IDEXX LaserCyte® Hematology Analyzer Case Study Book and Technical Guide

Practice what's possible®

IDEXX LABORATORIES

Fold out for normal dot plot comparison

Feline

Red Blood Cells

White Blood Cells

Feline

Canine

Red Blood Cells

White Blood Cells

Canine

Equine

Red Blood Cells

White Blood Cells

Equine

IDEXX LaserCyte® Hematology Analyzer Case Study Book and Technical Guide

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Introduction

Dennis DeNicola, DVM, PhD, DACVP, Chief Veterinary Educator, Clinical Pathologist

Dr. DeNicola completed his DVM in 1978 and his PhD in 1981, both at Purdue University. For more than 20 years, he served as an educator in clinical and surgical pathology. In addition, he directed the clinical pathology laboratory as well as the primary cytology and surgical pathology service at the veterinary school laboratory and ran a private pathology service for 15 years. A speaker at more than 150 national and international education symposia, Dr. DeNicola also has authored or co-authored more than 150 publications in various aspects of veterinary clinical pathology.

There have been tremendous technological advances in hematology instrumentation over the last 10–20 years. This advancement has paralleled decreased cost of production, which has resulted in a series of advanced yet affordable hematology analyzers for the veterinarian in clinical practice.

Analyzers vary dramatically in performance related to their underlying technology and quality of data generated. IDEXX introduced the first laser flow cytometry-based hematology analyzer for veterinary medicine, the LaserCyte® Hematology Analyzer, to the veterinary market in 2002. The LaserCyte analyzer parallels the performance of reference laboratory analyzers in both technology and scope of results.

As might be expected, with any advanced instrumentation, there is an increase in information that is being presented to the veterinarian. This new information allows the veterinarian to go beyond their previous training in hematology and gain valuable insight from additional parameters. This guide will help you better understand the basics of this technological evolution and how to use the information now available from your in-house hematology analyzer. Additionally, it will serve to help you understand how to maximize the value of and rapidly validate the hematology data collected.

The question “Why do I need a laser flow cytometry-based hematology analyzer?” is reasonable. Many people have survived for years without in-house hematology analyzers and many have relied on more basic technology like the IDEXX VetAutoread™ Hematology Analyzer or any of the many impedance technology analyzers currently on the market. However, the veterinarian has the same reason for advancing as we have in academic and reference laboratories: the need for the highest-quality laboratory data available.

While impedance technology sorts cells by size alone, a laser flow cytometry-based hematology analyzer evaluates not only the size and shape of a cell, but also the cell’s intracellular density and complexity as well as nuclear lobularity and density, providing a more complete and accurate assessment. This is especially important with a blood sample from a nonhealthy pet, where alteration in size (such as a larger, immature neutrophil or reactive lymphocyte) is often seen and could lead to cellular misclassification if judged on size alone.

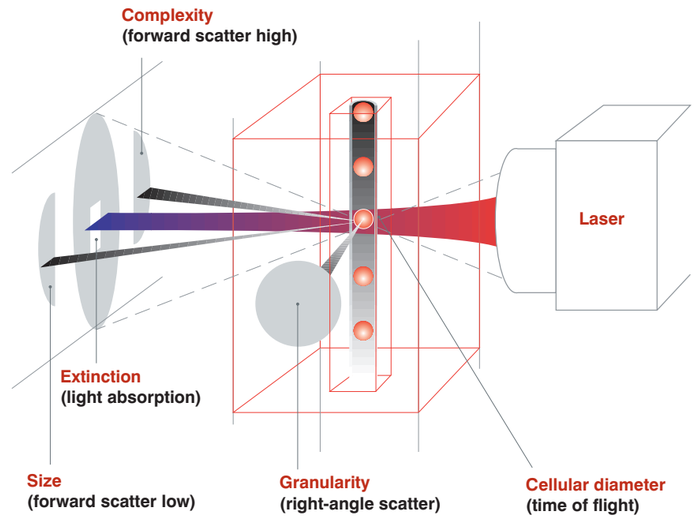
The advantages of the LaserCyte® Hematology Analyzer outlined in this guide are hopefully clear and concise and should serve as a valuable resource to you for a long time. It should be noted that even with all the advances we have made in technology, microscopic evaluation of the blood film is ALWAYS critical. This is one area however, where technician time is saved with the advances in technology. A much greater amount of time must be spent on blood film review with the more basic technologies. The blood film review with the advanced data collection from the LaserCyte analyzer is now limited to less than 1–3 minutes total. Only rapid data validation and cell morphology change recognition is required.

We have included ten clinical case studies demonstrating the interpretation of the LaserCyte data, the presentation of different dot plot patterns that are possible in an average veterinary practice, and the inclusion of example blood film pictures that correlate with the data generated. We hope these cases help solidify the value of collecting data from an advanced hematology analyzer like the IDEXX LaserCyte® Hematology Analyzer.

Dennis B. DeNicola, DVM, PhD, DACVP

Laser Flow Cytometry

The LaserCyte® Hematology Analyzer uses reference laboratory technology to analyze blood samples. The analyzer does so by focusing a laser beam on each individual cell and quantifying the scatter of light on four separate detectors. Simultaneously, it measures the amount of time it takes a cell to travel through the laser beam.



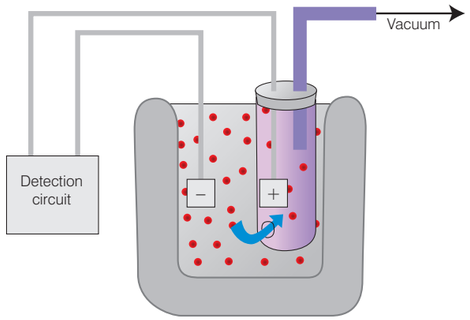
This cell travel time is referred to as the “time of flight” and it provides data on the diameter of the cell. Think of a flashlight as an analogy for the laser. Passing a golf ball in front of the light would be quicker than passing a basketball through the same light. While the time of flight, or cellular diameter, is being measured, four other detectors are measuring the quantity of light bouncing off of a cell or ball in this analogy. Continuing the analogy, the dimpled design of a golf ball would refract light differently than the seamed design of a basketball. In this analogy the golf ball would be classified as a different “cell” from the basketball. The four detectors on the LaserCyte analyzer in essence are measuring many of the same qualities that a pathologist would examine when looking at a blood film. These qualities include size, complexity, granularity and light absorption. With this information, the LaserCyte analyzer can analyze red blood cells and most important, arrive at an absolute reticulocyte count as well. In addition, it can analyze the full five-part white cell differential, providing the data needed for a more complete diagnosis.

Laser Flow Cytometry Offers Many Advantages over Other Methods

- Because a laser flow cytometry-based analyzer evaluates multiple parameters (nuclear and cytoplasmic material) of red blood cells, white blood cells and platelets, it produces more accurate and reliable counts than other systems.
- Clumped cells or platelets can be detected and ignored, which prevents interference with other cell counts.
- Large platelets (frequently encountered in cats) can be distinguished from erythrocytes due to difference in the scatter of light caused by the platelet granules.
- Laser flow cytometry-based analyzers are able to rapidly count large numbers (>200,000) of erythrocytes, which are needed to produce accurate and repeatable reticulocyte counts.

Impedance Technology Used by Most Other Systems

- Older technology based on the Coulter Principle of the 1950s.
- Blood cells pass through an electrically charged aperture generating a “pulse.”
- Cell counts are determined by the number of pulses measured in a known volume of blood.
- The electrical pulse is proportional to the cell volume. Therefore, size alone determines blood cell types (red, white and platelet). Good for speed yet tends to be less accurate.
- Typically offer red blood cell parameters without reticulocyte data. While parameters most often include a three-part differential, a three-part differential cannot determine the difference among the granulocytes (neutrophils, eosinophils, basophils), masking important data for a full diagnosis.



Limitations of Impedance Technology

- Lack of any reticulocyte data.
- Inability to produce a complete differential count. Impedance counters typically group granulocytes into one category.
- Relatively poor ability to differentiate among white blood cells and to differentiate white blood cells from nucleated red blood cells.
  - White blood cell count must be corrected for nucleated red blood cells.
- The overlap in size between feline platelets and erythrocytes leads to overestimates of the erythrocyte count and underestimates of the platelet numbers.
- Only nuclear material is analyzed not cytoplasmic.
- Aggregates of platelets may be counted as white blood cells; this is especially problematic in cats.
- White blood cell counts are determined after lysing red blood cells; failure of red blood cells to lyse completely produces falsely elevated white blood cell counts.
  - Erythrocytes with Heinz bodies don’t lyse; hence, companion animals with large numbers of Heinz bodies may have falsely elevated white blood cell counts, hemoglobin measurements and red blood cell indices (MCH, MCHC).
  - Polychromatophils (immature nonnucleated erythrocytes) are more resistant to lysing and lead to falsely elevated white blood cell counts.

Understanding Dot Plots

Dot plots are a visual representation of the complete blood count. They are beneficial for quickly interpreting and verifying results. Only the IDEXX LaserCyte® Hematology Analyzer provides these valuable tools.

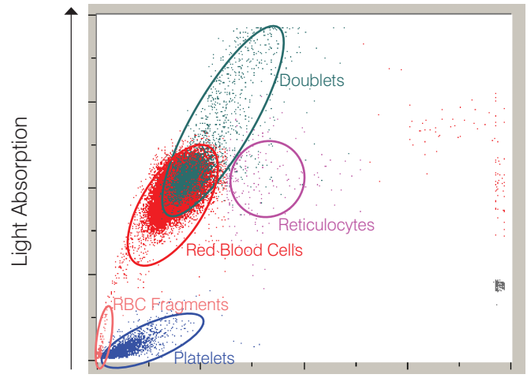
Dot Plots Are Cell Groupings

Each dot represents a single cell as it is analyzed by the device. Two separate runs occur presenting different-looking dot plots. One run is used primarily for collecting data on red blood cells, reticulocytes and platelets, and the other run is used primarily for collecting data on white blood cells. The different cellular elements of the blood appear as distinct clouds of dots and when the definition of the cloud is diminished or intensified this indicates variability within that particular cellular population, which could indicate an abnormality. The greater the abnormality, the greater the potential variation from normal. A blood film review will provide additional information. For example, if the clouds of dots are more dense than normal, an increased count for that particular cell will likely be evident in a blood film.

The dot plots are important pieces of information because the numerical data on the patient’s results page are derived from the dot plot. These plots, like the blood film, give a quick recognition of many abnormalities as well as confirmation of distribution of leukocytes. Conditions like reticulocytosis or increases and decreases in platelet counts stand out prominently in the visual presentation of a dot plot and will immediately increase confidence in the numerical data presented in the report.

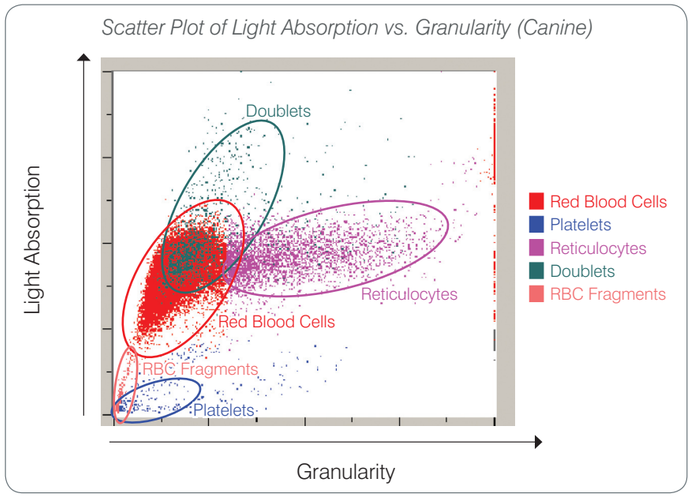
So how does the LaserCyte analyzer create dot plots? The analyzer collects information on each cell passing through the laser beam (see page 1) and examines characteristics of each cell in a multidimensional perspective. For practical purposes, these analyses are presented in a two-dimensional view for your review.

Scatter Plot of Light Absorption vs. Granularity (Canine)



In essence, the red blood cell (RBC) dot plot shows *x* and *y* axes. The vertical *y* axis measures the amount of laser light that is absorbed by a cell. A red blood cell is larger than a platelet and it spends more time in front of the laser beam and absorbs more light. This type of cell falls higher on the *y* axis than platelets. The horizontal *x* axis on the RBC dot plot correlates with the granularity of the cell. In the red blood cell dot plot, the most “granular” cell would be the reticulocyte, which has granular precipitates in the cell due to new methylene blue staining. Therefore, both light absorption and granularity aid in the distinct separation of red blood cells and platelets. An example of a dot plot from a dog with a high number or reticulocytes is shown on the following page; note the increased presence of the magenta-colored reticulocyte dots to the right of the mature red blood cells.

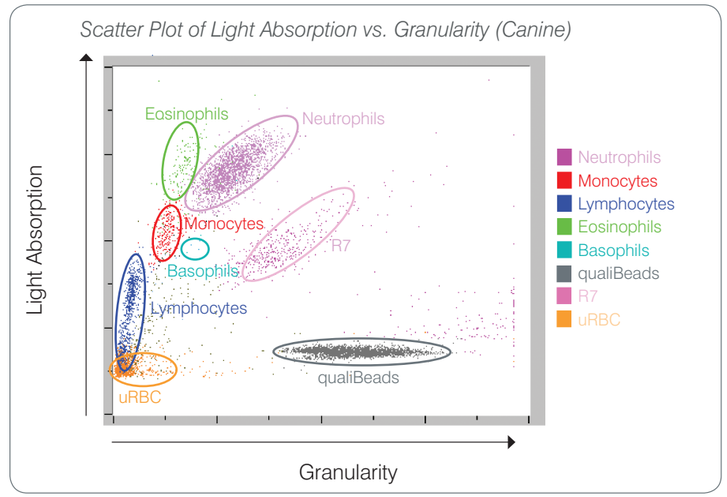
Red Blood Cell Classification



On the red blood cell run, the LaserCyte analyzer classifies the following populations:

- **Red Blood Cells (RBCs)—**The red blood cells (erythrocytes) are primarily responsible for carrying oxygen to tissue cells and carrying carbon dioxide from those cells. The RBC population is colored **red**.
- **Platelets—**Platelets (thrombocytes) play an integral role in the processes of primary and secondary hemostasis leading to the formation of clots. Due to their smaller size, they spend less time in front of the laser beam, absorb less light and therefore fall closer to the bottom on the *y* axis. Platelets are colored **blue**.
- **Reticulocytes—**Reticulocytes are immature red blood cells that contain ribosomal RNA. The CBC5R tube contains new methylene blue, which precipitates and stains the RNA. The reticulocytes are larger than many of the RBC population and more granular because of the RNA and therefore fall to the right of the RBC population. The dot plot at the top of this page shows an extreme example. On the dot plots, the reticulocytes are colored **magenta**.
- **Doublets—**Doublets are two discreet red blood cells that are in close proximity to one another when they are examined by the laser beam. They are mapped as one event but counted as two cells. These cells are colored **green**.
- **RBC Fragments**—Red blood cell fragments are portions of red blood cell membranes from broken cells. The particles have a similar size to platelets but refract light differently and are therefore located to the left of the platelet population. The red blood cell fragments are colored **pink**.

