Boule

Veterinary hematology analyses: reference ranges

Veterinary hematology differs from human hematology, in that the hematopoietic cells differ between animals species and breeds. In contrary to mammals, amphibians, reptiles and birds have nucleated red blood cells (RBCs) and platelets (PLTs). Birds also have heterophils, an additional group of white blood cells (WBCs), and reptiles have azurophils, thought to be a monocyte type of WBC that is mononuclear. Cell size may also vary largely between species. For example, the volume of the RBCs of sheep is about 19 fL, whereas in elephants, the RBC volume is about 120 fL. These differences pose challenges to veterinary hematology.

The importance of set reference ranges

As animals cannot speak and explain their symptoms, the blood count is important in investigations of underlying diseases. Automated hematology analyzers are frequently used in clinical laboratories to assess and monitor health condition of patients. Compared with manual microscopy, the automated analyzer is more time and cost efficient.

Although WBC and PLT populations vary less among mammals, different species may respond differently to inflammation and reference ranges are critical for interpretation of the results. The "normal" range should reflect the "reference" of 95% of the animals. Most automated hematology analyzers have pre-set reference ranges based on literature references or clinical studies. However, it is recommended that each clinic establishes its own reference ranges. When determining the reference values, the samples source should be considered, as cell counts might vary between regions, breeds, ages, and genders and can be affected by factors such as medication, diet, stress, and pregnancy (2).

How is a result outside the reference values interpreted?

When a result is outside the reference range for the tested population, indication of a possible clinical significance should be considered. As reference method to automated cell count, manual microscopy is often used.

As the reference range represents 95% of the "normal" of a population, 5% (or 1/20) samples will naturally fall outside of established range. As an example, if 20 analyses are performed on 100 "normal" animals of a certain population, only 36 of these animals would be considered "normal". As an abnormal result should always be considered as clinically significant, the accuracy of the result should be challenged, especially if the results do not seem to match the clinical signs from the patient. A re-run of the test can give a good indication of a tentative diagnosis. The possibility of a sampling error (e.g., due to clotting or poorly mixed sample) should also be considered, as should external methodological or instrumental errors (e.g., expired reagents) (2).

Table 1. Normal reference ranges provided with Exigo H400 for 12 pre-set species (1)

