IDEXX **VetAutoread**™
Hematology Analyzer Casebook

IDEXX LABORATORIES

Casebook

An explanation of numerous analyzer reports as compiled by:

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IDEXX gratefully acknowledges the efforts of Dr. Allan Hart (deceased), Laboratory Director, New Haven Central Hospital for Veterinary Hospital and Rufus James, Chief Laboratory Technician, New Haven Central Hospital for Veterinary Medicine, for the explanation of the numerous analyzer reports that were compiled for this casebook.

To commemorate the memory of Dr. Hart, IDEXX has established the Allan Hart Scholarship for senior veterinary students who show exceptional proficiency in clinical pathology.

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

IDEXX LABORATORIES

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Buffy Coat Profiles 1–4: Normal Dog, Cat, Horse and Cow

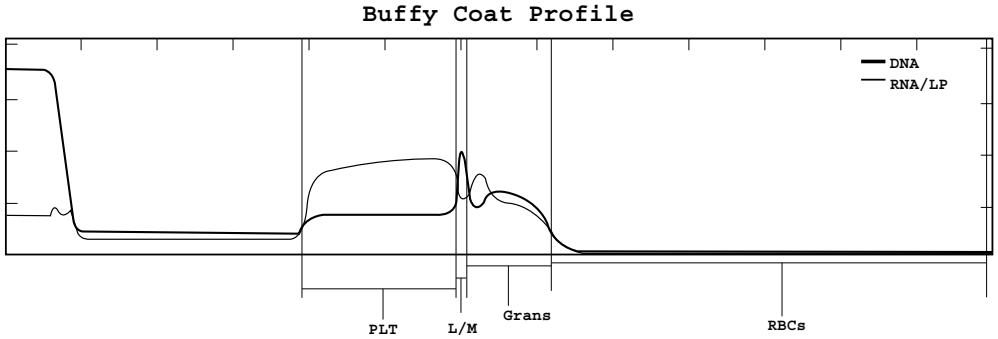
Examples of each species “typical” or normal buffy coat profile are reproduced below. Begin your review of each buffy coat profile on the right, in the red blood cell (RBC) area. Normal, mature RBCs contain neither DNA nor RNA, therefore, both the thick DNA and thin RNA/LP lines are on the baseline as viewed from right to left. The start of the granulocyte (Grans) layer is indicated by the abrupt upward climb of both the DNA and RNA/LP lines, where the acridine orange dye has been bound to the DNA and RNA/LP in the cells. The small rise and fall in the RNA/LP line, near the interface of granulocytes and the L/M layer, represents eosinophils. Eosinophils in the dog have a certain degree of autofluorescence that allows them to be identified by the IDEXX VetAutoread™ Hematology Analyzer.

Note the symmetrical slope on both the forward and back side of the L/M layer. This slope is typical of an L/M population dominated by lymphocytes. The normally small population of monocytes is concealed in the larger lymphocyte population. Leaving the L/M population, the RNA/LP line begins to dominate in the platelet layer. Following the platelet layer, both the DNA and RNA/LP lines drop down, but do not reach the baseline because of free dye fluorescing in the plasma. On the far left, there is a rise that represents the top of the float and the end of the analysis. This area should be free of cells. These are the simplest and most typical buffy coat profiles, and contain the basics for viewing the rest of the buffy coat profiles.

Buffy Coat Profile 1: Dog with Mild Eosinophilia

Species: Dog

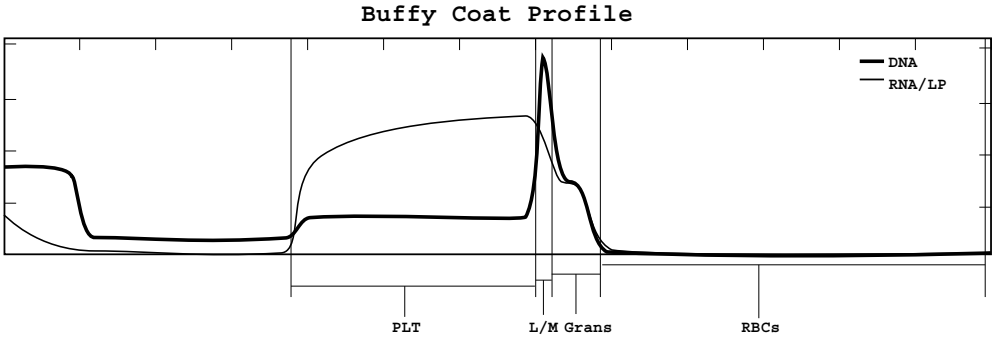
Test		Results	Units	Reference Range	Indicator (LOW / NORMAL / HIGH)
HCT	=	47.6	%	37.0 - 55.0	
HGB	=	15.8	g/dl	12.0 - 18.0	
MCHC	=	33.2	g/dl	30.0 - 36.9	
WBC	=	11.8	x10⁹/L	6.0 - 16.9	
GRANS	=	9.5	x10⁹/L	3.3 - 12.0	
%GRANS	=	81	%		
NEUT	~	7.8	x10⁹/L	2.8 - 10.5	
EOS	~	1.7	x10⁹/L	0.5 - 1.5	
L/M	=	2.3	x10⁹/L	1.1 - 6.3	
%L/M	=	19	%		
PLT	=	341	x10⁹/L	175 - 500	



Buffy Coat Profile 2: Normal Cat

Species: Cat

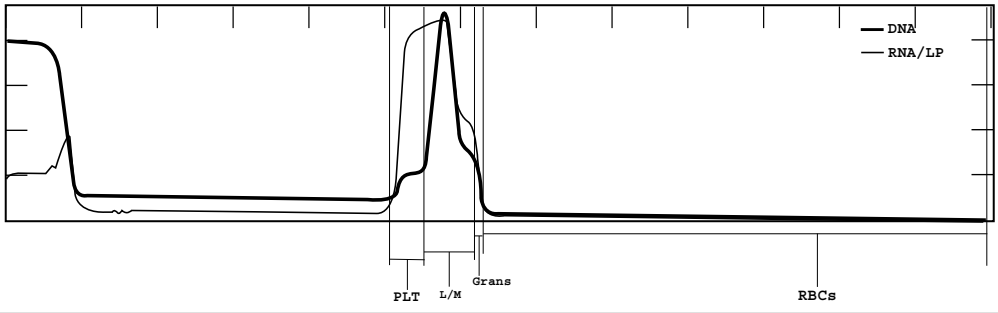
Test		Results	Units	Reference Range			Indicator (LOW / NORMAL / HIGH)
HCT	=	37.5	%	24.0	-	45.0	
HGB	=	12.8	g/dl	8.0	-	15.0	
MCHC	=	34.1	g/dl	30.0	-	36.9	
WBC	=	6.5	x10⁹/L	5.0	-	18.9	
GRANS	=	4.4	x10⁹/L	2.5	-	12.5	
%GRANS	=	68	%				
L/M	=	2.1	x10⁹/L	1.5	-	7.8	
%L/M	=	32	%				
PLT	=	549	x10⁹/L	175	-	500	



Buffy Coat Profile 3: Normal Horse

Species: Horse

Test		Results	Units	Reference Range			Indicator (LOW / NORMAL / HIGH)
HCT	=	35.2	%	32.0	-	52.0	
HGB	=	12.3	g/dl	11.0	-	19.0	
MCHC	=	34.9	g/dl	30.0	-	36.9	
WBC	=	6.9	x10⁹/L	6.0	-	12.5	
GRANS	=	3.7	x10⁹/L	2.8	-	8.0	
%GRANS	=	54	%				
L/M	=	3.2	x10⁹/L	2.1	-	7.0	
%L/M	=	46	%				
PLT	=	154	x10⁹/L	90	-	350	

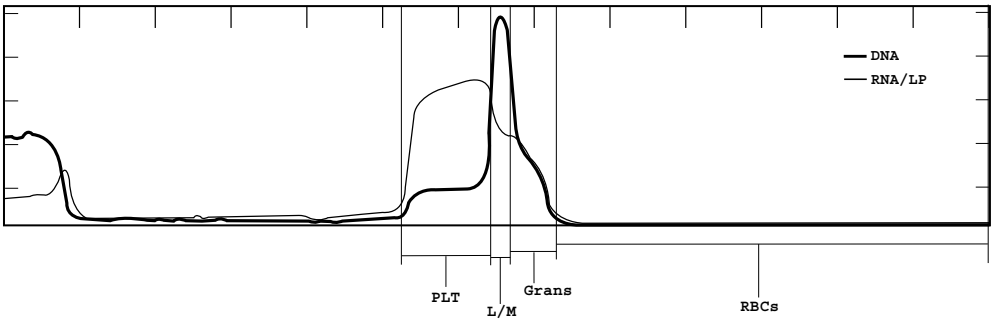


Buffy Coat Profile 4: Normal Cow

Species: Cow

Test		Results	Units	Reference Range			Indicator (LOW / NORMAL / HIGH)
HCT	=	31.9	%	25.0	-	42.0	
HGB	=	11.1	g/dl	8.0	-	14.0	
MCHC	=	34.8	g/dl	27.0	-	34.0	
WBC	=	7.6	x10⁹/L	4.0	-	12.0	
GRANS	=	2.9	x10⁹/L	2.0	-	6.0	
%GRANS	=	38	%				
NEUT	~	2.2	x10⁹/L	1.5	-	4.0	
EOS	~	0.7	x10⁹/L	0.5	-	1.5	
L/M	=	4.7	x10⁹/L	3.0	-	7.5	
%L/M	=	62	%				
PLT	=	365	x10⁹/L	175	-	500	

Buffy Coat Profile

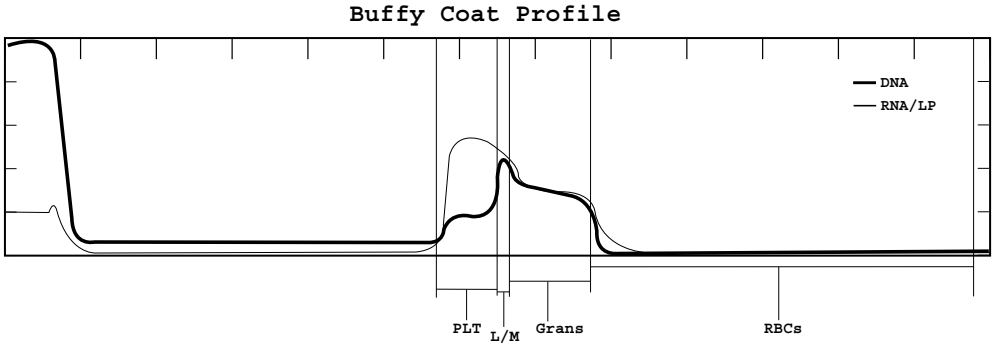


Buffy Coat Profile 5: Nonregenerative Anemia—Mild

This dog is anemic, with an Hct of 23.3%. There is a reticulocyte count of 0.6%. A rise in RNA indicates that a small population of reticulocytes are present. Since this dog needs a reticulocyte count of >2.0% to begin to replace the RBCs, it is nonregenerative. The buffy coat profile is clear and typical. There is a normal granulocyte population at 9.3 x 10^9/L, and reasonable platelets at 136 x10^9/L.

Species: Dog

Test		Results	Units	Reference Range			Indicator
							LOW NORMAL HIGH
BUN	=	56	mg/dl	5.0	-	23.0	
CREA	=	6.5	mg/dl	0.40	-	1.50	
PHOS	=	7.2	mg/dl	2.50	-	5.50	
HCT	=	23.3	%	37.0	-	55.0	
HGB	=	8.4	g/dl	12.0	-	18.0	
MCHC	=	36.1	g/dl	30.0	-	36.9	
WBC	=	11.0	x10^9/L	6.0	-	16.9	
GRANS	=	9.3	x10^9/L	3.3	-	12.0	
%GRANS	=	85	%				
NEUT	~	8.6	x10^9/L	2.8	-	10.5	
EOS	~	0.7	x10^9/L	0.5	-	1.5	
L/M	=	1.7	x10^9/L	1.1	-	6.3	
%L/M	=	15	%				
PLT	=	136	x10^9/L	175	-	500	
Retics	~	0.6	%				

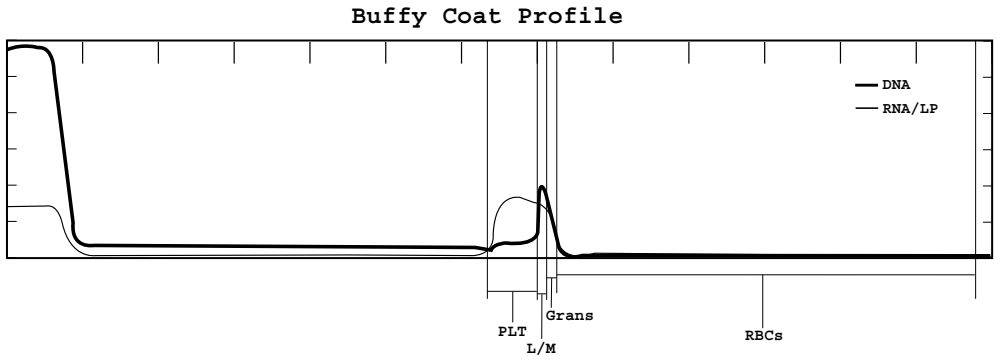


Buffy Coat Profile 6: Nonregenerative Anemia—Pancytopenia

This cat, with an Hct of 19.6%, is anemic. There are no reticulocytes, indicating this anemia is nonregenerative. There is also a granulocytopenia at 1.1 x10^9/L and a thrombocytopenia at 77 x10^9/L. All the cells made in the bone marrow depressed. This is pancytopenia. The buffy coat profile shows a very small, but typical, pattern.