White Blood Cell Classification



After the LaserCyte analyzer counts and classifies the RBCs, platelets and reticulocytes, the analyzer performs a rinsing cycle and then aspirates additional blood to prepare a dilution for the white blood cell (WBC) investigation. The LaserCyte analyzer classifies the following leukocyte populations:

- **Neutrophils—**Neutrophils are generally the largest of the white blood cell populations. Neutrophils are the primary defense against infection and are phagocytic. The neutrophil population is typically the densest population and as seen in the following case studies, the dot plot representation of this density can quickly reveal inflammatory and infectious changes that may warrant further investigation. The neutrophils are classified above and to the right of the monocyte population in **purple**.
- **Monocytes—**Monocytes are responsible for regulating the inflammatory response and phagocytosis. Monocytes are typically larger then lymphocytes, so they absorb more laser light. They are also more granular than lymphocytes and are found above and slightly to the right of the lymphocytes. The population is colored **red**.
- **Lymphocytes—**Lymphocytes are an integral part of the immune system and are important in producing antibodies and cytokines. Lymphocytes are small relative to the other major white blood cell populations and because smaller objects tend to absorb less laser light, they are found on the lower half of the *y* axis. The lymphocyte population is colored **blue**.
- **Eosinophils—**Eosinophils are associated with allergic diseases and parasitic infection by responding to histamine, which is released when parasitic antigens or the allergens bind to mast cells. There is a great deal of variation in the granularity of these cells from species to species. The differences in granularity affect the scatter of light and therefore affect the position, from species to species, of the populations relative to the other white blood cells (see equine dot plot on page 6). Eosinophils are colored **green**.
- **Basophils—**Basophils contain both heparin, which is important for inflammation as it prevents coagulation, and histamine, which is associated with hypersensitivity reactions. Basophils comprise the smallest of the major white blood cell populations classified by the LaserCyte analyzer. These cells are found directly to the right of the monocytes and below the neutrophils. The population is very small. The basophil population is colored **turquoise**.

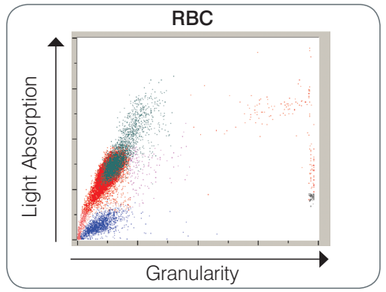
Non-WBC Events

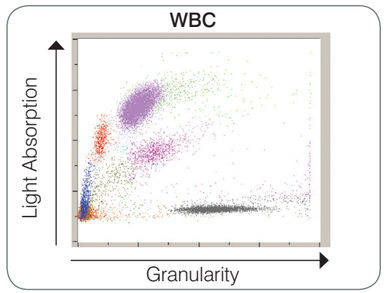
- **R7—**Degraded cellular population left over from red blood cell run. The LaserCyte analyzer correctly removes this population of cells from the overall leukocyte count. The R7 population is colored **magenta**.
- **uRBC—**The unlysed red blood cell (uRBC) population is composed of red blood cells that did not lyse prior to the white run. The uRBC population is colored **orange**.
- **qualiBeads® technology—**Each CBC5R tube contains a known quantity of qualiBeads. The LaserCyte analyzer counts the qualiBeads on both the red blood cell and white blood cell runs as a quality assurance check for each individual run. If the analyzer counts too few or too many qualiBeads, the sample run will be flagged to indicate a potential problem with that portion of the analysis. The qualiBeads population is colored **gray**.

Other Species

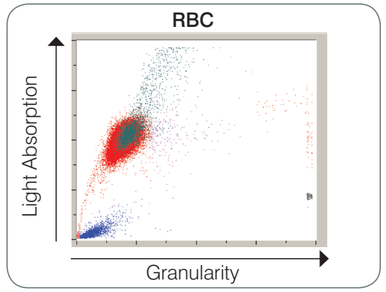
Because each species has unique cells, the LaserCyte analyzer produces different dot plots for each one. Here are typical dot plots for cats, dogs and horses.

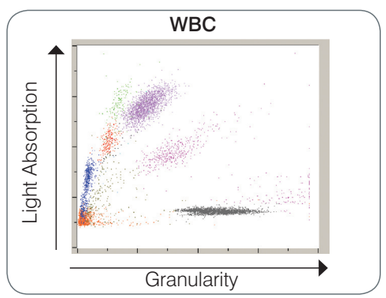
Feline



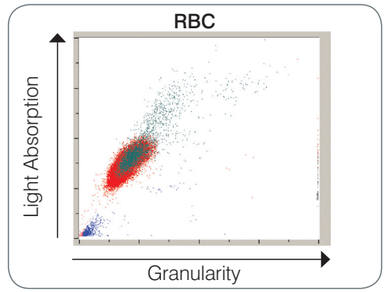


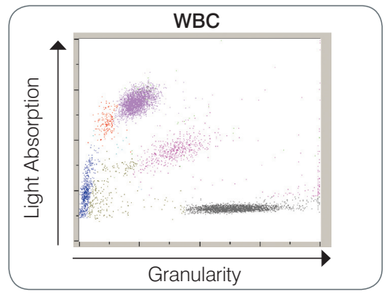
Canine

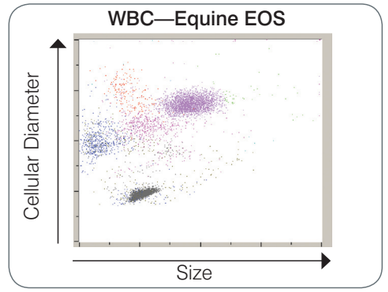




Equine







Note: Due to granular variations, equine eosinophils are classified on a different two-dimensional scatter plot.

How to Print Dot Plots:

1. From the Home screen, tap **Instruments**.
2. Tap the **LaserCyte** tab.
3. Tap the **LaserCyte Diagnostics** button.
4. When the message "*Warning: These options are advanced diagnostic functions that should only be used under the guidance of IDEXX Technical Support personnel.*" appears, tap **OK** to continue.
5. Tap the **Data** tab.
6. Tap **Print**.
7. Select the patient for whom you want to print dot plots and tap **View Records** and then **OK**.
8. Select the run you want to print and then tap **Print**.

IDEXX Laboratories
In-house Laboratory Report Example

IDEXX LABORATORIES

In-house Laboratory

Patient: Mandy
Species: Adult Canine
Client: Elizabeth Prescott

Doctor:
Client ID: 83921

Hematology

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Measure of RBC mass—severity of anemia	RBC	= 6.39	M/µL	(5.50 – 8.50)	6.27
	HCT	= 45.5	%	(37.0 – 55.0)	44.9
	HGB	= 14.1	g/dL	(12.0 – 18.0)	14.3
Description of RBC population	MCV	= 71.1	fL	(60.0 – 77.0)	71.6
	MCH	= 22.06	pg	(18.50 – 30.00)	22.81
	MCHC	= 31.0	g/dL	(30.0 – 37.5)	31.8
Objective measure of variation in RBC size	RDW	= 15.3	%	(14.7 – 17.9)	
Objective measure of regeneration	%RETIC	= 0.5	%		
	RETIC	= 30.8	K/µL		
Total number of leukocytes used in conjunction with differential for accurate interpretation	WBC	= 9.87	K/µL	(5.50 – 16.90)	11.3
Complete five-part leukocyte differential essential for accurate interpretation	%NEU	= 66.5	%		67.9
	%LYM	= 19.0	%		20.8
	%MONO	= 9.9	%		8.6
	%EOS	= 4.6	%		2.7
	%BASO	= 0.1	%		0.1
	NEU	= 6.56	K/µL	(2.00 – 12.00)	7.67
	LYM	= 1.87	K/µL	(0.50 – 4.90)	2.35
	MONO	= 0.98	K/µL	(0.30 – 2.00)	0.97
	EOS	= 0.45	K/µL	(0.10 – 1.49)	0.30
	BASO	= 0.01	K/µL	(0.00 – 0.10)	0.01
Total number of platelets	PLT	= 241.	K/µL	(175. – 500.)	325.
Description of platelet population	MPV	= 6.34	fL		9.23
	PDW	= 17.3	%		16.1
	PCT	= 0.2	%		0.3

Top Five Questions to Ask When Interpreting RBC, WBC and Platelet Counts

Source: Alan Rebar, Fred Metzger. Interpreting hemograms in cats and dogs. *The Veterinary CE Advisor*. December 2001.

Red Blood Cell Counts

Data includes red blood cell count, hematocrit, hemoglobin, red blood cell indices (MCV, MCH, MCHC, RDW) and reticulocyte count (percent and absolute).

1. **Is red blood cell mass increased (polycythemia), decreased (anemia) or normal?**
   Answered by red blood cell mass indicators (red blood cell count, hematocrit, hemoglobin).

2. **If red blood cell mass is decreased, is the anemia regenerative or nonregenerative?**
   Regeneration is confirmed with absolute reticulocyte count.

3. **If regenerative, is the mechanism blood loss or hemolysis?**
   - History, signs and physical exam are key to differentiation.
   - Reticulocyte counts >200,000/μL are highly supportive of hemolysis; lower reticulocyte counts can be associated with hemolysis or blood loss.

4. **If nonregenerative, can the mechanism be determined without bone marrow evaluation?**
   Anemia of inflammatory disease, FeLV infection, iron deficiency, renal failure.

5. **If red blood cell mass is increased, is the polycythemia relative or absolute?**
   - Relative polycythemia (due to dehydration) is the most common form.
   - Absolute polycythemia may be further classified as primary or secondary.

White Blood Cell Counts

All leukocytes, including neutrophils, lymphocytes, monocytes, eosinophils and basophils must be counted.

1. **Inflammation? (WBC count can be low, normal, or high)**
   Persistent eosinophilia, monocytosis and a neutrophilic left shift, alone or in combination, suggest inflammation.

2. **Stress? (pain, Cushing's syndrome, cancer)**
   - Lymphopenia is most consistent leukocyte change.
   - Eosinopenia, mild neutrophilia and mild monocytosis are possible.

3. **Demand for macrophages? (foreign bodies, IMHA, cancer, fungal infections, tissue necrosis)**
   Monocytosis.

4. **Systemic hypersensitivity? (parasites, heartworm, allergies, asthma)**
   Persistent eosinophilia and/or basophilia.

5. **Leukocyte morphologic changes on blood film?**
   - Presence of toxic or immature neutrophils on the blood film supports inflammation.
   - Presence of reactive lymphocytes indicate systemic antigenic stimulation.

Platelet Counts

Data includes total platelet count and platelet indices (MPV, PDW, PCT).

1. **Is the platelet count normal?**
   Normal platelet counts are critical for primary and secondary hemostasis.

2. **Is the platelet count decreased?**
   - Platelet clumping (blood film review) can cause false low platelet counts.
   - If true thrombocytopenia, investigate for decreased production (bone marrow failure), increased consumption (coagulation like DIC, inflammation), increased peripheral blood destruction (immune-mediated, infectious), sequestration (hypersplenism).

3. **Are there clinical signs of thrombocytopenia?**
   - Persistent thrombocytopenia with platelet counts below reference interval (20,000–40,000/μL) needed before petechiae is seen.
   - If petechiae seen with no thrombocytopenia, investigate platelet function with buccal mucosal bleeding time (BMBT) test.

4. **Is bone marrow needed to identify cause of a thrombocytopenia?**
   - In most species, except the cat, large platelets indicate bone marrow response to a peripheral demand; thrombocytopenia is likely a problem in the periphery (increased consumption or destruction) rather than due to bone marrow failure.
   - If bone marrow is needed, perform both a cytologic and core biopsy histologic evaluation.

5. **Is the platelet count increased?**
   - Rarely seen in veterinary medicine; confirm with blood film review.
   - Investigate for possible chronic blood loss and myeloproliferative disease (platelet leukemia).

Case Studies

The recommendations contained in the following case studies are intended to provide general guidance only. As with any diagnosis or treatment, you should use clinical discretion with each patient based on a complete evaluation of the patient, including physical presentation and complete laboratory data. With respect to any drug therapy or monitoring program, you should refer to product inserts for a complete description of dosages, indications, interactions and cautions.

Immune-Mediated Hemolytic Anemia

Maggie, 3-year-old, spayed female cocker spaniel

Case Study

History and Chief Complaint: Maggie presented with acute onset lethargy, anorexia and exercise intolerance. She is current on immunizations and is on monthly heartworm preventative and gastrointestinal (GI) parasite and tick/flea prophylaxis. There is no history of dietary indiscretion.

Abnormal Findings on General Physical Exam: Maggie is very lethargic. Mucous membranes are pale and there is mild icterus. Body temperature is 104°F and there is a grade 2/6 systolic murmur.

Differentials: Acute hemolytic anemia (possible immune-mediated), chronic heart failure, chronic valvular disease, Lyme disease, ehrlichiosis, anaplasmosis, neoplasia, systemic lupus erythematosus, glomerulonephritis, pancreatitis, chronic-active hepatitis

Diagnostic Plan: Because of the severity of the disease presentation and the wide clinical differential, a complete blood count (CBC), general clinical chemistry profile including electrolytes, complete urinalysis, SNAP® 4Dx® Test, thoracic and abdominal radiographs, and saline agglutination test and Coombs' test pending preliminary findings were requested.

Chemistry Findings: Liver—There is mild hepatocellular injury (increased ALT); cholestasis is likely based on the increased total bilirubin, bilirubinuria and ALKP; however, prehepatic jaundice (hemolytic disease) and nonspecific increase ALKP must be considered also. **Miscellaneous changes**—A nonspecific increase in amylase and a mild hyperglycemia most likely physiologic due to a glucocorticoid influence are present.

Other Significant Findings: Saline agglutination test—Positive. **Radiographs**—Mild hepatosplenomegaly was noted.

Diagnosis: Immune-mediated hemolytic anemia (IMHA)

Treatment/Monitoring Plan: Immunosuppressive doses of prednisone and azathioprin with judicious tapering of prednisone by 1/3 daily dose every 3 weeks after CBC returns to normal

Prognosis: Guarded

Hematology Findings:

Red Blood Cells

A moderately severe and strongly regenerative (reticulocytosis) anemia characterized as a normocytic and normochromic anemia is evident. Blood film review reveals the presence of many spherocytes as well as moderate polychromasia and anisocytosis supporting the reticulocytosis observed. Low but significant numbers of nucleated red blood cells were identified; however, since flow cytometry was used for the analysis, these did not interfere with the total leukocyte count. Agglutination was suggested on the blood film.

White Blood Cells

There is a relatively marked leukocytosis characterized by neutrophilia and monocytosis. This distribution was supported with the blood film review and, in addition, hyposegmented neutrophils were present in circulation.

Platelets

Platelets are adequate in number and this is supported with the blood film review. Large platelets were easily identified on the blood film and this was supported by increased MPV and PDW (typically less than 15–17 fL and 15–18%, respectively).

