VETSCAN® HM5

Hospital Resource Guide

zoetis

Welcome

to the VETSCAN® HM5 Hospital Resource Guide

This guide is designed to give you everything you need to get the most out of the VETSCAN HM5 haematology system. Throughout the chapters listed, you will find links to supplemental resources to help address questions.

We hope you find this guide useful. And as always, contact Diagnostic Technical Support for further assistance at:

0345 300 8034

DXSupport.UK@zoetis.com

Need guidance on a treatment plan?

Confirm results and a path forward for complex cases with remote specialist consultations—at no additional charge for Zoetis Diagnostics customers.*

ZoetisDx.com

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*Requires the use of ZoetisDx.com, the VETSCAN® FUSE or VetScan HUB and at least one Zoetis Diagnostics instrument or service.

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The VETSCAN HM5 is a fully automated, 5-part differential haematology analyser displaying a comprehensive 22-parameter complete blood count (CBC) with cellular histograms on an easy-to-read touch screen. Its reliable performance, elegant design, ease of use and minimal maintenance with automated reminders make it the optimal veterinary haematology system.

Reliable performance for full CBC analysis

Automated CBC analysis on a wide range of species

- Flexibility to analyse different species
- Ideal for veterinary clinics, including mixed and large animal facilities
  - Five-part differential for 6 species: alpaca, cat, cattle, dog, horse and llama
  - Three-part differential for 9 species: ferret, goat, guinea pig, mouse, pig, primate,* rabbit, rat and sheep

Histograms to complement differentials

- Blood cell populations are graphically represented by cellular histograms
- Can easily verify differential cell counts, help identify uncommon disease processes or check sample integrity at a glance

*For research use only.

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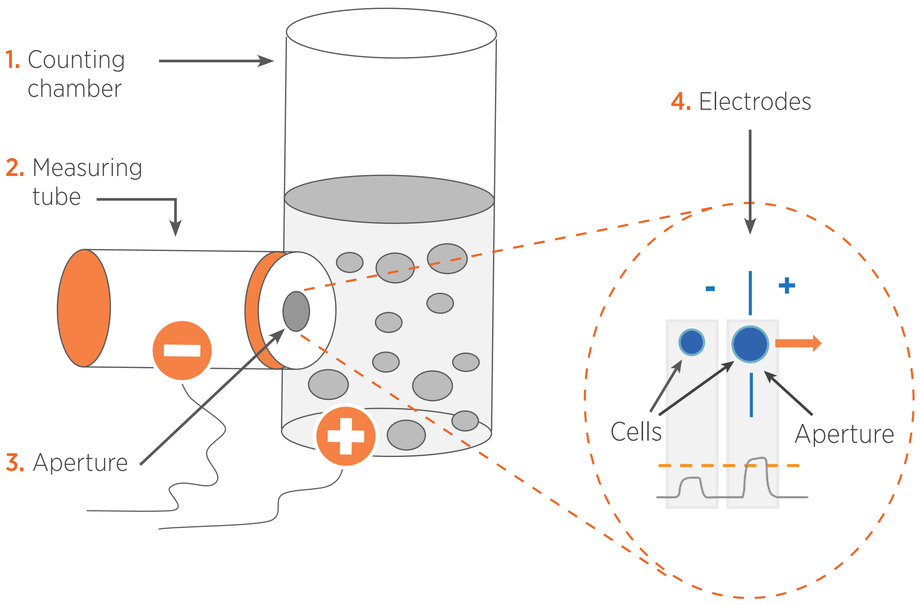
Most automated haematology analysers used in veterinary medicine are either impedance counters or laser flow cytometers or contain a combination of the two.

The VETSCAN HM5 uses impedance technology for the counting and categorisation of cells.

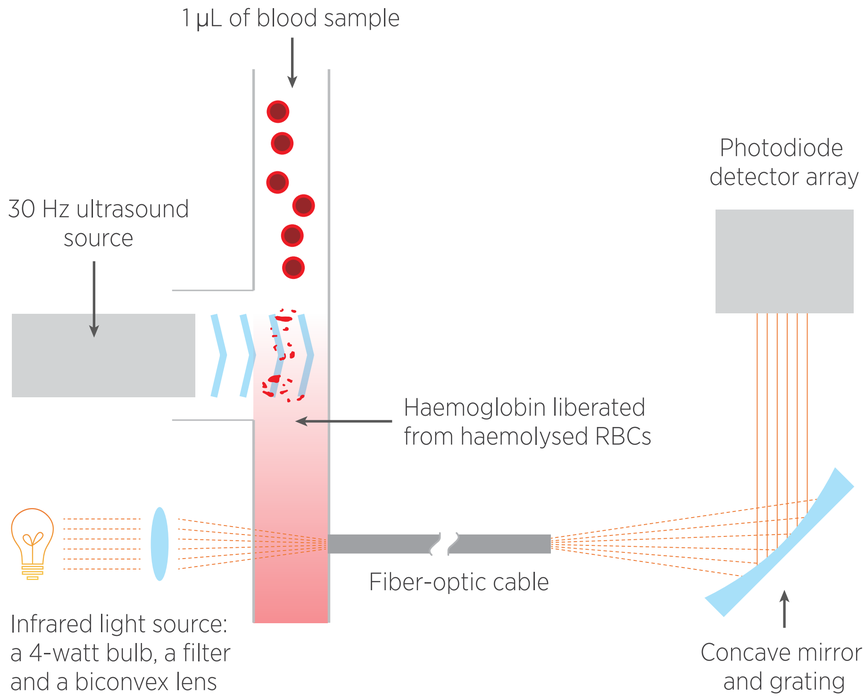
Impedance technology

- In impedance counters, particles (cells) in a blood sample are suspended in an electrolyte solution and passed through an aperture that connects 2 chambers—1 containing a positive electrode and 1 containing a negative electrode
- Passage of a particle through the channel invokes a brief change in the electrical impedance between the electrodes that is proportional to the size of the particle
- Cells pass through the aperture to be classified by voltage emitted
- Measured values from impedance counters include RBC count, MCV, PLT count, WBC count and a 3- to 5-part WBC differential
- From the measured values, additional values—notably MCHC and HCT—are calculated



Haemoglobin (HGB) spectrophotometry measurement

- Spectrophotometry measures free HGB (from the lysed RBC and any amount that was already present in the plasma)



Used with permission from Alex Yartsev.

HCT=hematocrit; MCHC=mean corpuscular hemoglobin concentration; MCV=mean cell volume; PLT=platelet; RBC=red blood cell; WBC=white blood cell.

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VETSCAN HM5 supports 15 species

Species listed here have been validated on the VETSCAN HM5.

- Those not included are not currently supported by Zoetis Diagnostics
- The VETSCAN HM5 cannot be used for avian/reptilian species
- Primate reference intervals are available for research purposes only

5-part differential

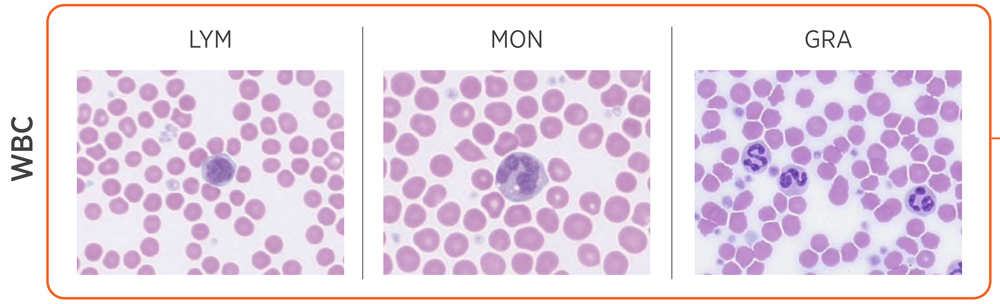
Dog	Cat
Horse	Cattle
Alpaca	Llama

3-part differential

Pig	Goat	Sheep
Rat	Ferret	Mouse
Guinea Pig	Rabbit	Primate*

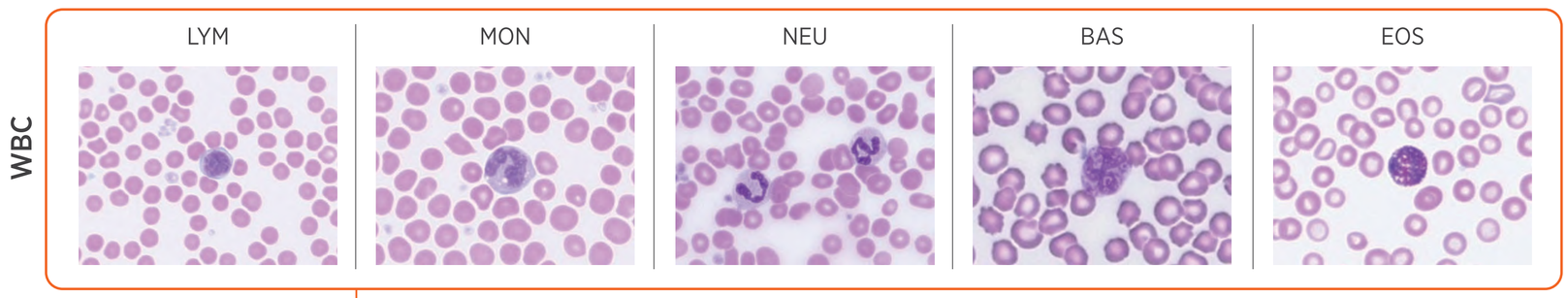
The truth behind the 3– and 5–part differential

Automated CBC analysers are generally classified as either 3-part or 5-part differential analysers.



3–Part WBC Differential[1]

Images obtained from VETSCAN IMAGYST™.



5–Part WBC Differential[2]

The ability of 5-part differential analysers to enumerate the less abundant cell types—ie, MON, EOS and BAS—separately, rather than as a mixed cell population, is a significant enhancement.

BAS=basophil; EOS=eosinophil; GRA=granulocyte; LYM=lymphocyte; MON=monocyte; NEU=neutrophil.
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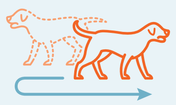
Patient preparation

These recommendations apply to all laboratories and instruments—whether point-of-care or send-out.

