White paper

Veterinary hematology analysis: understanding histograms

Hematology analysis provides a cost-efficient tool for animal health screenings and disease investigations. A complete blood count (CBC) can give indication of a variety of diseases and conditions that affect blood cells, such as anemia, infection, thrombocytopenia, or leukemia. This white paper aims to provide an understanding of the cell count results visualized in histograms by an automated hematology analyzer.

Introduction

Through a process called hematopoiesis, blood cells are derived from pluripotent hematopoietic stem cells in the bone marrow, where they mature before being released into circulation (Fig 1). The release of mature cells into circulation and their migration to various tissues are the result of organs communicating through the endocrine signaling system. The presence of immature cells in the bloodstream can therefore be an indication of a bone marrow failure. Additionally, many factors can make normal cells appear abnormal, including inflammation and infection, and abnormal cells can change back to normal cells if the underlying cause is removed or resolved.

A CBC test evaluates the cells in circulation, including the oxygen-carrying red blood cells (erythrocytes), the platelets (thrombocytes) that help clot the blood, and the white blood cells (leukocytes) of the immune system.

Hemoglobin-rich red blood cells (RBC) are the most common blood cells. Their main function is to carry inhaled oxygen from the lungs to the tissues and organs of the body and remove carbon dioxide that is carried back to the lungs and exhaled.

The primary function of platelets (PLTs) is to prevent bleeding. They interact with each other and other cells, such as white blood cells (WBC), to search for sites of injury, where they become activated.

As part of the CBC, a WBC differential count is often conducted. WBC types, morphology, and presence vary with animal species, but in many mammals with normal blood, about 60% of the WBCs are neutrophil (NEU) cells. NEUs help fight bacteria, and a high count is often associated with bacterial infections. Lymphocyte (LYM) cells, accounting for about 30% of all WBCs, help fight viruses. A high LYM count can therefore indicate a viral infection. The remaining 10% comprises monocyte (MON), eosinophil (EOS), and basophil (BAS) cells, typically associated with parasite infections or allergies.

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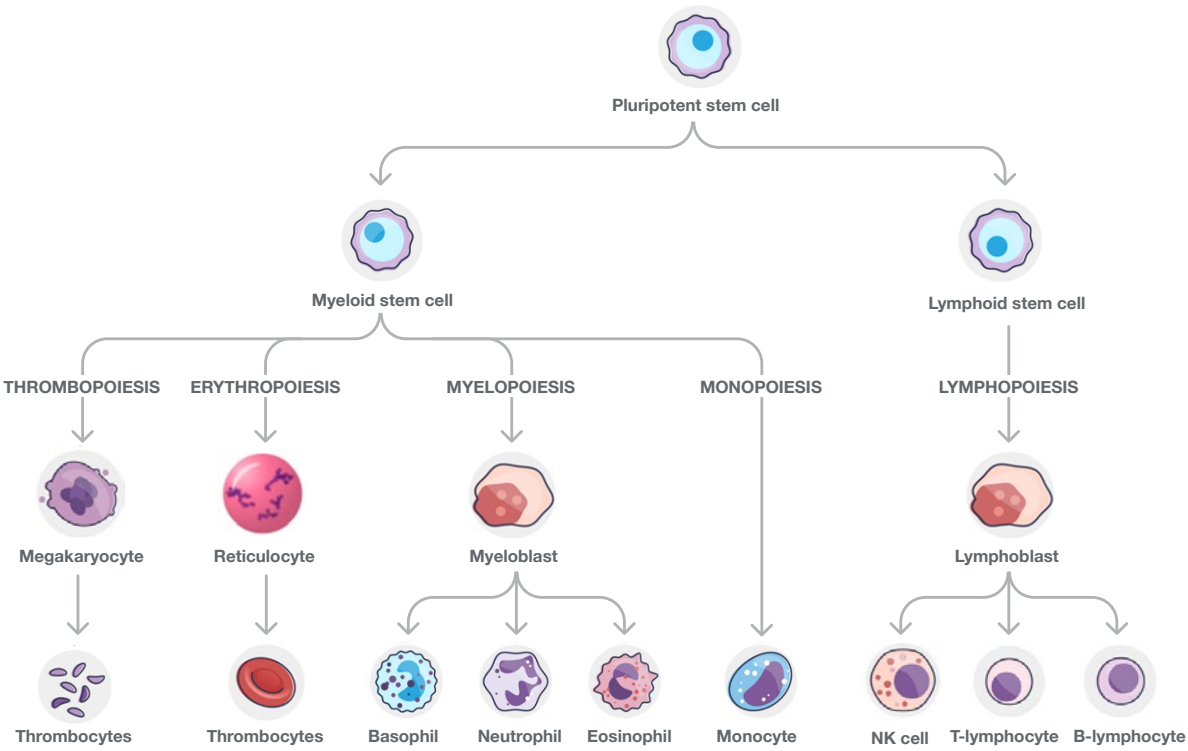


Fig 1. Overview of the hematopoietic blood cell production process, which is growth factor-dependent and strictly regulated to maintain steady-state blood levels. Abnormally high or low levels are associated with different types of disease conditions.