IDEXX LABORATORIES

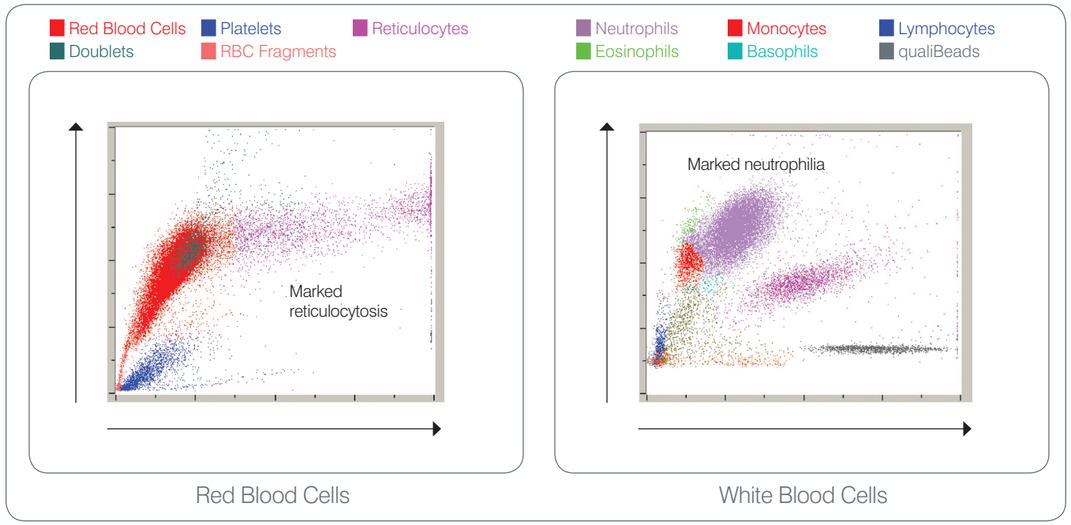
In-House Laboratory

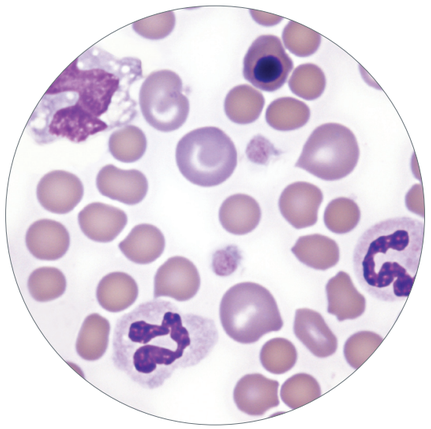
Patient: Maggie — Doctor: Smith
Species: Adult Canine
Client: Thomas Henry — Client ID: 17516

Hematology

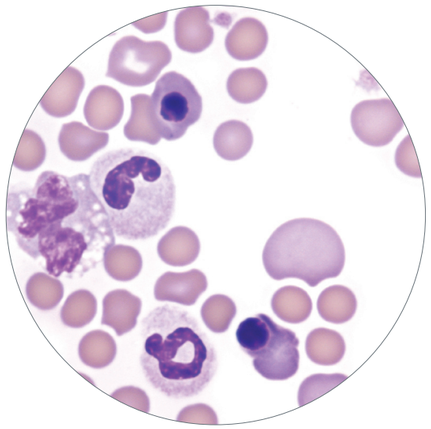
12/5/2007 12:14:30 PM — LaserCyte® — 7/18/2007

RBC	3.22	M/µL	LOW	(5.50 – 8.50)		7.57
HCT	22.6	%	LOW	(37.0 – 55.0)		50.3
HGB	8.1	g/dL	LOW	(12.0 – 18.0)		18.4
MCV	70.1	fL		(60.0 – 77.0)		66.5
MCH	25.26	pg		(18.50 – 30.00)		24.32
MCHC	36.1	g/dL		(30.0 – 37.5)		36.6
RDW	20.4	%	HIGH	(14.7 – 17.9)		14.9
%RETIC	6.8	%				0.3
RETIC	218.8	K/µL				22.5
WBC	75.36	K/µL	HIGH	(5.50 – 16.90)		8.04
%NEU	86.6	%				61.3
%LYM	3.3	%				22.4
%MONO	8.5	%				13.8
%EOS	1.2	%				2.3
%BASO	0.5	%				0.2
NEU	65.24	K/µL	HIGH	(2.00 – 12.00)		4.93
LYM	2.46	K/µL		(0.50 – 4.90)		1.80
MONO	6.39	K/µL	HIGH	(0.30 – 2.00)		1.11
EOS	0.90	K/µL		(0.10 – 1.49)		0.18
BASO	0.37	K/µL	HIGH	(0.00 – 0.10)		0.01
PLT	188.	K/µL		(175. – 500.)		220
MPV	20.35	fL				9.95
PDW	21.1	%				17.1
PCT	0.4	%				0.2





Blood film—RBC: 3+ spherocytosis, 2+ polychromasia, 2+ anisocytosis, 1 nRBC (top right); **WBC**: 2 neutrophils, 1 monocyte; **PLT**: large platelets



Blood film—RBC: 3+ spherocytosis, 2+ polychromasia, 2+ anisocytosis, 2 nRBC (top center); **WBC**: 2 hyposegmented neutrophils, 1 monocyte; **PLT**: large platelets

Chronic Lymphocytic Leukemia

Dani, 14-year-old, neutered male Lhasa Apso

Case Study

History and Chief Complaint: Dani presented for a routine wellness appointment. The owner reported that he may be somewhat less active over the last few weeks, but otherwise Dani is doing well for his age. The dog is on monthly heartworm preventive and flea/tick prophylaxis.

Abnormal Findings on General Physical Exam: Dani is bright, alert and responsive. Mucous membranes are mildly pale/pink and there is nuclear sclerosis of both eyes. There is moderate tartar and a grade 4/6 systolic murmur. On abdominal palpation, there is mild cranioventral organomegaly.

Differentials: Chronic cardiac (valvular) disease; hepatic and/or splenic enlargement due to metabolic, inflammatory or neoplastic disease; hyperadrenocorticism

Diagnostic Plan: Even though Dani is not presented as an emergency, the differential for hepatosplenomegaly is broad and may indicate a multisystemic illness. A complete minimum database for diagnostic purposes and for additional testing direction as well as for establishment of baseline data values is warranted. A complete blood count (CBC), general clinical chemistry profile including electrolytes, complete urinalysis and SNAP® 4Dx® Test were requested. Thoracic and abdominal radiographs were requested because of the organomegaly identified during the physical exam.

Hematology Findings:

Red Blood Cells

There is a mild, normocytic, normochromic anemia that is best characterized as regenerative anemia based on the reticulocytosis observed. The normal hemoglobin measurement, in light of the mild decrease in RBC count and hematocrit, suggests a mild intravascular hemolytic process and the presence of cell-free hemoglobin in the sample. Blood film review reveals a mild decreased RBC density supporting the mild anemia as well as mild polychromasia supporting the reticulocytosis observed. There is moderate poikilocytosis with moderate acanthocytosis and rarely seen fragmented erythrocytes supportive of a hemolytic process potentially involving the liver.

White Blood Cells

The most significant leukocyte abnormality is the finding of a marked leukocytosis characterized by a marked lymphocytosis. Blood film review confirms the lymphocytosis; the majority of these lymphocytes are normal to slightly increased in size and appear morphologically normal to slightly reactive. No significant atypism is seen, but the marked lymphocytosis is strong support for chronic lymphocytic leukemia.

Platelets

Normal

IDEXX LABORATORIES

In-house Laboratory

Patient:	Dani		Doctor:	Smith
Species:	Geriatric Canine			
Client:	Tory	Williams	Client ID:	Canine - Dani

Hematology

1/4/2008 12:16:53 PM				LaserCyte®		7/28/2006
RBC	5.46	M/µL	LOW	(5.50 – 8.50)		6.82
HCT	35.3	%	LOW	(37.0 – 55.0)		42.4
HGB	14.3	g/dL		(12.0 – 18.0)		15.9
MCV	64.7	fL		(60.0 – 77.0)		62.2
MCH	23.44	pg		(18.50 – 30.00)		23.31
MCHC	36.3	g/dL		(30.0 – 37.5)		37.5
RDW	16.2	%		(14.7 – 17.9)		14.9
%RETIC	2.5	%				0.4
RETIC	134.7	K/µL				27.3
WBC	48.80	K/µL	HIGH	(5.50 – 16.90)		6.75
%NEU	35.2	%				70.8
%LYM	54.6	%				13.8
%MONO	7.5	%				13.5
%EOS	2.6	%				1.6
%BASO	0.1	%				0.4
NEU	17.18	K/µL	HIGH	(2.00 – 12.00)		4.78
LYM	26.65	K/µL	HIGH	(0.50 – 4.90)		0.93
MONO	3.67	K/µL	HIGH	(0.30 – 2.00)		0.91
EOS	1.26	K/µL		(0.10 – 1.49)		0.11
BASO	0.04	K/µL		(0.00 – 0.10)		0.03
PLT	208.	K/µL		(175. – 500.)		490
MPV	9.72	fL				
PDW	17.0	%				
PCT	0.2	%				

Chemistry Findings: The primary abnormality in the serum chemistry profile was a moderate to marked increase in ALKP, a moderate increase in GGT and a mild increase in ALT. Changes are supportive of cholestasis (ALKP and GGT) with mild hepatocellular injury (ALT). No significant abnormalities are observed in other parameters. **Urinalysis**—No significant abnormalities. **SNAP® 4Dx® Test**—Negative.

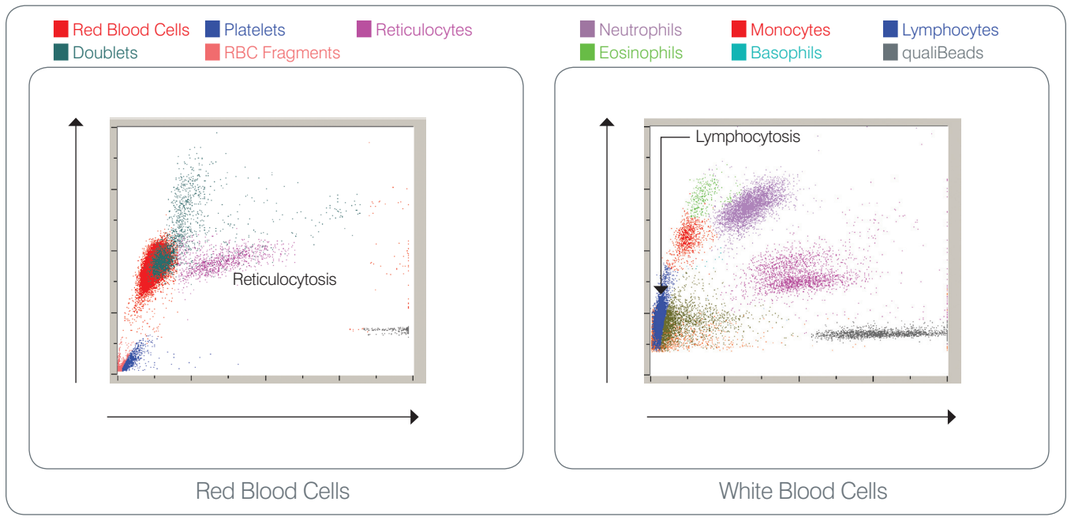
Thoracic and Abdominal Radiographs: Mild hepatosplenomegaly

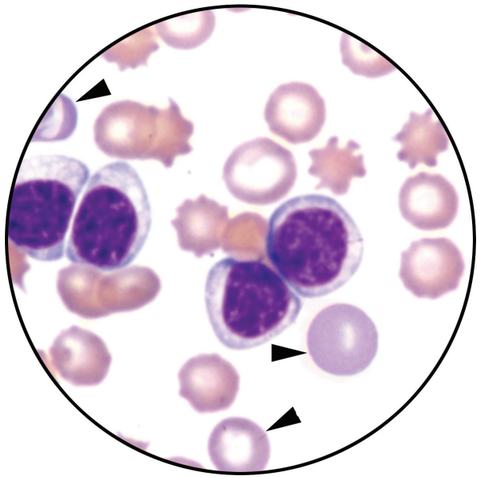
Cytology and Histopathology: Cytologic and histologic evaluation of a liver biopsy specimen revealed diffuse organ infiltration with the lymphoid leukemic cell population. Fine needle aspirates of the spleen revealed a similar monotonous population of malignant small lymphocytes.

Diagnosis: Chronic lymphocytic leukemia with widespread organ dissemination

Treatment/Monitoring Plan: After consultation with a veterinary oncologist, Dani was started on an appropriate chemotherapeutic protocol.

Prognosis: Guarded to poor





Blood film—RBC: 2+ poikilocytosis with acanthocytes (top right), 2+ polychromasia (arrowheads), 1+ anisocytosis; **WBC**: 4 reactive-appearing lymphocytes; **PLT**: none seen in this field of view

Pyometra

Sherri, 5-year-old female bulldog

Case Study

History and Chief Complaint: Sherri presented with a two-week history of intermittent lethargy, polyuria and polydipsia.

Abnormal Findings on General Physical Exam: Sherri is obese. Mucous membranes are mildly pale and pink. The abdomen was tense on abdominal palpation. Temperature was 105°F.

Differentials: Both systemic and local disease including diabetes mellitis, Cushing's disease, hypothyroidism, renal failure, glomerulonephritis, pyometra, pancreatitis and peritonitis were considered.

Diagnostic Plan: Because of potential systemic disease, a complete blood count (CBC), general clinical chemistry profile including electrolytes, complete urinalysis, fecal examination, tick-borne disease investigation and survey radiographs were requested for a minimum database and further direction.

Hematology Findings:

Red Blood Cells

There is a minimal, normocytic, normochromic, nonregenerative anemia. Findings are consistent with the commonly seen anemia associated with inflammatory disease (see White Blood Cells).

White Blood Cells

There is a moderate leukocytosis characterized primarily by marked neutrophilia and marked monocytosis with a low within reference interval lymphocyte count. Changes are supportive of inflammatory disease with a probably superimposed glucocorticoid influence ("stress").

Platelets

Platelet numbers are mildly decreased and few enlarged platelet forms are present, which is supportive of a bone marrow response to a peripheral demand for platelets.

IDEXX LABORATORIES

In-house Laboratory

Patient:	Sherri	Doctor:	Smith
Species:	Adult Canine		
Client:	Jane Rutgers	Client ID:	2705

Hematology

12/5/2007 1:46:21 PM — LaserCyte®

RBC	=	4.92	M/µL	LOW	(5.50 – 8.50)		5.98
HCT	=	36.7	%	LOW	(37.0 – 55.0)		44.9
HGB	=	12.5	g/dL		(12.0 – 18.0)		16.1
MCV	=	74.6	fL		(60.0 – 77.0)		75.1
MCH	=	25.39	pg		(18.50 – 30.00)		26.92
MCHC	=	34.0	g/dL		(30.0 – 37.5)		35.9
RDW	=	15.9	%		(14.7 – 17.9)		16.6
%RETIC	=	0.3	%				0.6
RETIC	=	13.7	K/µL				35.9
WBC	=	46.85	K/µL	HIGH	(5.50 – 16.90)		6.22
%NEU	=	79.8	%				69.9
%LYM	=	3.2	%				11.6
%MONO	=	14.9	%				15.8
%EOS	=	1.8	%				2.0
%BASO	=	0.3	%				0.6
NEU	=	37.37	K/µL	HIGH	(2.00 – 12.00)		4.35
LYM	=	1.49	K/µL		(0.50 – 4.90)		0.72
MONO	=	7.00	K/µL	HIGH	(0.30 – 2.00)		0.98
EOS	=	0.86	K/µL		(0.10 – 1.49)		0.13
BASO	=	0.13	K/µL	HIGH	(0.00 – 0.10)		0.04
PLT	=	143.	K/µL	LOW	(175. – 500.)		255
MPV	=	10.77	fL				8.70
PDW	=	19.3	%				16.6
PCT	=	0.2	%				0.5

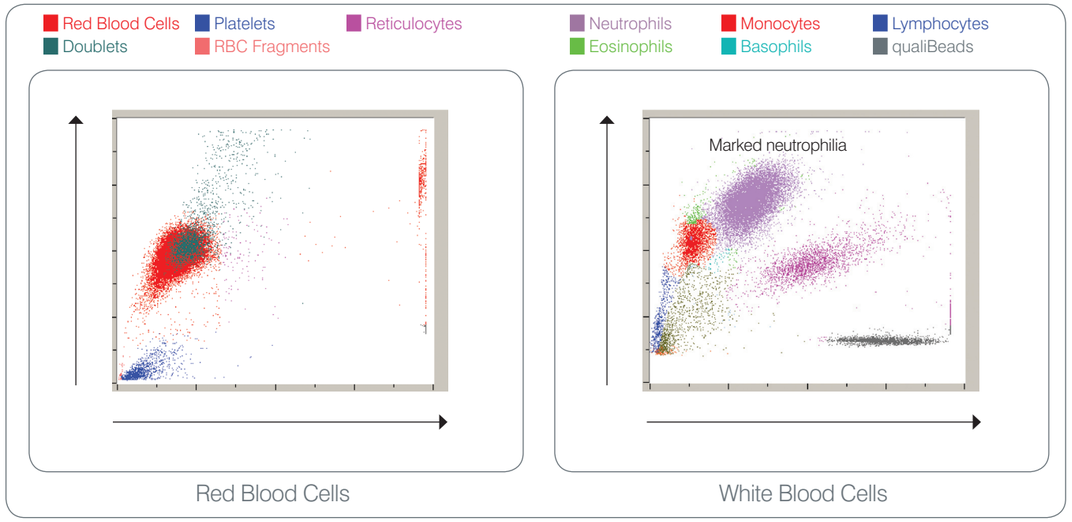
Chemistry Findings: Kidney—The minimal increased BUN and creatinine in conjunction with a urine sample with a specific gravity of 1.045 is supportive of prerenal azotemia most likely associated with dehydration/decreased renal perfusion. **Proteins**—The increased total protein and globulin support active inflammation; however, all proteins are high or high within the reference interval (albumin), which may be due in part to dehydration. This component is likely partially masked by the fact that albumin is decreased associated with the inflammatory process (negative acute phase reactant). **Liver**—There is a minimal increased ALT suggesting minimal hepatocellular injury and there is a nonspecific minimal increased ALKP.

