

ANEMIA



ANEMIA

Definition and diagnosis¹

Anemia is a pathologic state characterized by a **decrease in red blood cells (RBC), blood hematocrit (HCT) or the amount of hemoglobin (HGB) below lower reference limits.**

Three mechanisms can cause anemia:

- ▶ Destruction of red blood cells (*hemolysis*)
- ▶ Blood loss (*hemorrhage*)
- ▶ Decreased erythrocyte production

Anemia is often a diagnostic challenge hence the importance of using a methodical approach, starting with the question: **is the anemia regenerative or not?**

In response to anemia, the body can initiate a restoration process to replace an adequate amount of circulating red blood cells and hemoglobin. This restoration involves **increased erythrocyte production** releasing reticulocytes into the bloodstream.



RETICULOCYTES

Definition and interpretation¹

Reticulocytes are **red blood cell precursors**, released by the hematopoietic bone marrow. In case of anemia, an increased number of reticulocytes in the bloodstream reflects in domestic carnivores a regeneration process aimed at returning to a normal amount of red blood cells.

Maturation of reticulocytes into erythrocytes takes 1 to 2 days in dogs, while life span of feline reticulocytes is more variable.

REGENERATIVE ANEMIA	NON-REGENERATIVE ANEMIA
<p>=> reticulocyte number exceeding the upper reference limit</p> <p>Anemia secondary to acute or chronic bleeding or hemolysis are usually regenerative.</p>	<p>=> reticulocyte number within reference interval</p> <p>Reduced or ineffective erythropoiesis i.e. due to missing stimulation of erythroid precursors in the bone marrow (i.e. erythropoietin lack in case of chronic renal failure), bone marrow damage or replacement, or destruction of RBC precursors can cause non-regenerative anemia.</p> <p>It should further be considered, that bone marrow needs up to 3-4 days to initiate a regenerative response. During this time, the anemia may seem non-regenerative but become regenerative after that.</p>

Species particularities



Horses very rarely release polychromatophilic erythrocytes from bone marrow, therefore attempting to establish peripheral blood reticulocytosis has not been finally valuable.

Healthy horses do not have circulating reticulocytes because maturation of the red blood cells take place in the bone marrow.



In cats there are two types of reticulocytes in the blood.

RETICULOCYTES



Aggregate reticulocytes

- On a wright stain (the "normal stain") only polychromatophils can be seen. Reticulocytes can only be seen using supravital stains like new-methylene blue stain.
- On an i.e. new methylene blue stain, aggregate reticulocytes show several dark blue clumps/aggregates
- The aggregate reticulocytes mature into punctate reticulocytes within approximately 12 hours.

>> In cats, **increased numbers of aggregate reticulocytes indicate onset of anemia 4-7 days ago.**

Punctate reticulocytes

- On an i.e. new methylene blue stain: reticulocytes show few, small blue inclusion-like dots
- Punctate reticulocytes circulate for at least several days before all the RNA is lost.

>> An anemic cat with **increased number of punctate reticulocytes indicates onset of anemia 2-4 weeks ago.**



Regenerative anemia

Anemia is said to be regenerative when the **bone marrow tends to compensate for the decrease in the amount of red blood cells and hemoglobin.**

Be careful, however, because:

- Reticulocytosis usually appears about 3-4 days after hemorrhage or hemolysis. Thus, it may be necessary to examine two reticulocyte counts one week apart to qualify anemia as regenerative.

However, a recent study in the *Journal of Veterinary Internal Medicine (JVIM)*² suggests that IRF is a reliable parameter for early detection of a regenerative process, i.e. before the onset of reticulocytosis, in case of anemia in dogs. With a sensitivity of 82% and a specificity of 93% based on an IRF result greater than 27, this parameter provides important additional information to the practitioner to diagnose early regenerative anemia.

- An initially regenerative anemia becomes hyporegenerative after two or three weeks of evolution. This may occur especially after chronic blood loss and subsequent development of an iron deficiency anemia.

Two pathological phenomena cause regenerative anemia: **acute and chronic hemorrhages and hemolysis.**

- **Hemorrhage** is the likely cause if bleeding is noted in the physical exam, and or if hematuria or coagulopathies are noted.
- **Hemolysis** can be suspected in case of hemolytic plasma, hyperbilirubinemia, bilirubinuria and even clinically notable jaundice.

Non-regenerative anemia

Anemia is said to be non-regenerative when there is a **reduced or inefficient erythropoiesis.** The distinction can be made between:

HYPOREGENERATIVE ANEMIAS



The reticulocytic count is within the norms, suggesting that the bone marrow retains basal red blood cell replacement activity.

AREGENERATIVE ANEMIAS



The reticulocyte count is below the usual lower value, suggesting that the bone marrow has decreased erythroblastic activity.

The classification of non-regenerative anemias can be based on results of the bone marrow examination:

LOW PRODUCTION MARROW



Deficiency of one or more hematopoietic cell lineages. Parvovirus and FeLV, for example, can both cause this type of anemia.

