Importance of eosinophil parameter on veterinary hematology analyzers

There is a growing trend towards the identification of eosinophils (EOS) for automated hematology systems. This article will give a background to EOS morphology, functions and some clinical aspects of high and low values including a case study.

Introduction

The white blood cells (WBC) are usually divided into their five subgroups: neutrophils (NEU), lymphocytes (LYM), monocytes (MONO), eosinophils (EOS) and basophils (BASO). The EOS are generally identified through morphology and toxic proteins. They differ between the various animal species which is important especially if to count using an automated hematology system (Fig. 1).

The EOS are able to phagocytose and kill bacteria, (less effectively than other granulocytes) though they are mainly known for being involved in parasitic infections and allergic reactions.

At sites in the body where antibody-antigen reactions are occurring they are mobilized. Through their extreme potent toxic proteins they are able to kill foreign matter such as parasites. However, these toxic proteins can also cause tissue damage. Such damages are seen in allergic diseases such as asthma, allergic rhinitis, eosinophilic pneumonia in dogs and eosinophilic granulomas in cats.

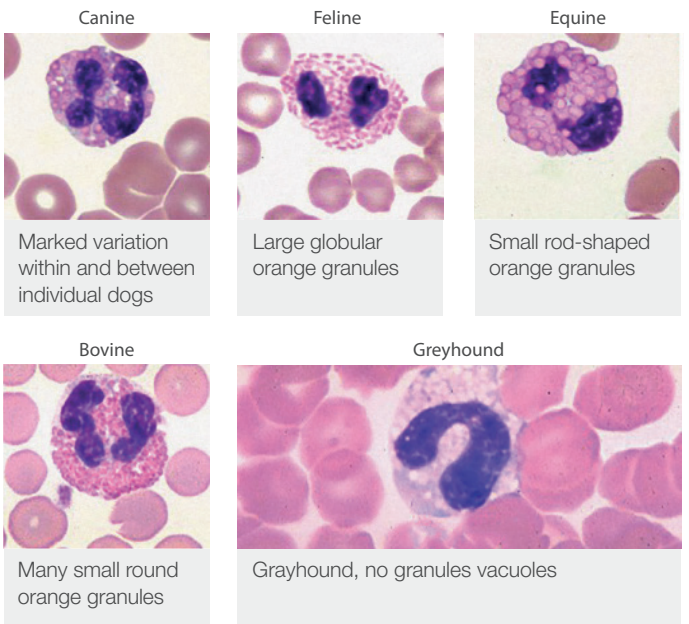


Fig 1. Morphology of eosinophils in four different species (dog, cat, horse and cow).

Table 1. Reference ranges for WBC differential among various species[1] (1)

	Total WBC ×10⁹/L	Neutrophils (band)	Neutrophils (segment)	Eosinophils	Basophils	Lymphocytes	Monocytes
Cat[1]	5.5-19.5	0-0.3	2.5-12.5	0-1.5	Rare	1.5-7.0	0-0.8
Cattle[1]	4.0-12.0	0-1.2	0.6-4.0	0-2.4	0-0.2	2.5-7.5	0.2-0.8
Dog[1]	6.0-17.0	0-0.3	3.0-11.5	0.1-1.2	Rare	1.0-4.8	0.2-1.4
Goat[1]	4.0-13.0	Rare	1.2-7.2	0-0.6	0-0.1	2.0-9.0	0-0.6
Horse (cold-blooded)[1]	6.0-12.0	–	–	–	–	–	–
Horse (warm-blooded)[1]	5.4-14.3	0-0.1	2.3-8.6	0-1.0	0-0.3	1.5-7.7	0-1.0
Pig[1]	11.0-22.0	–	–	–	–	–	–
Sheep[1]	4.0-12.0	Rare	0.7-6.0	0-1.0	0-0.3	2.0-9.0	0-0.8
Human[2]	4.0-11.0	–	2.5-7.5	0.04-0.44	0.01-0.1	1.5-3.5	0.2-0.8

[1] Summarized literature data from Schalm's Veterinary Hematology. 4th ed. Philadelphia: Lea & Febiger, 1986
[2] Data adepted from Essential Hematology. 4th ed. Oxford: Blackwell Sciences Ltd, 001.