|	Dog	Cat	Horse	Cow	Sheep	Goat	Pig	Mouse	Rat	Rabbit	Ferret	New world camel
RBC ($\times 10^{12}$/L)	5.50 – 8.50	5.00 – 11.00	6.50 – 12.50	5.00 – 10.00	9.00 – 15.00	8.00 – 18.00	5.00 – 8.00	6.50 – 10.10	7.21 – 8.45	5.20 – 6.80	6.80 – 11.80	11.30 – 16.90
MCV (fL)	60.0 – 72.0	39.0 – 50.0	36.0 – 52.0	40.0 – 60.0	25.0 – 40.0	16.0 – 25.0	50.0 – 56.0	42.3 – 55.9	55.8 – 62.2	64.6 – 76.2	43.0 – 51.0	20.1 – 27.5
HCT (%)	37.0 – 55.0	25.0 – 45.0	32.0 – 52.0	24.0 – 46.0	27.0 – 45.0	22.0 – 38.0	32.0 – 50.0	32.8 – 48.0	43.6 – 48.6	36.6 – 47.4	46.0 – 57.0	28.0 – 39.0
RDW (abs)	35.0 – 65.0	20.0 – 43.0	24.0 – 37.0	0.1 – 250	0.1 – 250	0.1 – 250	0.1 – 250	0.1 – 250	0.1 – 250	0.1 – 250	0.1 – 250	0.1 – 250
RDW (%)	12.0 – 17.5	14.0 – 18.5	16.0 – 21.0	0.0 – 99.9	0.0 – 99.9	0.0 – 99.9	0.0 – 99.9	0.0 – 99.9	0.0 – 99.9	0.0 – 99.9	0.0 – 99.9	0.0 – 99.9
HGB (g/dL)	12.0 – 18.0	8.0 – 15.0	11.0 -19.0	8.0 – 15.0	9.0 – 15.0	8.0 – 12.0	10.0 – 16.0	10.0 – 16.1	13.2 – 16.4	11.5 – 15.1	15.2 – 17.7	12.6 – 17.8
MCH (pg)	19.5 – 25.5	12.5 – 17.5	13.5 – 19.5	11.0 – 17.0	8.0 – 12.0	5.2 – 8.0	17.0 – 21.0	13.7 – 18.1	17.7 – 20.1	21.1 – 24.5	0.0 – 99.9	0.0 – 99.9
MCHC (g/dL)	32.0 – 38.5	31.0 – 38.5	34.0 – 40.5	31.0 – 38.5	31.0 – 34.0	30.0 – 36.0	30.0 – 34.0	29.5 – 35.1	31.4 – 33.6	29.5 – 33.9	32.0 – 38.0	42.0 – 46.5
PLT ($\times 10^{9}$/L)	200 – 500	200 – 500	100 – 350	100 – 800	200 – 800	–	100 – 900	250 – 1540	250 – 1200	250 – 610	250 – 1000	–
MPV (fL)	5.5 – 10.5	8.0 – 12.0	5.5 – 11.0	0.0 – 99.9	0.0 – 99.9	–	0.0 – 99.9	0.0 – 99.9	0.0 – 99.9	0.0 – 99.9	0.0 – 99.9	–
WBC ($\times 10^{9}$/L)	6.0 – 17.0	5.5 – 19.5	5.5 – 12.5	4.0 – 12.0	4.0 – 12.0	4.0 – 13.0	11.0 – 22.0	2.6 – 10.1	7.2 – 12.6	6.3 – 10.1	5.6 – 10.8	7.2 – 21.4
NEU/GRA ($\times 10^{9}$/L)	3.5 – 12.0	2.8 – 13.0	3.0 – 7.0	0.6 – 4.0	0.7 – 6.0	1.2 – 7.2	3.2 – 13.2	0.4 – 2.0	1.3 – 4.1	1.5 – 3.2	0.6 – 7.0	4.6 – 16.3
LYM ($\times 10^{9}$/L)	0.9 – 5.0	1.0 – 7.0	1.5 – 5.0	2.5 – 7.5	2.0 – 9.0	2.0 – 9.0	4.5 – 13.0	1.3 – 8.4	5.0 – 9.1	3.4 – 7.0	1.7 – 4.7	1.0 – 7.8
MONO ($\times 10^{9}$/L)	0.3 – 1.5	0.2 – 1.0	0.2 – 1.0	0.0 – 0.8	0.0 – 0.8	0.0 – 1.0	0.2 – 2.0	0.0 – 0.3	0.1 – 0.5	0.1 – 0.5	0.0 – 0.4	0.0 – 1.0
EOS ($\times 10^{9}$/L)	0.1 – 1.5	0.1 – 1.0	0.1 – 1.0	–	–	–	–	–	–	–	–	–

Exigo™ H400 hematology analyzer

Exigo H400 supports unlimited number of profiles, which enables creating profiles for certain breeds of the same species, such as warm-blooded and cold-blooded horses, or for greyhounds and "other" dog breeds.

Physiological variations in white blood cell concentration

White blood cell count is one of the most important parameters of the complete blood count (CBC) and is used to identify immune system-related diseases. However, the patient's physiological state should be considered when interpreting the results, as many factors can affect the WBC count (2):

- Age has a large impact on WBC. Young animals tend to have higher LYM and total WBC values than adults. For cattle, a decrease in WBC with age is usually observed.

- Gender can sometimes affect the normal range for WBC. For instance, stallions have lower WBCs than mares of the same age.

- Breed is an important factor for variation. For example, the NEU count of greyhounds are lower compared to other breeds, and their EOS cells resemble banded NEU cells (Figure 1). Moreover, warm-blooded horses have a higher WBC count than cold-blooded horses.

- Physical exercise, acute stress, and excitation typically increase the WBC count (mainly related to an increase in NEU).

