in cortisol. The highest ALP increases are seen in hyperadrenocorticism, termed Cushing's disease, or when the dog is on corticosteroids due to increased production of this enzyme. Increased ALP in cats always reflects hepatic disease; steroids do not cause an elevation of this enzyme in the cat.

- ALT (alanine aminotransferase) - enzyme found primarily in the liver cells, termed hepatocytes. ALT increases reflect hepatocyte damage from may causes.
- Amylase-enzyme produced in the pancreas that digests carbohydrates in the gastrointestinal tract. Elevated levels can but don't always indicate pancreatic inflammation.
  AST(aspartate aminotransferase) - enzyme found in skeletal and heart muscle and liver. When elevated with ALT, most likely reflects liver disease. AST elevations alone support muscle disease.

Bilirubin – bilirubin is produced by the liver from old red blood cells and is excreted in the urine and stool. Bilirubin is increased in some types of liver disease, particularly gallbladder disease and in autoimmune hemolytic anemia due to destruction of red blood cells in the circulation. When bilirubin levels in the blood reach a certain level, we will see a yellow coloring to non haired areas like the gums and ears. This is called jaundice or icterus. An increase in bilirubin will also color the urine a deeper yellow (see section on urinalysis)
BUN (blood urea nitrogen) - BUN is a waste product of protein metabolism It is produced by the liver and excreted by the kidneys. Decreased levels can indicate hepatic dysfunction but can also be seen with consumption of a low protein diet. Elevated values are seen with dehydration, kidney disease and with high protein levels in the diet or in the gut such as seen with gastrointestinal bleeding. A urinalysis (specifically the specific gravity) is necessary to determine if the elevation in BUN (and creatinine) is due to kidney disease or dehydration.

Calcium-calcium in the blood stream comes from bones. A hormone called parathyroid hormone (PTH) from the parathyroid gland (small glands next to the thyroid in the neck) regulate blood levels of calcium. High blood calcium, termed hypercalcemia, can reflect disorders of PTH including a hyperactive parathyroid gland, termed hyperparathyroidism, malignancy, or idiopathic hypercalcemia (cause unknown) seen in the cat. In these conditions, the phosphorus level may be low. Low blood calcium, termed hypocalcemia, can occur due to a malfunctioning parathyroid gland resulting in hypoparathyroidism but other conditions such as eclampsia (nursing pets) and antifreeze toxicity can lower calcium levels.

Chloride – chloride, sodium and potassium are electrolytes. They serve many different functions within the body. Low values of chloride, termed hypochloremia, are most often seen with vomiting. High values suggest dehydration or loss of water from the body.

Cholesterol - sterol in the blood. Cholesterol is produced in the liver, and low values, termed ypocholesterolemia, can be seen with hepatic dysfunction, gastrointestinal disease, and hypoadrenocorticism or Addison's Disease. High cholesterol values can be seen with diabetes, hypothyroidism and Cushing's Disease. In the advanced stages of glomerular disease, diagnosed by finding high proteins in a urine that shows no evidence of inflammation (high urine protein:creatinine ratio), cholesterol can be elevated, and albumin decreased. With ascities, this triad of signs is termed nephrotic syndrome.

CO2-reflects bicarbonate (HCO3) levels in the blood. Low levels support acidosis, high levels alkalosis. CO2 can be helpful in some instances but measurement of blood gases using an arterial or venous sample is more accurate. Blood gas analyzers are usually available at emergency and/or specialty veterinary hospitals.

Creatinine - creatinine is a waste product from muscles. It is eliminated by the kidneys. As with BUN, creatinine increases with both dehydration and kidney disease thus the urine concentrating ability, determined by urine specific gravity, must be analyzed to determine cause of an increased creatinine. In thin cats, creatinine may be falsely decreased due to their reduced muscle mass.

Creatinine Kinase – creatinine kinase or CK is released from damaged muscles. CK elevations support some type of acute muscle damage including heart muscle. Transient and sometimes significant elevations can be seen with injections and blood draws.

Globulin - blood protein that most often increases due to inflammation but also can increase with rickettsial disease and with neoplasia. Protein electrophoresis can help us determine if the globulins are elevated due to inflammation or neoplasia.

Glucose- blood sugar. Hyperglycemia, support a diagnosis of diabetes. However, stress in the cat can result in transient hyperglycemia. Pancreatitis can cause hyperglycemia. Hypoglycemia, low blood sugar level, can be seen with starvation, an insulin producing tumor and other neoplastic conditions, liver dysfunction, and systemic infection, sepsis.

Lipase – produced by the pancreas. Elevations may indicate pancreatic inflammation but can be seen with kidney and gastrointestinal disease. Lipase and amylase elevations are not helpful in the diagnosis of pancreatitis in the cat.