There is a trend towards EOS measurement due to requirements for more detailed information about medical condition (Table 2). The EOS can either be measured by automated hematology counter through impedance or laser. To read more about the differences between these measuring systems, see white paper on **Hematology analyzers: 3-part or 5-part, that is the question** (2).

Table 2. Medical information indicated by 3-part versus 4-part hematology screening analyzers

3-part hematology systems	4-part hematology systems
Differentiate granulocytes, lymphocytes and monocytes/ midcells	Differentiate neutrophils, lymphocytes, monocytes and eosinophils
Allow identification of conditions such as: – Anemia – Infection – Leukemia	Allow more detailed information on conditions such as: – Allergy – Bacterial infection – Parasite infections – Certain cancers Parasites are very common in animals and therefore eosinophils is an important parameter to monitor

Increased eosinophil count

Increased number of EOS, eosinophilia, can be a non-specific observation in some disorders such as gastrointestinal tract inflammation, inflammatory processes in the lungs or skin etc. Moreover, it is often temporary and not persistent eosinophilia. When high EOS are detected, the patient should be tested again at a later time to see if the high EOS is persistent or if it was only temporary.

Persistent eosinophilia could be observed in the following conditions (3):

- **Parasitic infestations** (especially with parasites that migrate in the body)
- **IBS** (Inflammatory bowel disease)
- **Pulmonary diseases**
- **Infectious diseases** (e.g. chronic upper respiratory disease, pneumonia, metritis, mastitis, lower urinary tract infection in dogs)
- The **recovery phase** of some infections
- Some **neoplastic conditions** (Mast cell tumors, lymphomas, myeloproliferative disorders, solid tumors)
- **Eosinophilic myositis and gastroenteritis**
- **Adrenal insufficiency (Addison's disease)**
- **Allergic reactions**, such as asthma, allergic dermatosis, food allergy, flee allergy.
- **Eosinophilic pneumonia** in dogs
- After **spleen extirpation** in dogs

1. **Eosinophilic myositis (EM)** includes a group of rare, clinically and pathological disorders where the EOS infiltrates the skeletal muscle. Parasitic infection is globally the most common cause (4).
2. **Eosinophilic gastroenteritis** is a rare disease characterized by food-related reactions, penetration of EOS in the GI tract, as well as an increase in the number of EOS in the blood (4).
3. **Adrenal insufficiency (Addison's disease)** is a hormonal disorder caused by a too low production of hormones from the adrenal gland (cortisol and aldosterone). Cortisol is important to help the dog combat stress, while aldosterone helps regulate the water and electrolytes (4).

Decreased eosinophil count

Low EOS, eosinopenia, can be caused by:

- **Stress**
- **Corticosteroid therapy**
- **Increased adrenal activity** caused by neoplasia or hyperplasia (Cushing's syndrome)

Exigo™ H400 and adaptations for accurate, cost efficient and simple diagnostics and EOS counts

Exigo H400 was designed to be a flexible system, allowing for multiple animal species to be sampled efficiently and by simple means. This is done by having 12 pre-installed profiles on the analyzer but with the possibility to add more profiles depending on the clinic or hospital needs. Each of the profiles are optimized with the software and hardware (not only normal ranges). The low sample volume of 20 µl also allows for smaller and/or dehydrated animals to be sampled.

Exigo H400 also has guiding pathology messages with hematological guidelines with clear recommended actions for pathological samples, as well as useful system information messages. To ensure a high quality EOS results the Exigo has a reagent-based EOS method which means that there is an added incubation of cells with the EOS-specific lyse and the EOS are counted in a separate measuring chamber and give a separate histogram to interpret along with the result (Fig. 2).

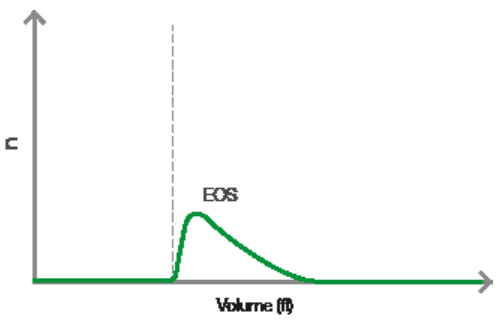


Fig 2. Eosinophil histogram provided with each eosinophil result in the Exigo H400.

