

# Introduction of veterinary hematology parameters: factors affecting parameters, clinical relevance and inter-species variations

Hematology is the most common diagnostic testing performed, tightly followed by clinical chemistry. To understand the results of a complete blood count (CBC) it is important to understand the meaning of each parameter, how the various cells are produced and where, what factors that may affect their concentrations and production. Veterinary blood is also more complicated than human diagnostics in that species have varying morphologies and factors affecting them.

All blood cells (white blood cells, red blood cells and platelets) are produced in the bone marrow from the Pluripotent stem cell. They then divide into various cell lineages and continue their maturation processes before being released into the blood stream.

Boule's own developed Exigo H400™ is an automated hematology system designed to be a robust, easy to use and high-quality instrument and prides itself in its extremely high precision and accuracy (1). The analyzer is especially tailored for clinics with small animals, dehydrated animals or with body fluid with some patented special features.

## Red blood cells and associated hematological parameters

The undifferentiated stem cells that they originate from produce all the blood cells including the platelets and white blood cells as well as the most abundant blood cell – the red blood cells.

### Where do they come from?

The bone marrow is the source for the production of the red blood cells (RBCs), also known as erythrocytes. The process from differentiating the stem cell to a mature red blood cell, also known as erythropoiesis, takes slightly different time in different species, but in cows this process takes around 100 hours. Once the RBCs are mature and released into the blood stream, their lifetime is not very long; approximately 110 days in dogs, 73 days in cats, 143 days in horses and 43 days in mice (2).

During erythropoiesis, the cell nucleus is gradually replaced by hemoglobin (HGB), giving the RBCs their characteristic biconcave shaped discs in dogs and slightly more rounded shape in cat and horses.

It is mainly through a hormone erythropoietin (EPO) produced by the kidneys, that the bone marrow is stimulated to produce more RBCs. When there is low oxygen detected in the blood (hypoxia), which can occur after a bleeding or at high altitudes, the kidneys respond by producing EPO.

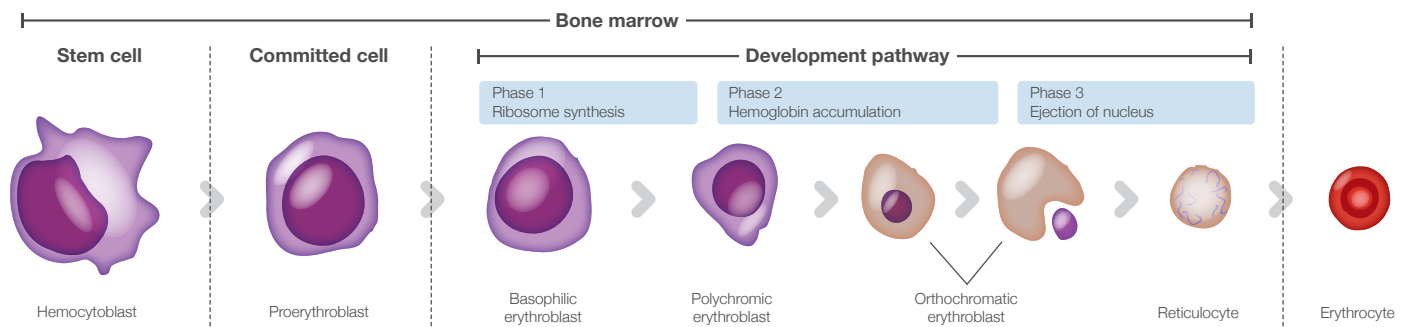


Fig. 1. Erythropoiesis and the various maturation stages of the RBCs.

## What is their function?

The RBCs have their main task to deliver oxygen from the lungs to the body tissues. This is possible due to the HGB present within the RBCs, which is built up of iron, porphyrin, globulin etc., and is able to bind the oxygen. The RBCs along with the HGB also indirectly remove carbon dioxide (CO<sub>2</sub>) from the body's tissues back to the lungs to be exhaled.

## How are they measured?

The main parameters can be measured for the mass of the RBCs:

- RBC concentration
- Mean cell volume (MCV), being an average of the diameter of the RBCs
- Hematocrit (HCT), the packed volume of the red blood cells
- HGB

In "normal" patients these should all follow each other whereas in anemic animals these parameters need to be investigated more in depth. See white paper on veterinary normal reference ranges (3).

## Red blood cell concentration

The RBC parameter is one of the fundamental ones also used in the calculations of MCV, MCH and MCHC. Earlier, RBC was calculated in the microscope manually through a Bürker chamber. However, with automated systems the methodological error has decreased from around 5-10% to less than 1%. It is however important that the automated systems are adapted for the various animal profiles to be tested as the size of the RBCs vary greatly.

Exigo H400 is especially adapted with software settings to work for various different animal profiles with varying size and membranes of RBCs. Moreover, the system is designed with a 60 µm capillary to be extra sensitive to small RBCs (1).

## Hemoglobin

The HGB count is one of the most common parameters requested by the laboratory, with the main reasons being to monitor RBC changes, liquid balance and anemia. HGB is quite an insensitive parameter which is not easily affected by handling procedures including storage and transportation. This makes it a reliable parameter.

Exigo H400 utilizes a non-toxic lysing reagent, which allows for a stable HGB complex that is then measured through absorption spectrophotometry at a wavelength of 535 nm and compared with a blank for each sample (1).

## Hematocrit

The HCT is the amount of RBC volume that is present in the total blood volume as a percent, meaning the packed cell volume of RBCs. It is also an important parameter in anemia where it is used to set the severity of the anemia, polycythemia and dehydration. The HCT can be measured either by an automated hematology count (as  $RBC \times MCV$ ) or if an EDTA blood tube is centrifuged the volume of RBCs to whole blood can also be measured. These two methods give slightly different results.