Species: Cat

Test	Results	Units	Reference Range	Indicator (LOW / NORMAL / HIGH)
HCT	19.6	%	24.0 - 45.0	
HGB	6.2	g/dl	8.0 - 15.0	
MCHC	31.6	g/dl	30.0 - 36.9	
WBC	3.4	x10^9/L	5.0 - 18.9	
GRANS	1.1	x10^9/L	2.5 - 12.5	
%GRANS	32	%		
L/M	2.3	x10^9/L	1.5 - 7.8	
%L/M	68	%		
PLT	77	x10^9/L	175 - 500	



Buffy Coat Profile 7: Disseminated Intravascular Coagulation (DIC)

Several results on this dog are flagged (see appendix A). The area flagged is the RBC/granulocyte interface. Flagging the RBC/granulocyte interface determination will automatically flag the WBC count and the % of the populations. The Hct shows an anemia at 18.5%, with an appropriate Hgb at 6.1 g/dL. The WBC count, the granulocyte count and the percentages all exhibit flags. The L/M population was read without flags.

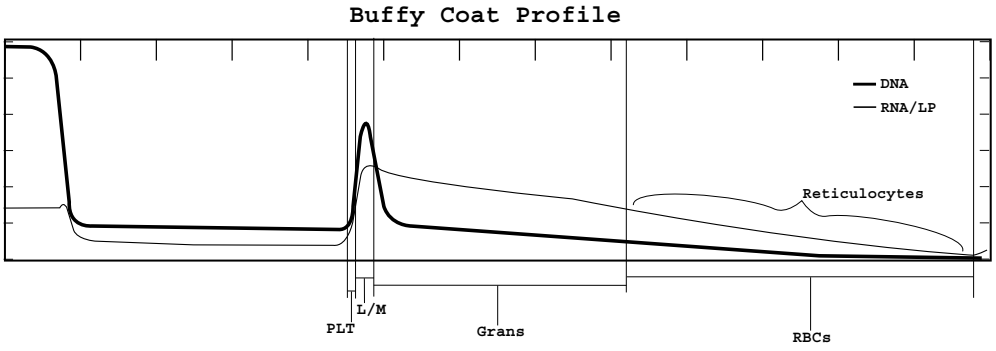
Follow the buffy coat profile from right to left along the RBC population. There is an immediate population of reticulocytes present that appears to extend off the float. About 1/3 of the way down the RBC area, a gradual increase of the DNA line begins. This is not typical of the abrupt increase seen when the granulocyte population is reached. The only cells that contain DNA in the RBCs are nRBCs. The area where the IDEXX VetAutoread Hematology Analyzer marked the beginning of the Grans is typical of the DNA granulocyte fluorescence usually seen when granulocytes are identified. The designation of 28.7 x10^9/L Grans may not be exact. Always confirm the presence and amount of nRBCs by a stained blood film.

This dog also had a low fibrinogen and a low total protein. This was a case of hemolytic and possible blood-loss anemia associated with DIC. The blood film contained nRBCs, including metarubricytes, rubricytes and prorubricytes, as well as many polychromatophils that correlate with the reticulocytosis noted.

Anytime it is suspected that nRBCs are present from the gradual increase of DNA instead of the abrupt climb of the granulocyte DNA—even if no flags are present—the operator should confirm the placement of the division between nRBCs and granulocytes by viewing a stained blood film.

Species: Dog

Test		Results	Units	Reference Range			Indicator (LOW / NORMAL / HIGH)
TP	=	3.5	g/dl	5.00	-	8.20	
HCT	=	18.5	%	37.0	-	55.0	
HGB	=	6.1	g/dl	12.0	-	18.0	
MCHC	=	33.0	g/dl	30.0	-	36.9	
WBC	#	31.2	x10^9/L	6.0	-	16.9	
GRANS	#	28.7	x10^9/L	3.3	-	12.0	
%GRANS	#	92	%				
L/M	=	2.5	x10^9/L	1.1	-	6.3	
%L/M	#	8	%				
PLT	=	21	x10^9/L	175	-	500	
Retics	>	4.0	%				
NRBCs		likely					



Buffy Coat Profiles 8–9: Granulocytopenia/Leukopenia

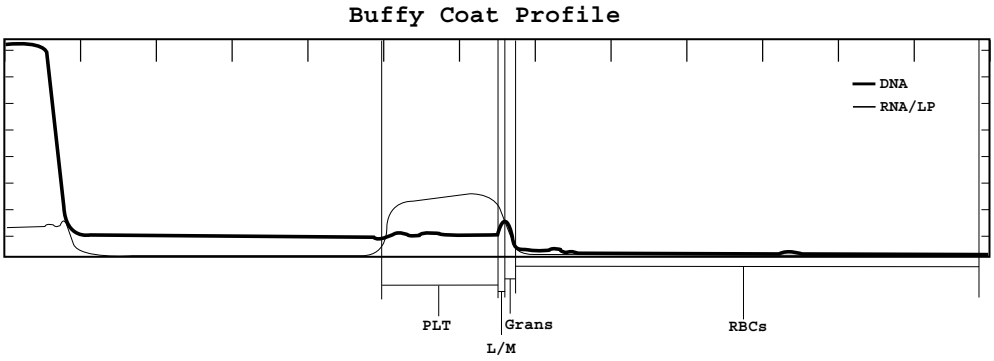
Two examples of granulocytopenia and leukopenia caused by parvovirus

Buffy Coat Profile 8: Granulocytopenia

The L/Ms are low normal, but the granulocytes are very low.

Species: Dog

Test		Results	Units	Reference Range			Indicator
							LOW NORMAL HIGH
HCT	=	37.4	%	37.0	-	55.0	
HGB	=	12.9	g/dl	12.0	-	18.0	
MCHC	=	34.5	g/dl	30.0	-	36.9	
WBC	=	2.1	x10^9/L	6.0	-	16.9	
GRANS	=	1.0	x10^9/L	3.3	-	12.0	
%GRANS	=	48	%				
L/M	=	1.1	x10^9/L	1.1	-	6.3	
%L/M	=	52	%				
PLT	=	269	x10^9/L	175	-	500	



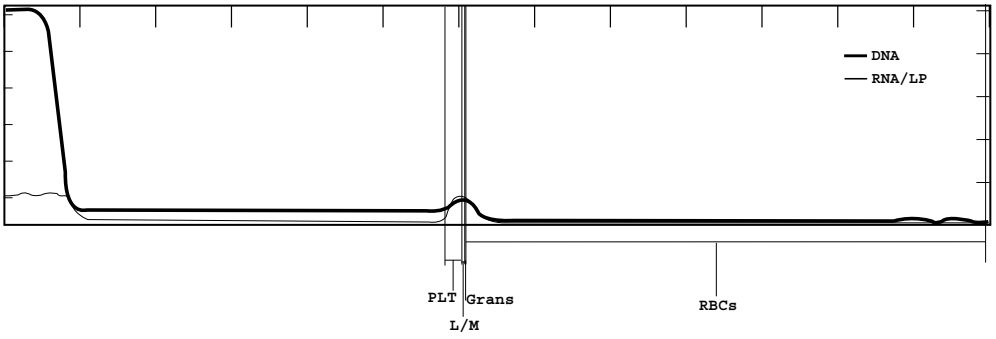
Buffy Coat Profile 9: Leukopenia

The cell populations have become so small that they cannot be repeated with consistency. Both the WBC profile and the platelet profile are severely diminished. It is difficult to determine the granulocytes from the L/Ms. Although the results are flagged, the populations and their totals are pathologically and critically depressed.

Species: Dog

Test		Results	Units	Reference Range			Indicator (LOW / NORMAL / HIGH)
HCT	=	29.3	%	37.0	-	55.0	
HGB	=	10.3	g/dl	12.0	-	18.0	
MCHC	=	35.2	g/dl	30.0	-	36.9	
WBC	#	.7	x10^9/L	6.0	-	16.9	
GRANS	#	.2	x10^9/L	3.3	-	12.0	
%GRANS	#	29	%				
L/M	#	.5	x10^9/L	1.1	-	6.3	
%L/M	#	71	%				
PLT	#	44	x10^9/L	175	-	500	

Buffy Coat Profile



Buffy Coat Profile 10: Granulocytosis with Monocytosis

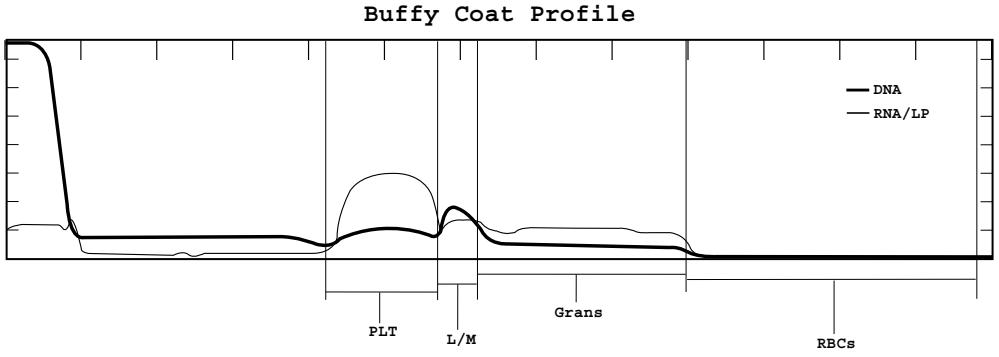
The blood count shows a leukocytosis with a granulocytosis and a borderline lymphocytosis/ monocytosis.

Examine the L/M layer. The shape of the L/M population as defined by the DNA line is different from the other L/M populations that normally appear. Instead of having a peak with two symmetrical sides, the right side of the L/M population shows a gentle slope while the left side of the population shows a steeper slope typical of most L/M populations. The monocyte layer is between the lymphocytes and the granulocytes. The gentle slope on the right is typical of the presence of monocytes in significant numbers. Confirm the results by examining a stained blood film.

This was a case of open pyometra. Although the granulocyte count was increased, it was not markedly high because most of the cells had migrated out of the infected uterus. The signs of marked inflammation—left shift noted on the blood film evaluation, monocytosis, rouleaux and high fibrinogen—defined the seriousness of the animal's state. In a closed pyometra, there is a locally intense accumulation of neutrophils within the uterus, and in many cases, there is a marked increase in neutrophils.

Species: Dog

Test	Results	Units	Reference Range	Indicator (LOW / NORMAL / HIGH)
HCT	33.9	%	37.0 - 55.0	
HGB	11.8	g/dl	12.0 - 18.0	
MCHC	34.8	g/dl	30.0 - 36.9	
WBC	28.2	x10⁹/L	6.0 - 16.9	
GRANS	22.0	x10⁹/L	3.3 - 12.0	
%GRANS	78	%		
L/M	6.2	x10⁹/L	1.1 - 6.3	
%L/M	22	%		
PLT	233	x10⁹/L	175 - 500	

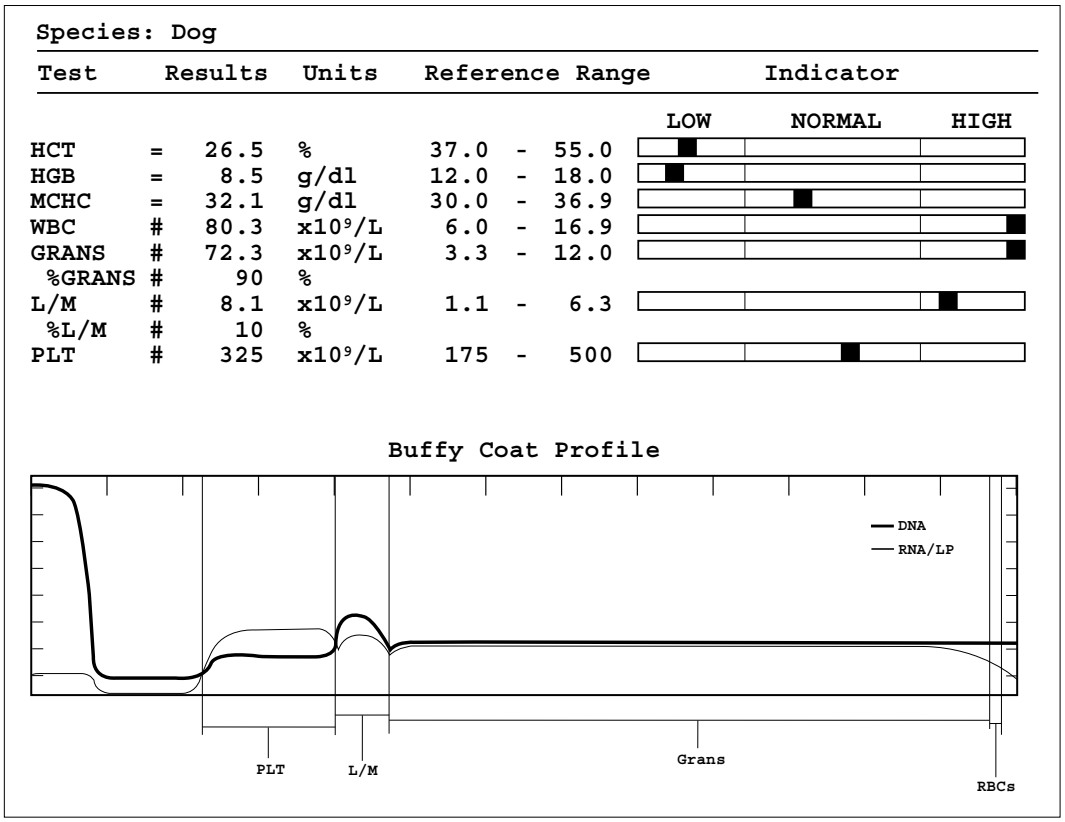


Buffy Coat Profiles 11–12: Leukocytosis Exceeding the Length of the Float

Starting from right to left, the DNA indication is never on the baseline. The DNA falls off the end of the float to the right, indicating the possibility of DNA-containing cells extending beyond the beginning of the float in the packed area of the RBCs. This can occur when there is incomplete separation of cell populations or when there are more DNA-containing cells (WBCs) than the float can hold. Examine the peripheral blood film to confirm.