Depending on the patient symptoms and testing to be performed, the Exigo is also a cost effective choice, allowing the user to choose between running either a 3 or 4-part analysis.

Case Study - West Highland White Terrier with recurrent Otitis Externa

One of the most common ear canal inflammatory disorder in dogs is called otitis externa. It can be caused by parasites, allergies or other foreign objects.

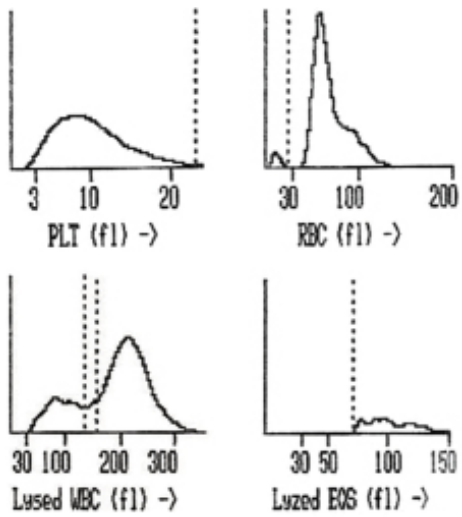
"An 11 year old West Highland white terrier had recurrent otitis externa. The right ear was red and he scratched at his ear. He had also red conjunctival membranes and licked his paws occasionally" (5).

Study design

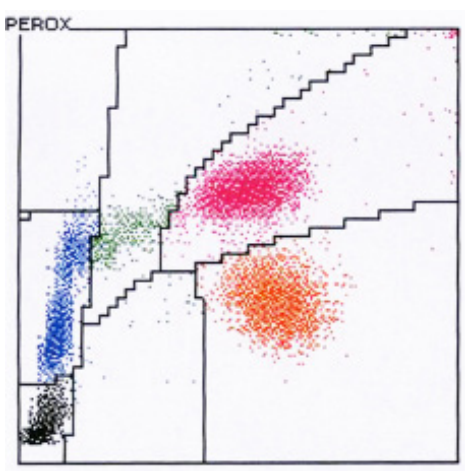
The following standards were used as guidance for study design:

- Validation, Verification, and Quality Assurance of Automated Hematology Analyzers; Approved Standard – Second Edition. CLSI H26-A2
- Measurement Procedure Comparison and Bias Estimation Using Patient Samples; Approved Guideline – Third Edition. CLSI EP09-A3
- Performance evaluation of in vitro diagnostic medical devices. EN 13612 (Fig. 3)

Graphic Reports



The Exigo eos report shows eosinophils based on lyse resistant cells larger than 70 fl in the Lysed EOS histogram at the bottom.



The Advia perox dot plot illustrates leukocytes in this blood based on size (y-axis) and peroxidase staining (x-axis). Eosinophils in this sample were well separated into a cell cluster of orange dots in the lower right. They appear small in size and slightly more peroxidase positive in the Advia system.

Laboratory Data - Exigo EOS (5)

Parameter	Patient	Reference Values
Platelet parameters		
Platelet count	326 × 10^9/L	(200–500)
MPV	9.8 fL	(5.5–10.5)
Red blood cell parameters		
RBC count	6.64 × 10^{12}/L	(5.5–8.5)
Hematocrit	45.1 %	(37.0–55.0)
MCV	67.8 fL	(60.0–72.0)
RDW	20.4 %	(12.0–17.5)
Hemoglobin	17.1 g/dL	(12.0–18.0)
MCH	25.7 pg 3	(19.5–25.5)
MCHC	37.9 g/dL	(32.0–38.5)
White blood cell parameters		
WBC count	9.8 × 10^9/L	(6.0–17.0)
Neutrophils	50.5 % 4.9 × 10^9/L	(3.5–12.0)
Lymphocytes	23.8 % 2.3 × 10^9/L	(1.2–5.0)
Monocytes	6.0 % 0.7 × 10^9/L	(0.3–1.5)
Eosinophils	19.7 % 1.9 × 10^9/L 3	(0.1–1.5)
Siemens Advia reported WBC Hematology parameters		
WBC count	10.3 × 10^9/L	(5.8–20.2)
Neutrophils	51.4 % 5.3 × 10^9/L	(4.3–9.1)
Lymphocytes	23.8 % 2.4 × 10^9/L	(2.0–4.7)
Monocytes	4.7 % 0.5 × 10^9/L	(0.2–2.0)
Eosinophils	19.5 % 2.0 × 10^9/L 3	(0.1–1.2)