## Reticulocytes

In Fig. 1 we see the maturation stages of the RBCs. One of the latest stages is the reticulocytes (RETICS). Morphology wise they are larger than the mature RBCs and have a nucleus containing RNA that can be stained blue. In dogs, RETICS take around 1-2 days to mature.

Table 1. Different species and their normal amounts of reticulocytes in the peripheral blood

Animal species	Retics present in peripheral blood	Comments
Dog, swine	< 2%	
Cat	2 types of retics, 2-17% punctuate RETICS	
Horse	0%	Even in severe anemia no retics are observed, however an increased MCV is usually seen
Cattle, sheep, goats	0%	
Guinea pig, rat, rabbit, mouse	Variable	Mice newborns can have 90% whereas adults 2-4%

## Mean cell volume

The MCV is also an important indicator for anemia. Being the mean volume of the RBCs it gives information on microcytosis (small RBCs), macrocytosis (large RBCs) or normocytic cell distribution. Due to the large variation of MCV among species it is important that the automated cell counter takes various species into account. Exigo H400 accounts for this.

## Red cell distribution width

The RDW is a value giving you the variation of the RBC size. In Exigo, H400 the RDW is calculated from the RBC size distribution curve and are only presented if the MCV value is displayed.

## Mean cell hemoglobin concentration

The MCHC is the average concentration of HGB in the total RBC mass. This means it is calculated as:  $MCHC = HGB/HCT$  or  $MCHC = HGB/(RBC \times MCV)$ .

The MCHC can also be used in anemia cases to classify them into normochromic (normal HGB concentration in the RBCs) or hypochromic (when the MCHC is low).

MCHC generally does not vary very much and is a good parameter for checking the stability of an automated hematology analyzer. Daily mean MCHC can be used to check the system over a period of time.

## Mean cell hemoglobin

The MCH is a less used parameter clinically and is the mean concentration of HGB in the RBCs and calculated by  $MCH = HGB/RBC$ . Usually the changes in MCH go hand in hand with observed changes in the MCHC.

## Differences among species?

There is a fundamental difference in the RBCs for birds, reptiles and fish compared with other species in that the RBCs still have their nuclei intact. This entails that the RBCs cannot be lysed by the reagent used with a normal automated hematology analyzer and hence, most automated cell counters cannot analyze blood from these animals. The most common technology used for counting the white blood cells is to utilize them being the only blood population with nuclei and lysing away all the RBCs. With fish, reptiles and birds this means that the un-lysed RBCs will be interfering with the total WBC count.

When it comes to camels, llamas and alpacas and related species, they also have some differences in their RBCs. The morphology here is ellipsoid and the cells are a lot more resilient to lysing. This may also pose problems for automated cell counters as well as their shape, potentially disturbing the MCV measurement which then directly has an impact on HCT and red blood cell distribution width (RDW).

Exigo H400 has solved the challenges of the camels and related species RBC counts by allowing for a longer lysing time to allow for the RBCs to properly lyse. Moreover, there is an MCV compensation and other software features that help ensure the MCV is accurate. As for the birds, reptiles and fish, the RBCs can still be measured in Exigo H400, though some other parameters will be compromised (mentioned further down).

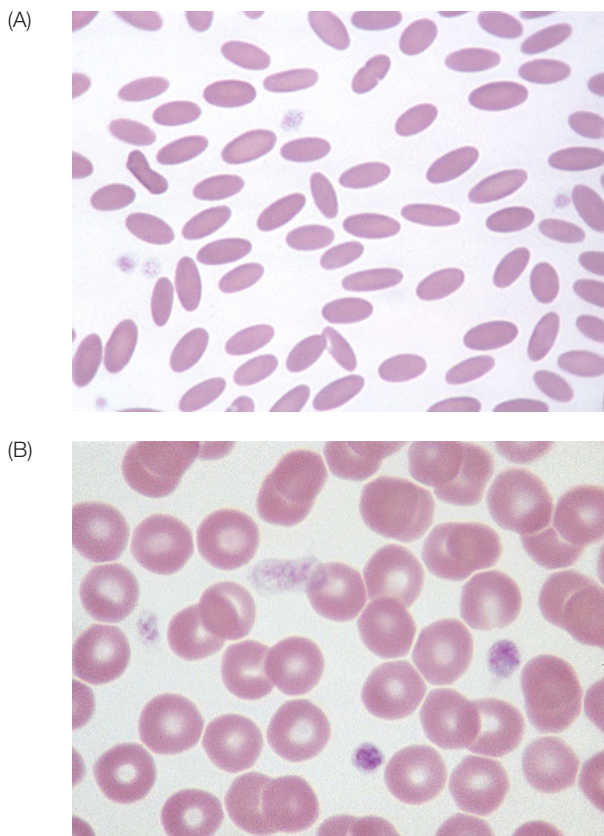


Fig. 2. (A) Blood smear from Llama showing the elliptical shaped RBCs

## Alterations in the red blood cells and associated hematological parameters

The most common clinical diagnosis associated with the red blood cells and the including parameters HGB, MCV, HCT, MCHC, RDW is anemia. Anemia is defined as a too low oxygen carriage capacity of the red blood cells. Anemia can be caused by different factors and be of different kinds. Anemia will be discussed in more detail further on.

### Alterations in RBC, HGB or HCT for clinical relevance (2)

#### 1. Low HGB, HCT or RBC

May indicate:

- Anemia – can have many causes such as bone marrow failure, EPO deficient, hemolysis, malnutrition, blood loss, etc.
- Late pregnancy
- Shock – through swelling of the spleen and hypotension
- Anesthesia/sedation – the spleen is more blood-filled
- Age – in younger animals these parameters tend to be lower and then increase with age

However, in some cases the results of low HCT may be false.