	Before the appointment	Rationale
	• Avoid feeding patients for 10 to 12 hours prior to appointment unless contraindicated • In horses and ruminants, fasting prior to haematology analysis is not required	• A postprandial sample may cause lipemic interference • Food consumption can cause fluctuations in haematology results—including HGB, MCH and MCHC[3]
	• Consider timing of patient appointment relative to when haematological testing will be completed • Understand that certain medications may impact test results	• Age-related changes can lead to artefacts in the blood sample for haematology testing—such as RBC crenation, WBC chromatin swelling, platelet clumping[4] • Several chemotherapeutic drugs, NSAIDs and antimicrobial medications are associated with haematological adverse drug events[5]
	• Avoid exercise and minimise excitement/fear prior to the appointment	Can cause: • Physiological leukocytosis[3] • Transient hyperglycemia in cats[6]
	At the clinic	**Rationale**
	• Minimise excitement/fear during the appointment • Consider the use of sedation and antianxiety medications to help decrease stress for anxious animals and enable safer and gentler restraint, when appropriate	Can cause: • Physiological leukocytosis[3] • Transient hyperglycemia in cats[6]
	• With a sick patient, anticipate that analyte results may be impacted	• Visually inspect for clots that can falsely impact cell counts and harm the analyser • Visual assessment of the sample preanalysis can highlight abnormalities (eg, haemolysis can indicate poor sample quality) or may indicate the presence of disease
	At time of sampling	**Rationale**
	• Good sample collection technique is critical (clean needle puncture of the vein, etc)	• Lack of good technique leads to an increased risk of clotted sample or haemolysis

MCH=mean corpuscular hemoglobin; NSAID=nonsteroidal anti-inflammatory drug.

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Keys to successful sample collection

The quality of the sample analysed is directly related to the quality of the result.

Avoid vein collapse when drawing samples	• Minimise suction on the syringe, and do not draw back too quickly
Prevent haemolysis	• Use the largest vein and needle appropriate for blood collection • Avoid use of any needle smaller than a 23 gauge (though certain exotic species may require a smaller needle) • Use minimal alcohol on fur/skin • Remove the needle from the syringe before dispensing into the blood tube unless using a closed vacuum blood collection system
Ensure the correct ratio of anticoagulant to blood	• Fill EDTA tube to manufacturer's sample fill line • Immediately after filling tube, cap the tube and invert 10-15 times to sufficiently mix with anticoagulant (more inversions would be needed in case of 0.25 mL, 0.5 mL or 1.3 mL tubes)
Ensure appropriate tube use	• Select tubes based on the testing requirements and size of patient (Microtainer®, 1.3 mL, 3 mL, 5 mL) • Ensure tubes have not expired • Always fill blood tubes in the correct order to avoid contamination — EDTA contamination of chemistry samples may affect electrolyte results and cause a falsely low Ca and falsely high K^+ — If improper tube-filling order occurs, the sample should be redrawn
Prevent unwanted blood clotting	• Do not hold off the vein for more than a few seconds before venipuncture • For feline samples collected from the medial saphenous vein, a vacuum blood collection system instead of a syringe is recommended
Do not allow samples to degrade	• Run samples as soon as possible after drawing

Ca=calcium; EDTA=ethylenediaminetetraacetic acid; K^+=potassium.

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Running a sample on the VETSCAN HM5[7]

DO's

- Always use an EDTA collection tube—no other tubes are validated
- Always fill the collection tube to the manufacturer's sample fill line
- Test as soon as possible after sample collection
- Refrigerate if it will be >20 minutes until sample can be run to preserve cell morphology
  - Allow sample to warm to room temperature prior to running on the VETSCAN HM5
- Reinvert the tube 10 to 15 times after the sample is collected AND again just prior to running on the VETSCAN HM5
- Create a blood smear shortly after collection—no need to stain or evaluate slide right away
- Check for blood clots before running the sample
- Select the correct species to be analysed, as the algorithms and cell sizes differ by species
  - If the original species selected was incorrect (eg, if a canine sample was run as a feline), rerun the sample under the correct species

DON'Ts

- Freeze sample
- Use if tube not filled to manufacturer's sample fill line—an incorrect ratio of EDTA to blood can affect results
- Run a sample with a visible blood clot
- Run a sample straight from the refrigerator without warming to room temperature

Spurious errors[8,9]

Keep in mind that anything that spuriously affects RBC or MCV will in turn spuriously affect MCHC and HCT. Anything that spuriously affects HGB will also affect MCHC. Inaccurate results are possible if the analyser is out of calibration.

Spurious results/errors	Cause	Prevention: Always include a blood smear to help with interpretation
↑ HGB	Haemolysis, lipemia, icterus, Heinz bodies, severe leukocytosis	Prevent haemolysis and lipemia
↑ MCV	Prolonged storage at room temperature, RBC swelling, autoagglutination, some regenerative anaemia cases	Run sample immediately or store in refrigerator
↑ MCHC	Haemolysis, lipemia, many Heinz bodies, storage at room temperature or false increase in HGB, marked spherocytosis	Most of these causes can be prevented with proper sample handling techniques
↓ PLT	PLT clumping, blood clots, macroplatelets	Prevent through proper sample handling, needle size and vein selection. Fill tube to manufacturer's sample fill line and invert tube immediately after collection and just prior to running on analyser
↑ RBC	Dehydration, macroplatelets	Consider patient health status and breed variations
↓ RBC	Blood clots, agglutination	Use proper sample handling to avoid blood clots. Never run a clotted sample

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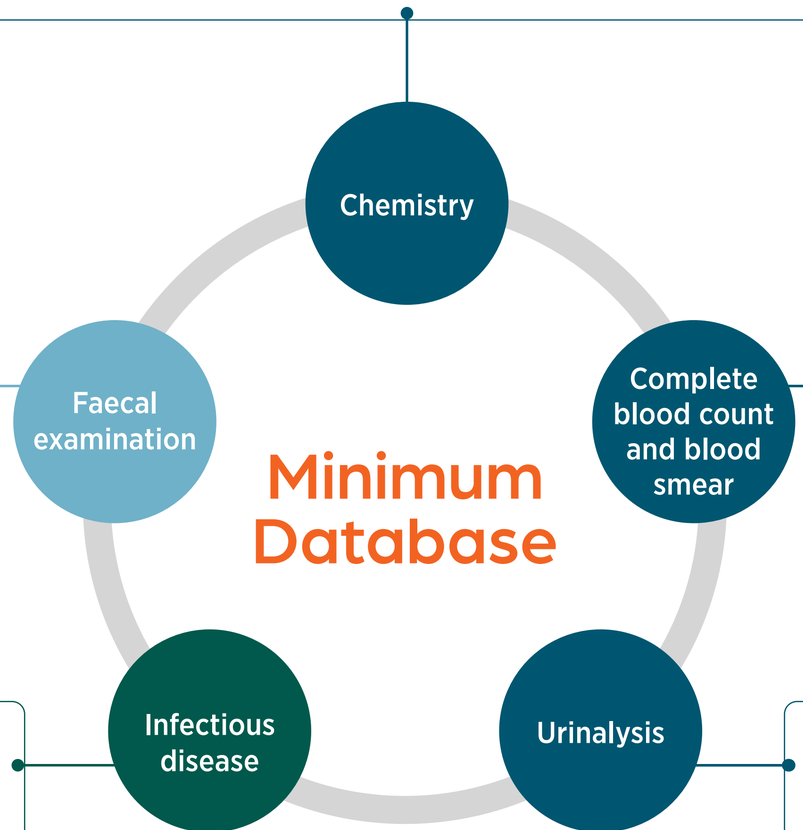
Define your laboratory testing minimum database[10]

Chemistry tests assess the function and condition of various body systems. Interpretation of chemistry test results should always be associated with the analysis of other sources of data, such as: patient's signalment, history, clinical signs and results of other diagnostic testing, especially haematology and urinalysis.



Faecal testing is part of a minimum database when medical history and physical examination are indicative of gastrointestinal disease and as part of a preventive healthcare screening depending on patient age and lifestyle.

Abnormalities in the blood cell counts and/or morphology can indicate presence of underlying disease and can sometimes be diagnostic.

Point-of-care infectious disease testing can complete a minimum database. Testing should be determined based on regional disease prevalence and patient lifestyle.

Several chemistry analytes are used to evaluate kidney function and hydration status. Urinalysis is critical to the evaluation of these analytes and determining whether any changes due to dehydration, kidney disease and/or lower urinary tract disease (eg, urinary tract infection) are present.

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A complete haematological picture includes the following CBC components:

1. Automated CBC (quantitative)
2. Blood smear (qualitative) Manual or using artificial intelligence (AI)
3. Packed cell volume (PCV)

Quantitative evaluation: automated CBC

The automated CBC is a diagnostic tool that classifies, enumerates and differentiates the different types of cells present in the peripheral blood. This **quantitative** evaluation of the blood provides different cell population counts and their associated indexes as well as graphic representations when performed on an automated analyser.

An automated CBC also includes a differential WBC count, which is a breakdown of the amount of each subpopulation of the WBC present in the total WBC population.

Since each WBC has a very specific function, the differential count may be used to identify abnormal levels of specific WBC subpopulations and may offer diagnostic information about specific underlying health conditions.

ZoetisDx

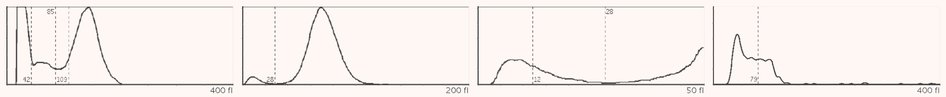
Clinic Name : Zoetis Demo Hospital
Requesting Doctor : Dr. Smith

Owner Name : Jenna Jones
Patient Name : Luna
Patient ID : 12345

Species : Canine
Breed : N/A

Hematology

Test	Ref Range	Units	Graph	08/27/21 19:17	10/04/22 16:02
WBC	6 - 17	10^9/l		13.79	13.93
LYM	1 - 4.8	10^9/l		2.76	2.48
MON	0.2 - 1.5	10^9/l		0.73	1.13
NEU	3 - 12	10^9/l		10.03	10.14
EOS	0 - 0.8	10^9/l		0.19	0.14
BAS	0 - 0.4	10^9/l		0.07	0.04
LYM%		%		20.0	17.8
MON%		%		5.3	8.1
NEU%		%		72.8	72.8
EOS%		%		1.4	1.0
BAS%		%		0.5	0.3
RBC	5.5 - 8.5	10^12/l		8.11	7.48
HGB	12 - 18	g/dl		16.8	15.6
HCT	37 - 55	%		52.56	53.71
MCV	60 - 77	fl		65	72
MCH	19.5 - 24.5	pg		20.7	20.9
MCHC	31 - 39	g/dl		32.0	29.1
RDWc	14 - 20	%		17.5	16.4
RDWs		fl		40.6	43.0
PLT	165 - 500	10^9/l		297	248
MPV	3.9 - 11.1	fl		11.0	10.3
PCT		%		0.33	0.25
PDWc		%		40.0	40.0
PDWs		fl		17.9	17.9



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Qualitative evaluation: blood smear

A blood smear is a **qualitative** part of the comprehensive CBC that is used to confirm results, assure quality, and may provide additional insights to guide diagnosis and treatment.[11-14] Microscopic examination of a blood smear is an essential part of the haematological picture, as it can provide vital diagnostic information that is not identified on the automated CBC.