The sample should be diluted (see Appendix B) with an equal portion of normal saline to reduce the number of cells to be analyzed. The larger the portions, the smaller the diluting error—use between 0.5 cc and 1 cc of each.

Buffy Coat Profile 11: Leukocytosis Exceeding the Length of the Float



Buffy Coat Profile 12: Leukocytosis Exceeding the Length of the Float (after Dilution)

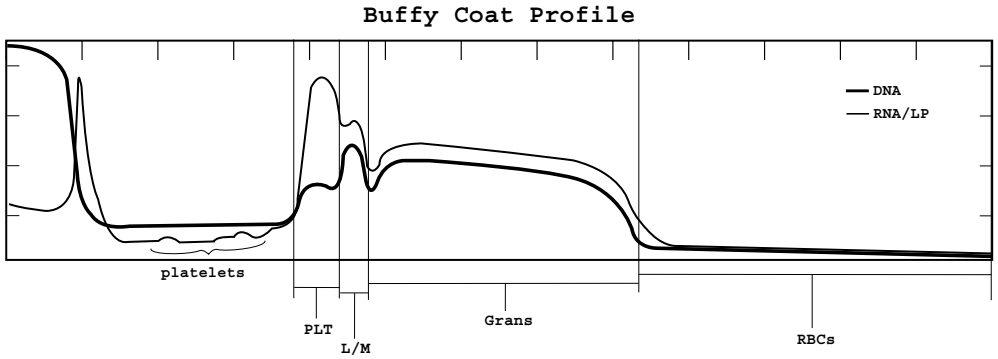
After dilution, a recognizable buffy coat profile is now present. There is a well-defined WBC population and a platelet population with some platelets aggregated at the end of the float. The Hct was 26.5% before the 1:1 dilution. It is now 13.9%. The dilution was well-executed. Multiply all the absolute numbers and platelets by 2x. Thus, the WBC count becomes 70.6 $x10^9$/L, the Grans count is 62.8 $x10^9$/L and the L/M is 7.8 $x10^9$/L. There is a slope to the right side of the L/M layer, suggesting the presence of monocytes, and this should be confirmed on the blood film. The platelets are greater than 116 $x10^9$/L times 2. Therefore, there are more than 232 $x10^9$/L platelets. Unless the platelets have been rising or falling, there is no need to go further.

Whenever it appears that DNA-containing cells may have extended beyond the limits of the float, do a dilution. In this case, there was also a large L/M population. If the L/M population had been <2.0 $x10^9$/L before dilution, the dilution could have lowered the size of the population to the point where it would have been flagged on the diluted sample. In that case, estimate the L/M population from the original buffy coat profile.

Use the Hct as a control for the accuracy of the dilution. Double all the absolute numbers and double the platelets. WBC counts >60.0 $x10^9$/L may exceed the float length.

Species: Dog

Test		Results	Units	Reference Range			Indicator (LOW / NORMAL / HIGH)
HCT	=	13.9	%	37.0	-	55.0	
HGB	=	4.4	g/dl	12.0	-	18.0	
MCHC	=	31.7	g/dl	30.0	-	36.9	
WBC	=	35.3	$x10^9$/L	6.0	-	16.9	
GRANS	=	31.4	$x10^9$/L	3.3	-	12.0	
%GRANS	=	89	%				
L/M	=	3.9	$x10^9$/L	1.1	-	6.3	
%L/M	=	11	%				
PLT	>	116	$x10^9$/L	175	-	500	



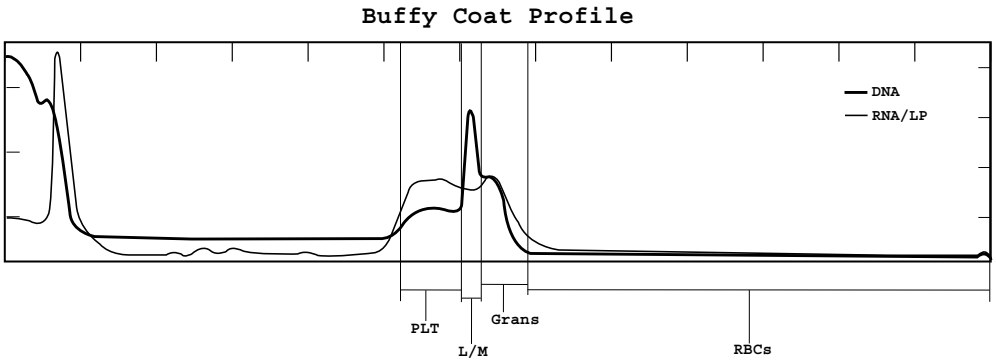
Buffy Coat Profile 13: Clumped Platelets

The IDEXX VetAutoread Hematology Analyzer measures platelets in the buffy coat starting at the end of the L/M area, moving left until the platelet population ends and the float area containing only plasma begins. In this analysis, the platelet count is indicated as >113 x10^9/L. There are platelets stacked on the left end of the float and, while they cannot be measured, those platelets are indicated by the “>” sign.

The platelet stack on this cat probably occurred because the blood draw was hurried, causing some activation and clumping of platelets.

Species: Cat

Test		Results	Units	Reference Range			Indicator
							LOW NORMAL HIGH
HCT	=	49.3	%	24.0	-	45.0	
HGB	=	14.9	g/dl	8.0	-	15.0	
MCHC	=	30.2	g/dl	30.0	-	36.9	
WBC	=	7.1	x10^9/L	5.0	-	18.9	
GRANS	=	4.8	x10^9/L	2.5	-	12.5	
%GRANS	=	68	%				
L/M	=	2.3	x10^9/L	1.5	-	7.8	
%L/M	=	32	%				
PLT	>	113	x10^9/L	175	-	500	



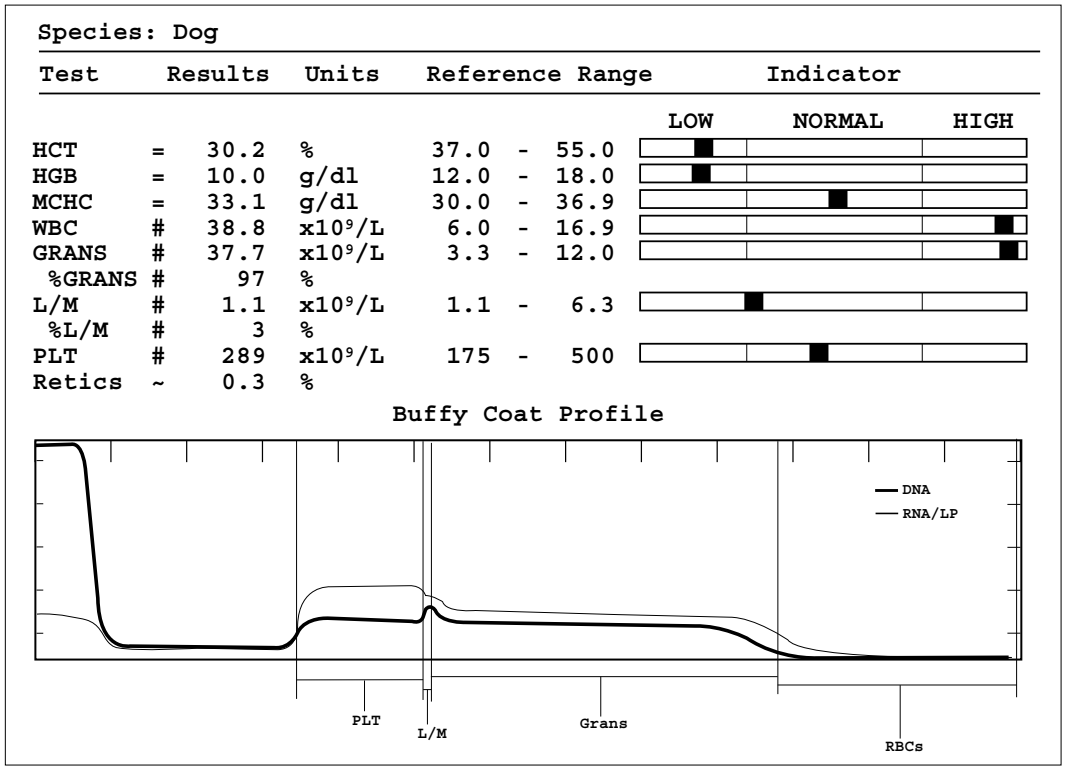
Buffy Coat Profile 14: Buffy Coat 3 or 6 Flags

Buffy coat 3 flags or buffy coat 6 flags will occur when the borders are indistinct between one or more of the WBC populations and platelets. The buffy coat profile should be examined for clinically useful information and to identify where the flags suggest the operator needs to expand the investigation of the sample. All information regarded as clinically useful should be confirmed by examination of a peripheral blood film.

The L/M layer may be indistinct because all the L/Ms may not have completed layering, allowing them to invade other layers.

Analyze this buffy coat profile starting from the right side. There is a normal baseline signal to start the RBC area. A rise in RNA indicates a small population of reticulocytes are present. The rise in the DNA of the granulocytes is clear. The granulocyte area is longer than normal, indicating an increase in cells. At the far left of the granulocyte area, just before the L/M indication, a change in the slope is seen.The DNA on the buffy coat profile begins to increase in the granulocyte area and appears to peak at the questioned beginning of the L/Ms. It continues and begins its downward slope in the indicated L/M area. As the DNA falls, the lipoprotein rises into a typical platelet layer. The length of the granulocyte population is consistent with a numerical population of 37.7 $x10^9$/L and is clinically acceptable. The platelet length is consistent with a count of 289 $x10^9$/L. There is a granulocytosis with a normal platelet count.

While you should respin the tube, you should also do a blood film to confirm the possible increase in either monocytes or banded neutrophils (Plate #6) and their significance.

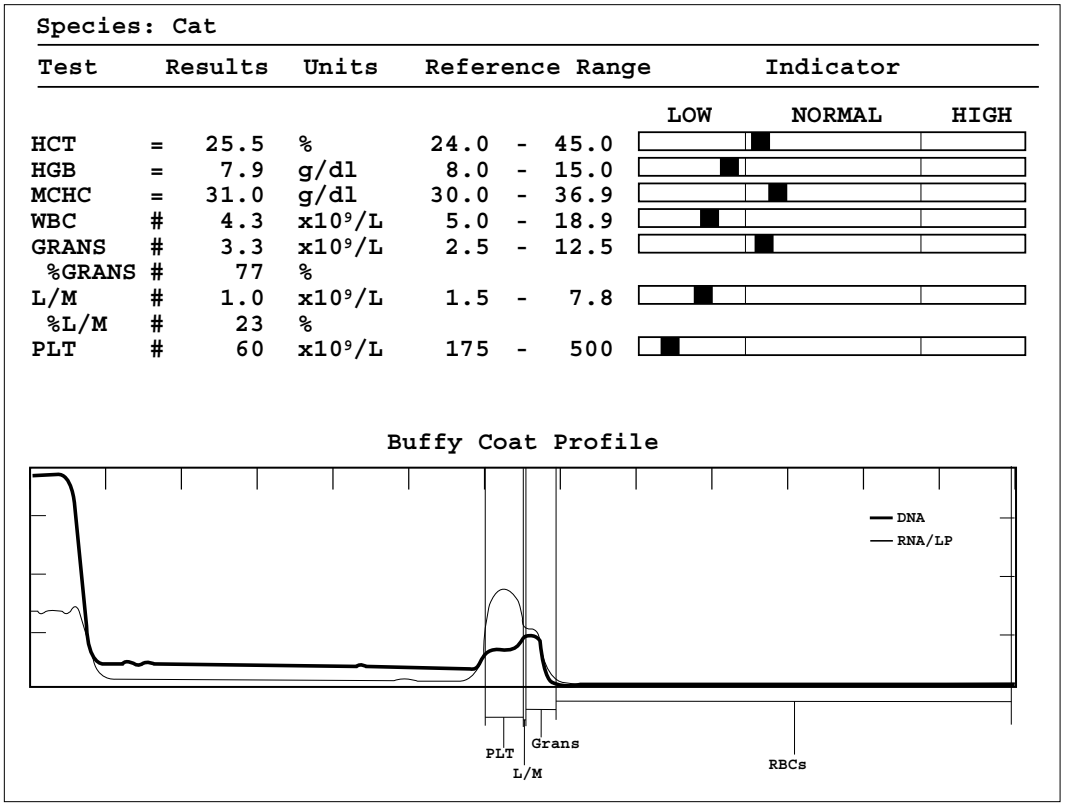


Buffy Coat Profile 15: Buffy Coat 1 with a Single WBC Population

The WBCs, granulocytes, L/Ms and platelets all have # flags present. In this buffy coat profile, one of the WBC populations is “missing,” and it is the L/M population. “Missing” L/Ms affect all of the parameters because the boundaries of the L/M affect the granulocytes and the platelets. Look at the buffy coat profile.