Potential reasons could include:

- Poor mixing of sample
- Hemolysis
- Low blood volume sampled in EDTA tube in relation to the concentration of EDTA

#### 2. High HGB, HCT or RBC

With a high concentration of RBC and elevated levels of HCT and HGB, the blood becomes “thicker” and it is less effective to transport oxygen.

Reasons might include:

- Activity, stress, fear – the spleen may here contract and release RBCs into the blood. This is especially apparent in warm-blooded horses where the HCT can increase from 35% to 50%. The reason is due to the large reservoir of 1/3 of the blood cells being stored in the resting state of the horse (1).
- Kidney disease – with high EPO
- Dehydration – such as diarrhea
- Anabolic steroid treatment
- Primary or secondary polycythemia – could be caused by high altitude, heart shunt, Cushing’s disease, etc.

However, in some cases the results of high HGB may be false.

Potential reasons could include:

- Lipemia
- Heinz bodies
- Extended sample storage – causes the RBCs to swell and the MCV and HCT increase
- High WBC count

The Exigo H400 compensates for high WBC counts using a compensation factor.

## Low MCV or MCHC

A decrease in the MCV (microcytosis) is generally associated with iron deficiency and/or chronic blood loss.

Hypochromacy (low MCHC) could be due to acute and chronic blood loss in anemic patients or also due to iron deficiency or hemolytic anemia. This parameter needs to be used in conjunction with MCV value.

## High MCV, RDW or MCHC

An increase in the MCV (macrocytosis) is a common sign in regenerative anemia as immature RBCs tend to be larger. Reasons could be B12 deficiency, folate deficiency or liver disease.

Increased RDW values (anisocytosis) can also be a sign of a regenerative anemia or just an increase in production of RBCs. Together with MCV, RDW can be used to distinguish between various anemia causes.

Due to RBCs in general being as concentrated with HGB as possible by the body, hyperchromic (high MCHC) is not a clinical phenomenon. This would most likely occur due to pre-analytical errors through old aged samples being tested or hemolysis in the sample.

## Anemia

Generally, anemia is associated with a low HGB, HCT and RBC count in a normal hydrated animal. Anemia is common, however, there are different forms of anemia which all have various causes and prognosis. Therefore, it is important to identify which type of anemia is present in a patient.

Other physical features of anemia include decreased appetite, increased heart rate and respiratory rates, pale mucous membranes, decreased strength etc.

To establish the prognosis of an anemic patient, the cause is needed to be found. This is usually found through patient history, clinical examinations, physical examinations and hematology parameters.

There are 2 subgroups that anemia is most commonly divided into: regenerative and non-regenerative. Regenerative anemia is where the bone marrow is responding to the anemia by producing more RBCs. Non-regenerative anemia is when the bone marrow responds inadequately to the increased need for RBCs.

Acute anemia may give very severe signs and symptoms to the patient even if the anemia is mild. Oppositely, chronic anemias can have milder symptoms due to adaptations of the heart and lungs to the chronic situation or even no symptoms in severe cases.

**Table 2.** Types of anemia and the underlying possible causes and symptoms associated (2)

Type of anemia	Causes	Symptoms
Non-regenerative anemia	<ol style="list-style-type: none"> <li>Decreased or inefficient production of RBC/HGB. Iron deficiency or bone marrow disturbance (causing decreased production of HGB or RBC)               <ol style="list-style-type: none"> <li>Anemia or inflammatory disease</li> <li>Neoplastic disease</li> <li>Nutritional deficiency (e.g. iron, copper, cobalt, folate)</li> <li>Aplastic or hypoplastic anemias</li> <li>Endocrine or metabolic disease</li> </ol> </li> </ol>	Pale mucous membranes, tachycardia, weakness, anorexia, enlarged spleen, fever, shock, hypotension etc.
Regenerative anemia	<ol style="list-style-type: none"> <li>Blood loss               <ol style="list-style-type: none"> <li>PLT disorders</li> <li>Gastrointestinal hemorrhage</li> <li>Coagulopathies</li> <li>Trauma or surgery</li> <li>Splenic rupture</li> </ol> </li> <li>Blood destruction (hemolysis)               <ol style="list-style-type: none"> <li>Immune-mediated disease</li> <li>Toxicities</li> <li>Intrinsic RBC defects</li> <li>Fragmentation</li> <li>Infections</li> </ol> </li> </ol>	<ol style="list-style-type: none"> <li>Blood loss could give symptoms of blood in vomit/nose/feces or hematomas.</li> <li>Hemolysis could be associated with icterus samples, red color of urine, larger spleen size, yellow color of mucous membranes.</li> </ol>

**Table 3.** Severity of anemia diagnosis through HCT% measure (2)

Severity	HCT %		
	Cat/ruminants	Dog	Horse
Mild	20–26	30–37	30–33
Moderate	14–19	20–29	20–29
Severe	10–13	13–19	13–19
Very severe	< 10	< 13	< 13

## Reticulocytes and anemia

Reticulocytes in the blood indicates a response to an EPO increase where the body is told to produce more RBCs. This can occur where blood loss is present, hemolytic conditions, etc. It is therefore important to see if an anemia is regenerative or not. High RETICS signifies regenerative anemia with a responsive bone marrow. If no anemia is present yet RETICS are high in concentration, this could potentially indicate low oxygen in the blood (hypoxemia). For cats, when the HCT is below 20% or 30% in dogs then a manual count of RETICS is recommended (2).

Apart from retics, there are other signs in the RBCs of regenerative anemia. These include:

- Nucleated RBCs
- Polychromasia – cytoplasm has a blue-pink color
- Anisocytosis – large difference in RBC sizes
- Basophilic punctuation
- Macrocytosis – MCV increase
- Howell-Jolly bodies – left over nuclear pieces in the cytoplasm are observed

In case of anemia, all of the morphologies of the RBCs should be microscoped. None of them can be identified by automatic hematology analyzers except anisocytosis (through RDW and RBC histogram) and macrocytosis (through a higher MCV).