Ideally, a blood smear evaluation should always be performed with every CBC, but it is vital that one be performed in the following clinical instances[8]:

- Anaemia (low RBC count)
- Thrombocytopenia (low PLT count)
- Neutrophilia or neutropenia (verify count and examine cells)
- Lymphocytosis
- Severe illness (eg, sepsis)
- Suspicion of parasites
- When certain warning flags are present on the automated CBC report

A **blood smear** enables the veterinarian to confirm results and assure quality and may provide additional insights to guide diagnosis and treatment.[11-14]

A blood smear evaluation should not be utilised as a replacement for an automated CBC, as automated analysers count thousands of cells for more precise and accurate data than manual cell counting. Machines must be properly maintained for consistent precision and accuracy.[15]

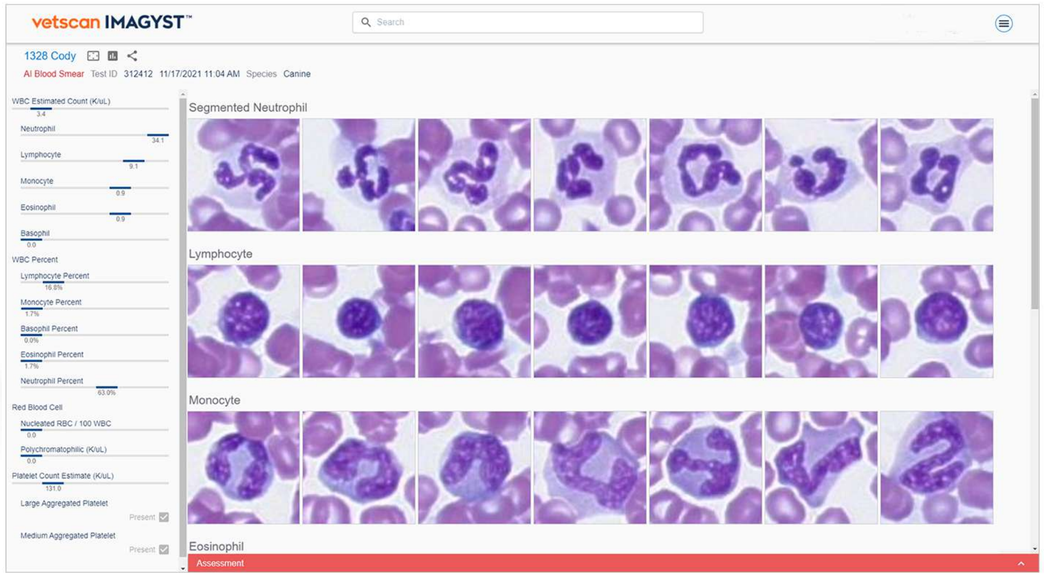

Figure: VETSCAN IMAGYST™ AI Blood Smear sample report

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Further quantitative evaluation: PCV/TS

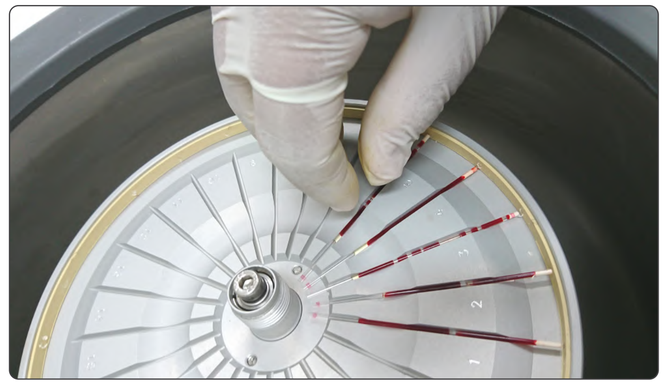
PCV is the direct centrifugal measurement of the percentage of the blood that consists of RBC. It is an accurate measurement with minimal inherent error (+/- 1%).[16]

HCT is a calculated value of the percentage of the blood that consists of RBC: HCT (%)=(RBC/µL) x MCV (fL/10).[17] Unlike PCV, the potential for error is greater with the HCT method because it is subject to MCV variation that can occur with certain haematological conditions (eg, agglutination) and/or improper sample handling (eg, excess EDTA, haemolysis or inadequate mixing).[18]

To verify that HCT was not affected by artefactual errors, perform a PCV measurement[19]

HCT (%) can also be estimated with this calculation, using the HGB result from the automated CBC report:

HGB x 3 (SI units: HGB x 0.3)



Zoetis Diagnostics delivers a complete haematology solution

A streamlined workflow makes it easy to get to the harder diagnoses

Start with VETSCAN HM5 for quantitative CBC results

Always supplement automated CBC with qualitative results from VETSCAN IMAGYST™ AI Blood Smear

Add on clinical pathologist review when needed*

Consult veterinary specialists for challenging cases directly through ZoetisDx†

AI=artificial intelligence; HCT=hematocrit; MCV=mean cell volume; PCV=packed cell volume; TS=total solids.
*If abnormalities are observed, expert review via digital image transfer is available. This can be done within the VETSCAN IMAGYST system. Additional costs may apply.
†Available in select markets.

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Responsible Patient Trending—**why perform**?

Due to biological variations, the best reference values are a pet's own diagnostic values over time, encompassing breed, age, sex and individual variation

- Most reference intervals represent results expected for 95% (19 of 20) of a healthy population, and therefore 1 of 20 healthy animals is expected to have a measured value outside of the reference interval[11]
- For these reasons, individual patient trending is more sensitive and better at detecting pathological changes than reliance on published reference values for chemistry and haematology[20]

Senior patients

- The common occurrence of physical exam and laboratory abnormalities in apparently healthy senior dogs and cats emphasises the need for regular health screening, including regular laboratory testing[12,13]
- Visit/exam frequency and testing recommendations should be based on patient's age, breed and lifestyle
- Senior and geriatric dogs and cats should be examined at least twice yearly to allow for earlier intervention of chronic disease
- Regular testing at geriatric equine annual examinations assesses overall health and may display any early signs of potentially serious disease, such as liver and kidney dysfunction or onset of metabolic disease[21]

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Keys to patient trending success

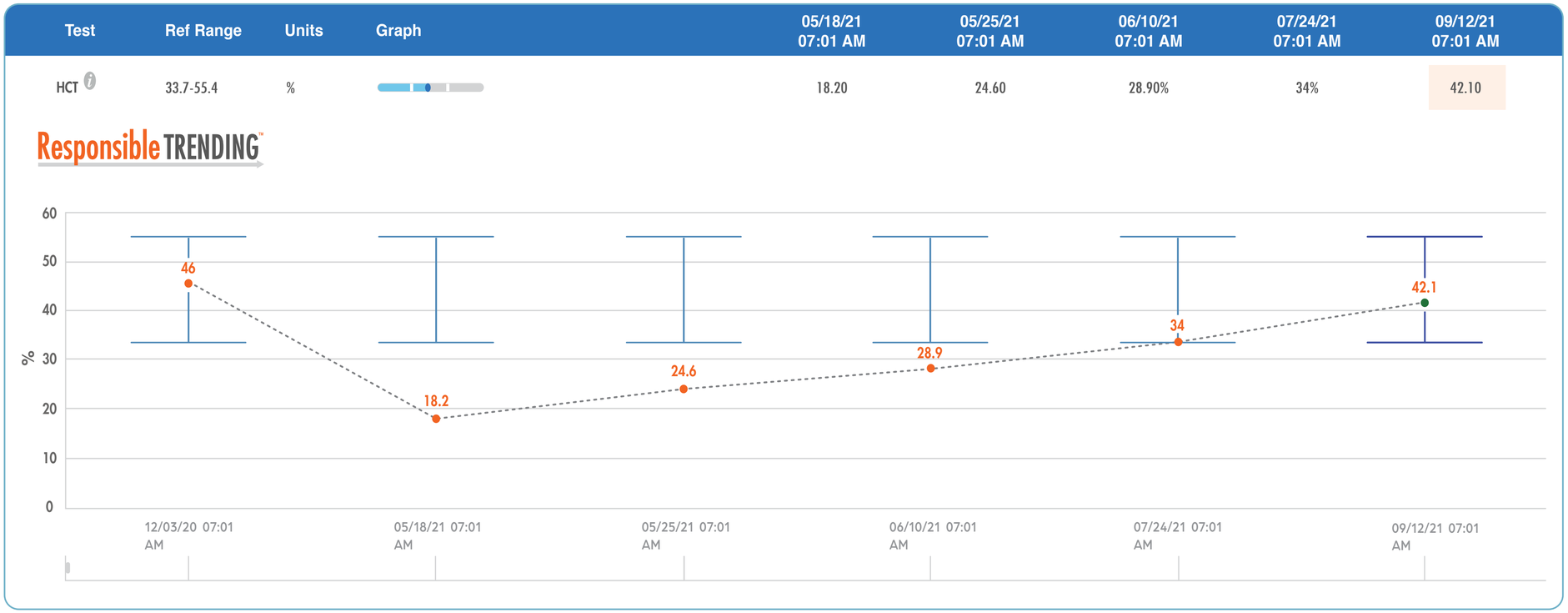
The best practice is to monitor a patient on the same analyser using the same analytical methods

Whenever comparing or trending analyte results, it is important to trend using best practices and responsible trending to have a consistent comparison. This practice includes:

- Using the same analyser every time, when possible
- Performing the test in the same way (sample type, number of hours pre- or posttreatment, fed or fasted state, etc)
- Keeping in mind that different assays and instruments have reference intervals that may differ among analysers and/or laboratories
- Performing a quality check or verifying with a different test, methodology or laboratory if a value does not match the clinical picture

What is responsible trending?