The first observation is that there appears to be only one WBC population. The IDEXX VetAutoread Hematology Analyzer has identified the population as being granulocytes, but because it does not have a reasonable size population of L/Ms to compare the DNA binding to, it flags the results. The L/M population, which is not clearly seen in the buffy coat profile, is calculated to 1.0 x10^9/L. The platelets are flagged because the division between the nonexistent L/Ms is vague. The L/Ms may well be less than 1.0 x10^9/L, but the effect on the platelets is clinically insignificant. A simple blood film confirms the analysis. Scan the blood film to see which is the major population and confirm that the other populations are severely depressed.

In this case, a manual differential was done, and the granulocytes were 98% and the L/Ms were 2%.



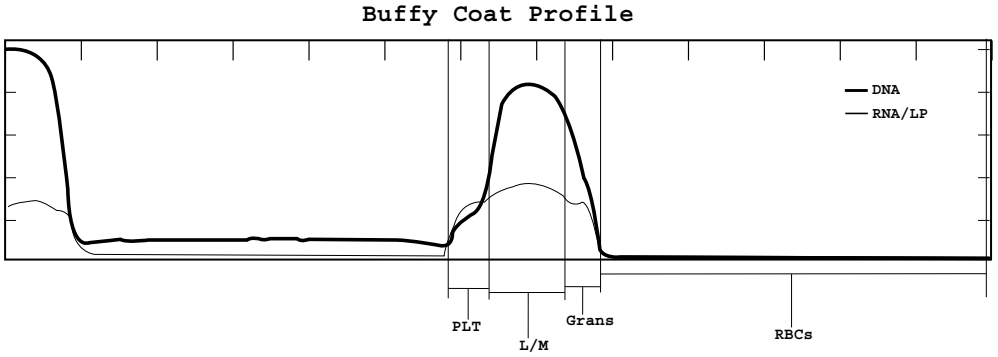
Buffy Coat Profile 16: Acute Lymphoid Leukemia

The buffy coat profile on this cat is dominated by an L/M population. The right and left sides of the L/M area are steep and symmetrical. This suggests that the cells are basically all lymphocytes. The blood film showed large, atypical cells. The cat had lymphoblastic leukemia.

When one population dominates an increase in the L/M population, the buffy coat profile does not indicate which population it is. A requisite examination of a peripheral blood film quickly identifies the dominant population and finalizes the diagnosis (Plate #15).

Species: Cat

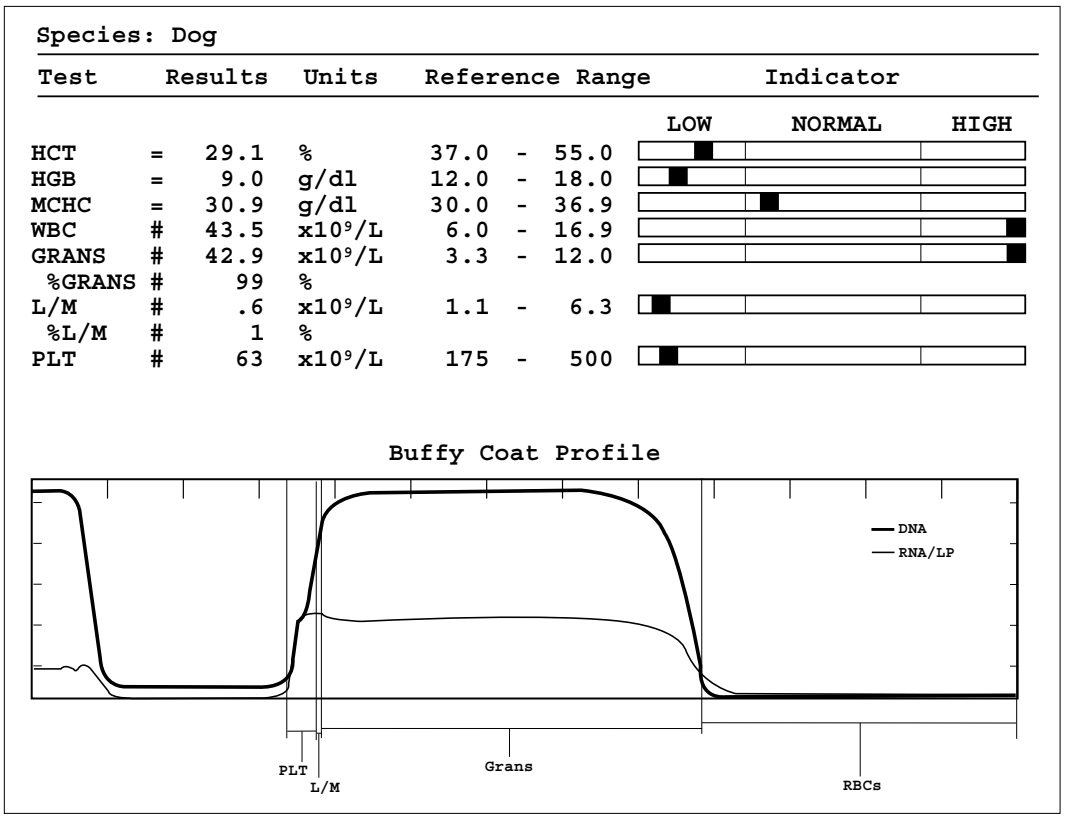
Test	Results	Units	Reference Range	Indicator (LOW / NORMAL / HIGH)
HCT =	11.1	%	24.0 - 45.0	
HGB =	3.4	g/dl	8.0 - 15.0	
MCHC =	30.6	g/dl	30.0 - 36.9	
WBC =	25.1	x10^9/L	5.0 - 18.9	
GRANS =	4.6	x10^9/L	2.5 - 12.5	
%GRANS =	18	%		
L/M =	20.5	x10^9/L	1.5 - 7.8	
%L/M =	82	%		
PLT =	67	x10^9/L	175 - 500	



Buffy Coat Profile 17: Undifferentiated Leukemia

There is a single population in which the intensity of the DNA binding appears extreme. The IDEXX VetAutoread Hematology Analyzer has identified this as granulocytes and flagged the results. The L/Ms may be low or nonexistent. The platelets are reduced.

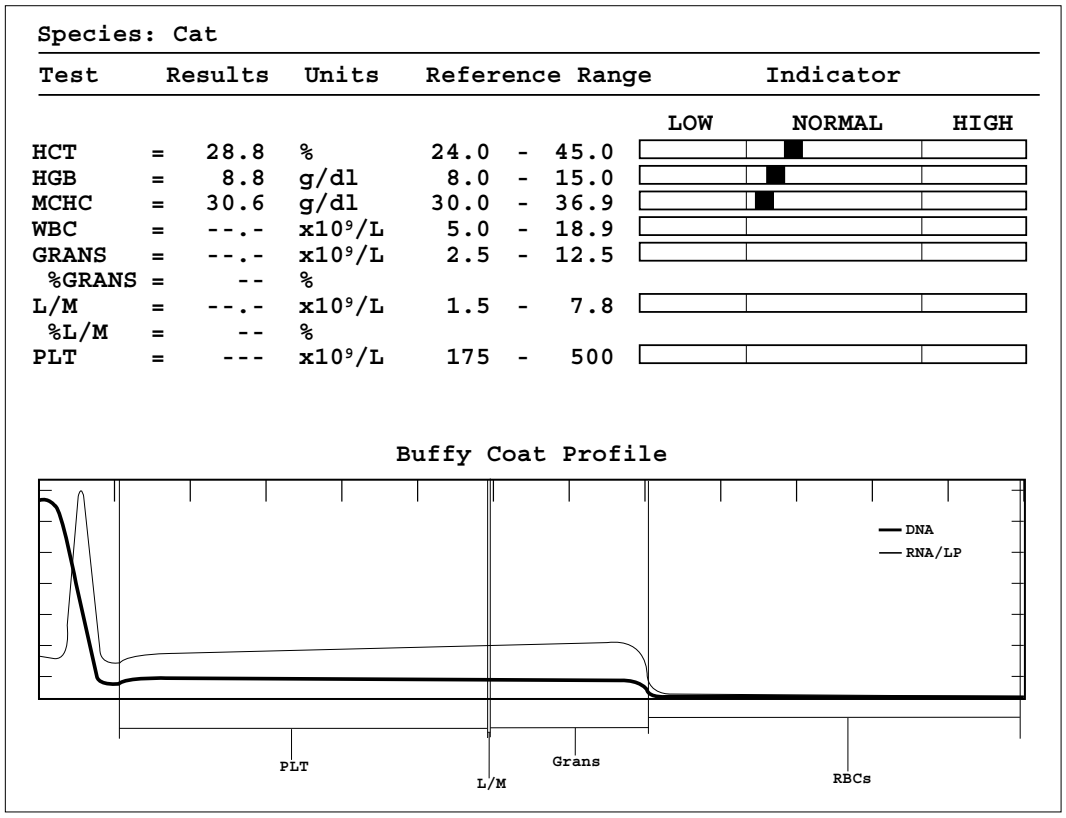
A peripheral blood film was examined (Plate #19). The cells were difficult to classify, but were large and contained numerous malignant criteria. The film was sent to a pathologist for an opinion. The cells were described as large, pleomorphic, unclassifiable blasts. A diagnosis of acute blastic leukemia of undetermined cell lineage was given.



Buffy Coat Profile 18: Feline Panleukopenia

From right to left, there is a normal RBC baseline without reticulocytes or nRBCs. The first population to appear is dominated by RNA/LP binding. Follow the RNA/LP population to the end of the float, where it ends in a typical platelet stack. This is a single population of platelets that contains only lipoprotein. There are no DNA populations, indicating there are no WBCs present. All single populations will result in flags or no reported results. The IDEXX VetAutoread Hematology Analyzer will always print out the buffy coat profile of the analysis, allowing the operator to carefully assess the presence or absence of cell populations in the buffy coat profile.

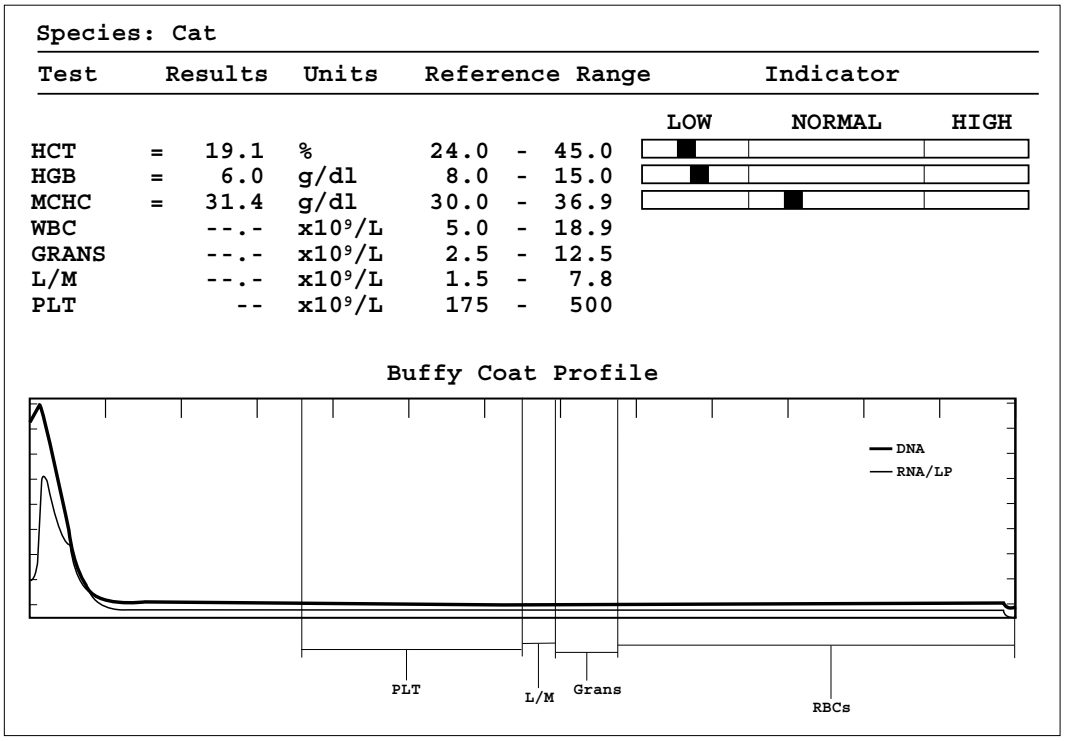
A blood film was examined. There was only one lymphocyte per 15 100x objective microscopic fields and no granulocytes. The kitten had feline panleukopenia (feline distemper).