- Late pregnancy can many times increase the WBC count.

- Food intake can give significant increase in WBCs for dogs and swine around 1–2 h after a meal. However, in horses, the opposite occurs with a slight almost negligible decrease and in cattle there is no observed difference.
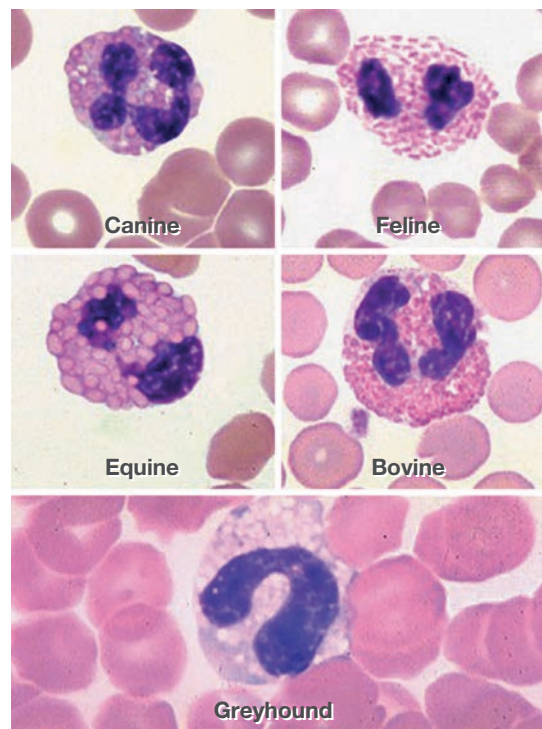
Fig 1. The eosinophil (EOS) appearance varies between (A) dogs, (B) cats, (C) horse, (D) cattle and (E) greyhounds.

Morphological changes of the immature neutrophils

The WBC are usually divided into five subpopulations: neutrophils (NEU), lymphocytes (LYM), monocytes (MONO), eosinophils (EOS) and basophils (BASO). However, it is not only the type of cell that is important, but also the lifetime stage morphology. Yet again we are back to a difficulty that hematology analyzers face. The five above named subpopulations can be identified but not specific morphological changes and stages in their maturation. Therefore, it is important that the instrument flags the sample as "abnormal" and a manual microscopy has then to be performed to verify the analyzers values as well as detect other morphological cases that cannot be addressed otherwise.

Immature neutrophils

Neutrophils make up the largest segment of the WBCs (in general, some species excluded). They are produced by the body in the bone marrow where they then mature until they are released into the blood stream as segmented neutrophils. Sometimes immature neutrophils are released into the blood circulation.

Immature neutrophils in circulation have a non-segmented nucleus compared to the mature NEU, and this gives it a characteristic morphology and they are therefore known as band neutrophils (other stages during their maturation are then metamyelocytes and myelocytes (Fig. 2). An increase in the immature neutrophils is known as a "left-shift". The left shift can be either degenerative or regenerative. A degenerative left shift signifies a severe inflammation and is many times linked to an acute bacterial infection (sepsis). A degenerative left shift is generally indicated by an increased ratio of immature to mature NEU regardless of the total WBC or NEU count. In a regenerative left shift, on the other hand, both the total NEU (neutrophilia) and band NEU counts are increased. Clinically, this means that the bone marrow has responded to the inflammation. A regenerative left shift is not as severe as a degenerative left shift (2).

Toxic changes

During and as a part of the inflammatory response, the immature neutrophils released from the bone marrow can have changes to their morphology, known as toxic changes, that identify them as immature. There are five main morphologies for toxic changes:

1. *Toxic granulation*
   In the cytoplasm, red granules can easily be detected (due to granules taking up the stain well during the manual microscopy procedure). This change is less seen in small companion animals but quite common in larger animals such as horses, cattle, camels, etc. (3).

2. *Döhle bodies*
   Döhle bodies are usually the first indication of a toxic change. As the rough endoplasmic reticulum (RER) come together in spirals, it cases blue aggregates that look quite linear. However, it is important to note that healthy cats can have a few small Döhle bodies. More importantly is to note that storage of blood can cause the formation of Döhle bodies from the neutrophils (3).