Responsible Trending™, available only on the ZoetisDx online platform, shows chronological test analyte results as a sequence of graphs. **This visual format provides a clear story of each patient's trends in test results over time—with results from different analysers displayed together but always relative to each analyte's reference interval on its respective analyser.**



Note: It is imperative when comparing results between different analysers or labs to interpret the raw value with respect to the reference interval provided and not the raw number due to inherent methodology differences.

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Introduction to histograms

Histograms are graphic representations of cell distribution and cell counts[22]

Histograms are the graphs that display on your results screen or appear on the printed haematology report, depending on the practice management software used. They are easily understood and can help to:

Verify differential cell counts

The graphical display of data provides information not available in the automated CBC and allows verification of the CBC's numerical results

Perform quality control check

Show sample integrity and potential abnormalities, so users can quickly decide whether results are meaningful or whether they need to draw a new sample

Identify uncommon disease processes

Identify uncommon disease processes that can affect cell morphology and distribution. These need to be confirmed visually with a blood smear

Histogram interpretation requires a thorough review of PLT, RBC and WBC curves for abnormalities

- Perform a blood smear for all patients who have any cell outside of the reference interval (range)
- Remember that a single haemogram is a snapshot in time and changes can occur rapidly
- Monitoring haemogram results frequently (every 12 to 24 hours) can be helpful in determining the course of an inflammatory response and response to treatment
- Always interpret results in conjunction with the clinical evaluation of the patient

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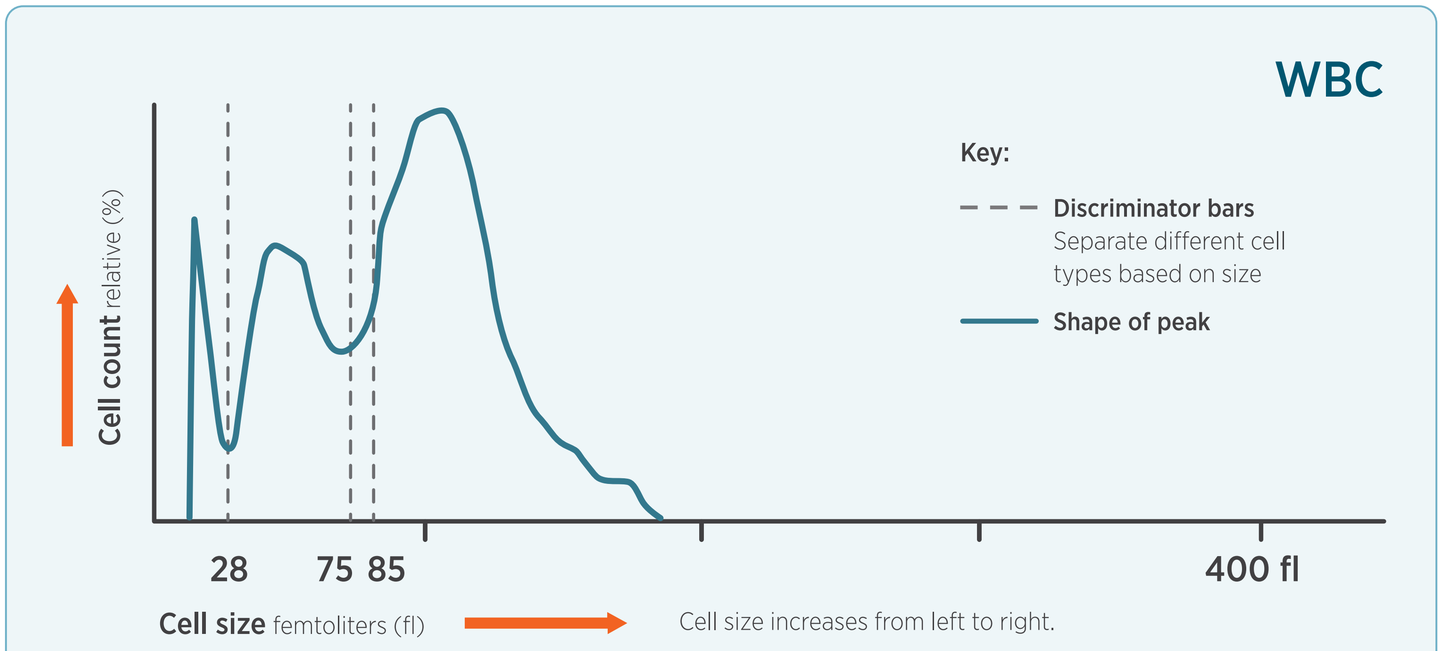
Interpreting histograms

Histogram interpretation requires a thorough review of PLT, RBC and WBC curves for abnormalities.

Elements of a histogram[23]



- The area under the curve is equal to the relative cell count for the specific sample run[24]
- Peak heights among samples/runs cannot be compared[25]

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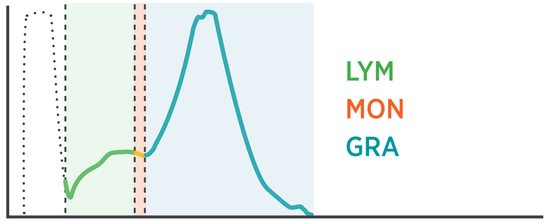
Normal histograms

WBC Histogram



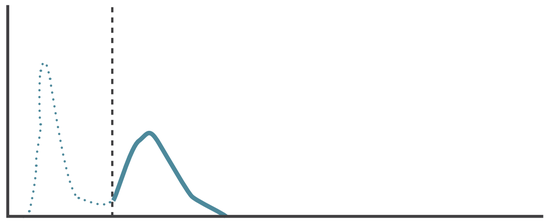
The peaks in the **WBC histogram**, separated by discriminators, correspond with LYM, MON and GRA. Debris (lysed RBC) from the WBC counting step may be seen to the left of the first discriminator.

LYM peak (left) is seen to the right of the first discriminator. In canines, the LYM peak starts on the low-to-mid portion along the y-axis, as shown, indicating lower populations of this cell type. In normal felines, peak starts on the mid portion of the y-axis due to higher relative number of LYM in cats

MON peak (centre) is seen to the right of the second discriminator and is typically shorter due to a smaller population relative to other WBC

GRA peak (right) is seen to the right of the third discriminator and is predominantly composed of neutrophils. It is the tallest and widest peak, indicating it is the most numerous WBC population

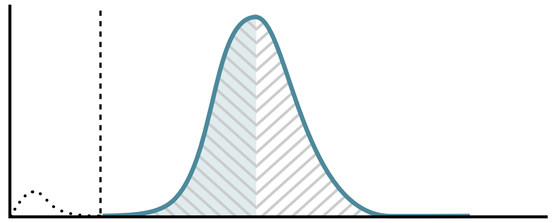
EOS Histogram



The **EOS histogram** varies and may be symmetrical or asymmetrical, jagged or smooth. Debris (lysed cells) from the EOS counting step may be seen to the left of the discriminator. It is not uncommon for the EOS discriminator to appear on the edge of the EOS peak.

- The eosinophils are counted separately from the other WBC types and are thus shown in a separate histogram
- Interpreting EOS by focusing on the numerical VETSCAN® HM5 data is recommended

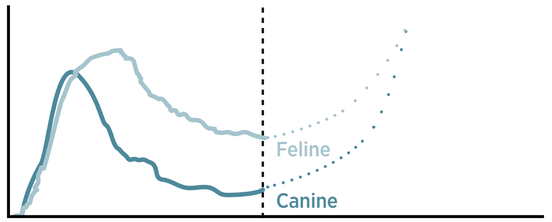
RBC Histogram



The **RBC histogram** in normal dogs and cats should present as an almost symmetrical, bell-shaped curve. The PLT peak can be seen to the left of the discriminator.

- The width of the curve relates to the RDWc
- An increased RDWc would show on the RBC histogram as a wider peak and mean that some of the RBC are either larger and/or smaller than normal
- RDWc measures anisocytosis, or RBC size variation

PLT Histogram



The **PLT histogram** begins with a sharp increase to a peak and tapers downward as cell size increases. This indicates that most PLT are small, with fewer large PLT. The RBC peak can be seen starting to the right of the discriminator.

- In felines and equines, the histogram tapers downward more slowly, indicating more large cells, commonly associated with mild PLT clumping
- The reported PLT number is the minimum number of free PLT counted by the analyser
- The PLT histogram should be evaluated whenever the PLT count is low and/or lymphocytes are elevated. A blood smear to confirm a low PLT count is also recommended

RDWc=red cell distribution width (percent).

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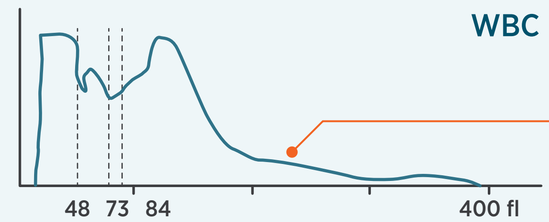
PLT clumping and anaemia[26]
Feline

Always confirm these results with a blood smear

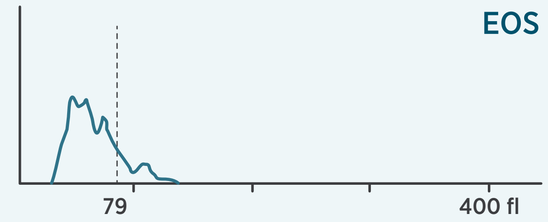
High WBC could be nucleated cells or clumped PLT

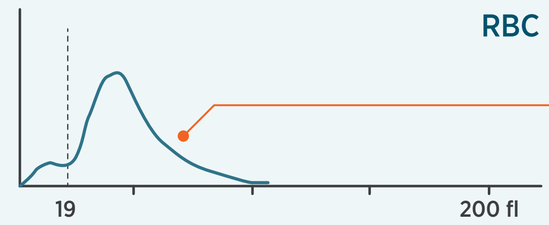
Elevated NEU

		RESULTS	UNITS	REF. RANGE
WBC	HIGH	22.39	10^9/l	5.5-19.5
LYM		3.7	10^9/l	1.5-7.0
MON		1.41	10^9/l	0.0-1.5
NEU	HIGH	17.2	10^9/l	2.5-14.0
EOS		0.08	10^9/l	0.0-1.0
BAS		0.01	10^9/l	0.0-0.2
LYM%		16.5	%	0.0-100.0
MON%		6.3	%	0.0-100.0
NEU%		76.8	%	0.0-100.0
EOS%		0.3	%	0.0-100.0
BAS%		0	%	0.0-100.0
RBC	LOW	2.07	10^12/l	5.0-10.0
HGB	LOW	3.3	g/dl	8.0-15.0
HCT	LOW	9.69	%	24.0-45.0
MCV		47	fl	39.0-55.0
MCH		16.1	pg	12.5-17.5
MCHC		34.3	g/dl	0.0-0.2
RDWc		26.8	%	
RDWs		49.2	fl	
PLT	LOW	78	10^9/l	300.0-900.0
MPV	LOW	11.2	fl	12.0-17.0
PCT		0.09	%	
PDWc		35.2	%	
PDWs		13.5	fl	