In addition to reporting the blood cell counts, an automated hematology analyzer determines a range of other parameters, such as hemoglobin (HGB) content; parameters related to cell size such as the mean corpuscle volume (MCV), mean PLT volume (MPV), RBC distribution width (RDW), and PLT distribution width (PDW); as well as calculated parameters such as the mean red cell hemoglobin (MCH = HGB/RBC), mean red cell hemoglobin concentration (MCHC = HGB/HCT), hematocrit (HCT = RBC × MCV), and plateletcrit (PCT = PLT × MPV).

Automated hematology analysis results

The cell counts are performed in parallel cycles on the automated hematology analyzer (Fig 2). A dilution reagent provides an isotonic environment for the RBC and PLT counts, while a hemolytic reagent lyses the RBCs to release HGB and maintain the morphology of white blood cells to facilitate the counting of these cells. The results are presented as parameter values as well as in histograms which are graphical representations of the numerical data of the different cell populations.

Histograms provide information about the average volume of RBC, PLT, and WBC cell populations, size distribution of the cell populations, and detection of subpopulations. The x-axis represents the volume of the cells in femtoliters (fL), whereas the y-axis represents their relative occurrence (number of cells) in percent (Fig 3).

The histograms constitute an important part of the results and should be carefully studied, as they provide valuable and important insights about the sample processed by the analyzer (Fig 4). A normal histogram starts and ends on the baseline.

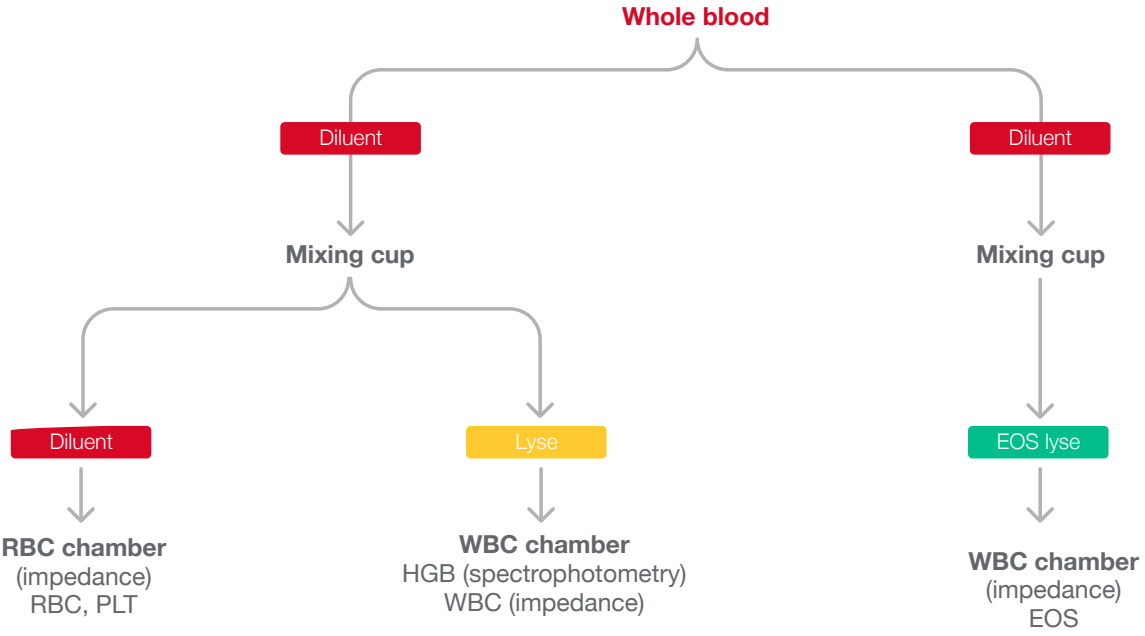


Fig 2. Measurement principle of Exigo™ H400 automated veterinary hematology system.