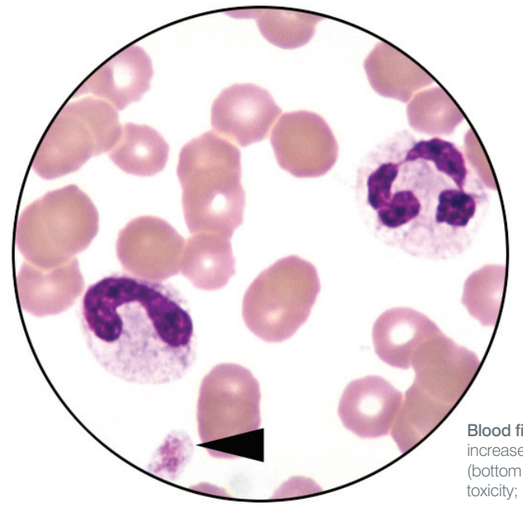
Diagnostic Imaging: Abdominal radiographs reveal the presence of a diffusely enlarged uterus. No other significant abnormalities are noted.

Diagnosis: Pyometra, closed

Treatment/Monitoring Plan: Fluid therapy, antibiotic therapy, ovariohysterectomy and postsurgical monitoring of CBCs and clinical chemistry values until within reference interval limits

Prognosis: Good





Blood film—RBC: normal morphology, increased Rouleaux; **WBC**: 1 immature (bottom left) and 1 mature neutrophil, 2+ toxicity; **PLT**: large platelets (arrowhead)

Intestinal Mass with Chronic Blood Loss

Max, 8-year-old, intact male mixed-breed dog

Case Study

History and Chief Complaint: Max presented with a two-month history of intermittent lethargy, weakness, anorexia, weight loss and diarrhea with melena. He is current on all immunizations and is on monthly heartworm preventive and flea/tick prophylaxis.

Abnormal Findings on General Physical Exam: Max is lethargic and has a thin body condition. Mucous membranes are pale pink and there is some discomfort on palpation of the cranioventral abdomen. Melenic soft stool was observed on rectal exam.

Differentials: Neoplasia (hepatic/splenic/intestinal/gastric), inflammatory bowel disease, lymphangiectasia, chronic pancreatitis, bacterial overgrowth, Lyme disease, ehrlichiosis, anaplasmosis

Diagnostic Plan: Complete blood count (CBC), general clinical chemistry profile including electrolytes, complete urinalysis, fecal analysis, SNAP® 4Dx® Test, SNAP® *Giardia* Test, abdominal radiographs

Hematology Findings:

Red Blood Cells

There is a mild to moderate microcytic (low MCV) regenerative (reticulocytosis) anemia with a low within reference interval MCHC. Changes suggest a developing iron deficiency, which was confirmed upon blood film evaluation; many hypochromic red blood cells were identified along with the polychromasia, which validates the reticulocyte count. The lack of a decreased MCHC may be due to red blood cell fragility and the presence of cell-free hemoglobin.

White Blood Cells

There is a mild leukocytosis characterized by a mild neutrophilia and monocytosis. Inflammation is most likely since there is no identified lymphopenia; however, a combination of inflammation and glucocorticoid influence ("stress") should be considered.

Platelets

Platelets are adequate in number, which is confirmed with the blood film review, but large and variably sized platelets are present indicating peripheral blood demand for platelets.

IDEXX LABORATORIES

In-house Laboratory

Patient: Max — Doctor: Smith
Species: Geriatric Canine
Client: Alyssa Kahn — Client ID: Canine - Max

Hematology

1/7/2008 11:18:55 AM — LaserCyte®

						5/28/2007
RBC	5.39	M/µL	LOW	(5.50 – 8.50)		6.05
HCT	28.2	%	LOW	(37.0 – 55.0)		43.8
HGB	8.7	g/dL	LOW	(12.0 – 18.0)		15.6
MCV	52.3	fL	LOW	(60.0 – 77.0)		72.4
MCH	16.11	pg	LOW	(18.50 – 30.00)		25.79
MCHC	30.8	g/dL		(30.0 – 37.5)		35.6
RDW	19.0	%	HIGH	(14.7 – 17.9)		16.2
%RETIC	2.5	%				0.2
RETIC	134.1	K/µL				18.2
WBC	21.75	K/µL	HIGH	(5.50 – 16.90)		8.09
%NEU	80.7	%				73.2
%LYM	7.6	%				14.8
%MONO	9.9	%				8.1
%EOS	1.8	%				3.9
%BASO	0.1	%				0.0
NEU	17.56	K/µL	HIGH	(2.00 – 12.00)		5.92
LYM	1.64	K/µL		(0.50 – 4.90)		1.19
MONO	2.16	K/µL	HIGH	(0.30 – 2.00)		0.66
EOS	0.38	K/µL		(0.10 – 1.49)		0.32
BASO	0.02	K/µL		(0.00 – 0.10)		0.00
PLT	295.	K/µL		(175. – 500.)		208
MPV	18.71	fL				10.58
PDW	19.3	%				17.0
PCT	0.6	%				0.2

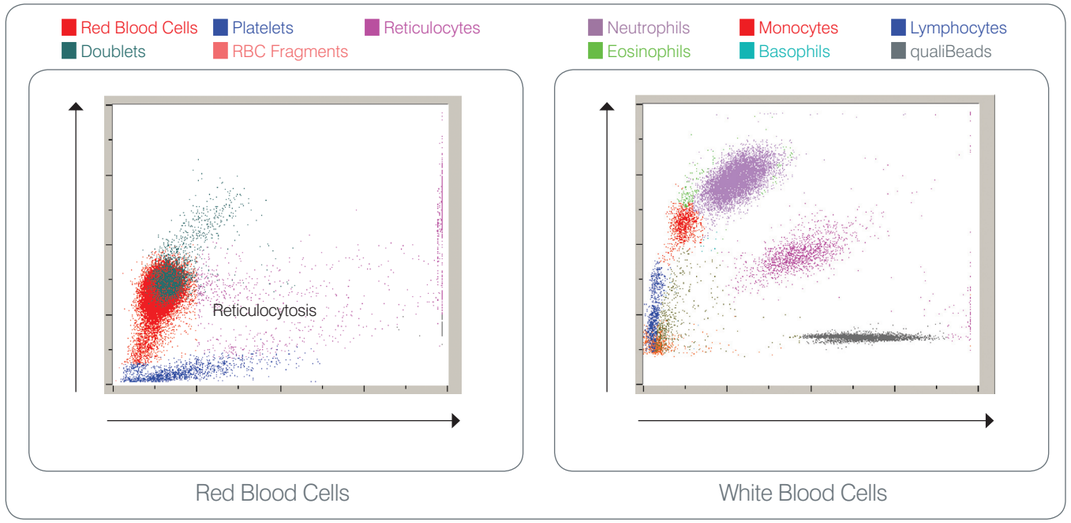
Chemistry Findings: The low total protein and albumin with a low within reference interval globulin are supportive of panhypoproteinemia as might be seen with chronic blood loss. Since this was a fasted blood sample, bleeding into the intestinal tract is suggested with the finding of an increased BUN (blood—high-protein diet) with a normal creatinine. The ALKP increase is mild and nonspecific.

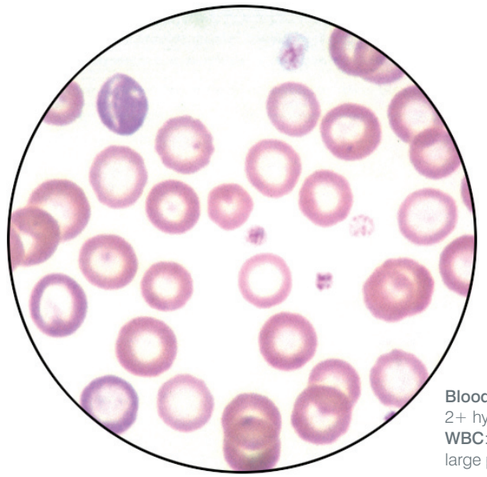
Other Significant Findings: **Fecal analysis**—negative. **SNAP® 4Dx® Test**—negative. **SNAP® *Giardia* Test**—negative. **Abdominal radiographs**—poorly defined area in the cranioventral abdomen: possible mass associated with liver, spleen, or intestines. **Abdominal ultrasound**—irregular small intestinal mass.

Diagnosis: **Intestinal mass**—chronic blood-loss regenerative anemia with developing iron deficiency (microcytic, hypochromic) secondary to a bleeding intestinal mass

Treatment/Monitoring Plan: Exploratory laparotomy with mass resection and anastomosis. Histopathology pending. Timely monitoring of CBC postoperatively.

Prognosis: Guarded pending histopathology results





Blood film—RBC: 2+ microcytosis, 2+ hypochromasia, 2+ polychromasia; **WBC**: none seen in this field of view; **PLT**: few large platelets, variably sized platelets

Hookworm-Induced Gastroenteritis and Regenerative Anemia

Brutus, 5-month-old German shepherd

Case Study

History and Chief Complaint: Brutus presented with an acute history of profuse diarrhea with melena and mild to moderate lethargy. Brutus has access to a large yard and has had several previous episodes of gastroenteritis secondary to presumptive dietary indiscretion, which responded to conservative therapy with bland diet and metronidazole. He is current on immunizations and was dewormed at 8, 12 and 16 weeks of age. There are no current medications or treatment. The owner has not been giving him monthly heartworm preventive.

Abnormal Findings on General Physical Exam: Mildly lethargic, thin body condition, mucous membranes moderately pale and pink, pendulous abdomen with gas/fluid palpated in bowel loops, and fecal staining of perineal area with evidence of melena

Differentials: Gastrointestinal parasitism, exocrine pancreatic insufficiency, hypoadrenocorticism, viral/bacterial gastroenteritis, hemorrhagic gastroenteritis, dietary indiscretion/foreign body, intussusception, rodenticide or other toxicity/food allergy/intolerance, secondary bacterial overgrowth

Diagnostic Plan: Complete blood count (CBC), general clinical chemistry profile with electrolytes, complete urinalysis, fecal analysis, SNAP® *Giardia* Test, SNAP 4Dx® Test

Hematology Findings:

Red Blood Cells

There is a moderate normocytic and normochromic anemia that is strongly regenerative based on the high reticulocyte count. The MCV is low within the reference interval even though there is strong regeneration. The MCHC is also low within the reference interval. These two findings are strongly supportive of developing iron deficiency. Microcytosis and hypochromasia supportive of developing iron deficiency are observed during the blood film review.

White Blood Cells

There is a moderate leukocytosis characterized by a moderate neutrophilia and mild monocytosis. Inflammation is likely but concomitant glucocorticoid influence ("stress") may be a contributing factor.

Platelets

There is a moderate thrombocytopenia that is confirmed with blood film review. The high PDW (typically less than 15–18%) suggests significant variation in size of platelets, which is also confirmed with blood film review. Large and variably sized platelets were noted.

IDEXX LABORATORIES

In-house Laboratory

Patient:	Brutus		Doctor:	Smith	
Species:	Puppy				
Client:	David	Williams	Client ID:	Canine - Brutis	

Hematology

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						12/01/2007
RBC	4.22	M/µL	LOW	(4.70 – 8.50)		6.16
HCT	25.9	%	LOW	(32.0 – 55.0)		39.5
HGB	8.0	g/dL	LOW	(10.3 – 18.0)		14.8
MCV	61.3	fL		(60.0 – 77.0)		64.1
MCH	19.00	pg		(18.50 – 30.00)		24.03
MCHC	31.0	g/dL		(30.0 – 37.5)		37.5
RDW	19.1	%	HIGH	(14.7 – 17.9)		16.3
%RETIC	6.0	%				0.9
RETIC	252.5	K/µL				55.4
WBC	33.63	K/µL	HIGH	(5.50 – 16.90)		11.50
%NEU	86.0	%				77
%LYM	3.8	%				7.9
%MONO	8.7	%				11.1
%EOS	1.2	%				3.8
%BASO	0.3	%				0.2
NEU	28.93	K/µL	HIGH	(3.00 – 12.00)		8.86
LYM	1.28	K/µL		(0.50 – 4.90)		0.90
MONO	2.92	K/µL	HIGH	(0.30 – 2.00)		1.28
EOS	0.41	K/µL		(0.10 – 1.49)		0.44
BASO	0.09	K/µL		(0.00 – 0.10)		0.03
PLT	62.	K/µL	LOW	(175. – 500.)		250
MPV	18.02	fL				9.23
PDW	33.4	%				18.9
PCT	0.1	%				0.2

Coagulation — IDEXX Coag Dx™

1/7/2008 4:58:22 PM

PT	12	(11 – 14)		13
aPTT	75	(60 – 93)		74

Chemistry Findings: Total protein, albumin and globulin were mildly decreased supportive of blood loss. ALKP was moderately high and phosphorus was mildly increased; however, this is common for young dogs with active bone growth (bone ALKP isozyme).

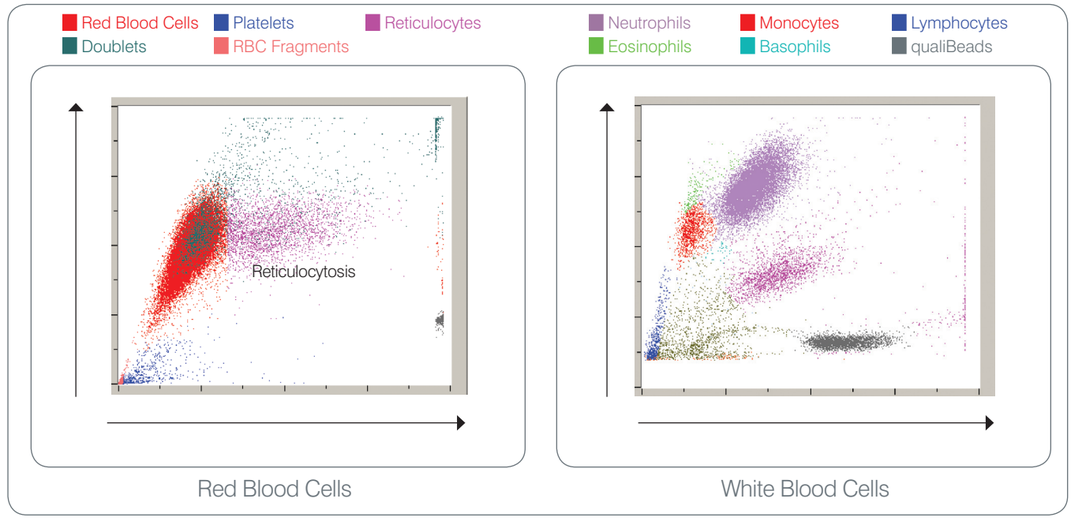
PT/aPTT (IDEXX Coag Dx™ Analyzer): The citrate PT and aPTT were both within reference interval limits (12 and 75 seconds, respectively), ruling out rodenticide toxicity or other acquired coagulopathy.