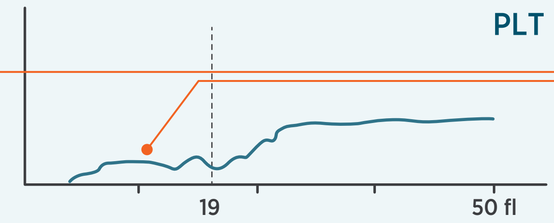


Long tail on WBC curve





Tail on RBC curve indicates higher amount of larger, immature RBC



Flattened PLT curve with an upward trend and low PLT count

In felines, RDWc >24% indicates increased variation of RBC size (anisocytosis)

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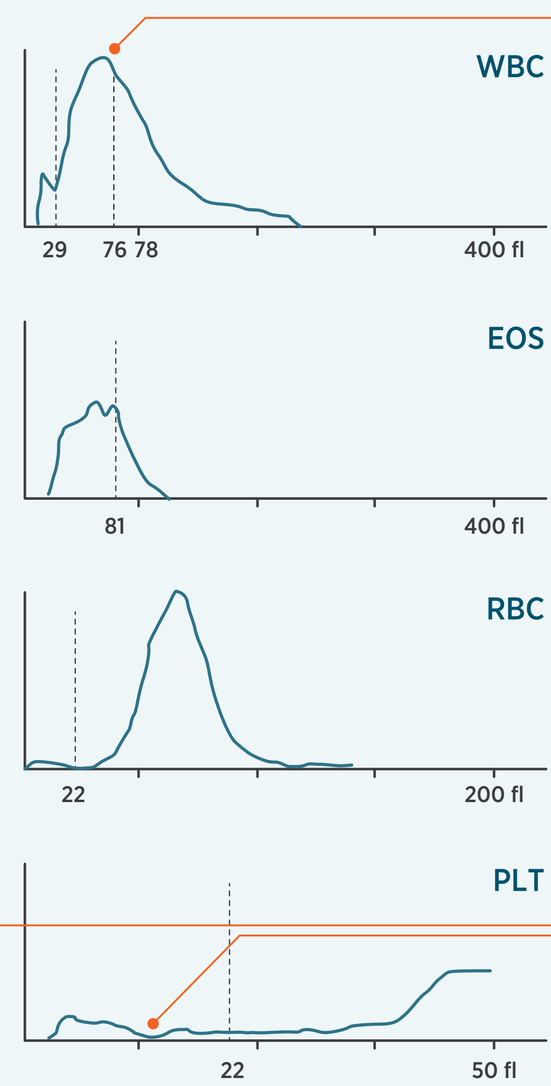
Classic leukemia[26]
Canine

Always confirm these results with a blood smear and potential pathology review

Very high total WBC

		RESULTS	UNITS	REF. RANGE
WBC	HIGH	115.94	10^9/l	6.0-17.0
LYM	HIGH	51.03	10^9/l	1.0-4.8
MON	HIGH	3.61	10^9/l	.02-1.5
NEU	HIGH	61.06	10^9/l	3.0-12.0
EOS		0.22	10^9/l	0.0-0.8
BAS		0.02	10^9/l	0.0-0.4
LYM%		44	%	0.0-100.0
MON%		3.1	%	0.0-100.0
NEU%		52.7	%	0.0-100.0
EOS%		0.2	%	0.0-100.0
BAS%		0	%	0.0-100.0
RBC	LOW	5.4	10^12/l	5.5-8.5
HGB		14.9	g/dl	120-18.0
HCT		37.21	%	37.0-55.0
MCV		69	fl	60.0-77.0
MCH	HIGH	27.6	pg	19.5-24.5
MCHC	HIGH	40.1	g/dl	31.0-39.0
RDWc		15	%	
RDWs		49.2	fl	
PLT	LOW	55	10^9/l	165.0-500.0
MPV		9	fl	3.9-11.1
PCT		0.05	%	
PDWc		38.3	%	
PDWs		14.5	fl	



Only one WBC peak

Clumping vs truly low PLT (ambiguous PLT curve)

M warning flag may appear (refer to manual)

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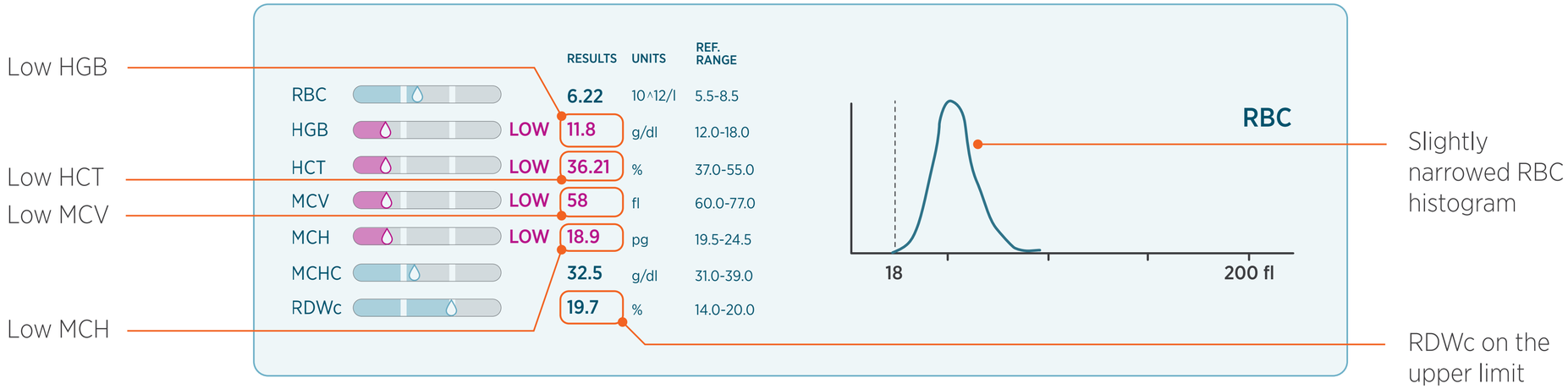
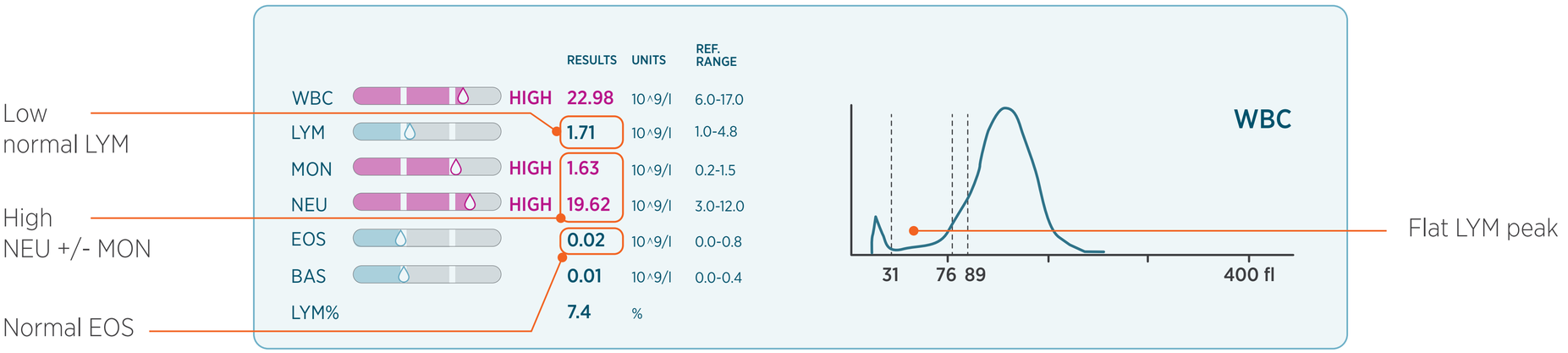
Microcytic anaemia[25]
Canine



Stress leukogram[26]
Canine



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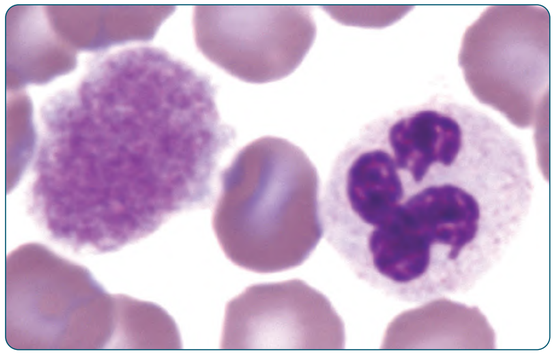
Inherited macrothrombocytopenia[14,27]

Inherited macrothrombocytopenia is due to a mutation in the gene encoding beta1-tubulin. This mutation has been identified in approximately 90% of Cavalier King Charles spaniels (CKCSs), as well as other breeds, including bichons frises, boxers, cairn terriers, Chihuahuas, cocker spaniels, English toy spaniels, Havanese, Jack Russell terriers, labradoodles, Labrador retrievers, Maltese, mixed breeds, Norfolk terriers, poodles and shih tzu.

Inherited macrothrombocytopenia on the VETSCAN® HM5[28,29]

Macrothrombocytes will not break into fragments when exposed to lyse solution and may cause inaccurate counts and/or instrument errors, particularly in analysers that use impedance technology as the primary measurement method. When such samples are analysed on the VETSCAN HM5, an "L" flag will often be displayed. While an "L" flag could suggest a potential lyse-resistant RBC anomaly or a problem with lyse delivery, in this case, the analyser is reporting macroplatelets as nonlysed lymphocytes.