INVADED MARROW



Tumor or fibroblast cells are visible on a myelogram. These cells are observed in hematopoietic tumors or idiopathic myelofibrosis for example.

Finally, there are so-called fluctuating anemias, fluctuating anemias, in which the reticulocyte count varies. They are particularly found in cats infected with FeLV.

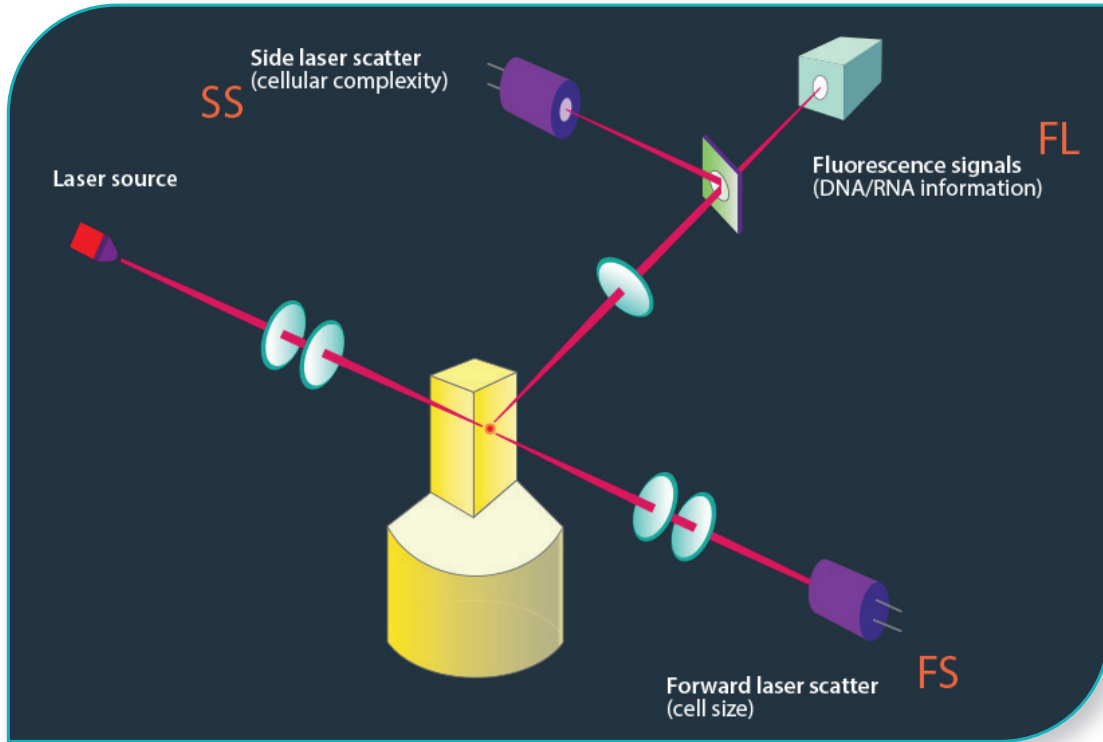


Figure 1

Element HT5+ laser scatter scheme.