3. *Cytoplasmic basophilia*
   Due to the rough endoplasmic reticulum (RER) and polyribosomes (translate mRNA into polypeptides) being scattered throughout the cytoplasm, the cytoplasm gets an irregular blue appearance. Depending on the number of NEU affected, the cytoplasmic basophilia can be classified as mild, moderate or severe (3).

4. *Cytoplasmic vacuolation*
   Due to the degranulation of lysosomes (organelle in cells that breaks down molecules) in the cytoplasm, blurry vacuoles can be seen (important to note is that clear vacuoles are not a feature of a toxic change) (3).

5. *Nuclear immaturity*
   Most difficult to detect in the microscope is the "nuclear immaturity", where the chromatin in the nucleus is lighter and not as tightly packed as usual.

Toxic changes are usually pointing towards an inflammation and therefore many times is observed together with a left shift (with immature NEU). However, left shift without toxic changes can occur in animals, for example, immune-mediated hemolytic anemia in dogs. Although rare, a toxic change without a left shift is also sometimes observed, for example, in the case of marrow dysplasia (3).
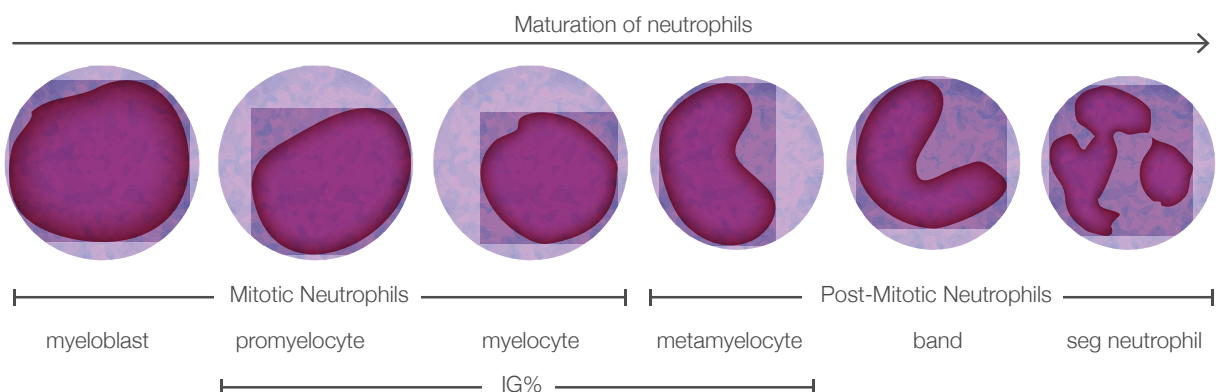

Maturation of neutrophils

Mitotic Neutrophils			Post-Mitotic Neutrophils		
myeloblast	promyelocyte	myelocyte	metamyelocyte	band	seg neutrophil

IG%

Fig 2. The maturation stages of segmented neutrophils.

Neutrophilic responses in different animal species

A phenomenon quite common among animals is known as a stress leukogram. This condition can occur when the animal is nervous, exited or scared. It is also known as the epinephrine-mediated response. Within short time, the WBC population can shift dramatically and alter many parameters of the CBC analysis. As the NEU response might differ between species, the stress leukogram as well as inflammatory responses can look different in various animals. Inflammation leukogram together with toxic neutrophils usually indicates a more severe inflammation.

1. Dogs

In general, both infections and stress tend to increase the NEU counts in dogs (typically to $10–30 \times 10^9$/L). A significant left shift of about 1×10^9/L band NEU is often seen in inflammation. It is possible to categorize the left shift as regenerative or degenerative based on the total WBC count where a value above normal total WBC indicates regenerative left shift and below WBC normal indicates a degenerative left shift.

Dogs generally do not give "stress leukograms", meaning that the blood status is not usually affected. However, if it happens, all different cell types (WBC, LYM, EOS, MON, NEU) tend to increase and be on the high end.