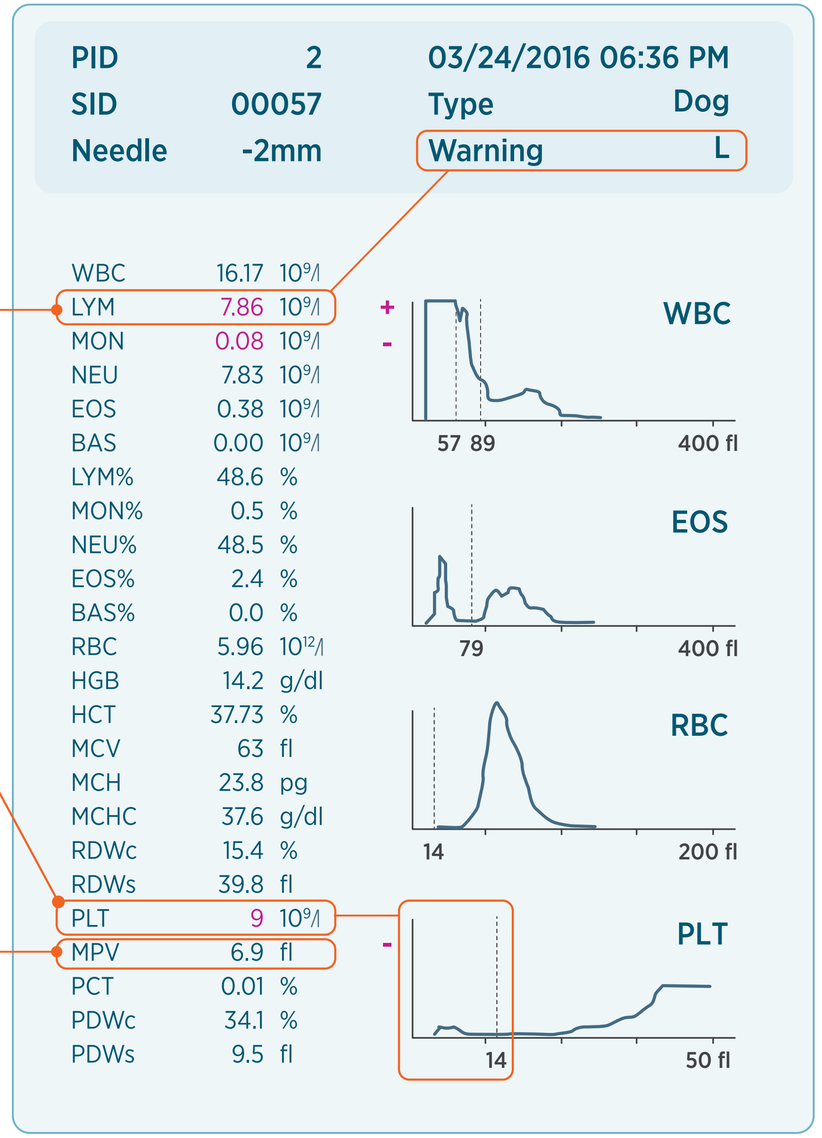


From chapter 7, Evaluation of haemostasis: coagulation and platelet disorders. Figure 7–26. In: Harvey JW, ed. *Veterinary Haematology: A Diagnostic Guide and Colour Atlas*. 1st ed. Elsevier Inc.; 2012:211.

A CBC from the VETSCAN HM5 for a patient with inherited macrothrombocytopenia will typically show an elevated LYM and a warning "L" flag

There will not be a significant PLT peak, and the PLT count will be reported as very low

The MPV may also be reported as normal



Note: Not every CKCS has macrothrombocytopenia. Those individuals with normal-size PLT will not show the features mentioned above.

MPV=mean platelet volume.

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Understanding classic leukogram patterns

Changes in total and differential leukocyte counts are usually grouped into patterns that facilitate interpretation. These patterns are:

		NEU	Left Shift	Toxic Δ*	LYM	MON	Inflammation
Stress leukogram	• A result of cortisol released by the adrenal gland • Occurs due to a wide range of processes — Systemic illness; metabolic disturbance; pain • Mimicked by corticosteroid therapy	↑	No	No	↓	↑ canine > feline	+/-
Physiological leukocytosis	• A result of epinephrine or norepinephrine release • Also called a flight-or-fight response • Most often seen in cats (of any age) and possibly in the young of other species • Usually transient and generally resolves about 30 minutes after the patient relaxes	↑	No	No	↑ (mostly feline)	Normal	Unusual
Inflammatory leukogram	• Represents the balance between tissue demand and bone marrow supply • May vary depending on source and severity of inflammation and timing of sample collection • NEU numbers may vary from severely depressed to markedly increased • A left shift indicates the presence of immature NEU — Usually, but not always, indicates an inflammatory leukogram • Inflammation is possible in patients without an inflammatory leukogram	**Mild/Chronic Inflammation**					
		↑	+/-	No	Normal or ↓	↑ (chronic)	Hopefully
		Acute Inflammation					
		↑	↑	Frequent	↓	Normal or ↑	Yes
		Overwhelming Inflammation					
		↓	↑ to ↑↑	Present	↓	No	Yes
Leukemoid reaction	• Characterised by a marked neutrophilic leukocytosis (>50,000 cells/µL) with a concurrent, orderly left shift • Toxic changes may or may not be present • Resembles granulocytic leukemia but is caused by another process • Also referred to as extreme neutrophilic (granulocytic) leukocytosis	↑↑↑	+/-	Occasional	Normal or ↓	Normal or ↑	?

*Δ=change.

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Understanding anaemia

Anaemia is one of the most common haematological abnormalities encountered in veterinary clinical practice. It is the manifestation of an underlying disorder, much like pyrexia, and not a diagnosis. It can be a primary sign of disease (eg, hemorrhage or immune-mediated hemolytic anaemia) or a marker of underlying disease (eg, cancer or chronic kidney disease). Therefore, even mild, asymptomatic anaemia should be investigated thoroughly to diagnose and treat the primary problem.

Anaemia is defined by a PCV, HCT, HGB or RBC count below the reference intervals for that species. Anaemia can be mild, moderate or severe and could be caused by an acute disease process or have been ongoing for a long time due to a chronic condition.

> *When evaluating an anaemic patient, haematology testing MUST include BOTH quantitative automated cell count + qualitative blood smear evaluation*

Once we receive abnormal RBC, HCT, HGB or PCV results, how do we proceed?

- Evaluate the haematology results in the context of the entire patient, including the patient's signalment and clinical status as well as the minimum database and other diagnostic tests
- Consider the potential for laboratory or sampling error
- If an automated count is performed and anaemia detected:
  - Confirm with a PCV, since this is the direct measurement of the proportion of blood comprised of RBC
  - Perform a blood smear to examine the RBC morphology and confirm automated cell counts to aid in determining a diagnosis and prognosis
- Remember, a comprehensive CBC includes an automated cell count along with a blood smear evaluation

Blood smear, anaemic patient[11-14]

Note: Anaemia can be masked by concomitant dehydration. Decreased measured erythrocyte parameters may also be observed when the total-body erythrocyte mass is normal but there is an expansion of the vascular space faster than the expansion of the total-body erythrocyte mass (relative anaemia).[30-32]

CBC=complete blood count; HCT=hematocrit; HGB=hemoglobin; PCV=packed cell volume; RBC=red blood cell.

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Further diagnostic testing to determine the underlying cause of anaemia[33]

Blood smear examination provides information about blood cell pathology and the potential for blood parasites not available with automated analysers. In addition, evaluation of RBC morphology can help pinpoint a diagnosis, determine the recommended treatment and monitor the response to treatment for anaemia.

Common RBC morphologies

IMHA	Regenerative anaemia	RBC damage due to microangiopathy*	Oxidative damage	Iron deficiency
Spherocytes	Anisocytosis	Schistocytes	Eccentrocytes	Schistocytes
Agglutination	Howell-Jolly bodies	Acanthocytes	Heinz bodies	Microcytes
Ghost cells	Polychromasia	Keratocytes	Spherocytes	Leptocytes

*Associated with neoplasia, disseminated intravascular coagulation, glomerulonephritis or vasculitis.

Consider the whole patient

Because anaemia is a manifestation of an underlying disorder and not a diagnosis, further diagnostic testing is usually necessary to determine the underlying cause. Additional diagnostic tests listed below should be utilised based on the differential diagnosis suggested by the classification of anaemia (see **Anaemia algorithm** on page 26).

Additional diagnostic tests

- Clinical chemistry profile/urinalysis +/- endocrine testing
- Virology, serology if infection is likely (eg, pyrexia, lymphadenopathy, etc)
- Bone marrow examination may reveal many diagnoses (eg, myelofibrosis, aplastic anaemia, bone marrow necrosis/inflammation, dyserythropoiesis, leukemia, metastatic neoplasia, myelodysplastic syndromes, etc)

IMHA=immune mediated hemolytic anaemia.

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Two ways to classify anaemia: bone marrow responsiveness

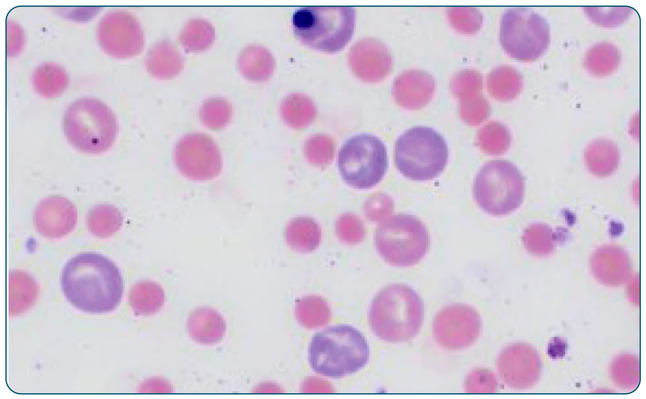
- Classification of anaemia in accordance with bone marrow responsiveness is based on the presence or absence of an increased number of immature erythrocytes in circulation (known as reticulocytosis, polychromasia) or erythroid hyperplasia in the bone marrow. Evaluation must be interpreted relative to the duration and severity of the anaemia[32]
- In most species, a RETIC count is considered the easiest, most reliable measure of marrow responsiveness. A notable exception is the horse, which releases few to no immature RBC into circulation; therefore, a bone marrow sample must be used to determine the erythroid response
  - **Note:** an automated RETIC count should always be verified with a blood smear to examine RBC morphology and to confirm the automated RETIC count

RETIC counts can be interpreted by either absolute or corrected counts to determine if regeneration exists

Absolute RETIC/PCM count/µL	=	(% RETIC or % PCM/100)	X	RBC count/µL
Corrected RETIC/PCM %	=	% RETIC or % PCM	X	Patient's PCV / average PCV for the species

- Interpretation must be made relative to the duration and severity of the anaemia. Simply relying on a reference interval may lead to misinterpretation of the erythroid response. See **Anaemia algorithm** on page 26 for examples

VETSCAN IMAGYST™ can identify polychromatophils



VETSCAN IMAGYST supports using a PCM count to classify the bone marrow response to an anaemia. RETIC and PCM are both immature red blood cells and have the same function but are identified using different staining methodologies.