Buffy Coat Profile 19: Results Absent Due to a Single Dominant Population Exceeding the Float Length

No results are reported on this blood count other than the RBC parameters.

On the far right of the buffy coat profile, the DNA line is above the baseline at the beginning of the analysis. This DNA signal continues basically unchanged until it reaches the end of the float on the far left, where it increases as a DNA fluorescence continuing above the float. This suggests a single population of WBCs that extends beyond the length of the float. Using a dilution (see Appendix B) it was determined that the single population was >150.0 x10^9/L cells. Examination of a peripheral blood film is necessary to identify the cell population (Plate #16). The cat had an acute lymphoid leukemia.



Appendix A: Buffy Coat Profile Alert Messages

When the properties of a blood sample have affected the reliability of the results, the IDEXX VetAutoread Hematology Analyzer is programmed to display and print alert messages that identify the parameters in question.

Values affected will have a "#" symbol next to them and should be verified by inspection of the buffy coat profile or an examination of a stained blood film. When platelet aggregation or cell clumping is the cause of a sample alert message, the sample must be redrawn to obtain accurate values.

Buffy Coat 1 Alert Message:

Granulocytes have not separated cleanly from the lymphocyte/monocyte layer and RBC layer. Confirm results with a blood film.

Buffy Coat 3 Alert Message:

The lymphocyte/monocyte layer has not separated distinctly from the other cell layers, possibly due to inadequate staining. Confirm differential results with a blood film.

Buffy Coat 4 Alert Message:

The buffy coat layers are inconsistent due to clumped platelets, granulocytes, an expired tube or a stain on the tube exterior. Remove the tube, carefully clean the exterior and retest. If the situation persists, obtain a fresh sample and retest.

Buffy Coat 6 Alert Message:

The buffy coat cell layers inconsistent. Respin the sample and retest. If the situation persists, redraw from the existing sample and retest. Examine the buffy coat profile to confirm any results accompanied by a # symbol.

Granulocytes 1 Alert Message:

The red cells have not separated cleanly from the granulocytes. To confirm granulocyte results, verify the proper location of the RBC/granulocyte boundary on the buffy coat profile or examine a blood film.

Hgb 1 Alert Message:

The presence of either immature RBCs or cells on top of the float may affect the hemoglobin measurement. To confirm this result, retest the sample using a 1:1 dilution with saline (see Appendix B) and review the RBC morphology.

PLT 1 Alert Message:

Platelets were found on top of float, possibly due to collection technique or the blood sample being more than 90 minutes old. The reported value represents the lower limit of the actual platelet number. If the situation persists, obtain a fresh sample and retest within 90 minutes.

Appendix B: Performing a Dilution

Use a 1:1 dilution of patient sample and normal saline. Use 0.5–1.0 cc of each to reduce error. Patient plasma can also be used in place of normal saline. Saline may cause platelet clumping, which will affect the platelet result.

Multiply all values—except the MCHC and WBC percentages—by 2 for correct results.

Appendix C: Hematology Techniques

Drawing a Quality Sample

All blood analysis requires a quality sample to produce quality results. This is especially true in hematology. Adequate samples can be obtained using a Becton Dickinson Vacutainer® Blood Collection System or a needle and syringe.

It is important to use a new syringe to preserve the quality of the sample. Syringes that have been washed do not have good suction, and any remaining moisture can damage cells or alter the sample. Ideally, the largest accessible vein that will allow free flow of blood should be used as the collection site. If a needle and syringe are used instead of a Vacutainer tube, draw back slowly, no faster than blood could flow naturally through the vein. If the flow stops, it may be because the vein has collapsed into the needle from too strong a draw. As a result, platelets can aggregate, forming of microclots that act as artifacts and can artificially lower platelet, RBC and WBC counts. Always draw enough sample to fill the EDTA tube. Inadequate filling of the EDTA tube will result in dilution of the sample and artificial lowering of cell counts. To transfer the blood from the syringe to the EDTA tube, let the vacuum draw the sample into the tube. If a small-gauge needle is used or hemolysis is a consistent problem, remove the rubber stopper from the tube and the needle from the syringe prior to expelling the blood into the tube. Mix gently and mix well.

Making and Viewing Blood Films

The IDEXX VetAutoread Hematology Analyzer requires the same commitment that any hematology analyzer requires: the user must understand the histogram of the instrument and be able to determine cell morphology. To accurately read and understand cell morphology requires a quality blood film.

To assist you in reading films, morphology examples of some of the more commonly occurring clinical conditions have been reproduced in Appendix G.

A blood film is an important complement to an automated CBC. Follow these easy steps to prepare a high-quality blood film.

1. Place a small drop of blood on a clean glass slide approximately 2 cm from one end of the slide.
2. Place a clean glass “spreader” slide in front of the drop of blood at an angle approximately 30° to the blood-film slide and back the “spreader” slide into the drop of blood.
3. Let the blood spread along the contact line between the two slides; this should take place quickly.
4. With a steady fluid movement, move the spreader slide down the entire blood-film slide, maintaining the 30° angle without lifting the spreader slide. Blood from the drop will follow the spreader slide, placing a thin film of blood on the other slide. The blood film should be 3–4 cm in length.
5. Let the blood film air-dry. Only apply forced air-drying when in a humid environment.

For specimens with low hematocrits (anemia), increase the angle between the slides to make a thicker blood smear. For specimens with high hematocrits (dehydration, polycythemia, etc.), decrease the angle between the slides to make a thinner blood smear.

Let the slide air dry and stain it with a Romanowsky-type stain, a modified “quick” stain or a Wright's Giemsa stain. After staining, allow the slide to air dry again. Do not wipe the slide as that can blur images, remove cells and add debris. Place the slide under a microscope on low power and scan for general impressions. Scan the feathered edge, the outer edges and the drop area for large cells (i.e., blasts, bands, monocytes, mast cells) and make note of them for future reference. Then, focus at high dry for detailed examination. Also, view the slide for clumps of cells, such as platelet clumps and WBC clumps that can alter cell counts. Then focus on high magnification for detailed examination and enumeration of the differential cell count. When performing cell counts, evaluate

cell morphology to the area behind the feathered edge where there is a monolayer of cells (a monolayer is defined as the area of the slide where approximately 50% of the red blood cells are touching). This will aid in correct identification of the cells.

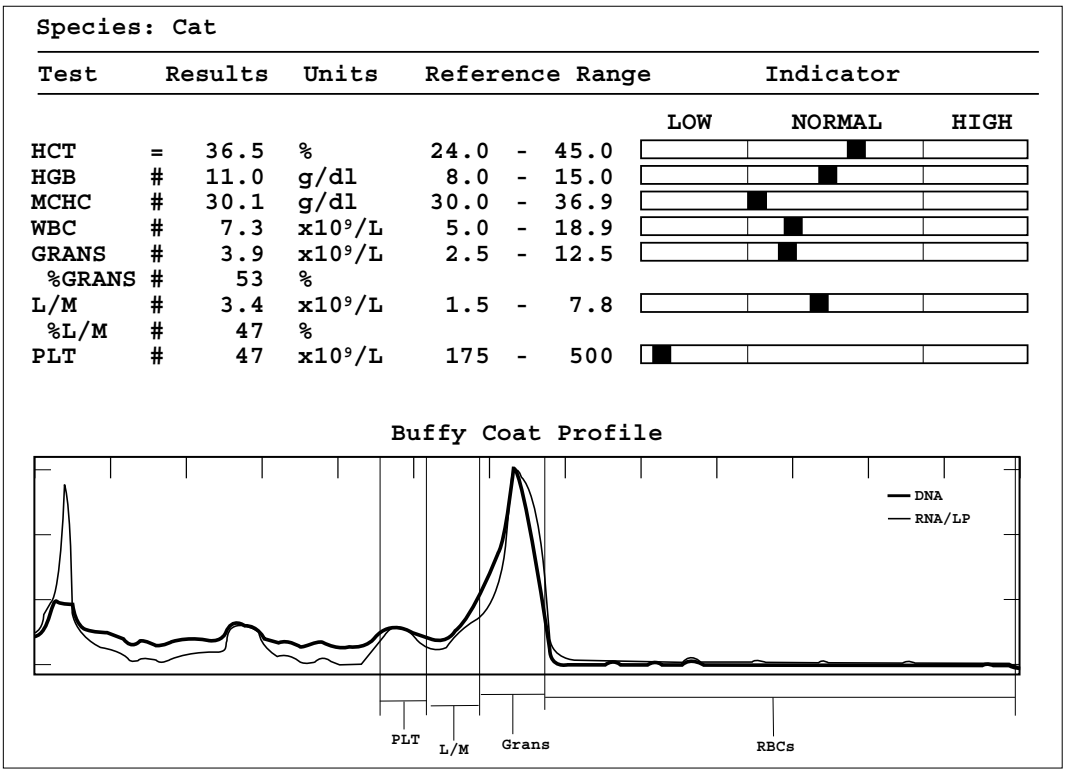
When performing counts, it is ideal to move in a battlement pattern—three fields over and three fields up—three across and three down. A 100-cell differential can quickly be counted, segregating the cells into neutrophils, lymphocytes, monocytes, eosinophils, basophils and others (blasts, mast cells). Neutrophils should be further categorized as segmented, bands or immature. Exact classification of immature neutrophils beyond the band state into metamyelocytes, myelocytes and promyelocytes is not necessary. Just determine the degree of left shift in cases of inflammation and make sure all of the correct maturation stages are present to rule out maturation abnormalities as seen with leukemia. After the count is complete, compare it to the low power scan to make sure any large cells have been accounted for accurately.

Morphology of WBCs should be examined for signs of toxicity to include the presence of Döhle bodies, basophilic and vacuolated cytoplasm, and toxic granulation. RBC morphology should be evaluated for staining intensity (central pallor, hypochromasia, polychromasia, spherocytes), size (microcytes, polychromatophils, macrocytes, spherocytes), shape (acanthocytes, schistocytes) and parasites (*Mycoplasma, Babesia, Cytauxzoon, Anaplasma*).

Appendix D: Damaged Samples

Buffy Coat Profile 20: Damaged Samples

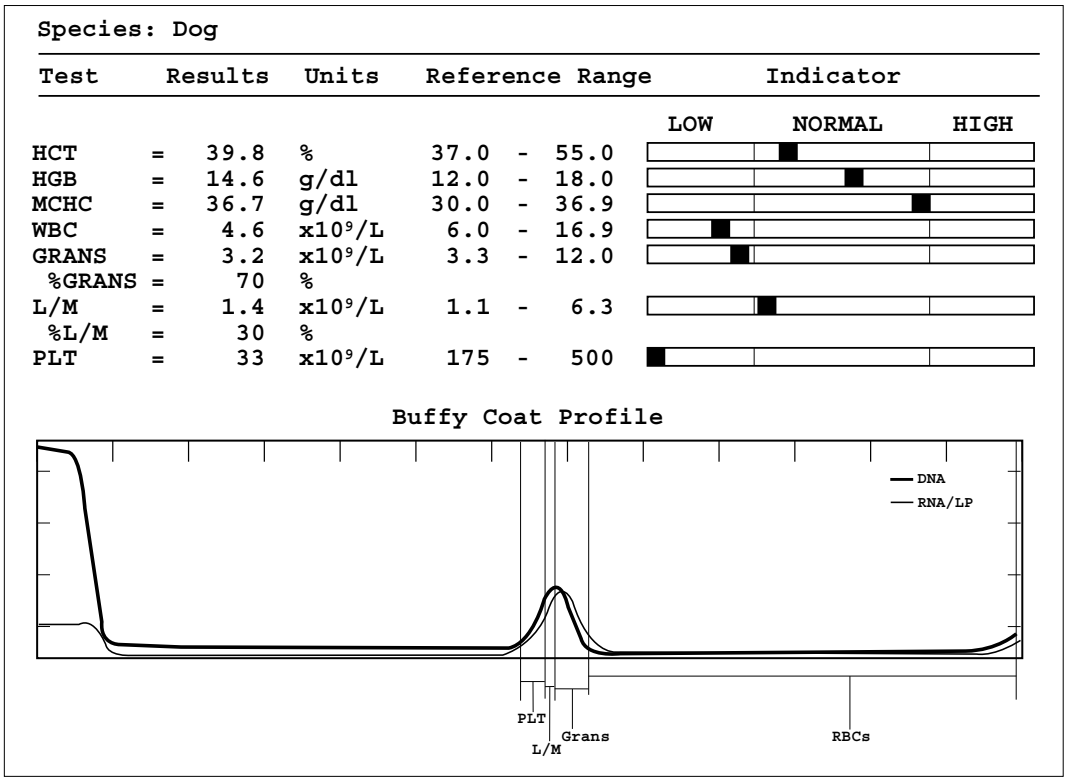
Compare the buffy coat profile below to the normal patterns of the previous buffy coat profiles. There is no similarity to any normal pattern. From right to left, the RBC area has small bumps of both DNA and RNA/LP and the entire area is above the baseline. Moving to the left, the first population seen has a very high rise in DNA and RNA/LP, and is not typical of the granulocytes seen in normal buffy coat profiles. Following this population, there is a series of rises and falls of DNA and RNA/LP until, finally, there is a high peak of RNA/LP on the end of the float. The commingled fluorescence represents a mixture of RBCs, WBCs and platelets. These aggregations portray small clots. None of the populations has separated clearly and there is a stack of platelets on the end of the float. This was blood damaged by poor sampling. There are small clots along the float. The IDEXX VetAutoread Hematology Analyzer has attempted to define populations, but the numbers are very questionable. The blood should be discarded and a new, nonclotted sample should be drawn.