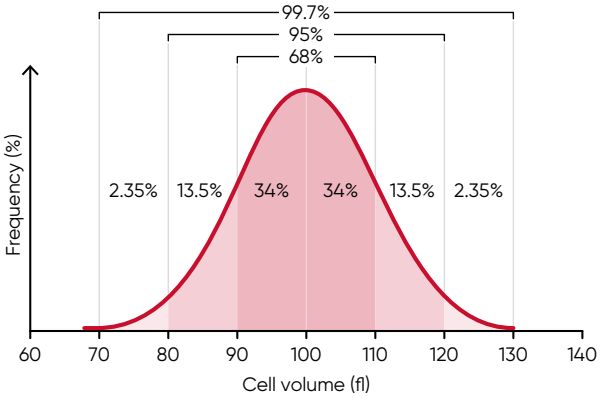


Fig 3. Cell variation in size within an individual population is called intra-individual biological variation. This variability has certain limits and is called Gaussian (or normal) distribution.

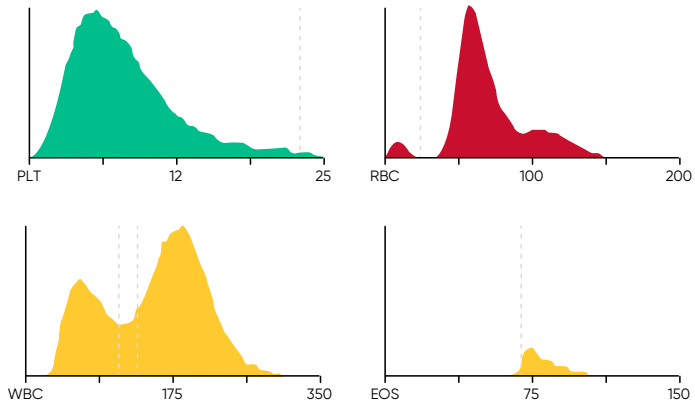


Fig 4. Exigo H400 displays histograms for platelets, red blood cells, white blood cells (LYM, MON, NEU) and eosinophils. Floating discriminators (dotted lines), that find the minima between the peaks, allow differential count of the WBC subpopulations, and prevent microcytic RBCs to be falsely counted as PLTs and elevated PLTs to interfere with the RBC count.

The RBC histogram

From the RBC histogram, the MCV and RDW values are calculated from the area under the curve (Fig 5). MCV is the average RBC volume, while RDW provides information about the degree of variation in RBC size (Fig 6). Examples of erythrocyte abnormalities are shown in Figures 7 to 10.

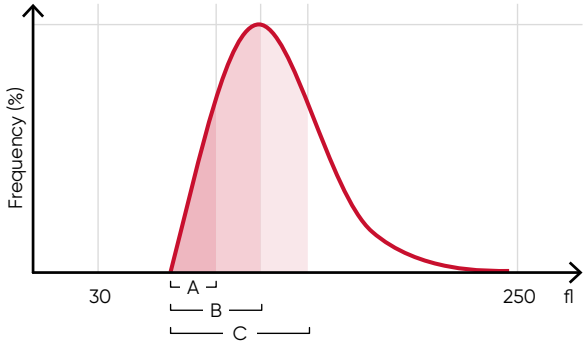


Fig 5. In Exigo H400, MCV is calculated as the sum of the size of all counted RBC pulses divided by the number of RBC pulses, RDW absolute value in fL (RDWa) is calculated as f(BAUC - AAUC), and RDW% is calculated as f((CAUC - AAUC)/BAUC), where f is the normalization factor.

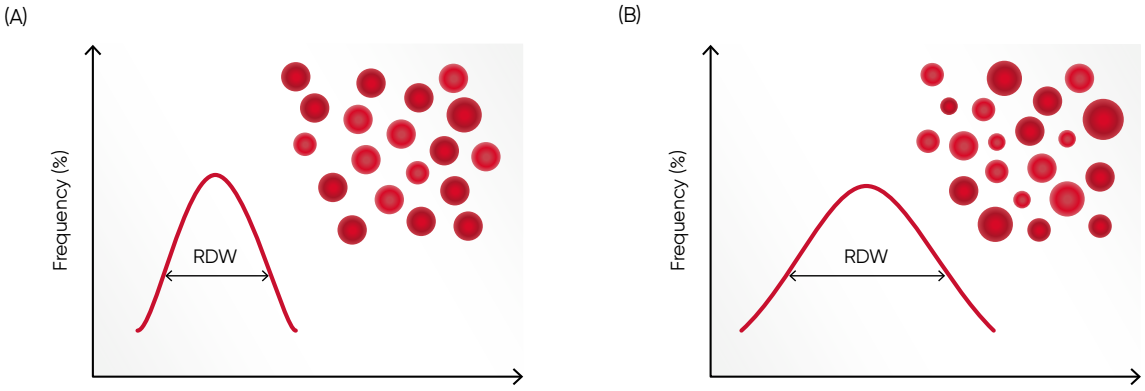


Fig 6. (A) Moderate anisocytosis (B) Pronounced anisocytosis (RDW > 20%).