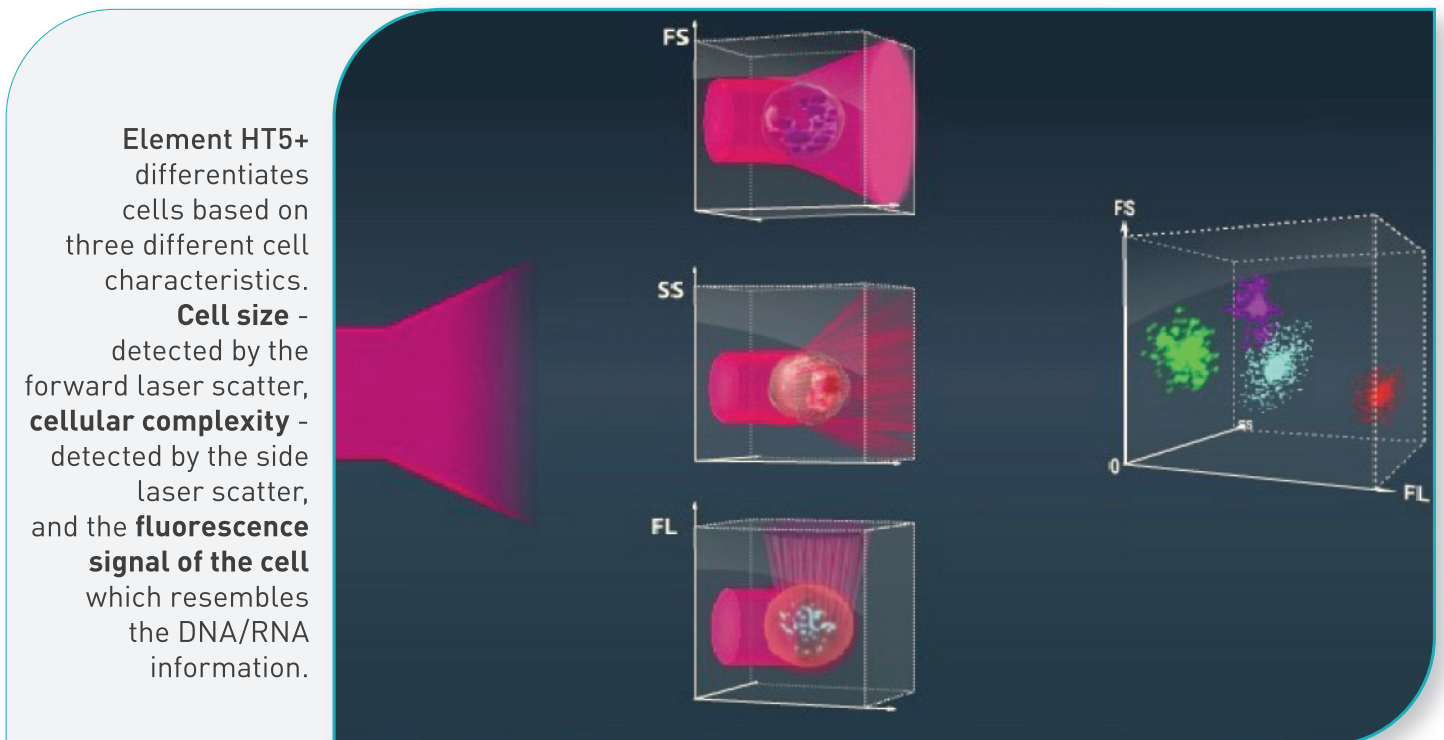


Figure 2

Element HT5+ SF-Cube Technology.

A 3-D-Scattergram is generated from the cell characteristics detected by 3 different scatter informations.

FS = Forward laser scatter, SS = Side Laser scatter, FL = Fluorescence signal.



The measurement of reticulocytes is the best way for a quantitative evaluation of increased erythropoiesis in anemic patients.

The **Element HT5+** provides an absolute reticulocyte number for dogs and cats.

Unlike red blood cells, reticulocytes contain RNA in their cytoplasm that is specifically stained by a fluorescent reagent.

The laser radiation emitted in the device makes it possible to reveal this fluorescence and therefore to count reticulocytes accurately.

Depending on the amount of RNA and therefore the degree of fluorescence emitted, reticulocytes are classified into different fractions LFR, MFR, and HFR. In addition, the Element HT5+ is calculating the IRF and detecting the RHE:

Low-Fluorescence Reticulocytes = LFR

The LFR fraction represent the amount of reticulocytes containing the smallest amount of RNA: they are the most mature fraction of reticulocytes.

Medium-Fluorescence Reticulocytes = MFR

The MFR and HFR fractions represent the share of reticulocytes containing the largest amount of RNA: these are young and immature reticulocytes.

High-Fluorescence Reticulocytes = HFR

Immature Reticulocytes Fraction = IRF

The IRF parameter is defined as a reflection of the reticulocyte maturation process. It is calculated from the most immature reticulocytes fractions: MFR and HFR.

Reticulocyte Hemoglobin Expression = RHE

The reticulocyte hemoglobin expression is determined quantitatively. This parameter indicates the hemoglobin content of the reticulocytes and thus determines the quantity (*erythropoietic activity*) and quality (*hemoglobin synthesis and uptake*) of the newly formed red blood cells. This is particularly useful for distinguishing the two most common anemias (iron deficiency anemia and «anemia of chronic disease»). It can also be used to distinguish true iron deficiency from «functional» iron deficiency (iron mobilization disorder). **RHE is independent of an acute phase response** and is therefore more suitable for the interpretation of an iron deficiency than ferritin and transferrin.⁴



CONCLUSION

Reticulocytes are immature red blood cells and their increase in anemic animals indicates a regenerative response of the bone marrow and is essential for the evaluation of anemic patients. They furthermore help us in differentiating regenerative from non-regenerative anemia and thereby narrow down the etiology of the disease.

- ▶ **Low Fluorescent Ratio (LFR)**
- ▶ **Middle Fluorescent Ratio (MFR)**
- ▶ **High Fluorescent Ratio (HFR)**

The sum of Middle and High Fluorescent Ratio represent the **Immature Reticulocyte Fraction (IRF)**, which is highly sensitive to the early ascent of reticulocytes and the response of the bone marrow hematopoietic function. IRF increases earlier than the absolute number of reticulocytes. In human medicine this parameter is long known as an early marker for regeneration of anemic patients.

The **Reticulocyte Hemoglobine Expression (RHE)** indicates the hemoglobin concentration of each reticulocyte, serving as a sensitive indicator for early reflection of iron status in erythrocyte production.^{3,4}

Therefore, with its additional parameters (#RETIC count, LFR, MFR, HFR, IRF, and RHE), our **Element HT5+** offers you an important diagnostic tool for the early evaluating anemic patients.



REFERENCES

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²Jung J-H, Yang Y, Seo D, Cho S, Choi G, Kim Y. Clinical utility of immature reticulocyte fraction for identifying early red blood cell regeneration in anemic dogs. J Vet Intern Med. 2023; 37(2): 484-489.

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