Buffy Coat Profiles 21–22: Improperly and Properly Drawn Samples Compared

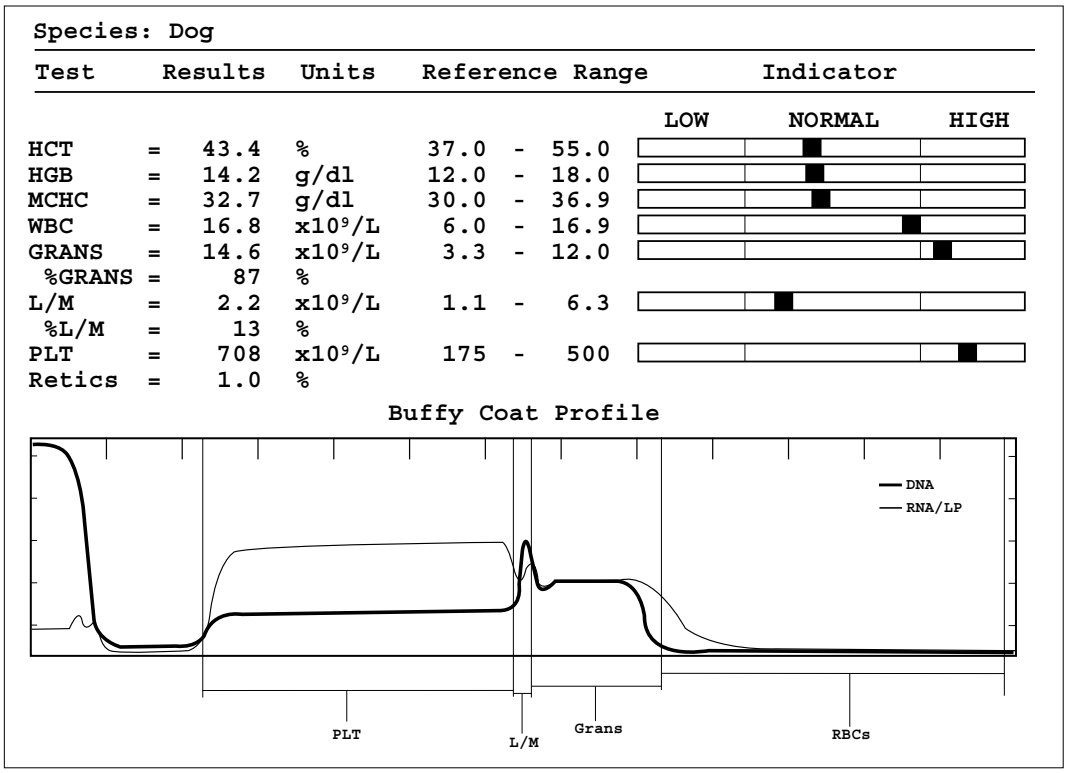
These samples are drawn 14 minutes apart from the same dog. In buffy coat profiles 21–22, there is a small clot seen on the far right at the start of the float RBCs. The WBC area is small, but definitive. There is a severe reduction in platelets. The rest of the sample reveals no visual damage and the results are given without flags. The EDTA tube had 0.25 cc in a 2-cc vacuum tube. The sample suffered from a combination of patient resistance and excitement, poor harvesting on the part of the phlebotomist and a severe underfill. This situation caused dilution of the sample and irritation to the cells from the concentration of EDTA and the technique of the phlebotomist. The platelets were mainly aggregated in the EDTA tube.

Buffy Coat Profile 21: Improperly Drawn Sample Demonstrating Incorrect Blood Counts



Buffy Coat Profile 22: Properly Drawn Sample

A blood sample properly drawn from the dog 14 minutes later shows the correct blood count. If the phlebotomist thinks the sample could be compromised, the sample should be redrawn.



Appendix E: Classifying Anemia

Anemias are classified by the response of the bone marrow to the current volume of RBCs. The best way to evaluate the bone marrow response is by a reticulocyte count. A reticulocyte contains remnant RNA from a developing RBC. With the IDEXX VetAutoread Hematology Analyzer, RNA binds acridine orange and, reticulocytes being the least dense of the RBCs, are found to the far left of the RBC area. A rise in RNA in that area of the RBCs represents a population of reticulocytes. Cats have two types of reticulocytes. The aggregate reticulocytes represent an active response by the bone marrow and contain a substantial amount of RNA that is detected by the IDEXX VetAutoread Hematology Analyzer. Punctate reticulocytes persist for extended periods of time in the peripheral blood and are not indicators of a regenerative marrow. They contain a minimal amount of RNA that is not reflected in the buffy coat profile. Only the aggregates are counted in the IDEXX VetAutoread Hematology Analyzer.

nRBCs occur in the far left of the RBC area and are reflected by a shoulder on the RBC graph. Because nRBCs contain both RNA and DNA, each line will have a shoulder. They will congregate just below the granulocytes and are the only RBC-containing DNA. Depending upon the number and type of nRBCs, they may cause flags and a buffy coat profile alert message asking the operator to confirm the appropriate placement of the RBC/granulocyte interface (see Buffy Coat Profile 7: Disseminated Intravascular Coagulation).

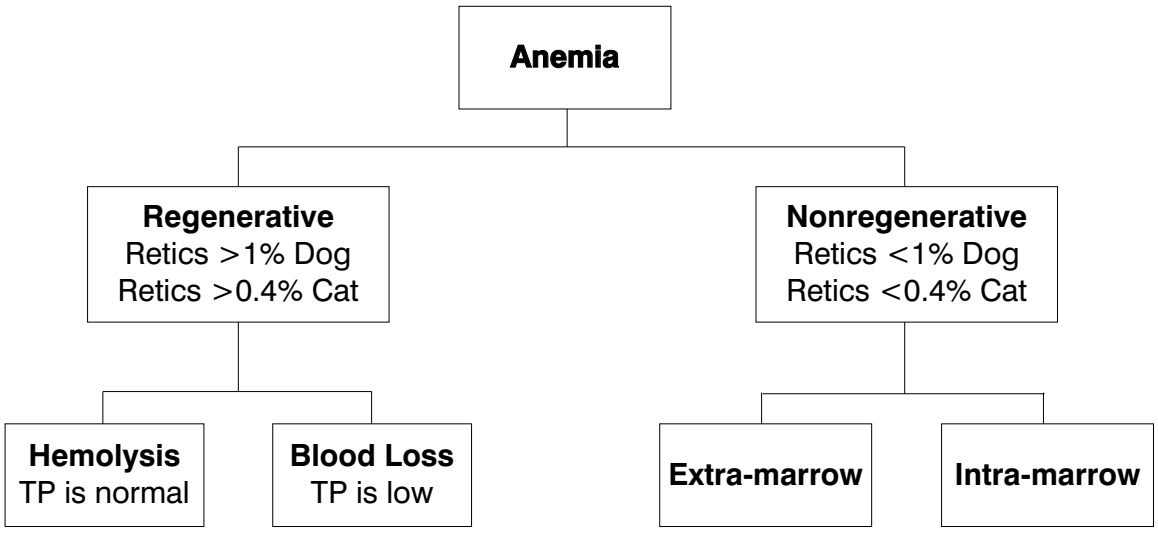
The following flow chart shows how to classify an anemia with the IDEXX VetAutoread Hematology Analyzer. First, define an anemia as regenerative or nonregenerative by the reticulocyte count. In the dog, reticulocytes of greater than 1% are regenerative with a normal Hct. The reticulocyte % as reported must be corrected to changes in the Hct. There is an inverse relationship between the Hct and the reticulocyte count. As the Hct goes down, the reticulocyte % must go up the same proportion to remain regenerative. Therefore, if a dog with a reticulocyte count of >1% is regenerative at a normal species Hct of 45%, then at an Hct of 22.5%, that same animal needs a reticulocyte count of >2% to remain regenerative.

In the cat, reticulocytes of greater than 0.4% are regenerative at an Hct of 40%. The same principles exist for correcting the reticulocyte count. The horse does not show reticulocytes in the peripheral blood. The cow will show them after severe, acute blood loss, but they disappear early in the recovery period. In the horse and cow, reticulocytes normally mature in the bone marrow. The horse is relatively unique in that reticulocytes are only rarely released from the bone marrow in extremely low numbers.

There are two major classes of regenerative anemias, blood loss and hemolysis. The total protein of the plasma may help in this sample classification. If blood loss has occurred, the animal will lose cells and serum; therefore, total protein evaluation of the animal soon after the blood loss incident may show a normal Hct and total protein. However, once there has been redistribution of extracellular fluid, the Hct and total protein will decline proportionally. If hemolysis is present, the RBCs are being destroyed within the blood vessel and the total protein is normal.

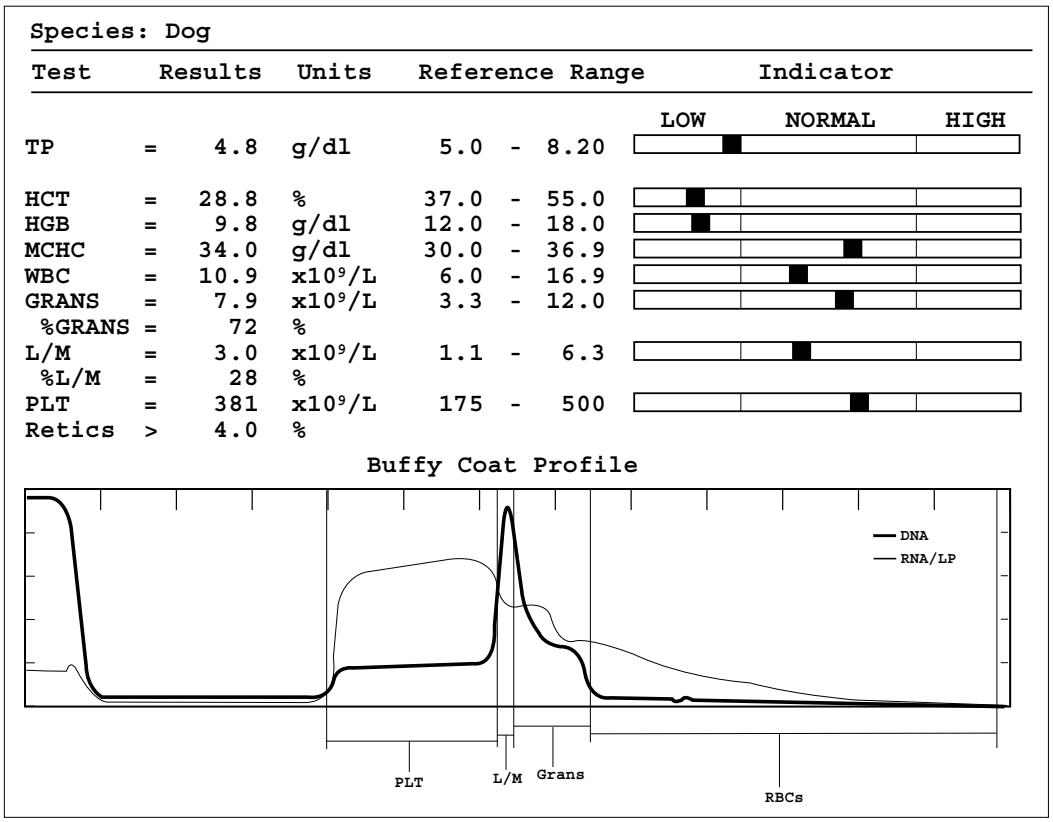
In the nonregenerative state, it is important to know whether the disease is affecting just the RBCs or other cell lines concurrently. If more than one cell line is diminished, there is possible bone marrow involvement.

To determine the cause of a pancytopenia, it would be important to do a bone marrow examination and combine that with the physical examination, history and CBC.