## Anemia investigation procedure (2)

1. CBC with emphasis on the RBC morphology, retics count, RBC associated parameters (MCV, RDW, MCHC). Both automated hematology together with a manual smear.
2. PLT count or at least an estimate and total plasma protein (TP) should be measured.
3. Biochemistry profile and endocrine testing could be done to look for metabolic or endocrine disturbances. Here the liver, kidney and thyroid are of special interest.
4. Serum concentration of iron and total iron binding capacity (TIBC) are usually tested if inflammation or iron deficiency is thought to be the cause.
5. A biopsy of the bone marrow or an aspirate could be done if nothing is abnormal of the above and it is more useful for non-regenerative anemias.
6. Specialized tests for immune disorders could also be done if applicable (direct Coombs test, antinuclear Antibody test).
7. If hemorrhage is thought to be the cause, coagulation, fecal tests, iron assays and body fluid tests can be performed.

## Polycythemia

When the blood becomes too concentration through dehydration or the spleen contracting and releasing more blood into the blood circulation, the HCT, HGB, RBC all become temporarily increased. This is relative polycythemia. Absolute polycythemia is when there is a permanent RBC increase. Absolute polycythemia may occur with uncontrolled RBC production in the bone marrow. This is very unusual in cats and dogs. EPO is usually low or normal and WBC and PLT are seen to be elevated.

There is also secondary polycythemia which is when there is an increase in the EPO levels in for instance heart or lung diseases

or kidney tumors producing EPO.

## Platelets and associated hematological parameters

### Where do they come from?

Platelets (PLTs) are the smallest blood cells, 5–7  $\mu\text{m}$  in diameter with no nucleus. They come from the same stem cell the myeloid stem cell, which is then differentiated into megakaryocytes that later bud off into platelets. The general life time is around 7 days (2).

### What is their function?

Platelets (PLTs) are important in the blood clotting mechanism of the blood where they minimize blood loss when a vessel is damaged by binding to the wall of the vessel and clumping together, forming thrombus. PLTs are also vital in many inflammation and healing processes as they interact with many other types of cells and by releasing various signaling substances.

### How are they measured?

PLTs are in general counted in parallel with the RBCs in automated hematology systems and are well separated from the RBCs due to their much smaller size. Manual count can be done using a hemocytometer and a microscope with phase-contrast functionality (2). Compared to automated instruments, manual PLT reading is quite inaccurate. PLT clumps also make manual count difficult on the hemocytometer.

Exigo H400 counts the PLTs using impedance technology and a smaller 60  $\mu\text{m}$  capillary to be extra sensitive at smaller RBCs and PLTs. Read more about this in the Exigo Tech note (1).

### Mean platelet volume

The mean platelet volume (MPV) is, like the MCV, the mean size of the diameter of the PLTs.

This parameter is interesting to investigate during low PLT counts (thrombocytopenia). MPV can be affected by many factors that will alter the values that increase the difficulty in the interpretations of this parameter at times. These factors are mentioned further down.

### Plateletocrit and platelet distribution width

The plateletocrit (PCT) and the platelet distribution width (PDW) are sometimes reported by automated hematology analyzers. These parameters are equivalent to HCT and RDW on the RBCs. The PCT (fraction of blood consisting of PLTs) and PDW

are not used frequently today for clinical use.

## Alterations in the platelets and associated hematological parameters

Low PLT values are a great risk for bleeding (hemorrhage) whereas a high PLT count gives a risk of blood clots (thrombosis).

### Low PLT count (2)

Low PLT (thrombocytopenia), is a common diagnosis and there are many potential reasons behind it. If there is a change in the PLT or megakaryocyte function it is instead known as thrombopathy. It is especially important to monitor the PLT levels before surgery to prevent the risk of bleeding. As with other hematology parameters, a low PLT value from an automated hematology system indicates that a blood smear should be performed to assess the morphology of the cells. It is at a later stage also possible to manually check on a blood smear the bone marrow to see the potential platelet production disturbances with the megakaryocytes.

Some causes of thrombocytopenia:

- Leukemia, lymphoma
- Immune thrombocytopenic purpura (ITP)
- Drugs: chemotherapeutic, estrogen, non-steroid anti-inflammatory drugs, sulfadiazine, etc. – this is as the PLTs and megakaryocytes are very sensitive to toxins and this results in a much reduced production.
- Other type of drugs such as heparine, sulfonamides, furosemide, gentamycin – these drugs attach to the membranes of the PLTs and this causes the immune system with white blood cells to be activated for phagocytosis and many of the PLT are destroyed.
- Aplastic anemia – the bone marrow is depressed causing low PTL
- Infections – the PLTs can be destroyed by the immune system or just have a decreased production due to the bacteria, virus, fungi, protozoa or nematodes (e.g. canine leishmania, equine infectious anemia, feline immunodeficiency virus, African swine fever virus, bovine virus diarrhea virus, ehrlichia (anaplasma), feline leukemia virus, etc.)
- Hemolytic anemia
- Malignant cells in the bone marrow
- Vaccination – certain vaccines have reported this side effect in some cases
- Disseminating intravascular coagulation (DIC)
- Cavalier King Charles Spaniel breed known to have macrocytic PLTs and fewer total counts – see WP regarding normal ranges (3)

There may also be falsely low platelets counted by automated hematology cell counters. Possible reasons for pseudothrombocytopenia include:

- Agglutination – this is a reaction that may occur with certain patient samples in the EDTA. PLT and certain PLT antibodies or other molecules are released when they come in contact with EDTA causing agglutination. Today there is no clinical significance of this but it may sometimes cause costly and time consuming diagnostics to investigate a false

thrombocytopenia. Therefore, it is important to check with manual microscope to avoid.