Image obtained from VETSCAN IMAGYST at 400x magnification, showing Wright's-stained blood smear of RBC. Immature RBC are shown in a purple-blue colour.

PCM=polychromatophil; PCV=packed cell volume; RBC=red blood cell; RETIC=reticulocyte.

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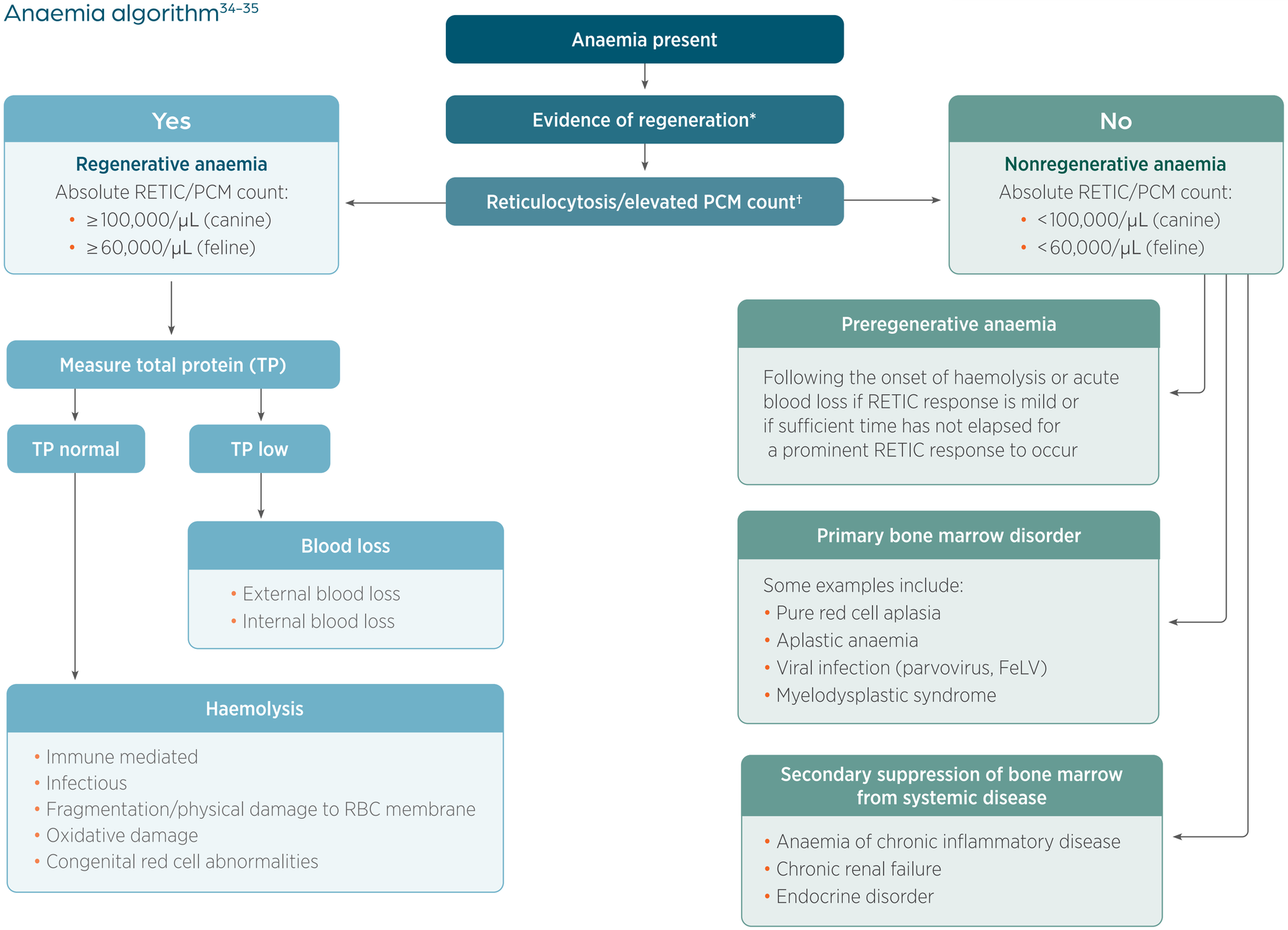
Two ways to classify anaemia: bone marrow responsiveness

See the full anaemia algorithm↗

Anaemia algorithm[34–35]



*Evaluation of the adequacy of the bone marrow regenerative response in the individual patient should also include consideration of severity and chronicity of the anaemia, suspected cause of the anaemia and potential for multiple causes contributing to the patient's anaemia. Trending the anaemia and RETIC/PCM count through sequential CBC may be helpful.

†RETIC/PCM counts supporting regeneration can be seen in nonanaemic patients. This may reflect a normal physiological response or a response to an increased need. Serial evaluations of the CBC should be done to rule out an emerging anaemia in these patients. RETIC or elevated PCM counts in the absence of anaemia (RAA) may indicate recovery from anaemia or may be associated with nonanaemic chronic hypoxia (eg, cardiovascular disease, pulmonary disease). RAA has also been observed in patients with gastrointestinal, inflammatory and neoplastic disorders and in dogs with osteoarthritis or receiving osteoarthritis treatments (eg, anti-inflammatory drugs, nutraceuticals).

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Two ways to classify anaemia: red blood cell indexes

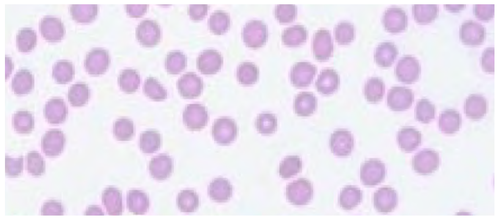
- In addition to RETIC or PCM counts, it is important to review the pertinent RBC parameters found on the automated CBC report: MCV and MCHC to describe trends in RBC size and HGB concentration to aid in classification of the anaemia
- See **Anaemia algorithm** on page 26 for additional information

Table: MCV classifications

MCV	Description	Common pathology
Decreased	Microcytic	• Iron deficiency • Hepatic portocaval vascular shunts • Normal breed variation (eg, Shiba Inu, Akita)
Normal	Normocytic	• Usually nonregenerative, poorly or early regenerative • "Early regenerative" refers to blood loss or blood destruction anaemia in which evidence of regeneration is not yet apparent because the bone marrow has not had time to respond to acute loss
Increased	Macrocytic	• Regeneration: bone marrow is responding and is releasing PCM/RETIC that are larger than normal • Congenital poodle macrocytosis • Hereditary stomatocytosis • Myelodysplasia • FeLV

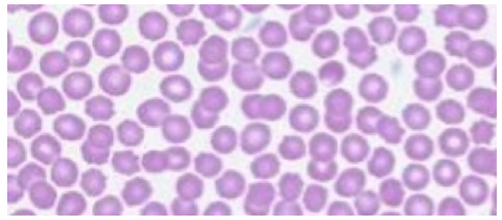
The 3 most important and relevant anaemia diagnostic patterns using RBC indexes are[34]:

Microcytic Hypochromic



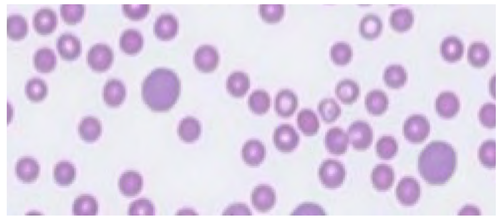
Usually due to iron deficiency anaemias

Normocytic Normochromic



Nonregenerative anaemias with residual normal erythrocytes

Macrocytic Hypochromic



Regenerative anaemias with large, young erythrocytes that are not fully hemoglobinized

Images obtained from VETSCAN IMAGYST™.

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Anaemia classification by red blood cell indexes[35]

Anaemia present

Microcytic

Hypochromic/normochromic

- Chronic iron deficiency
- Portosystemic shunts (often not anaemic)
- Anaemia of inflammatory disease (usually normocytic)
- Hepatic lipidosis in cats (usually normocytic)
- Normal Akita and Shiba dogs (not anaemic)
- Familial dyserythropoiesis of English springer spaniels (rare)
- Hereditary elliptocytosis in dogs (rare)
- Spurious when PLT are included in erythrocyte histograms
- Spurious in dogs with persistent hyponatremia (not typically anaemic)

Normocytic

Normochromic

- Preregenerative anaemia
- If RETIC response is mild or if sufficient time has not elapsed for a prominent RETIC response to occur
- Early iron deficiency anaemia before microcytes predominate
- Chronic inflammation and neoplasia (sometimes slightly microcytic)
- Chronic renal disease
- Endocrine deficiencies
- Selective erythroid aplasia
- Aplastic and hypoplastic bone marrow
- Lead toxicity (may not be anaemic)
- Cobalamin deficiency

Macrocytic

Hypochromic

- Regenerative anaemias with marked reticulocytosis
- Hereditary stomatocytosis in dogs (often slight reticulocytosis)
- Increased erythrocyte osmotic fragility in Abyssinian and Somali cats (reticulocytosis is usually present)
- Spurious with prolonged storage of blood sample

Normochromic

- Regenerative anaemias (decreased MCHC is not always present)
- FeLV infections with no reticulocytosis (common)
- Myelodysplastic syndromes
- Nonregenerative immune-mediated anaemia and/or myelofibrosis in dogs
- Poodle macrocytosis (not anaemic)
- Hyperthyroid cats (slight macrocytosis without anaemia)
- Folate deficiency (rare)
- Spurious with erythrocyte agglutination
- Spurious in cats and dogs with persistent hypernatremia (maybe hypochromic)

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Running alternative fluids on the VETSCAN HM5

Identify in-clinic nucleated cell counts* in alternative fluids (including pleural, peritoneal and synovial fluids) with the VETSCAN HM5. Characterise exudate samples to diagnose and identify potential inflammation, infections and neoplasia.