Buffy Coat Profile 23: Regenerative Anemia—Hemorrhage

Moving from right to left on the RBC area, there is an increase in the RNA/LP line. The granulocytes show the typical DNA increase at the RBC/granulocyte interface. The RBC layer contains a distinct population defined by the rise in the RNA/LP line to the beginning of the granulocytes. RBCs containing RNA/LP are reticulocytes. Reticulocytes with numbers beyond 4% will be marked as >4%. This dog is anemic, with an Hct of 28.8%. The reticulocyte count is >4% and the total protein is low at 4.8 g/dL. This is a regenerative anemia potentially associated with hemorrhage.



Buffy Coat Profile 24: Regenerative Anemia—Hemolytic

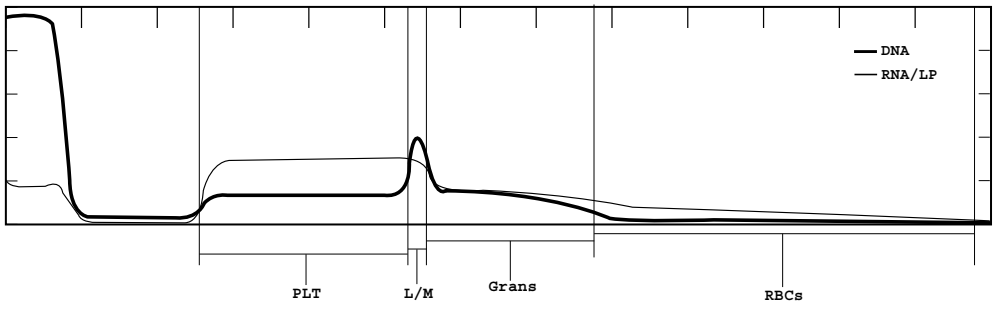
This dog is anemic at 30.8%. The reticulocyte count is regenerative at >4% and the total protein is normal at 6.8 g/dL. There is a granulocytosis.

This was a case of hemolytic anemia. Canine hemolytic anemias are commonly immune-mediated. The blood film should be examined for spherocytes (Plate #5) or for other morphologic clues to the underlying cause.

Species: Dog

Test		Results	Units	Reference Range			Indicator
							LOW NORMAL HIGH
TP	=	6.8	g/dl	5.0	-	8.20	
HCT	=	30.8	%	37.0	-	55.0	
HGB	=	9.7	g/dl	12.0	-	18.0	
MCHC	=	31.5	g/dl	30.0	-	36.9	
WBC	=	21.4	x10^9/L	6.0	-	16.9	
GRANS	=	19.5	x10^9/L	3.3	-	12.0	
%GRANS	=	91	%				
L/M	=	1.9	x10^9/L	1.1	-	6.3	
%L/M	=	9	%				
PLT	=	472	x10^9/L	175	-	500	
Retics	>	4.0	%				

Buffy Coat Profile



Appendix F: Fibrinogen

Fibrinogen is a good, simple inflammatory parameter to use in veterinary medicine. In the IDEXX VetAutoread Hematology Analyzer,* it is measured by a repeatable heat precipitation method that reports accurate results in the high and low ranges for all species.

Fibrinogen Normals

Dog:	0.1–0.25 g/dL 100–250 mg/dL
Cat:	0.1–0.25 g/dL 100–250 mg/dL

Horse:	0.1–0.4 g/dL 100–400 mg/dL
Bovine:	0.2–0.5 g/dL 200–500 mg/dL

*The IDEXX VetAutoread Hematology Analyzer measures fibrinogen. The interpretation or use to which that measurement is applied is not an IDEXX test, but are standard applications derived from veterinary literature.

There are numerous proteins in the blood, such as fibrinogen, albumin, immunoglobulins, coagulation proteins and acute phase proteins. The largest and most abundant proteins are albumin, immunoglobulins and fibrinogen. Protein measurements can be made from plasma or serum, however, the clotting process in serum formation uses and traps coagulation proteins and fibrinogen, respectively. The total protein (TP) can be measured on a refractometer. If plasma is used from an IDEXX VetTube™, heparinized microhematocrit tube or EDTA tube, all proteins will be present. If the TP determination is done from a serum separator tube or a red-topped tube, fibrinogen and other coagulation proteins are not present. The subtraction of measured serum albumin from the TP will give a relatively accurate total globulin determination.

Fibrinogen is an acute phase protein in many animals, and blood levels will begin to rise within hours of tissue injury. The amount of the fibrinogen response is different in each species, but is usually proportional to the amount of inflammation. In an acute condition, the amount of fibrinogen increases and the longer it remains increased, the more guarded the prognosis becomes. In a favorable response, the fibrinogen rapidly returns to normal ranges. In chronic disease, the fibrinogen increases and remains elevated as long as the disease is present.

Fibrinogen is produced in the liver along with albumin and other inflammatory proteins. Fibrinogen occurs in smaller amounts than the other body proteins. Fibrinogen increases in the cow are commonly in the area of 1000 mg/dL and can be >2000 mg/dL in severe inflammation. In the horse, when fibrinogen levels are high, they typically do not reach the limits of the cow. In inflammation, dog and cat fibrinogen is commonly lower than in the cow or horse. Fibrinogens in the 400–500 mg/dL range are seriously high, while >250–400 mg/dL are commonly seen in the average inflammatory disease state. The value of the fibrinogen is significant in conditions such as chronic infections, where destruction and replacement of WBCs have balanced over the elongated disease course and returned to normal numbers, but the fibrinogen remains high, indicating the need to continue treatment or surveillance. Another remarkable use is to identify a nonspecific inflammation after all other routine biochemical and hematological results have analyzed normal. It gives the practitioner a positive result to report to the owner and a reason to continue the investigation.

Fibrinogen is an excellent test to add to a presurgical/anesthesia screen to guard against danger with a low-cost profile. Unless there is a predetermined reason for a high fibrinogen level, an increase needs to be investigated. A common reason for fibrinogen increase in these screens is periodontal disease, where the infection may be greater than expected. These animals are candidates for antibiotic treatment.

In its everyday use, fibrinogen is part of the coagulation cascade. The loss of fibrinogen will result in the inability of the blood to clot properly. This happens occasionally with disseminated intravascular coagulation (DIC).

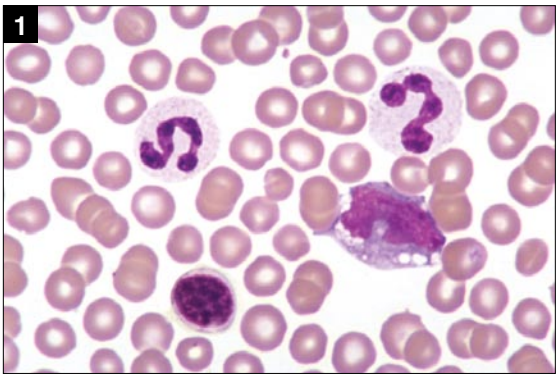
In domestic animals, DIC has a relatively low incidence and usually presents as a secondary disease following recovery from a severe inflammatory condition. In DIC, the body begins to attempt to clot blood in spite of a nonhemorrhagic situation. The clots are only partly formed and break down, causing a rapid loss of fibrinogen and platelets, along with a rise in fibrin-degradation products (FDPs). A fibrinogen markedly below the reference interval (<60 mg/dL) and low platelets with the patient bleeding is typical of DIC. DIC can be fatal unless found early and addressed appropriately. The underlying cause should be identified and treated aggressively. The disease process is rapid, and early diagnosis is essential.

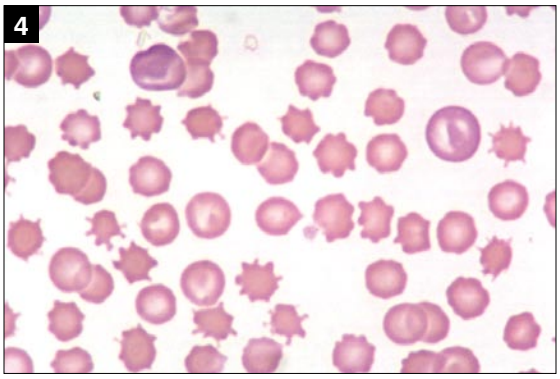
Causes of increased fibrinogen include, but are not limited to:

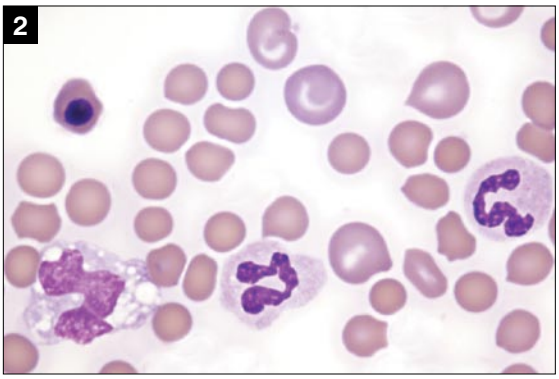
- Viral and bacterial infections
- Traumatic injuries
- Malignancies**
- Kidney disease
- Surgery
- Heart disease
- Post-abortion
- Some poisons
- Canine pregnancy

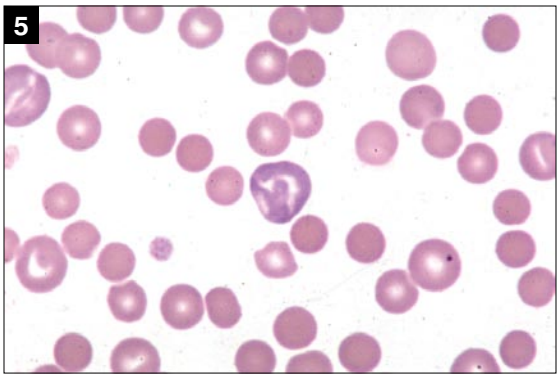
**Circulating malignancies with no solid tumor state may have normal fibrinogens.