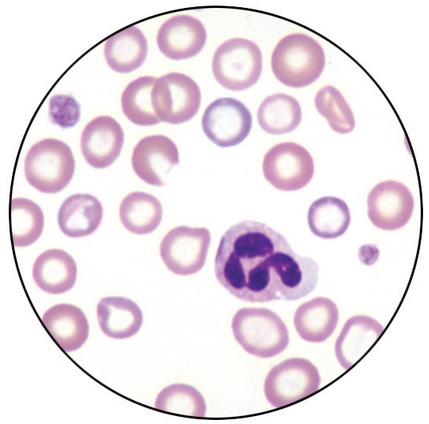
Other Significant Findings: Fecal analysis—Hookworms (*Ancylostoma*). **SNAP® *Giardia* Test**—Negative. **SNAP® 4Dx® Test**—Negative.

Diagnosis: Hookworm-induced gastroenteritis with secondary regenerative blood-loss anemia and developing iron deficiency

Treatment/Monitoring Plan: Fenbendazole and metronidazole, bland diet and CBC repeated at timely intervals until within redundant reference interval limits.

Prognosis: Good





Blood film—RBC: 2+ polychromasia, 2+ hypochromasia, 2+ microcytosis, 1+ anisocytosis; **WBC**: 1 neutrophil, 1+ toxicity; **PLT**: large platelets, variably sized platelets

Nonregenerative Anemia Secondary to Renal Failure

Betty, 15-year-old, spayed female domestic shorthair

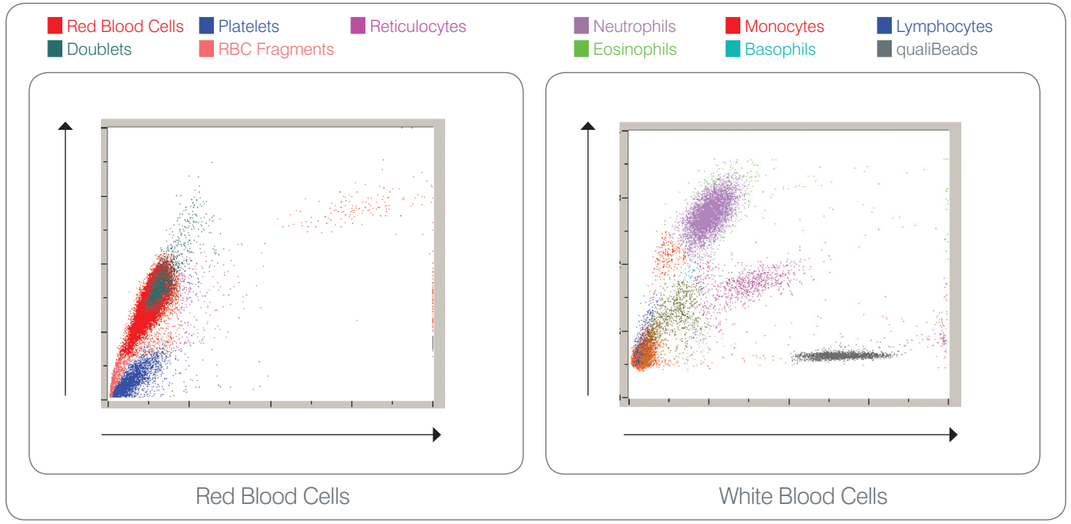
Case Study

History and Chief Complaint: Betty is an indoor/outdoor cat that is current on all vaccines. She has a one-month history of intermittent anorexia, weight loss, polyuria/polydipsia and lethargy.

Abnormal Findings on General Physical Exam: There is mild to moderate lethargy, moderately pale mucous membranes, thin body condition and loss of periorbital fat pads. There is an uremic odor to the breath. Kidneys are small and irregular on abdominal palpation.

Differentials: Chronic renal failure, lymphosarcoma/other neoplasia, inflammatory bowel disease, feline leukemia virus, feline immunodeficiency virus, feline infectious peritonitis virus, hyperthyroidism

Diagnostic Plan: Complete blood count (CBC), general clinical chemistry profile, complete urinalysis, SNAP® T_4 Test, SNAP® FIV/FeLV Combo Test, and thoracic and abdominal radiographs were planned.



Hematology Findings:

Red Blood Cells
There is a moderately severe, normocytic, normochromic, nonregenerative anemia. Blood film review reveals moderate poikilocytosis with few burr cells.

White Blood Cells
No significant quantitative or morphologic abnormalities are noted.

Platelets
Platelets are normal in number and there are few enlarged platelet forms present, but this is an equivocal finding in the cat.

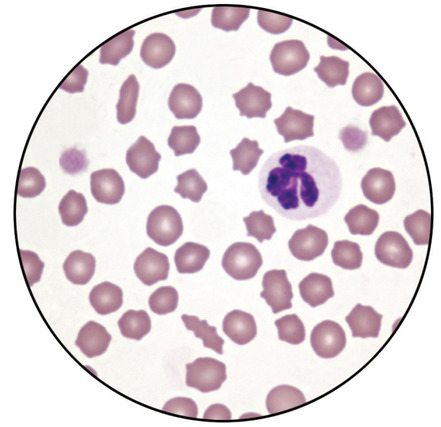
IDEXX LABORATORIES

In-house Laboratory

Patient: Betty
Species: Geriatric Feline
Client: Martha Jones
Doctor: Smith
Client ID: Feline - Betty

Hematology

5/5/2007 11:24:47 AM					LaserCyte®	2/5/2006
RBC	=	3.84	M/µL	LOW	(5.00 – 10.00)	6.14
HCT	=	18.4	%	LOW	(30.0 – 45.0)	30.0
HGB	=	6.2	g/dL	LOW	(9.0 – 15.1)	9.6
MCV	=	47.9	fL		(41.0 – 58.0)	48.9
MCH	=	16.15	pg		(12.00 – 20.00)	15.64
MCHC	=	33.7	g/dL		(29.0 – 37.5)	32.0
RDW	=	20.9	%		(17.3 – 22.0)	18.1
%RETIC	=	0.5	%			0.8
RETIC	=	19.2	K/µL			49.1
WBC	=	13.80	K/µL		(5.50 – 19.50)	15.28
%NEU	=	89.5	%			91.2
%LYM	=	4.5	%			5.1
%MONO	=	3.4	%			1.3
%EOS	=	2.1	%			2.3
%BASO	=	0.5	%			0.1
NEU	=	12.36	K/µL		(2.50 – 12.50)	13.94
LYM	=	0.61	K/µL		(0.40 – 6.80)	0.79
MONO	=	0.47	K/µL		(0.15 – 1.70)	0.20
EOS	=	0.29	K/µL		(0.10 – 0.79)	0.35
BASO	=	0.07	K/µL		(0.00 – 0.10)	0.01
PLT	=	371.	K/µL		(175. – 600.)	442
MPV	=	15.62	fL			16.38
PDW	=	19.9	%			18.6
PCT	=	15.6	%			0.7



Blood film—RBC: 2+ poikilocytosis, few burr cells, 1+ anisocytosis; **WBC** : 1 neutrophil; **PLT**: large platelets

Chemistry Findings: Kidney—There is an increased BUN, creatinine and phosphorus indicating decreased glomerular filtration. This, in combination with the finding of a nonconcentrated urine (specific gravity of 1.010), is supportive of renal azotemia. **Electrolytes**—Electrolyte values are within reference interval limits; however, the potassium level is on the extreme low end of the reference interval and total body potassium decrease must be considered.

Other Significant Findings: Thyroid—T_4 levels are within the reference interval; there is no support for hyperthyroidism. **SNAP® FIV/FeLV Combo Test**—Negative. **Thoracic and abdominal radiographs**—Small irregular kidneys were noted.

Diagnosis: Chronic renal failure with secondary nonregenerative anemia

Treatment/Monitoring Plan: Treatment was concentrated on fluid therapy, dietary modification (low protein) and potassium supplementation. Serial monitoring of renal parameters, potassium and CBC is warranted.

Prognosis: Guarded

Eosinophilia and Moderate Reticulocytosis Secondary to Fleas

Felix, 2-year-old, intact male Maine Coon

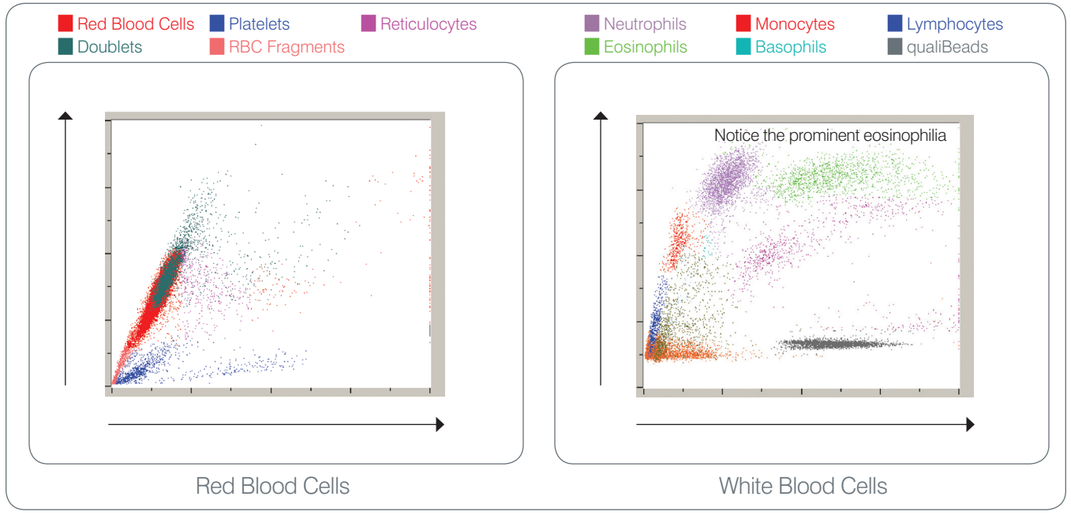
Case Study

History and Chief Complaint: Felix presented for a wellness appointment and immunizations. He is strictly an indoor cat, but he did escape from the house two weeks ago for several hours. There have been no medical complaints; Felix is doing well.

Abnormal Findings on General Physical Exam: Mucous membranes are slightly pale and a small amount of flea dirt is noted at the base of the tail. No live fleas were seen. No other significant abnormalities were noted.

Differentials: Flea infestation, possible mild flea-induced anemia

Diagnostic Plan: Complete blood count (CBC), SNAP® FIV/FeLV Combo Test and fecal analysis were planned.



Hematology Findings:

Red Blood Cells

No quantitative erythrocyte abnormalities are noted, but there is mild nonspecific poikilocytosis and mild anisocytosis evident on the blood film. In addition, there is mild polychromasia, which supports the finding of a mildly increased reticulocyte count. Although there is no anemia, it is noted that the hematocrit on this animal one year previously was 42.1%. Current findings are supportive of a mild regenerative response by the marrow.

White Blood Cells

There is a moderate eosinophilia. Other leukocytes are within reference interval limits. No morphologic abnormalities are noted.

Platelets

Platelets are normal in number and enlarged platelets are seen, but this is an equivocal finding in the cat.

IDEXX LABORATORIES

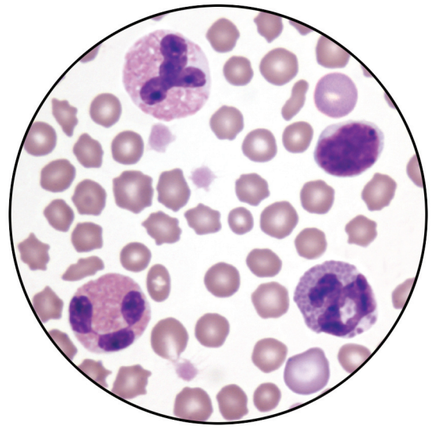
In-house Laboratory

Patient: Felix — Doctor: Smith
Species: Adult Feline
Client: Bob Jackson — Client ID: Feline - Felix

Hematology

7/28/2006 12:16:53 PM — LaserCyte® — 3/28/2005

RBC	=	7.47	M/µL	(5.00 – 10.00)		8.21
HCT	=	37.0	%	(30.0 – 45.0)		42.1
HGB	=	12.8	g/dL	(9.0 – 15.1)		14.2
MCV	=	49.6	fL	(41.0 – 58.0)		51.3
MCH	=	17.20	pg	(12.00 – 20.00)		17.30
MCHC	=	34.7	g/dL	(29.0 – 37.5)		33.7
RDW	=	20.9	%	(17.3 – 22.0)		19.9
%RETIC	=	0.9	%			0.7
RETIC	=	69.6	K/µL			57.5
WBC	=	10.10	K/µL	(5.50 – 19.50)		6.33
%NEU	=	51.3	%			54.7
%LYM	=	11.5	%			30.5
%MONO	=	9.4	%			6.3
%EOS	=	27.2	%			7.9
%BASO	=	0.6	%			0.6
NEU	=	5.19	K/µL	(2.50 – 12.50)		3.46
LYM	=	1.16	K/µL	(0.40 – 6.80)		1.93
MONO	=	0.95	K/µL	(0.15 – 1.70)		0.40
EOS	=	2.74	K/µL	(0.10 – 0.79)	HIGH	0.50
BASO	=	0.06	K/µL	(0.00 – 0.10)		0.04
PLT	=	230.	K/µL	(175. – 600.)		220
MPV	=	15.07	fL			18.2
PDW	=	19.5	%			19.0
PCT	=	0.4	%			0.4



Blood film—RBC: 2+ anisocytosis, 1+ poikilocytosis;
WBC: 2 eosinophils, 1 lymphocyte, 1 basophil (bottom right);
PLT: large platelets

Other Significant Findings: SNAP® FIV/FeLV Combo Test—Negative. **Fecal analysis**—Negative.