- Platelet aggregates – PLTs can join together to form large clumps called aggregates. This occurs outside the body (i.e. in vitro) in the EDTA tubes. The longer the samples are stored in the EDTA tubes the more aggregates can be formed. This issue is most common in cows and cats and therefore automated cell counters are not always useful for the PLT count here. To help identify aggregates on a cell counter it is important to look at the histogram and the distribution curves for both PLT/RBC but also the WBC curve. This is as the clumps of cells may make the PLT population small and the cells may appear larger and shift towards the RBC curve. Moreover, the larger aggregates may be mistaken for lymphocytes (causing falsely high lymphocyte count) and therefore interfere with the WBC histogram.
- Satellitism – here neutrophils (sometimes monocytes) and platelets bind together to form a larger complex. The PLTs look like satellites surrounding the WBC. Potentially a shift in the neutrophil histogram curve towards the right can be seen as these are counted as larger cells.

These pseudothrombocytopenias can sometimes be avoided by using other anticoagulant than EDTA, such as sodium citrate.

### High PLT count (2)

Increase in PLT above the normal range is known as thrombocytosis. In general there is no symptoms or dangers with a moderate increase, however, a large increase may cause blood clots (thrombosis) which can be very severe. Some of the potential causes of increased PLT counts:

- Chronic myeloid leukemia (CML)
- Polycythemia vera
- Recent surgery or other accident/bleeding – PLT production has been activated
- Acute or chronic infections
- Inflammatory reactions
- Chronic bleedings – for instance with iron deficiency
- Myeloproliferative disorders
- Cushing's disease or glucocorticoid therapy

Just like there is a potential for falsely low PLT counts there are also potential reasons that you can get falsely high PLTs. These may include:

- Fragments – from cells decaying
- Automatic cell counters having electrical disturbances and microbubbles in the reagent interfering
- Repeated puncture of a vein in the same place
- Microcytosis – when MCV is very low for the RBCs, some of the RBCs may interfere with the PLT count. Due to the large concentration of RBCs compared to PLTs in the blood there will usually be no noticeable affect on the RBC value.

### Low MCV count (2)

The average size of the PLTs can be seen with:

- Chemotherapy
- Decreased PLT production
- Increased destruction of PLTs

- Megaloblastic anemia
- Aplastic anemia

### High MCV count (2)

If the size of the PLTs increase in general, it usually indicated a better functioning of the PLTs. It can be caused by:

- Increased PLT production
- CML
- ITP
- Myelodysplastic syndrome (MDS)

Once again there is a potential for false MPV:

- Fragments – decaying RBCs
- Water absorption – EDTA tends to swell platelets with time. Especially during the first 2 hours (1).

## White blood cells and associated hematological parameters

### Where do they come from?

The white blood cells (WBCs, also known as leukocytes) are in general split into 5 subpopulations: neutrophils (NEU), lymphocytes (LYM), eosinophils (EOS), monocytes (MONO) and basophils (BASO). There are also immature forms of each of the subpopulations that can occur in certain circumstances.

All subpopulations are produced in the bone marrow from

the same initial pluripotent stem cell. They then undergo slightly different paths (Fig. 3). The NEU, EOS and BASO are all three known as granulocytes as they all have granules in their cytoplasm and they have a more similar production path in the bone marrow. The lymphocytes can also be formed in lymphoid tissues (thymus, lymph nodes, spleen).

### What is their function?

The WBCs are the body's defense troop for foreign matter and microbes, they are important in inflammatory response, as well as the "cleaners", removing dead or damaged cells and tissue.

In general, an inflammation in the body will increase the WBCs (leukocytosis). Too many WBCs may however be harmful to the individual with a too strong inflammatory response.

Too few WBCs (leukopenia) is also detrimental and can be deadly as it can result in increased risk of infection where the body is not capable of removing or handling it.

NEU cells are the first line of defense in the blood. They can easily migrate into tissues to phagocyte (a way to destroy microbes and other material by internalizing them) bacteria and other foreign matter where needed. They are able to find these locations through infections and inflammations in the tissues releasing signaling substances that are recognized by the NEU. As this occurs, the bone marrow will in parallel activate the production of more NEU into the blood. The immature NEU are known as band NEU. You can read more about them in the WP