2. Cats

Unlike dogs, cats tend to often give "stress leukograms". This is usually shown by high LYM values (lymphocytosis) when it is induced by excitement. When stress is involved, it usually is indicated by a neutrophilia (high NEU) without a left shift and, in some cases, also by low EOS values (eosinopenia) and low LYM values (lymphopenia).

An inflammatory leukogram in cats is associated with WBC of $25–40 \times 10^9$/L, where more than 1×10^9/L of band neutrophils signifies a substantial left shift.

The inflammation is either regenerative or degenerative depending on the WBC count, if above normal indicating regenerative and normal or below indicating degenerative.

3. Horses

The inflammatory leukogram of horses is less affected by large NEU increases as compared to dogs and cats. However, NEU counts of $10–20 \times 10^9$/L can be seen in infections, and banded NEU above 0.3×10^9/L signifies a substantial left shift.

If the WBC is above normal, or sometimes within reference range, the left shift is regenerative. Temporary neutropenia might instead indicate a serious condition and in such a case, the number of banded NEU are usually almost the same as the segmented NEU, together with a low WBC count.

A "stress leukogram" can also be seen in horses during hard exercise and excitement. Here, the stress leukogram is usually indicated by an increase in WBC to $12–15 \times 10^9$/L, without a left shift and with a normal to low LYM count.

4. Swine

The WBC for swine is usually high. During infection, however, a large increase in the NEU (typically $25–35 \times 10^9$/L) can be observed.

5. Cattle

Cows are slightly different than other ruminant animals and mammals in that their peripheral blood usually have more LYM than NEU. A WBC between $15–25 \times 10^9$/L is considered a significant leukocytosis and above 25×10^9/L it is severe. For cattle, the WBC differential into the five subpopulations, especially LYM and NEU counts, is more important than the total WBC count. To aid in diagnosis, the fibrinogen count in cow serum can be an additional marker for inflammation.

In case of inflammation, LYM is decreased and NEU is either decreased or slightly increased, the total WBC count is usually within the normal range or even lower than normal. Usually, a significant left shift (> 0.3×10^9/L band NEU) is observed.

On the other hand, in chronic inflammations, the WBC can be as high as 20×10^9/L and accompanied by a neutrophilia and a left shift of more than 0.3×10^9/L band NEU.

The stress leukogram for cows tends to be associated with WBC of $8–18 \times 10^9$/L, without left shift and with lymphopenia and eosinopenia. Excitement and an epinephrine-mediated response, on the other hand, can give WBC counts of $15–27 \times 10^9$/L, without a left shift and LYM, EOS and MONO values within their normal ranges.

Examples of other parameters also affected by physiological variations

It is not only the WBCs that can be affected by physiological variations. Many other parameters of the CBC can also be affected.

For example, Cavalier King Charles spaniels are known to have large and few platelets, so called, macrocytic thrombocytopenia (2). These platelets can many times be so large that they are counted erroneously as RBCs in automated hematology counters.

Red blood cells (RBCs), the hemoglobin value (HGB) and the hematocrit (HCT) levels can also be affected by anxiety, stress and activity by the spleen in animals. For example, spleen contractions can stimulate the release of RBCs into the blood vessels, increasing these red blood cell counts. This phenomenon is particularly prominent among horses, in which the HCT can increase up to 40%. Especially warm-blooded horses have a spleen that acts as a large storage for the RBCs, giving a much wider normal reference range for HCT, HGB and RBC. All these three red cell parameters are also affected by age (most frequently in all species being lower values in younger animals), late pregnancy, malnutrition, etc.

Conclusion

Veterinary hematology is challenged by both inter- and intraspecies variations. To interpret a CBC, it is important to know the normal reference range for the specific population to be tested. Conditions such as stress or excitement might also affect the parameter values. With Boule's Exigo H400 analyzer, normal reference ranges are pre-set for 12 animal profiles. By addition of more profiles, reference ranges should be checked with local reference labs or university labs or possibly literature values.

References

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