Diagnosis: Eosinophilia associated with fleas with a secondary mild decrease in red blood cell mass (decreased hematocrit compared to baseline) with an appropriate bone marrow response

Treatment/Monitoring Plan: Flea control of cat and environment, timely monitoring of serial CBCs

Prognosis: Good

Regenerative Anemia Secondary to *Mycoplasma* Infection

Lily, 3-year-old, spayed female domestic shorthair

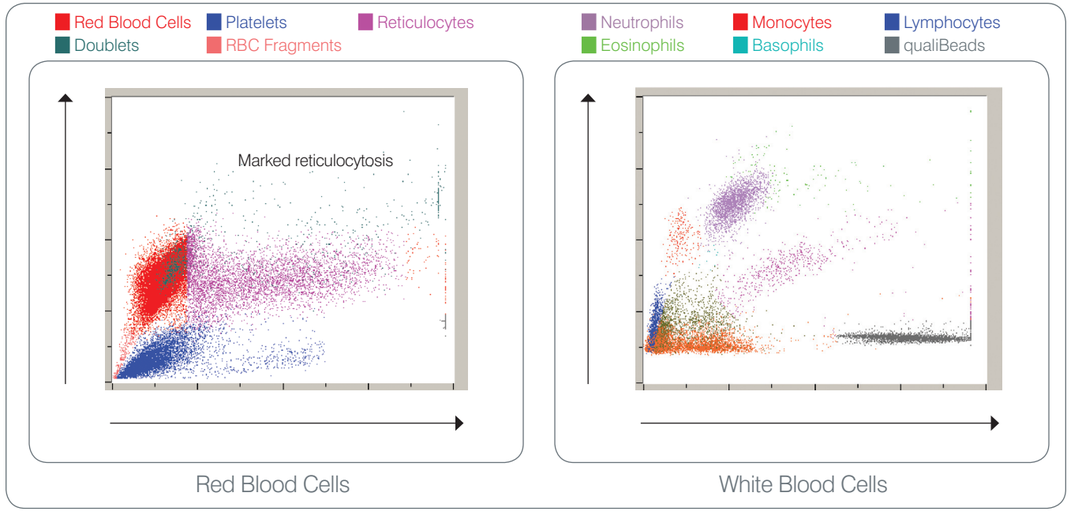
Case Study

History and Chief Complaint: Lily presented with a 3- to 4-day history of lethargy and anorexia. She is an indoor/outdoor cat and she is not current on immunizations. No previous medical history.

Abnormal Findings on General Physical Exam: Lethargic and mucous membranes are pale. Temperature was 104°F.

Differentials: FeLV, FIV, other viral (e.g., panleukopenia) infections, *Mycoplasma haemofelis*, FIP, occult bite/fight wound abscess

Diagnostic Plan: Complete blood count (CBC), general clinical chemistry profile, SNAP® FIV/FeLV Combo Test, fecal analysis



Hematology Findings:

Red Blood Cells

There is a marked normocytic and normochromic anemia. The marked reticulocytosis supports marked regenerative anemia and the degree of regeneration suggests hemolytic anemia. Peripheral blood film examination reveals numerous red blood cells with epicellular, tiny, pale-blue organisms consistent in morphology with *Mycoplasma haemofelis* (previously *Haemobartonella*) organisms.

White Blood Cells

Normal

Platelets

Normal

IDEXX LABORATORIES

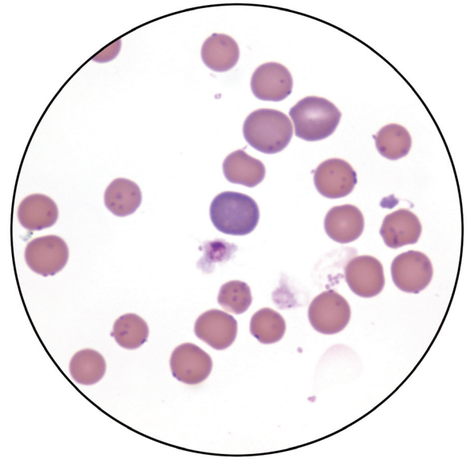
In-house Laboratory

Patient:	Lily	Doctor:	Smith
Species:	Adult Feline		
Client:	Betty Kaoub	Client ID:	6930

Hematology

12/5/2007 5:27:03 PM — LaserCyte®

RBC	=	2.40	M/µL	LOW	(5.00 – 10.00)
HCT	=	13.2	%	LOW	(30.0 – 45.0)
HGB	=	4.7	g/dL	LOW	(9.0 – 15.1)
MCV	=	55.0	fL		(41.0 – 58.0)
MCH	=	19.53	pg		(12.00 – 20.00)
MCHC	=	35.5	g/dL		(29.0 – 37.5)
RDW	=	19.9	%		(17.3 – 22.0)
%RETIC	=	13.3	%		
RETIC	=	319.9	K/µL		
WBC	=	7.62	K/µL		(5.50 – 19.50)
%NEU	=	71.0	%		
%LYM	=	19.6	%		
%MONO	=	5.6	%		
%EOS	=	3.4	%		
%BASO	=	0.3	%		
NEU	=	5.41	K/µL		(2.50 – 12.50)
LYM	=	1.50	K/µL		(0.40 – 6.80)
MONO	=	0.43	K/µL		(0.15 – 1.70)
EOS	=	0.26	K/µL		(0.10 – 0.79)
BASO	=	0.02	K/µL		(0.00 – 0.10)
PLT	=	548.	K/µL		(175. – 600.)
MPV	=	18.61	fL		
PDW	=	17.5	%		
PCT	=	1.0	%		



Blood film—RBC: 2+ polychromasia, 2+ anisocytosis, 3+ *Mycoplasma haemofelis* organisms; **PLT**: large platelets, variably sized platelets

Chemistry Findings: Primary changes are found in the liver panel. Moderate increase in ALT supports mild to moderate hepatocellular injury, the mild increase in ALKP and GGT support cholestasis and the increased total bilirubin supports cholestasis and/or hemolytic disease as is noted previously.

Other Significant Findings: **SNAP® FIV/FeLV Combo Test**—negative. **Fecal analysis**—negative.

Diagnosis: Severe regenerative anemia secondary to *Mycoplasma haemofelis* infection

Treatment/Monitoring Plan: Appropriate supportive care and antibiotic therapy

Prognosis: Good pending response to therapy

Salmonellosis and Gastroenteritis

Billy, 5-year-old quarter horse gelding

Case Study

History and Chief Complaint: Billy was purchased one year ago, the previous CBC results were originally requested as part of the prepurchase examinations. There is a two-day history of anorexia, lethargy, acute abdominal pain and profuse diarrhea.

Abnormal Findings on General Physical Exam: Billy is moderately lethargic. There is pyrexia and fecal staining under the tail. Dehydration (estimated at 5–8%) and tachypnea are noted.

Differentials: Potomac horse fever, clostridial diarrhea, salmonellosis, toxicity

Diagnostic Plan: Because of Billy's severe clinical presentation and potential systemic illness, a complete blood count (CBC), general clinical chemistry profile with electrolytes, complete urinalysis, blood gas analysis, lactate measurement, fecal analysis and possible fecal culture, and *Salmonella* PCR were indicated.

Hematology Findings:

Red Blood Cells

There are no quantitative or morphologic (blood film review) changes noted; however, in light of the dehydration indicated on physical examination, the potential of hemoconcentration and masked anemia should be considered and the red blood cells should be reevaluated following fluid replacement therapy.

White Blood Cells

The primary changes include leukopenia characterized by a low normal neutrophil count and a lymphopenia.

Platelets

No significant platelet abnormalities are noted.

IDEXX LABORATORIES

In-house Laboratory

Patient:	Billy	Doctor:	Smith
Species:	Adult Equine		
Client:	Gaymond Rutgers	Client ID:	7555

Hematology

12/3/2007 4:44:29 PM — LaserCyte® — 12/3/2006

RBC	=	8.35	M/µL		(6.80 – 12.90)		8.68
HCT	=	38.9	%		(32.0 – 53.0)		40.1
HGB	=	14.7	g/dL		(11.0 – 19.0)		15.1
MCV	=	46.6	fL		(37.0 – 58.0)		46.2
MCH	=	17.50	pg		(12.30 – 19.90)		17.40
MCHC	=	37.6	g/dL		(31.0 – 38.6)		37.7
RDW	=	19.4	%		(17.0 – 21.0)		19.1
WBC	=	4.59	K/µL	LOW	(5.40 – 14.30)		7.5
%NEU	=	65.5	%				53
%LYM	=	25.1	%				41
%MONO	=	6.9	%				4.1
%EOS	=	2.0	%				1.6
%BASO	=	0.6	%				0.3
NEU	=	3.01	K/µL		(2.26 – 8.50)		3.98
LYM	=	1.15	K/µL	LOW	(1.50 – 7.70)		3.08
MONO	=	0.32	K/µL		(0.10 – 1.00)		0.31
EOS	=	0.09	K/µL	LOW	(0.10 – 1.00)		0.12
BASO	=	0.03	K/µL		(0.00 – 0.03)		0.02
PLT	=	122.	K/µL		(90. – 350.)		141
MPV	=	6.86	fL				14.2
PDW	=	17.3	%				17.5
PCT	=	0.1	%				0.2

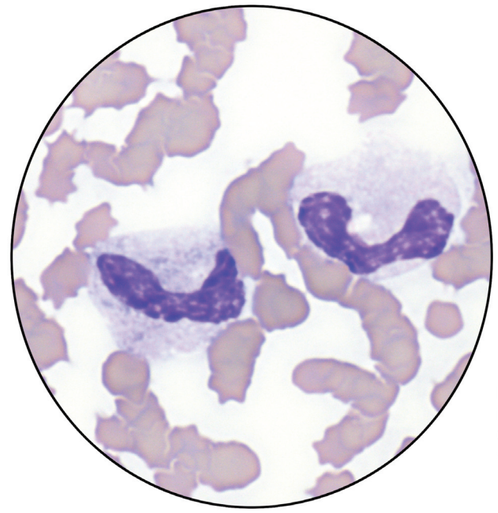
Chemistry Findings: Total protein—Albumin and globulin are all high and the albumin:globulin ratio is decreased. The increased albumin is most likely related to the dehydration and the other protein changes are supportive of inflammation. **Acid-base/Electrolytes**—Blood gas analysis revealed a titrational metabolic acidosis with a decreased sodium and proportional decrease in chloride along with a decreased HCO_3^- and an increased anion gap. Changes support the presence of unmeasured anions and lactic acidosis is most likely.

Other Significant Findings: Lactate—The increased lactate suggests decreased tissue perfusion and is supported by the acid-base disturbance noted above; findings support a guarded prognosis. **Fecal culture (serial)**—positive for salmonella. **Salmonella PCR**—positive.

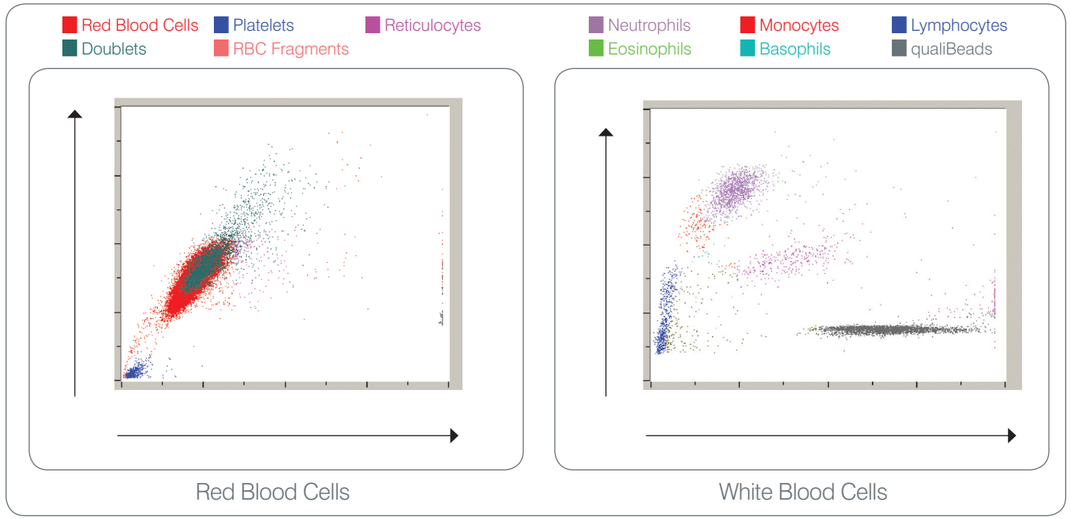
Diagnosis: Salmonellosis, gastroenteritis, severe overwhelming inflammation

Treatment/Monitoring Plan: Supportive care, fluid and antibiotic therapy, timely monitoring of CBC

Prognosis: Guarded to poor



Blood film—RBC: 2+ poikilocytosis, increased Rouleaux; **WBC**: 2 immature (band) neutrophils, 3+ toxicity; **PLT**: none seen in this field of view



Heinz Body Hemolytic Anemia Secondary to Red Maple Toxicosis

Jackie, 5-year-old Thoroughbred mare

Case Study

History and Chief Complaint: Jackie presented with a 24-hour history of lethargy, tachycardia, tachypnea and anorexia. The owner noted dark-brown urine on the bedding. Her paddock has a large red maple tree that has been losing leaves (autumn). The owner did not observe any consumption of leaves.

Abnormal Findings on General Physical Exam: Jackie is moderately lethargic and has pale, dark-brown mucous membranes; mild icterus; shallow, rapid breathing; moderate tachycardia (heart rate at 80 bpm); and pyrexia with a temperature of 103.6°F.

Differentials: Equine infectious anemia (EIA), equine granulocytic ehrlichiosis (EGE), immune-mediated hemolytic anemia, red maple toxicosis, onion toxicosis

Diagnostic Plan: Complete blood count (CBC), fibrinogen, general clinical chemistry profile with electrolytes, complete urinalysis, blood gas analysis, lactate measurement, Coggins test for EIA

Hematology Findings:

Note: Freshly collected blood appeared brown, indicating possible methemoglobinemia. The plasma appeared slightly orange-red indicating probable hemoglobinemia.

Red Blood Cells
There is a moderate (compared to normal HCT of 48%) normochromic, normocytic anemia. The blood film review reveals moderate anisocytosis, many Heinz bodies and keratocytes, as well as few schistocytes.

White Blood Cells
Normal

Platelets
Normal

IDEXX LABORATORIES

In-house Laboratory

Patient: Jackie — Doctor: Smith
Species: Adult Equine
Client: Kyle Jacobs — Client ID: 199728

Hematology
10/5/2007 4:32:33 PM — LaserCyte®

RBC	=	6.05	M/µL	LOW	(6.80 – 12.90)
HCT	=	29.7	%	LOW	(32.0 – 53.0)
HGB	=	11.4	g/dL		(11.0 – 19.0)
MCV	=	49.1	fL		(37.0 – 58.0)
MCH	=	18.86	pg		(12.30 – 19.90)
MCHC	=	38.4	g/dL		(31.0 – 38.6)
RDW	=	18.5	%		(17.0 – 21.0)
WBC	=	7.45	K/µL		(5.40 – 14.30)
%NEU	=	52.0	%		
%LYM	=	42.0	%		
%MONO	=	4.3	%		
%EOS	=	1.6	%		
%BASO	=	0.2	%		
NEU	=	3.87	K/µL		(2.26 – 8.50)
LYM	=	3.13	K/µL		(1.50 – 7.70)
MONO	=	0.32	K/µL		(0.10 – 1.00)
EOS	=	0.12	K/µL		(0.10 – 1.00)
BASO	=	0.01	K/µL		(0.00 – 0.03)
PLT	=	175.	K/µL		(90. – 350.)
MPV	=	8.68	fL		
PDW	=	17.5	%		
PCT	=	0.2	%		

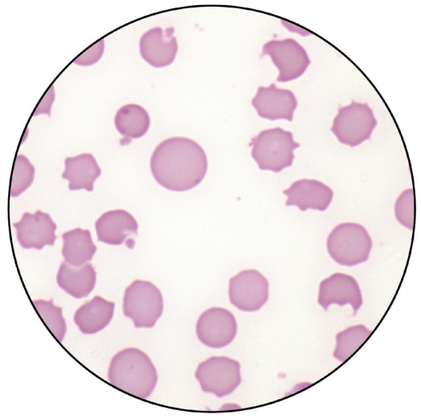
Chemistry Findings: Proteins—Total protein, albumin and globulin are all high, and the albumin:globulin ratio is decreased. The increased albumin is most likely related to the dehydration and the other protein changes are supportive of inflammation. **Acid-base/Electrolytes—**Blood gas analysis revealed a titrational metabolic acidosis with a decreased sodium and proportional decrease in chloride along with a decreased HCO_3^- and an increased anion gap. Changes support the presence of unmeasured anions and lactic acidosis is most likely, which was confirmed with the increased lactate level; the increased lactate suggests decreased tissue perfusion. Findings support a guarded prognosis.