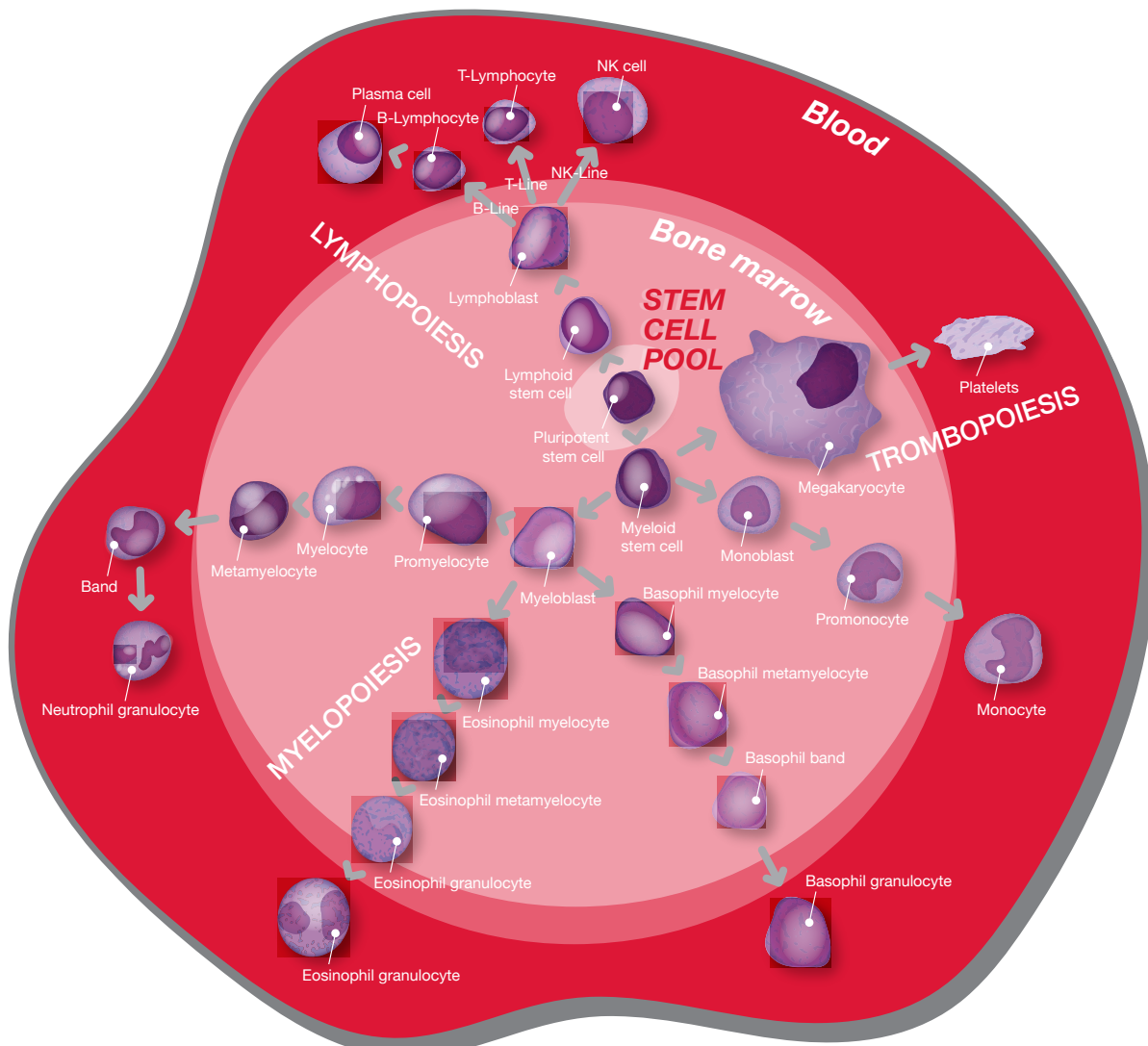


Fig. 3. Hematopoiesis of the white blood cells.

regarding normal reference ranges (3).

LYM cells are the second line of defense and control the production of antibodies. They also can destroy cells by cytotoxicity. There are two main categories of LYM cells: B-lymphocytes and T-lymphocytes.

Monocytes (also known as macrophages) are also able to undergo phagocytosis to remove and destroy matter and microbes. The MONO cells are important for the communication between the different WBC subgroups during immune defense.

EOS are also able to undergo phagocytosis though not as effectively. They are attracted to locations with antigen-antibody reactions and have potent proteins that they use to destroy parasites. These proteins are highly toxic and can also cause damage to the host tissue as well and this is an example of what occurs during allergies.

BASO cells are also active in allergies where they have histamine and heparin in their cytoplasm that they can release when they come in contact with IgE-receptors (immunoglobulin E) for antigens. They are very rare cells in general and when an increase is seen in BASO, an increase in EOS usually also occurs.

### How are they measured?

- WBC and the differential count (NEU, LYM, EOS, MONO, BASO) can be measured by impedance, laser and other techniques or microscopy based on their different morphologies and characteristics. When counting them automatically using impedance the different cell types are divided into subgroups based on their size. A lyse reagent is added to the blood and all membranes are disrupted (RBCs and WBCs) and the WBC are the only cells with nuclei that are then categorized according to size.
- In laser technology the WBC's are categorized using light scatter. The intensity of the scattered light reflects the cell size and internal structure, while the low-angle signal shows cell size and the middle- and high-angle signals show intracellular (nucleus and cytoplasm) information (5).
- Microscopy allows for many more developmental stages and cell types to be distinguished with the staining that no automated counter today can identify (examples including toxic changes, echinocytes, RBC cellular changes, bacteria, PLT clumping, Heinz-bodies, etc.).

Manual microscope counting, even though it is the reference method, does show higher CV% at around 10% whereas with automated counters the CV% is usually less than 3%. Due to limited cell identifications however of automated counters, they are screening systems that identify abnormal samples that need to be further checked by microscopy.

Exigo H400 measures the concentration of the WBC and differential using floating discriminators and impedance. Read more in the Exigo Tech note (1).

### Differences among species?

There are fundamental differences between species when it comes to the WBC subgroups and counts. Birds and fish differ from mammals with the subgroups, for instance having heterophils as another differential subgroup making automated counting difficult. The fact that they have nucleated RBCs

and PLTs (also reptiles) automated cell counters have another difficulty when trying to count the WBCs.

### Reference values for WBC and WBC differential

For the reference values and physiological variations in the WBC's, see WP on normal reference ranges (3).

## White blood cell

### Granulocytes – neutrophils, eosinophils and basophils

The NEU, EOS and BASO are all grouped together as granulocytes, named after their characteristic granules present in their cytoplasm. These cells migrate via the blood to tissues where they mainly perform their functions. Most of the body's granulocytes are not present in the blood, but many are attached to the blood vessel walls and will not be counted in the CBC analysis unless they are released into the blood through signaling factors such as stress hormones. Other granulocytes are also stored in a reserve pool present in the bone marrow and in the production site. These will also be made available to the body if needed. This is one of the reasons that the granulocyte value can give huge variation in values just within the hour, potentially doubling or tripling. To get the most accurate value during blood sampling, which the normal range is based on, the patient needs to be calm and relaxed.

Read more about neutrophils and their maturation in WP on normal reference ranges (3).