Validated for synovial fluid in horses[36†]

Synovial fluid procedure

1. Add a flake of hyaluronidase‡ (enzyme concentrate) to sample in a serum top tube, mix and store at room temperature for 10 minutes
2. Run the undiluted sample under the appropriate species setting on the VETSCAN HM5
3. Observe the total WBC number, which is useful in the determination of whether the WBC count is normal or abnormal
4. Microscopically evaluate a stained smear for a leukocyte differential and identification of nonleukocyte cells and/or infectious organisms
5. Perform a Soak Cleaning cycle (blue HM5) using HemaClean after every synovial fluid sample run

Validated for pleural and peritoneal fluid in dogs[36†]

Pleural and peritoneal fluid procedure

1. Run undiluted sample in a serum tube under the appropriate species setting on the VETSCAN HM5
2. Observe the total WBC number, which is useful for classification of fluids and provides the total nucleated cell count
3. Perform a total protein concentration on a refractometer
4. Microscopically evaluate a stained smear for a leukocyte differential and identification of nonleukocyte cells and/or infectious organisms

Contact Diagnostic Technical Support 24/7 for assistance with interpreting VETSCAN HM5 troubleshooting reports

0345 300 8034

DXSupport.UK@zoetis.com

*VETSCAN HM5 analysers are not intended to provide accurate differential cell counts for alternative fluid analysis samples.

†For results for species and/or fluids not validated, fluid samples may be run. However, precision and accuracy are not available, and results will not be supported by Zoetis Diagnostics.

‡Hyaluronidase from Sigma Chem Co, Part No. H3506, 100 mg bottle.

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VETSCAN HM5 reference intervals, SI units[7]

Parameter	Units	DOG	CAT[37]	HORSE	CATTLE	SHEEP*	GOAT*
WBC	10^9 cells/l	6.0 - 17.0	3.5 - 20.7	5.4 - 14.3	4.0 - 12.0	4.0 - 12.0	4.0 - 13.0
LYM	10^9 cells/l	1.00 - 4.80	0.83 - 9.10	1.50 - 7.70	2.50 - 7.50	2.00 - 9.00	2.00 - 9.00
MON	10^9 cells/l	0.20 - 1.50	0.09 - 1.21	0 - 1.50	0 - 0.84	0 - 0.75	0 - 0.50
NEU (GRA)	10^9 cells/l	3.00 - 12.00	1.63 - 13.37	2.30 - 9.50	0.60 - 6.70	(0.70 - 7.30)	(1.20 - 8.00)
EOS	10^9 cells/l	0 - 0.80	0.02 - 0.49	0 - 1.00	0.10 - 1.00	---	---
RBC	10^{12} cells/l	5.5 - 8.5	7.7 - 12.8	6.8 - 12.9	5.0 - 10.0	9.0 - 15.8	5.5 - 8.5
HCT	%	37.0 - 55.0	33.7 - 55.4	32.0 - 53.0	24.0 - 46.0	27.0 - 45.0	37.0 - 55.0
HGB	g/l	120 - 180	100 - 170	110 - 190	80 - 150	90 - 150	120 - 180
MCV	fL	60 - 77	35 - 52	37 - 59	40 - 60	28 - 40	60 - 77
MCH	pg	19.5 - 24.5	10.0 - 16.9	12.3 - 19.7	11.0 - 17.0	8.0 - 12.0	19.5 - 24.5
MCHC	g/l	310 - 390	270 - 350	310 - 390	300 - 360	310 - 340	310 - 340
RDWc	%	14.0 - 20.0	18.3 - 24.1	N/A	N/A	N/A	N/A
PLT	10^9 cells/l	165 - 500	125 - 618	100 - 400	100 - 800	100 - 800	200 - 500
MPV	fL	3.9 - 11.1	8.6 - 14.9	N/A	N/A	N/A	N/A

Reference intervals are determined from a population of healthy adult animals of a given species for a given test.

VETSCAN HM5 reference intervals continue on the next page.

---: EOS not included in 3-part differential.
N/A: Reference interval data not available for these parameters
*3-part differential with GRA.

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VETSCAN HM5 reference intervals, SI units[7] (cont'd)

Parameter	Units	RABBIT*	FERRET*	GUINEA PIG*	RAT*	MOUSE*†	PIG*	ALPACA	LLAMA
WBC	10^9 cells/l	3.0 - 11.5	2.0 - 10.0	5.0 - 17.0	2.1 - 19.5	6.0 - 15.0	11.0 - 22.0	6.0 - 30.0	8.0 - 23
LYM	10^9 cells/l	2.00 - 9.10	0.40 - 6.50	2.00 - 15.00	2.00 - 14.10	3.40 - 7.44	5.50 - 11.10	1.00 - 20.00	1.00 - 6.00
MON	10^9 cells/l	0 - 0.50	0.10 - 0.70	N/A	0 - 0.98	0 - 0.60	0.66 - 1.32	N/A	N/A
NEU (GRA)	10^9 cells/l	(0 - 2.80)	(0.80 - 4.50)	(1.00 - 11.00)	(0.10 - 5.40)	(0.50 - 3.80)	(5.00 - 10.00)	3.00 - 20.00	5.00 - 24.00
EOS	10^9 cells/l	---	---	---	---	---	---	N/A	N/A
RBC	10^{12} cells/l	5.0 - 9.0	7.8 - 13.0	4.8 - 6.3	5.3 - 10.0	7.0 - 12.0	5.0 - 8.0	8.0 - 20.0	10.0 - 17.0
HCT	%	36.0 - 50.0	36.0 - 56.0	30.0 - 44.0	35.0 - 52.0	35.0 - 45.0	32.0 - 50.0	25.0 - 45.0	25.0 - 50.0
HGB	g/l	130 - 160	120 - 180	80 - 150	140 - 180	120 - 160	100 - 160	90 - 210	110 - 180
MCV	fL	57 - 70	40 - 48	50 - 90	50 - 62	45 - 55	50 - 68	15 - 35	20 - 35
MCH	pg	17.5 - 23.5	13.5 - 16.5	12.0 - 13.0	16.0 - 23.0	11.1 - 12.7	17.0 - 21.0	7.5 - 13.5	10.0 - 14.0
MCHC	g/l	300 - 380	320 - 350	300 - 360	310 - 400	220 - 320	300 - 340	300 - 450	300 - 450
RDWc	%	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
PLT	10^9 cells/l	218 - 641	96 - 776	200 - 600	500 - 1000	200 - 450	325 - 715	N/A	N/A
MPV	fL	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

VETSCAN HM5 reference intervals continue on the next page.

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*3-part differential with GRA.
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VETSCAN HM5 reference intervals, common units[7]

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LYM	10^9 cells/l	1.00 - 4.80	0.83 - 9.10	1.50 - 7.70	2.50 - 7.50	2.00 - 9.00	2.00 - 9.00
MON	10^9 cells/l	0.20 - 1.50	0.09 - 1.21	0 - 1.50	0 - 0.84	0 - 0.75	0 - 0.50
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EOS	10^9 cells/l	0 - 0.80	0.02 - 0.49	0 - 1.00	0.10 - 1.00	---	---
RBC	10^{12} cells/l	5.5 - 8.5	7.7 - 12.8	6.8 - 12.9	5.0 - 10.0	9.0 - 15.8	5.5 - 8.5
HCT	%	37.0 - 55.0	33.7 - 55.4	32.0 - 53.0	24.0 - 46.0	27.0 - 45.0	37.0 - 55.0
HGB	g/dl	12 - 18	10 - 17	11 - 19	8 - 15	9 - 15	12 - 18
MCV	fL	60 - 77	35 - 52	37 - 59	40 - 60	28 - 40	60 - 77
MCH	pg	19.5 - 24.5	10.0 - 16.9	12.3 - 19.7	11.0 - 17.0	8.0 - 12.0	19.5 - 24.5
MCHC	g/dl	31 - 39	27 - 35	31 - 39	30 - 36	31 - 34	31 - 34
RDWc	%	14.0 - 20.0	18.3 - 24.1	N/A	N/A	N/A	N/A
PLT	10^9 cells/l	165 - 500	125 - 618	100 - 400	100 - 800	100 - 800	200 - 500
MPV	fL	3.9 - 11.1	8.6 - 14.9	N/A	N/A	N/A	N/A

Reference intervals are determined from a population of healthy adult animals of a given species for a given test.

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LYM	10^9 cells/l	2.00 - 9.10	0.40 - 6.50	2.00 - 15.00	2.00 - 14.10	3.40 - 7.44	5.50 - 11.10	1.00 - 20.00	1.00 - 6.00
MON	10^9 cells/l	0 - 0.50	0.10 - 0.70	N/A	0 - 0.98	0 - 0.60	0.66 - 1.32	N/A	N/A
NEU (GRA)	10^9 cells/l	(0 - 2.80)	(0.80 - 4.50)	(1.00 - 11.00)	(0.10 - 5.40)	(0.50 - 3.80)	(5.00 - 10.00)	3.00 - 20.00	5.00 - 24.00
EOS	10^9 cells/l	---	---	---	---	---	---	N/A	N/A
RBC	10^{12} cells/l	5.0 - 9.0	7.8 - 13.0	4.8 - 6.3	5.3 - 10.0	7.0 - 12.0	5.0 - 8.0	8.0 - 20.0	10.0 - 17.0
HCT	%	36.0 - 50.0	36.0 - 56.0	30.0 - 44.0	35.0 - 52.0	35.0 - 45.0	32.0 - 50.0	25.0 - 45.0	25.0 - 50.0
HGB	g/dl	13 - 16	12 - 18	8 - 15	14 - 18	12 - 16	10 - 16	9 - 21	11 - 18
MCV	fL	57 - 70	40 - 48	50 - 90	50 - 62	45 - 55	50 - 68	15 - 35	20 - 35
MCH	pg	17.5 - 23.5	13.5 - 16.5	12.0 - 13.0	16.0 - 23.0	11.1 - 12.7	17.0 - 21.0	7.5 - 13.5	10.0 - 14.0
MCHC	g/dl	30 - 38	32 - 35	30 - 36	31 - 40	22 - 32	30 - 34	30 - 45	30 - 45
RDWc	%	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
PLT	10^9 cells/l	218 - 641	96 - 776	200 - 600	500 - 1000	200 - 450	325 - 715	N/A	N/A
MPV	fL	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

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