Osmolality – osmolality is a measure of the concentration of substances such as sodium, chloride, potassium, urea, glucose, and other ions in blood. An increase in osmolality can help determine if a toxin such as ethylene glycol (antifreeze toxicity) has been ingested.

Phosphorus – phosphorus and calcium levels in the blood are controlled by PTH. Phosphorous increases in the blood in kidney disease, generally in chronic renal disease, and also in dehydration. Cats with hyperthyroidism can have higher phosphorus levels. A low calcium level and high phosphorus level is consistent with hypoparathyroidism, diagnosed by a low PTH level. A high calcium and low phosphorus level in the face of a high PTH level diagnoses hyperparathyroidism.

Potassium – an electrolyte. High levels of potassium, termed hyperkalemia, can be seen with acute renal failure (such as seen with antifreeze toxicity), lower urinary tract obstruction, and with Addison's Disease. Hyperkalemia can be life threatening as it can cause a dangerously low heart rate, termed bradycardia. Low blood potassium, termed hypokalemia, is seen most often in cats due to decreased appetite or but can present in dogs and cats with potassium wasting through renal and gastrointestinal disease. Hypokalemia can cause severe muscle weakness.

Sodium - one of the major salts in the body fluid, sodium is important in the body's water balance and the electrical activity of nerves and muscles. High sodium values can be seen with dehydration and water loss. Low sodium values, termed hyponatremia, can be seen with vomiting and loss from the gastrointestinal tract, kidney disease, and with Addison's disease.

Total Protein – total protein is made up of albumin and globulin. Total protein can be elevated when either one is elevated but if both are elevated, dehydration/water loss is generally the cause. A decrease in both albumin and globulin may reflect gastrointestinal disease or hemorrhage. Clinical findings will help determine which cause is more likely.

Total T4 – tetraiodothyronine or T4. Basic test for hypothyroidism (dog) and hyperthyroidism (cat).Various illnesses can cause a low T4 (nonthyroidal disease) which can make a diagnosis of hypothyroidism in an ill or stressed dog difficult. In the older cat, concurrent disease with hyperthyroidism such as kidney disease can push the Total T4 down into the mid to high normal range. Thus confirmatory testing may be needed to accurately diagnose hyperthyroidism in a cat for which the total T4 level is not elevated.

Groups of Tests by Organ System

Although a basic screen provides the most information, we often group tests by the organ systems they are testing, for example:

- Kidney Disease - BUN, creatinine, phosphorus, potassium, urinalysis
- Liver Disease - ALT, AST, ALP, GGT, bilirubin, albumin, cholesterol, BUN

- Gastrointestinal Disease - albumin, globulin, cholesterol
- Pancreatic Disease – amylase, lipase

Additional Testing
After reviewing the results of screening tests, we will narrow our differential list. Some potential diseases we were considering now are ruled-out or seem less likely, others more likely. Perhaps our screening tests have given us the diagnosis or enough information to make a tentative diagnosis. But often we need additional testing to get that diagnosis or further refine our differential list.

Here are some additional tests that we might run and their corresponding conditions:

- ACTH Response Test - used to diagnose Cushing's Disease and Addison's Disease
- Low Dose Dexamethasone Response Test (LDDS) - used to diagnose Cushing's Disease
- Urine Cortisol: Creatinine - Screening test for Cushing's Disease
- Baseline Cortisol: Screening test for Addison's Disease
- Insulin test - Test done to rule-out insulinoma, a cause of hypoglycemia
- Ionized Calcium and Parathyroid Hormone (PTH) level - paired tests to diagnose disorders of the parathyroid gland, hypoparathyroidism resulting in hypocalcemia and hyperparathyroidism resulting in hypercalcemia
- Bile Acid Panel/single bile acid test - test of liver function
- Trypsin-Like Immunoreactivity- Test for Exocrine Pancreatic Insufficiency
- Pancreatic Lipase (cPL, fPL) - used to diagnose pancreatitis
- Folate and Cobalamine Levels: used to diagnose gastrointestinal disease/malabsorption/bacterial overgrowth in the gut
- (Gastrointestinal Function testing: cPL or fPL, TLI, folate/cobalamin)
- Thyroid Stimulating Hormone (TSH) testing - used to help diagnose hypothyroidism and hyperthyroidism in the cat after a Total T4 is run.
- Urine protein:creatinine ratio: used to diagnose glomerular disease when there is protein in the urine and the sediment is inactive (ie. no WBC's, not discolored due to hematuria)
- SDMA (symmetric dimethylarginine): Test of glomerular filtration, assist in diagnosis and staging of chronic renal disease in additional to UA, BUN, creatinine.