### Lymphocytes

Lymphocytes, like granulocytes, stem from the bone myeloid marrow stem cell, they then take a different path via a lymphoid stem cell to a lymphoblast before differentiating into several (at least 10) different subpopulations. These different subpopulations of LYM have different functions and the sizes range is quite large and their life expectancy can be either just a few days or several years depending on the type. The characteristics and morphology many times can not distinguish these different LYM subtypes, immunological methods to see the antigens present on their membranes are required to identify them. The two main subgroups of LYM are known as B-lymphocytes and T-lymphocytes.

1. The B-LYM are developed and matured in the bone marrow and liver whereas the T-LYM are developed in the thymus. Apart from the blood vessels there are also lymphatic vessels. These are where the LYM are also circulating through, not only the blood.

The B-LYM cells may also become activated and further differentiate into Plasma cells. These cells look similar to BASO in the cytoplasm and have a lighter color close to the nucleus (see Fig. 3). Their function is to release antibodies (Ig, immunoglobulins). Their presence in the blood is very rare.

2. T-LYM are involved with both communications with other WBC cells for cell-mediated immune responses by creating signals to them to "tag" the microbes or foreign matter but may also destroy infected cells. The B-LYM on the other hand are involved with antibody production through developing plasma cells and part of the memory cells which store the information on how to produce the antibodies for



certain antigens from a previous infection or vaccination.

## Monocytes

MONO are much more similar to the granulocyte group of WBCs, mainly through their ability to phagocytose microorganisms. When the MONO are circulating in the blood stream they are in a more immature stage until they are activated and locate to the target tissues where they differentiate further.

## Alterations in the white blood cells and associated hematological parameters

Leukocytes are sensitive to storage which may alter the values when analyzing. Moreover, whole blood from sick patients is even more fragile and it is even more important to analyze the samples as quickly as possible after sampling. In automated analyzers, the LYM cells are the most sensitive to being destroyed and miscounted whereas in manual microscope it is the granulocytes.

### Low white blood cell counts (2)

Low WBC (leukopenia), can be caused by very many reasons and can either be due to a decrease in NEU or LYM or all types of WBC subgroups (see more specifics further down for the various cell types). Some reasons include:

- Liver or spleen disease
- Severe infection – using up most of the NEU cells
- Decreased WBC production – infection, fibrosis, tumor, radiation, etc.
- Collagen vascular disease
- Bone marrow depression

### High white blood cell counts

High WBC, (leukocytosis) can also be caused by various reasons. It is important here to look at the various subgroups of the differentiation separately. Some general reasons include infection, leukemia, extreme physical stress, tissue damage, inflammatory disease, etc.

### Low neutrophil counts (2)

Low NEU (neutropenia), means that a risk for infection grows, especially when under  $0.5 \times 10^9/L$ . it can be caused by:

- Virus infections – e.g. parvovirus, FLV, FIV, equine infectious anemia, bovine virus diarrhea virus etc.
- Aplastic anemia
- Myelofibrosis
- Inflammations – severe from bacteria's with for example sepsis, toxemia or tuberculosis. Could also come from fungal infections or immune-mediated diseases.
- Spleen – an enlarged spleen
- Autoimmune diseases
- Neoplasia – together with bone marrow depression
- Estrogens
- Drugs, chemical agents – sulfa drugs, corticosteroids, benzene, lead, mercury etc.
- Vitamin B12 or folic acid deficiency
- Anaphylactic shock

- Radiation – ionizing
- Cachectic conditions

### High neutrophil counts (2)

Some reasons for high NEU include:

- Cortisone treatment
- Stress
- Strong exercise or excitement – effect of adrenaline in body. This is a transient neutropenia.
- Tissue damage
- Bleeding or acute hemolysis
- Virus infections – e.g. feline infectious peritonitis
- Leukemoid reaction and myeloid leukemia
- Acute/chronic bacterial infection – local infections such as empyema, pyometra, etc.
- Leukocyte adhesion deficiency – seen in Irish setter dogs and Holstein cattle

### Left shift, toxic changes

Left shift is a clinical term pointing to an increase in immature WBCs, in particular band NEU. Toxic changes in the NEU are changes occurring to the cells that are highly important for clinical diagnosis.

See WP on normal reference ranges (3) for more detailed information on specifics and how these are triggered and affect the patient.

### Low eosinophil counts (2)

Low EOS values may come from:

- Corticosteroid therapy
- Infections
- Stress
- Cushing's syndrome and adrenal activity

### High eosinophil counts (2)

High EOS counts (eosinophilia), can many times be short lived. Therefore, a high EOS count should preferably be re-checked a few hours later to see if it is persistent. If it is long lasting eosinophilia then reasons may be:

- Allergies – e.g. asthma, food allergies, urticaria, etc.
- Parasitic infections – e.g. Ascaris and Trichinella. Eosinophilia is however usually not seen with intestinal parasites
- Pneumonia – in dogs with eosinophilic pneumonia
- Addison's disease
- Spleen removal – in dogs
- Recovery phase after infections
- Neoplastic conditions
- Eosinophilic myositis
- Eosinophilic gastroenteritis

### High basophil counts (2)

High BASO (basophilia), usually occurs simultaneously with eosinophilia for instance in allergies. However, basophilia is very

rare. In dogs it may be associated with heartworm or neoplasia or high lipid concentration.

### Low lymphocyte counts (2)

Low LYM (lymphopenia), can be associated with:

- Viral infections – usually acute ones such as canine hepatitis, parvovirus, FLV, canine distemper, etc.
- Stress

### High lymphocyte counts (2)

High LYM (lymphocytosis), can be associated with:

- Leukemia
- Addison's disease
- Post vaccination
- Age – young animals
- Infection recovery
- Chronic antigen infection

When the LYM become activated by antigen stimulation they become known as "reactive lymphocytes". Their morphology differs some from the LYM as they are larger and have a BASO like cytoplasm and sometimes even nucleoli. If there is an increase in the reactive LYM it suggests an increased antibody

production and that the individual has had strong antigen stimulus to activate the LYM cells, such as after vaccination.