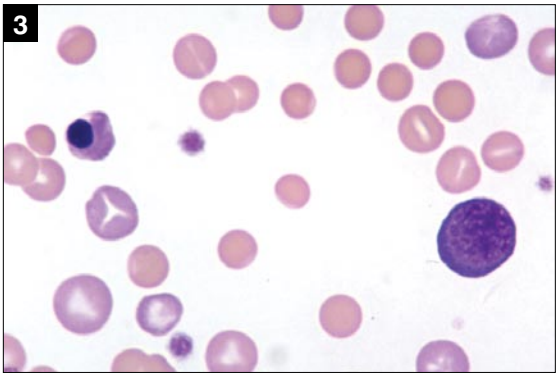
Appendix G: Morphology





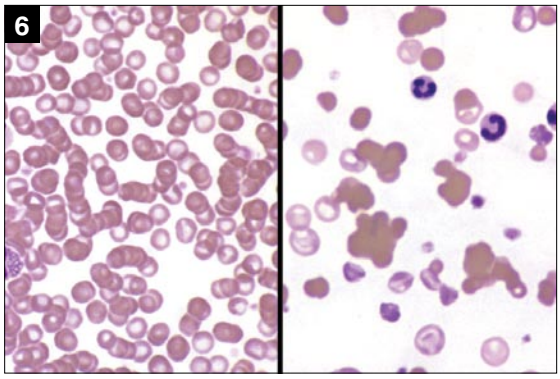


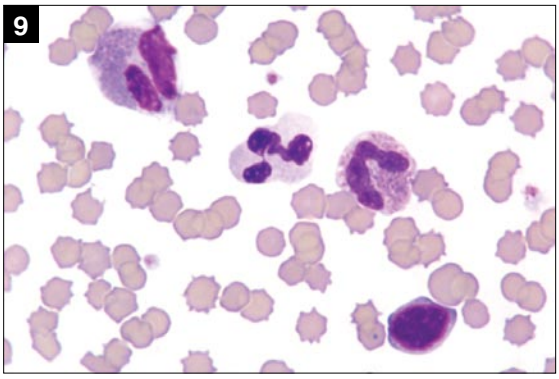


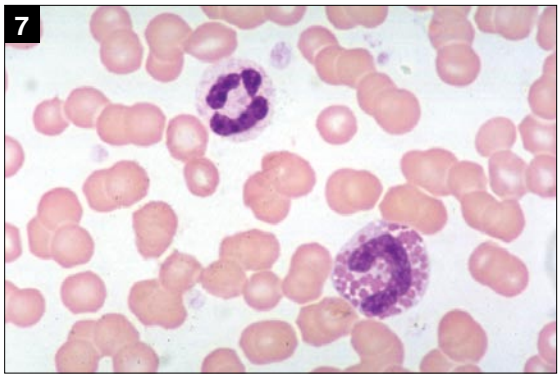


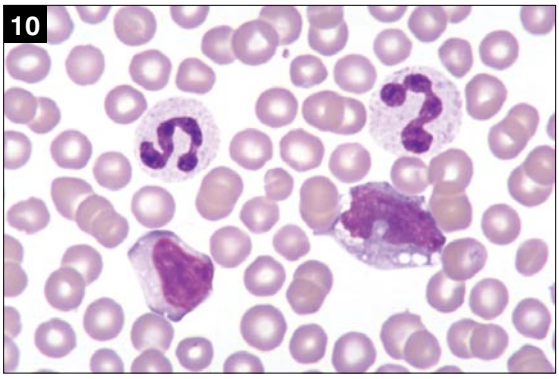
Legend

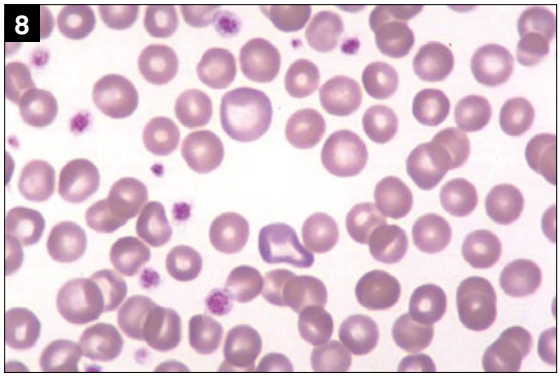
1. **Dog:** Blood film from a healthy dog with two segmented neutrophils, one monocyte and one lymphocyte
2. **Dog:** Regenerative anemia with several polychromatophils, one metarubricyte, two neutrophils and a monocyte
3. **Dog:** Regenerative anemia with several polychromatophils, one metarubricyte (center left) and one early rubricyte (center right)
4. **Dog: Acanthocytosis.** These are erythrocytes with 2–10 blunt, fingerlike projections of variable size that should not be confused with crenated erythrocytes with multiple short, sharp projections of uniform size. Acanthocytes are formed when there are altered phospholipid:cholesterol ratios in the plasma affecting the amount of lipids in the cell membrane. Liver disease, occasionally splenic disease (including hemangiosarcoma) and other metabolic disorders, should be considered in a differential diagnosis list.
5. **Dog: Spherocytosis.** Spherocytes are smaller, denser erythrocytes with no central zone of pallor. When present in significant numbers without other poikilocytosis, they are supportive of extracellular immune-mediated hemolytic disease. In this field of view, there are many polychromatophils supportive of a regenerative immune-mediated hemolytic anemia.





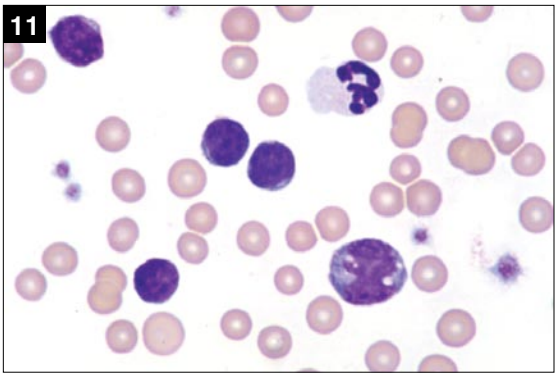


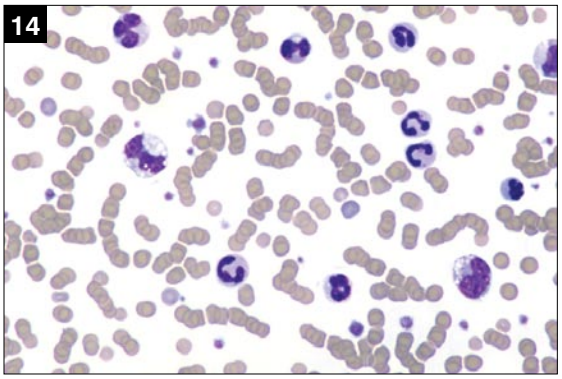


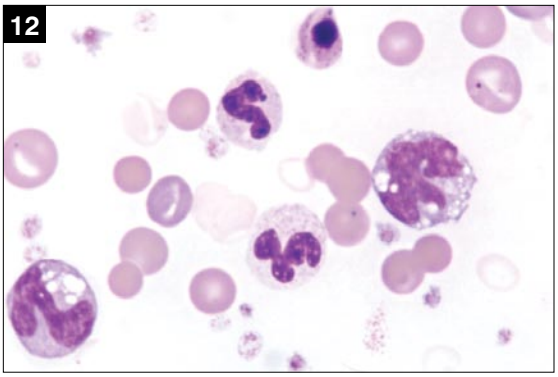


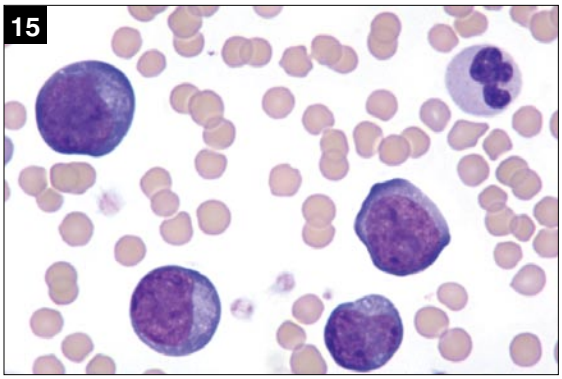
Legend

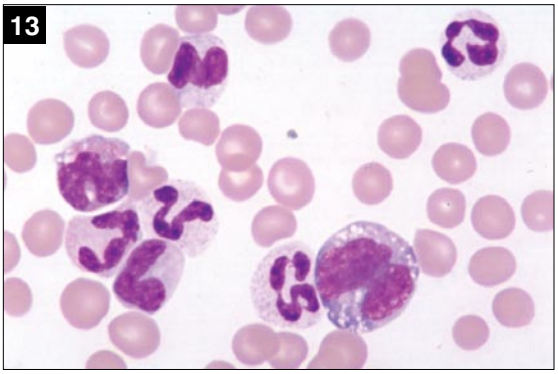
6. **Dog: Rouleaux (left) and agglutination (right).** Rouleaux is the organized linear array ("stack of coins") associated with a weak bond between cells. Rouleaux increases with inflammation and the presence of abnormal proteins. Agglutination is the irregular, three-dimensional clumping of erythrocytes associated with cross-linking of surface-associated immunoglobulins. Agglutination may be seen with immune-mediated hemolytic disease, but must be confirmed with a saline agglutination test to differentiate from marked rouleaux.
7. **Dog: Neutrophil (top) and eosinophil (bottom).** Eosinophils from different species have different-sized and -shaped red-orange granules. Dog eosinophils have generally round and variably sized granules. Eosinophils are commonly seen with allergic/hypersensitivity responses or with tissue-phase parasites.
8. **Dog.** Normal-sized platelets are present in this field of view. These are nonnucleated plates in mammals that play a critical role in coagulation. The presence of large platelet forms in most species (primary exception is the cat) is an indication of a bone marrow response to a peripheral demand for platelets.
9. **Cat.** Four leukocytes, including (top left to bottom right) a basophil, neutrophil, eosinophil and lymphocyte, are present. There is mild nonspecific poikilocytosis in the erythrocytes.
10. **Dog.** Two mildly toxic neutrophils, a reactive lymphocyte and a normal monocyte are present.





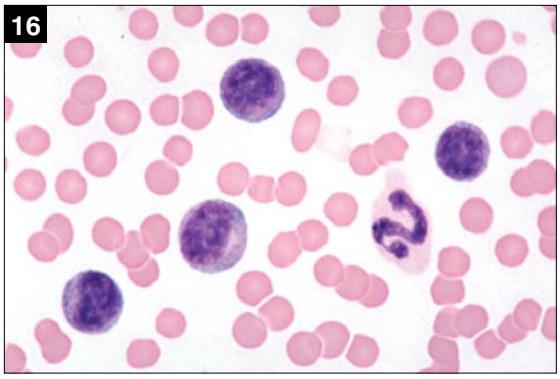


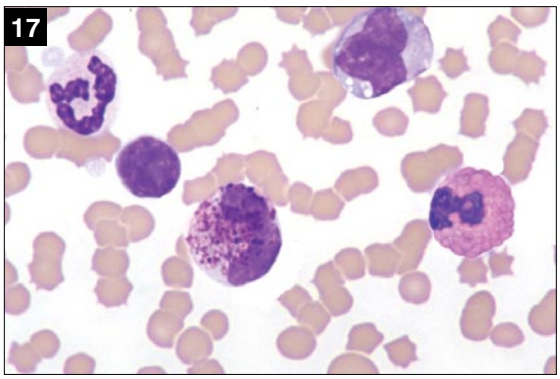


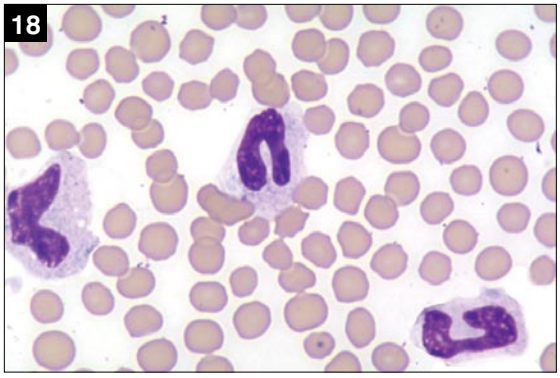


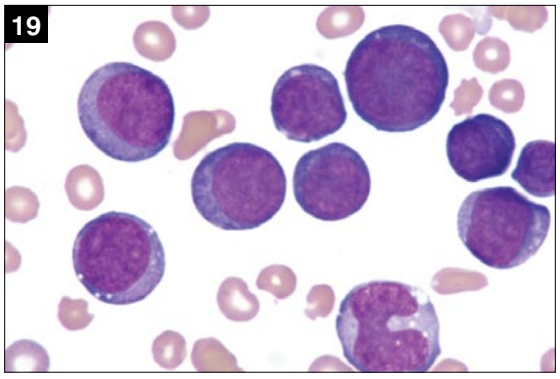
Legend

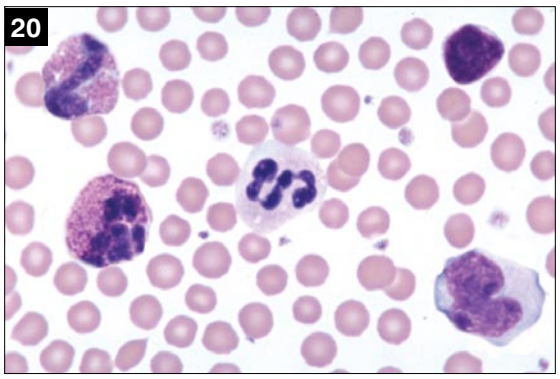
11. **Cat.** Four lymphocytes, one neutrophil and one monocyte are present. The buffy coat profile showed a large L/M layer. The blood film shows that the primary leukocyte is the lymphocyte, confirming lymphocytosis.
12. **Dog.** This blood film from a dog shows immune-mediated hemolytic anemia (IMHA). In addition to the anemia and decreased erythrocyte density, there are multiple abnormalities, including spherocytosis, anisocytosis, Howell-Jolly bodies, polychromasia and ghost erythrocytes. There is a significant leukocytosis, characterized by a neutrophilia (two neutrophils present) and monocytosis (two monocytes present).
13. **Dog.** This blood film is consistent with a leukocytosis, characterized primarily by a neutrophilia with a left shift (presence of immature neutrophil forms). Two monocytes and six neutrophil forms are present. The report showed a small L/M (1.0) layer and a large granulocyte (32.0) layer, and was flagged (#) with a buffy coat 3 message. The blood film is consistent with the report and the numbers can be accepted as clinically correct.
14. **Cat.** This cat had a WBC count of 70.6. In the buffy coat profile, the L/M signature indicated an increase of monocytes. The blood film confirms a neutrophilia, a monocytosis and rouleaux formation, which all support an active inflammatory process.
15. **Cat.** Acute lymphoid leukemia. Four large immature-appearing lymphocytes and one neutrophil are present. The L/M count was 26.4 x 10^9/L.











Legend

16. **Cat: Acute lymphoid leukemia.** The total number of lymphocytes in this case was >150 x 10^9/L.
17. **Horse.** Each of the normal leukocytes of a horse, including (left to right) a neutrophil, lymphocyte, basophil, monocyte and eosinophil, are present in this field of view. These leukocytes are surrounded by normal-appearing erythrocytes and there is moderate rouleaux present, which is typical of horse blood.
18. **Horse.** Three toxic immature neutrophil forms are present in this horse with colic. Toxic changes of increased blue-staining and granularity of the cytoplasm are the primary morphologic changes.
19. **Dog: Acute undifferentiated leukemia.** Numerous immature and poorly differentiated leukocytes were circulating in the peripheral blood.
20. **Cow.** Normal leukocytes from a peripheral blood film of a cow. An eosinophil (top left), basophil (bottom left), neutrophil (center), lymphocyte (top right) and monocyte (bottom right) are present.

Notes

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Notes

Do you have a question about a buffy coat profile from a case at your clinic?

Owners of the IDEXX VetAutoread™ Hematology Analyzer are encouraged to discuss their questions with an IDEXX Customer Support representative. Simply fax your results printout with the buffy coat profile to us toll-free at 1-800-248-2483. Be sure to include your name and telephone number on the printout. A representative will call you back as soon as possible to discuss your concerns.

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