Advia is the Advia 2120 hematology instrument produced by Siemens that was also used in the laboratory. Reference values for the Advia are from Moriz A, Fickenscher Y, Meyer K, Failing K, Weiss DJ: Canine and feline hematology reference values for the Advia 120 hematology system. Vet Clin Pathol 2004, 33:32-38.

Fig 3. Laboratory data from Exigo and Siemens Advia.

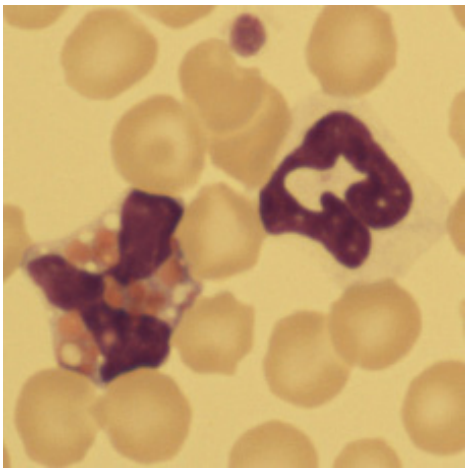


Fig 4. Photomicrograph shows an eosinophil (left) and segmented neutrophil (right) in this blood sample.

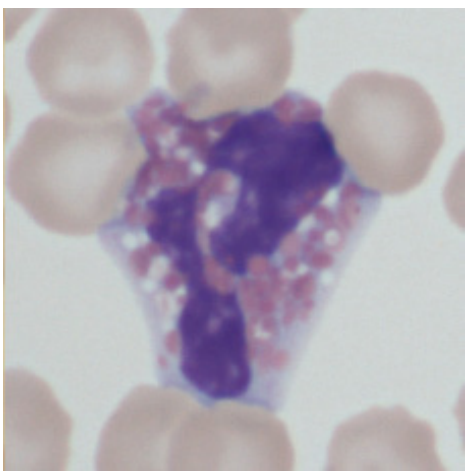


Fig 5. Photomicrograph shows an eosinophil.

Interpretation

"The West Highland white terrier dog had signs of an allergy in having red conjunctival membranes, red ear and that it licked his paws and scratched his ears. The eosinophilia in the blood supports an allergic cause to his clinical signs. The two automated hematology instruments had very similar results (Table 3).

The manual differential leukocyte count (22% eosinophils) was slightly higher than the automated counts, but there were several lysed (damaged) leukocytes in the blood smear which makes the manual count less accurate. Manual differential leukocyte counts are more imprecise than automated instruments. But the similar manual count also supports that the automated counts were accurate."

Submitted by Harold Tvedten, DVM, PhD, Dipl. ACVP.

Conclusion

EOS are becoming a more important and sought-after parameter for more detailed information about medical conditions in animals. With more existing knowledge today about inter- and intra-species variations in the morphology and functions of the EOS cells, they are more easily classified and understood in terms of clinical relevance.

To read more about the features and functions of the other WBC subgroups, see white paper on the introduction of veterinary hematology parameters (2).

References

1. Gröndahl, G. Veterinary Hematology – An introduction. Boule Diagnostics, 33267, Edition 4 (2019).
2. Whitepaper: Hematology analyzers: 3-part or 5-part, that is the question. Boule Diagnostics, 31183, Edition 1 (2019).
3. White paper: Introduction of veterinary hematology parameters: factors affecting parameters, clinical relevance and inter-species variations. Boule Diagnostics, 34071.
4. Caroline Mansfield, BSc, BVMS, MACVSc, MVM, DECVIM-CA. Eosinophilic Diseases of Dogs. World Small Animal Veterinary Association World Congress Proceedings, (2008).
5. Harold Tvedten, DVM, PhD, Dipl. ACVP.

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Boule Diagnostics AB, Domnarvsgatan 4, SE-163 53 Spånga, Sweden

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