Other Significant Findings: Coggins test (EIA)—Negative.

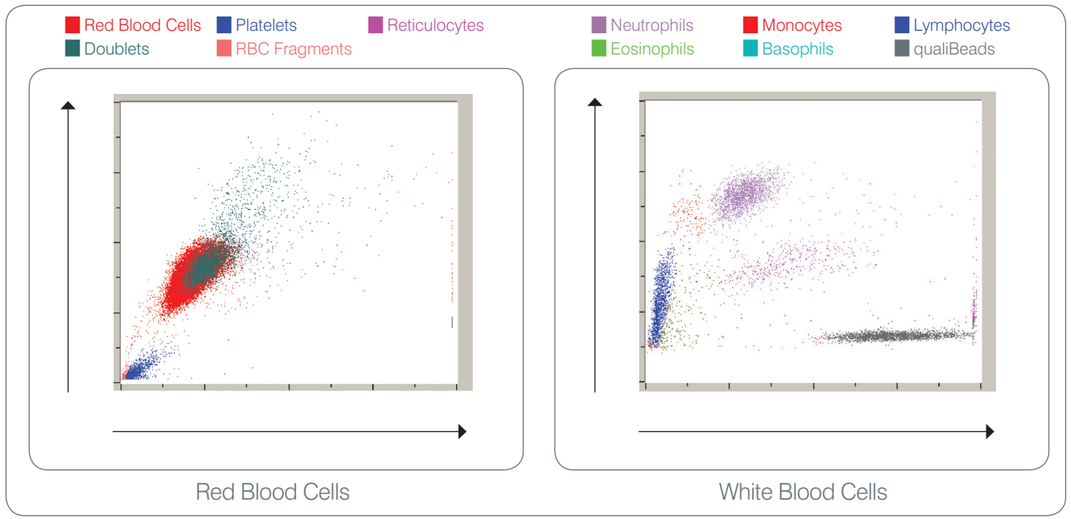
Diagnosis: Acute Heinz body hemolytic anemia (probable intravascular and extravascular) due to severe oxidant stress; probable red maple toxicosis

Treatment/Monitoring Plan: Supportive care, fluid and antibiotic therapy, timely monitoring of CBC findings

Prognosis: Guarded to poor



Blood film—RBC: 2+ anisocytosis, 2+ poikilocytosis, 2+ Heinz bodies; **WBC**: none seen in this field of view; **PLT**: none seen in this field of view



Technology

IDEXX LaserCyte® Hematology Analyzer Specifications

Manufactured by:	IDEXX Laboratories, Inc.
Technology:	Laser flow cytometry: 5-part differential and reticulocyte count
Reported Parameters (24):	RBC, HCT, HGB, MCV, MCH, MCHC, RDW, Reticulocytes (absolute # and %), WBC, NEU #, %NEU, LYM #, %LYM, MON #, %MON, EOS #, %EOS, BAS #, %BAS, PLT, MPV, PDW, PCT
Sample Volume:	95 µL whole blood with EDTA anticoagulant is used per evaluation (500 µL of whole blood in a 13 mm x 75 mm IDEXX VetCollect® tube is required for automated sampling)
Time to results:	~ 10–12 minutes
Species:	Canine, feline, equine
Maintenance:	No daily maintenance required
Internal Control:	Each CBC5R tube has a set number of qualiBeads®. qualiBeads are known particles with specific characteristics that are used to verify pipetting accuracy and laser performance.
Calibration:	Performed at IDEXX Laboratories
Data Storage Capacity:	Unlimited
Dimensions (W x D x H):	34 cm x 36 cm x 33 cm (13.4 in x 14.2 in x 13 in)

Analysis Principles of the LaserCyte® Analyzer

The LaserCyte Analyzer Uses Two Technologies to Measure Parameters

1. Laser Flow Cytometry

Four Detectors and Measured Time of Flight in Front of Laser Beam

Detectors

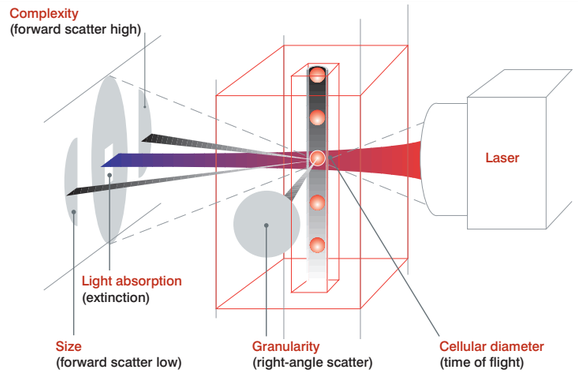
Cellular diameter (TOF)

Complexity (FSH)

Size (FSL)

Granularity (RAS)

Light absorption (EXT)



Parameters

RBC
Reticulocyte
Platelets

WBC

MPV
(derived from PLT peak mean)

RDW
(derived from coefficient of variation of RBC population)

MCV
(derived from peak mean)

PDW
(derived from coefficient of variation of platelet population)

Lymphocytes
%LYM

Basophils
%BASO

Neutrophils
%NEU

Monocytes
%MONO

Eosinophils
%EOS

Analysis

$$HCT = \frac{RBC \times MCV}{10}$$

$$MCH = \left(\frac{HGB}{RBC}\right) \times 10$$

$$\%RETIC = \frac{100 \times RETIC}{RBC}$$

$$PCT = \frac{MPV \times PLT}{10000}$$

$$MCHC = 1000 \times \frac{HGB}{MCV \times RBC}$$

2. Spectral Absorption

Two LEDs

Red Blood Cell Run

White Blood Cell Run

Blue LED

Intracellular HGB

Comparison of HGB measurements from the LEDs provides a more accurate HGB reading

Blue Green LED

HGB post RBC lysis

Reported HGB

Key: Cellular diameter (also known as time of flight—TOF)
Complexity (also known as forward scatter high—FSH)
Size (also known as forward scatter low—FSL)
Granularity (also known as right-angle scatter—RAS)
Light absorption (also known as extinction—EXT)

Red Blood Cell

White Blood Cell

LaserCyte® Analyzer Message Codes

Automated cell counters have two main objectives. First, they must examine the various components of a blood sample and return the appropriate red blood cell count, white blood cell count, platelet count and various cellular indices. Second, they must prompt the user with a message in the event that the cell counts cannot be fully analyzed. For example, if the white blood cell morphology includes cells that the analyzer cannot fully categorize, the device must return a message suggesting a blood film should be reviewed for confirmation.

The LaserCyte analyzer message codes signal the user that an abnormal cell or group of cells is present and it cannot be characterized in the normal hemogram. These message codes act as internal controls to remind the doctor that a sample must be examined under a microscope. In the vast majority of cases, this microscopic review process will take less than 1–3 minutes. A manual leukocyte differential will be needed only rarely.

Code: DB 1/2

Full Text: Differential algorithm issues. Confirm with blood film.

Consequences: LYM, MONO, %LYM, %MONO are flagged with "*".

Explanation: Sample-related message; the patient's white blood cell morphology made it difficult to separate the lymphocytes and monocytes. Evaluate the blood film to confirm the values.

Code: DB 1/3

Full Text: Differential algorithm issues. Confirm with blood film.

Consequences: NEU, MONO, %NEU, %MONO are flagged with "*".

Explanation: Sample-related message; the patient's white blood cell morphology made it difficult to separate the monocytes and neutrophils. Evaluate the blood film to confirm the values.

Code: DB 1–5

Full Text: Differential algorithm issues. Confirm with blood film.

Consequences: LYM, MONO, NEU, EOS, BASO, %LYM, %MONO, %NEU, %EOS, %BASO are flagged with "*".

Explanation: Sample-related message; the patient's white blood cell morphology made it difficult to separate the cells. Evaluate the blood film to confirm the values.

Code: DB 2/8

Full Text: Differential algorithm issues. Confirm with blood film.

Consequences: WBC, LYM, %LYM, %MONO, %NEU, %EOS, %BASO are flagged with "*".

Explanation: Sample-related message; the patient's white blood cell morphology made it difficult to separate the cells. Evaluate the blood film to confirm the values.

Code: DB 3/4

Full Text: Differential algorithm issues. Confirm with blood film.

Consequences: NEU, EOS, %NEU, %EOS are flagged with "*".

Explanation: Sample-related message; the patient's white blood cell morphology made it difficult to separate the neutrophils and eosinophils. Evaluate the blood film to confirm the values.

Code: DB 5

Full Text: Abnormal differential. Confirm with blood film.

Consequences: BASO, %BASO are flagged with "*".

Explanation: Instrument- or sample-related message; the %BASO value is >2.5% (very rare). Evaluate the blood film to verify the basophil results.

Code: DB 7

Full Text: Differential algorithm issues. Confirm with blood film.

Consequences: WBC, NEU, EOS, %LYM, %MONO, %NEU, %EOS, %BASO are flagged with "*".

Explanation: Sample-related message; the patient's white blood cell morphology made it difficult to separate the neutrophils and eosinophils. Evaluate the blood film to confirm the values.

Code: DB 10

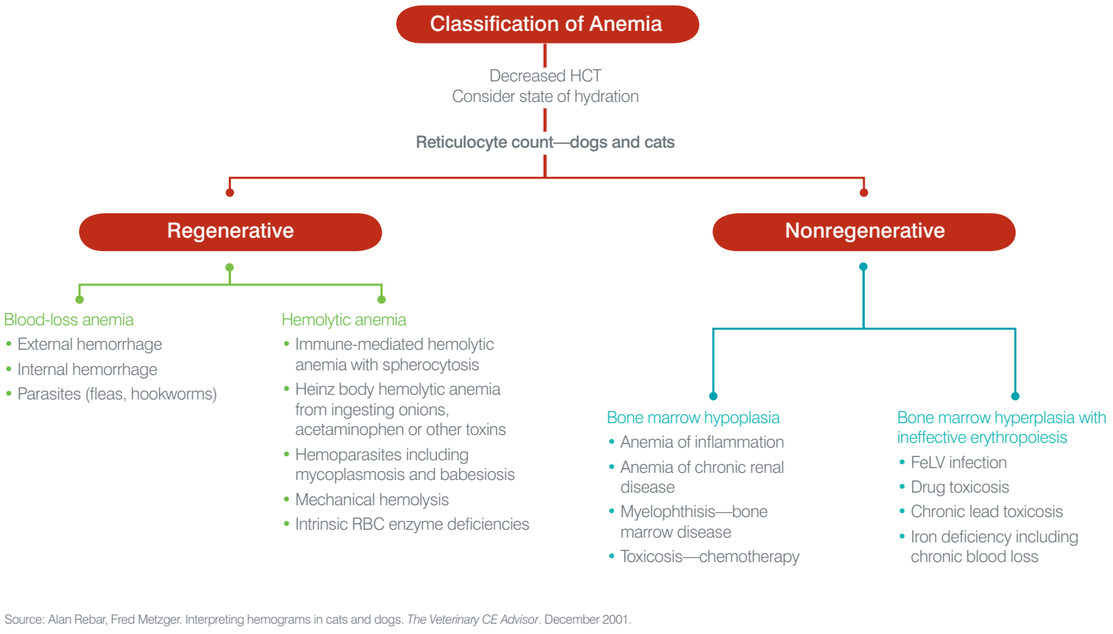
Full Text: Possible rate analysis issue. Confirm differential with blood film and WBC.

Consequences: WBC, LYM, MONO, NEU, EOS, BASO, %LYM, %MONO, %NEU, %EOS, %BASO are flagged with "*".

Explanation: Sample-related message; during analysis, a significant number of the white blood cells decayed and were not included in the WBC count. This message is usually due to a patient's white blood cells being more fragile than normal. Internal temperature may be the cause. Evaluate the blood film to confirm the values.

Reference Materials

Classification of Anemia



Source: Alan Rebar, Fred Metzger. Interpreting hemograms in cats and dogs. *The Veterinary CE Advisor*. December 2001.

Leukocyte Profiles

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Commonly Encountered Leukocyte Profiles and Their Potential Differential Patterns

Leukocyte type	Minimal inflammation	Mild inflammation	Moderate inflammation	Established inflammation	Overwhelming inflammation	Glucocorticoid influence (stress)	Epinephrine influence (excitement)
Mature neutrophil	N	N to ↑	↑ to ↑↑	↑ to ↑↑↑	↓ to ↓↓↓	↑ to ↑↑	N to ↑
Band neutrophil	N	N to ↑	↑ to ↑↑	N to ↑	↑	N	N
Lymphocyte	N	N to ↓	↓ to ↓↓	N to ↑	↓↓	↓↓	N to ↑
Monocyte	N	N to ↑	N to ↑↑	N to ↑↑	N	N to ↑	N
Eosinophil	N	↑ to ↓	↑ to ↓	N to ↓	↓	↓	N
Basophil	N	N to ↑	N to ↑	N to ↑	N	N	N

N = no change
↑ = minimal to mild increase
↓ = minimal to mild decrease
↑↑ = moderate increase
↓↓ = moderate decrease
↑↑↑ = marked increase
↓↓↓ = marked decrease

Imprecision of Canine Manual Differential Leucocyte Count: Effect of Smear, Observer and Number of Counted Cells

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Proceedings from: European Society of Veterinary Clinical Pathology (ESVCP) 8th Annual Congress; September 5–8, 2006; Cambridge, UK.

Background and Objective:
The manual differential leucocyte count (MDLC) is considered the gold standard to which automated differential counts are compared. However, MDLC imprecision is estimated statistically based on two following assumptions:

- Leucocyte distribution is homogenous throughout the smear.
- One smear is representative of the blood.

We investigated if leucocyte prepartition was homogenous on 3 canine blood smears and assessed the possible effects of smear, observer and number of counted cells on MDLC.

Material:
Leucocyte prepartition: Blood smears from 3 "normal" dogs were divided into 4 columns and 4 lines delimiting 16 areas (excluding feather edge), in which all cells were counted and differentiated at x200 or x400.

Differential count imprecision: All MDLC were blinded. Five routine MDLC (100 cells counted) were performed by three observers (two trained, one less trained) in 10 smears from one "normal" dog and 10 from one dog with leucocytosis. In the same 20 smears, MDLC was determined by counting 100, 200, 300, 400 and 500 cells by one observer.

Statistical analysis: Khi² and ANOVA, using Excel and Systat.

Results:
Leucocyte prepartition: In the 3 smears, there were significant differences in MDLC in some areas, columns or lines, compared to the whole smear, without a common pattern.

Differential count imprecision: Coefficient of variation (CV) of neutrophil count was <7% for the three observers, but eosinophil, basophil, lymphocytye and monocyte counts CV were above 48, 100, 19 and 41% respectively. CVs were slightly lower when counting 200 or more cells but remained high (27, 86, 13 and 36% respectively with 500 cells counted). Smear effect was statistically significant for lymphocytes and monocytes in one dog, and for lymphocytes and neutrophils in the other. Observer effect was significant in one dog for monocyte count.

Conclusion:
MDLC is an imprecise method except for neutrophils.