- Corticosteroid treatment
- Drugs, radiation – through bone marrow depression
- Acute bacterial infections – systemic ones
- Cushing's or higher adrenal activity

### High monocyte counts (2)

The MONO counts are usually low, but if they instead increase to give a monocytosis, possible associations include:

- Chronic diseases
- Age – older animals
- Monocytic leukemia – dogs
- Some infections – listeriosis in swine, etc.
- Corticosteroid treatment
- Cushing's disease or higher adrenal activity

### Species specific differences in interpretation of WBC differential

## General WBC differential interpretations

Table 4. General interpretations of prognosis for patients with various observed WBC differential counts (2)

Observed WBC differential counts								Comment and prognosis
WBC	NEU	Left shift	Toxic NEU	Band NEU	LYM	EOS	MONO	
	3	Mild				3		Mild infection, body can handle
	3	Slight			4	4		Moderate/severe infection with stress
3	3	3						More serious but well-functioning bone marrow
	3				4	4		May indicate a more serious condition with concurrent lymphopenia
	4	3						More immature than mature NEU, degenerative left shift, meaning a grave condition
			3					Guarded prognosis
	3	No			4	4		Indicates stress, pain or steroids
							3	Chronic disease
4 falling				4	3	3		Good prognosis sign
		Degenerative						Bad prognosis
					4 falling or persistent			Bad prognosis

See WP on normal reference ranges (3).

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## Conclusion

Veterinary blood differs a lot within morphology and behavior between species, and it is therefore important to understand the factors affecting the parameters for various species.

Exigo H400 automated hematology analyzer is designed to be tailored to customers in need of various profiles and has many software and hardware features in place to account for these differences.

## Glossary

**Anisocytosis** – when there is a larger than normal difference in RBC sizes in a patient.

**Aplastic anemia** – autoimmune disease where the body is not capable of producing enough blood cells.

**Band neutrophil** – immature form of a neutrophil where the nucleus is not segmented and is curved.

**Basophilic punctuation** – when there is several basophilic granules dispersed in the cytoplasm of RBCs in the peripheral blood.

**B-lymphocyte** – lymphocyte subgroup developed in the bone marrow (mammals) responsible for the adaptive immune system. They secrete antibodies but they can also later become memory cells, storing antigen-specific antibodies in case the same foreign matter attacks the immune system again.

**Echinocytes** – (also known as burr cells) this is when RBCs have an abnormal cell membrane with spiky projections.

**Erythrocyte** – another name for a red blood cell

**Erythropoiesis** – the process of red blood cell production, from a stem cell to a mature red blood cell.

**Erythropoietin (EPO)** – A hormone (also known as haematopoietin) produced in the kidney in response to hypoxia (low oxygen levels). EPO stimulates the red blood cell production in the bone marrow.

**Heinz bodies** – presences of denatured HGB in the RBCs which is not seen with routine blood staining.

**Hemolytic anemia** – where the anemia stems from the break down of RBCs, hemolysis.

**Howell-Jolly bodies** – when you have basophilic nuclear leftovers (parts of DNA) circulating inside RBCs in the blood. RBCs should not have a nucleus, but in certain conditions this

may occur.

**Immature granulocytes** – granulocytes (eosinophils, neutrophils and basophils) in their immature stages of development usually found in the bone marrow but sometimes in the peripheral blood. These stages can include metamyelocytes, myelocytes and promyelocytes.

**Left shift** – increase in immature WBCs, in particular band NEU.

**Leukemia** – groups of blood cancers leading to high abnormal cells in the blood, such as blasts.

**Macrocytosis** – increase in RBC size with the same HGB level (MCV increase).

**Megaloblastic anemia** – is where the production of RBCs lacks the possibility of DNA synthesis, meaning the cell later cannot divide. Therefore it is also known as macrocytosis with larger cells.

**Microcytosis** – RBCs are smaller than usual (low MCV).

**Nucleated RBCs (nRBC)** – when mammal RBCs contain a cell nucleus. This is normal in immature stages of RBCs, such as in progenitor cells but can also occur in pathological conditions and also in newborn infants.

**Phagocytosis** – when a cell uses its plasma membrane to engulf another large particle.

**Plasma cell** – B-lymphocytes may differentiate further into plasma cells that secrete large amounts of antibodies due to an antigen stimulus to neutralize or destroy the foreign matter.

**PLT clumping** – when platelets aggregate together forming “clumps”

**Polychromasia** – A condition where many immature RBCs are released into the blood stream and these cells have a cytoplasm with a grey-blue-pink color.

**Polycythemia** – a disease where the HCT is increased, either via an increase in the RBCs or a decrease in plasma volume.

**Reticulocyte** – immature red blood cells that develop and mature in the bone marrow before being released into the blood stream and within a day maturing into RBCs. These cells still contain ribosomal RNA that is visible with a stain under microscope.

**RNA** – ribonucleic acid, similar to DNA it is a nucleic acid and building block for life but found in a single strand (mainly). It is also involved in expressing and regulating genes.

**T-lymphocyte** – lymphocyte subgroup which is developed in the thymus important in either killing cells that are infected by e.g. virus with cytotoxicity or by communicating with other WBCs to signal them to a site of infection.

**Toxic change in NEU** – includes three main findings in a blood smear: **toxic granulation**, **Döhle bodies** and **toxic vacuolization**. Toxic granulation is where segmented NEU have a build-up of dark, big granules in the cytoplasm. Döhle bodies are where the NEU get gray-blue colored cytoplasmic inclusions in NEU, band NEU or metamyelocytes present close to the cell wall. Toxic vacuolization is where you have unstained areas of the cytoplasm of NEU.

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WPE34071-3 12/2019