Evaluation of the LaserCyte: An In-house Hematology Analyzer for Dogs and Cats

Bettina Wenger-Riggenbach, Mike Hässig, Regina Hofmann-Lehmann, Hans Lutz
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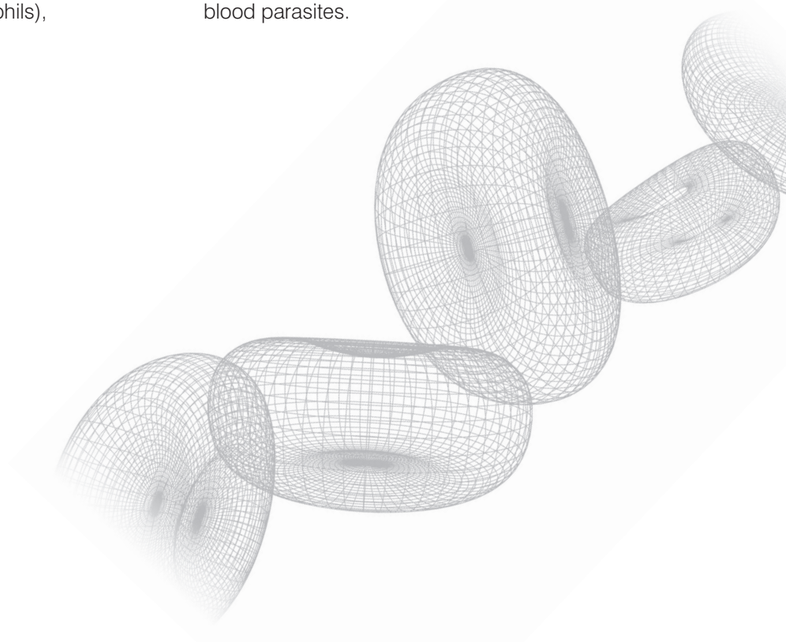
Proceedings from: 12th Congress of the International Society of Animal Clinical Biochemistry; May 22–26, 2006; Istanbul, Turkey.

Abstract:
In the present study, the LaserCyte instrument, a fully automated flow cytometer for use in veterinary practice, was evaluated for dogs and cats. Precision (coefficient of variation, CV) for red blood cell (RBC) parameters was ≤3.9%, for reticulocytes between 14.9 and 102%, for white blood cells (WBC) between 3 and 9.5% for neutrophils between 3.9 and 6.5%, for lymphocytes between 7 and 17.9%, for monocytes between 4.9 and 13.1%, for eosinophils between 10.4 and 32.1%, for basophils between 7.8 and 32%, for platelets between 3.1 and 13.2 and for platelet indices between 0 and 28.2%. The range linearity extended the reference ranges. The agreement with reference methods (coefficient of correlation, r) were ≥0.96 (RBC), ≥0.94 (hematocrit), ≥0.96 (hemoglobin), ≥0.95 (mean corpuscular volume), ≥0.94 (WBC), ≥0.93 (neutrophils), ≥0.77 (lymphocytes), ≥0.77 (monocytes), ≥0.29 (eosinophils), ≥0.03 (basophils), ≥0.13 (reticulocytes) and ≥0.86 (platelets). The LaserCyte allowed the correct assessment of RBC and WBC parameters with respect to clinical relevance in the majority of samples. Lymphocytopenia was detected in only 51 out of the 89 cases and monocytopenia in one out of 11 cases, The reticulocyte counts were correctly estimated in 85 out of 149 cases. It was concluded that the LaserCyte allowed reliable determination of the RBC parameters, WBCs, neutrophils in both species and platelets in dogs. Based on its capabilities to reliably determine feline platelets and of the parameters mentioned above, this instrument is considered a useful in-house analyzer for the veterinary practice. Qualitative microscopic assessment of blood smears is still necessary for detecting abnormal cell morphologies, certain cell precursors and blood parasites.



Comparison of Reticulocyte Counts to Mean Corpuscular Volume and Mean Corpuscular Hemoglobin Concentration in Anemic Dogs

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Proceedings from: 12th Congress of the International Society of Animal Clinical Biochemistry; May 22–26, 2006; Istanbul, Turkey

Background:
Anemia, a common abnormality in small animal medicine, is first classified as being regenerative or nonregenerative to aid in the identification of the underlying cause. Microscopic evaluation of routine-stained blood films or new methylene blue-stained blood samples for the recognition of polychromasia and reticulocytosis, respectively, can provide subjective information to allow this first classification. Although the use of in-house hematology analyzers is increasing in popularity in veterinary medicine, most veterinary practices that perform in-house hematology do not routinely look at blood films. Recent automation in in-house laser flow cytometry-based hematology analyzers can provide objective reticulocyte counts. Classically, the RBC indices, mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) have been used in the morphologic classification of anemias in both humans and domestic animals; their measurements became readily available with the widespread use of impedance-based hematology analyzers. An increased MCV (macrocytosis) and a decreased MCHC (hypochromasia) support a regenerative response; however, this profile may also be noted in nonregenerative responses and because they represent mean changes in these erythrocyte parameters, they are less sensitive than observing polychromatophils on the blood film or counting reticulocytes manually or with automated analyzers. The potential for having an MCV and MCHC within reference interval limits during regeneration is high.

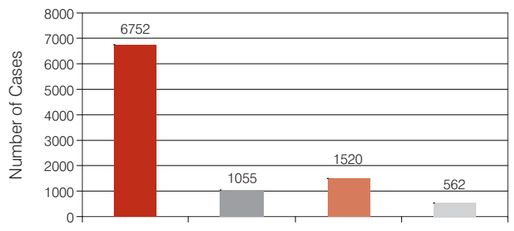
Objective:
Identification of regenerative anemia in the dog reportedly can be accomplished by blood film inspection for polychromasia, evaluating red cell indices and determining reticulocyte counts. The classic erythrocyte indices profile for regeneration is macrocytosis and hypochromasia. The objective of this study was to compare mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) changes to an absolute reticulocyte count in a series of anemic dogs to determine the frequency of a "classic" erythrocyte indices profile during a regenerative response.

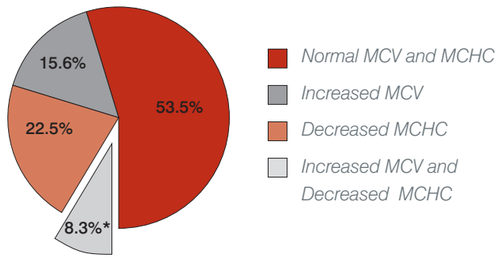
Materials and Methods:
All data from canine complete blood count (CBC) submissions to 14 IDEXX Reference Laboratories (IRL) in the United States from 01-01-05 through 03-31-05 were collected. A hematocrit value <35% was used to indicate anemia and an absolute reticulocyte value of >60,000/µL was used to indicate regeneration. In dogs classified as having a regenerative anemia, the number and percentage of cases with increased MCV, decreased MCHC, and both increased MCV and decreased MCHC values outside of the IRL reference intervals were determined.

Results:
Collected data are summarized in the table below. During the three-month evaluation period, 203,939 comprehensive CBC data sets including absolute reticulocyte counts were complete enough for inclusion in this study. Anemia was observed in 18,975 (9.3%) of these cases and 6,752 (3.3%) of the cases had reticulocyte counts greater than 60,000/µL. Among the regenerative cases, 1,055 (15.6%) had increased MCV, 1,520 (22.5%) had decreased MCHC and only 562 (8.3%) had both an increased MCV and a decreased MCHC.

Conclusion:
For routine CBC evaluations in the anemic dog, changes in erythrocyte indices are an unreliable predictor of regeneration. A blood film analysis and reticulocyte count are needed for a more accurate assessment of regeneration. The absolute reticulocyte count is the most objective measure of regeneration in the dog.

Erythrocyte Indices Distribution Among Canine Regenerative Anemias





Only 8.3% of the cases had both an increased MCV and a decreased MCHC making this combination an **unreliable predictor of anemia.*

Eosinophil Count in Cats by Manual Methods, the Hemavet® 950 and LaserCyte® Analysers

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Proceedings from: European Society of Veterinary Clinical Pathology (ESVCP) 8th Annual Congress; September 5–8, 2006; Cambridge, UK.

Eosinophilia is an uncommon haematological change in cats but has high medical significance in various diseases. Most blood analysers have not been validated for eosinophil counting in animals. This study was designed to compare two manual methods and two analysers, the Hemavet® and LaserCyte®.

Eosinophil count was performed in 62 EDTA-K3 feline blood samples by manual count on blood smear, manual count after Phloxine B 1% stain, the Hemavet 950 (impedance technology, Drew Scientific, UK) and the LaserCyte (flow-cytometry technology, IDEXX Laboratories, USA) analysers. Results were compared by Passing Bablok's regression procedure and Bland Altman plot.

Total WBC was much higher with the Hemavet than with the LaserCyte (paired t-test <0.001, mean difference of 3.9 x 10^9/L WBC).

The agreement between eosinophil manual count on blood smear (x) and the analysers (y) was poor for the Hemavet and good for the LaserCyte. The Passing Bablok's equations were [CI 95%]:

$y = 0.867\ [0.518–1.305]\ x + 0.009\ [-9.202–0.117]$
for the Hemavet

$y = 0.981\ [0.775–1.268]\ x + 0.220\ [0.116–0.244]$
for the LaserCyte

Correlation was better with the LaserCyte ($r = 0.65$) than with the Hemavet ($r = 0.32$). There was no significant difference in eosinophil count between Phloxine and Hemavet (paired t-test >0.05), whereas it was significantly higher with the LaserCyte (paired t-test <0.001).

The clinical classification of values as "normal" or "high" at the 1.5 x 10^9/L threshold showed discrepancies according to method, but this probably results from the differences of total WBC count.

This study suggests flow cytometry gives a better estimate of eosinophil counts in cats than impedance technology. However, it must be investigated in larger numbers of eosinophilia cases.

Total Leukocyte Count in Cats: Comparison of Results Obtained by Manual Count and Six In-house Analysers

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Proceedings from: 17th ECVIM-CA Congress; September 13–15, 2007; Budapest, Hungary.

There is a regular increase in the number of veterinary in-house haematology analysers and of the number of constituents measured. However, most analysers have not been validated. Leukocyte counts have been reported to be less reliable in cats than in dogs but few studies have been done to evaluate the applicability of the in-house analysers in cats. The objective of this study was to compare manual leukocyte counts with results of six in-house analysers in clinically normal and diseased cats.

Sixty cats were sampled on K3-EDTA tubes and all analyses were performed within six hours. Duplicate manual counts were performed using Unopette Leukocytes kits and a Malassez chamber. Blood specimens were analyzed in duplicate on VetABC (Scil), MS9/5 (Melet Schloesing), Abacus Junior (Diatron), Medonic (Boule), Hemavet 950 (Drew Scientific) and LaserCyte® (IDEXX). Repeatability of the manual method was evaluated by 10 repeats in a low, a normal and a high leukocyte count specimens. Assessment of precision of the analysers was assessed with control samples or with information from the manufacturer. Comparisons were based on calculations of correlation coefficient, Passing-Bablok regression analysis and Bland-Altman diagram of difference.

Manual leukocyte counts ranged from 1.55 to 34.85 10^9/L; for technical reasons some samples could not be analysed by all the machines. Correlation coefficients were 0.81, 0.86, 0.79, 0.97, 0.85 and 0.96 with VetABC, MS9/5, Abacus Junior, Medonic, Hemavet 950 and LaserCyte respectively. Passing-Bablok equations were $y = 0.96x + 0.25$, $y = 1.18x + 0.18$, $y = 1.40x + 0.28$, $y = 1.15x + 0.26$, $y = 1.10x + 0.31$ and $y = 0.99x + 0.68$ respectively. Analytically different results were obtained in 25/58 (VetABC), 30/55 (MS9/5), 41/58 (Abacus Junior), 31/54 (Medonic), 28/52 (Hemavet 950) and 20/57 (LaserCyte), respectively. Medical misinterpretation would have been obtained in 4/58 (VetABC), 10/55 (MS9/5), 16/58 (Abacus Junior), 5/54 (Medonic), 7/52 (Hemavet 950) and 2/57 (LaserCyte) respectively. The largest differences were observed when platelet aggregates were present.

Leukocyte counts in diseased cats are not accurately measured by all in-house analysers. The large size of feline platelets, leukocyte aggregates and small platelet aggregates could explain all the false leukocyte counts observed in this study. Impedance analysers are more or less sensitive to platelet aggregates, which is not the case of flow cytometry.

Evaluation of a Point-of-Care Hematology Analyzer in Dogs and Cats Receiving Anticancer Chemotherapy

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Proceedings from: Veterinary Cancer Society Conference; October 27–30, 2005; Huntington Beach, California.

Introduction:

The new in-house hematology analyzer LaserCyte® (IDEXX Laboratories, Westbrook, Maine) performs a complete blood count (CBC) with a five-part differential and reticulocyte count using laser-flow cytometry. The aim of this study was to compare results generated by the LaserCyte with those obtained by standard methods in dogs and cats undergoing chemotherapy.

Methods:

Dogs and cats in the study had one of these neoplasms: LSA, CLL, OSA, MH, MCT, HSA, FSA, malignant melanoma, or carcinoma. Chemotherapeutic protocols consisted of single agent or combinations of doxorubicin, vincristine, cyclophosphamide, L-asparaginase, cytosine arabinoside, CCNU, gemcitabine, carboplatin, chlorambucil, melphalan, actinomycin D, methotrexate, prednisone and suramin. We evaluated 370 samples from 83 dogs and 70 from 23 cats on the LaserCyte and CellDyn-3500® (Abbott Diagnostics, Abbott Park, Illinois) with a manual differential count.

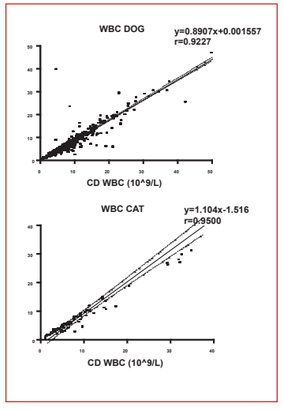
Results:

Correlation coefficients (dogs/cats) were 0.95/0.93 for hematocrit (HCT), 0.93/0.95 for total white blood cells (WBC), 0.93/0.94 for neutrophils and 0.93/0.71 for platelet (PLT) count. All CBCs where leukopenia, neutropenia, or anemia were detected with standard methods had the same feature with LaserCyte. Thrombocytopenia was detected with LaserCyte in 93% (dogs) and 26% (cats) of the thrombocytopenic samples detected with standard methods.

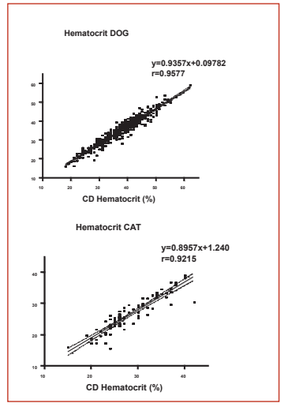
Conclusions:

The LaserCyte is a reliable hematology analyzer for dogs and cats on chemotherapy. It had excellent correlation for HCT, PLT, WBC and neutrophil counts, and accurately detected anemia, leukopenia, or neutropenia. The sensitivity of the instrument for thrombocytopenia was lower (predominantly feline), but acceptable. In general, automated measurements of feline platelets can be highly variable method to method.

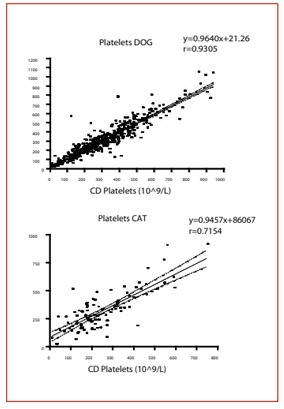
Result



Result



Result



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