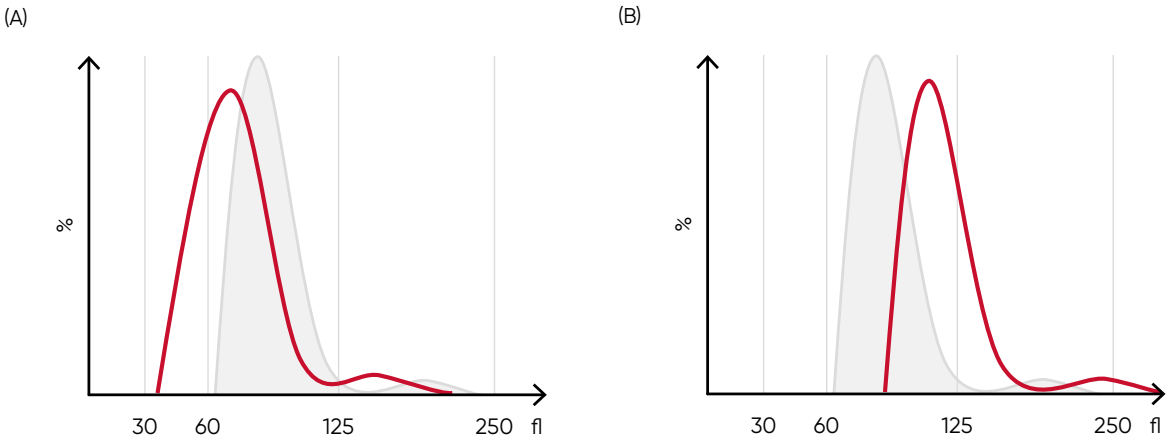
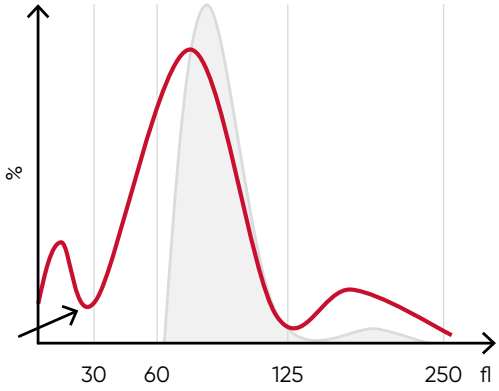


Fig 7. (A) A shift of the RBC histogram to the left gives an indication of microcytic RBCs (smaller than normal). (B) A shift of the RBC histogram to the right gives an indication of macrocytic RBCs (larger than normal).

(A)



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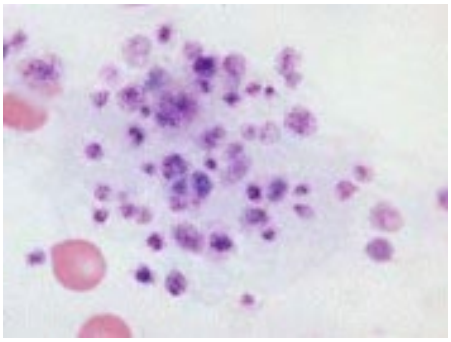

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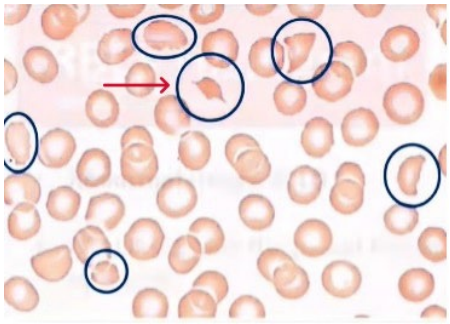

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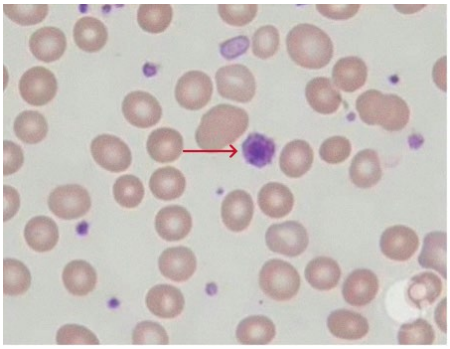

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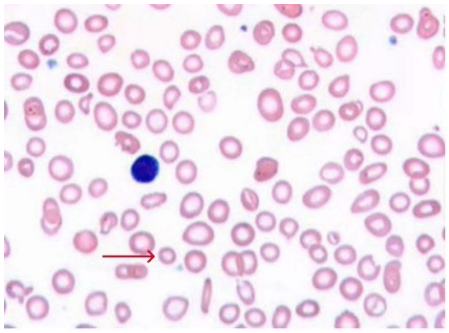
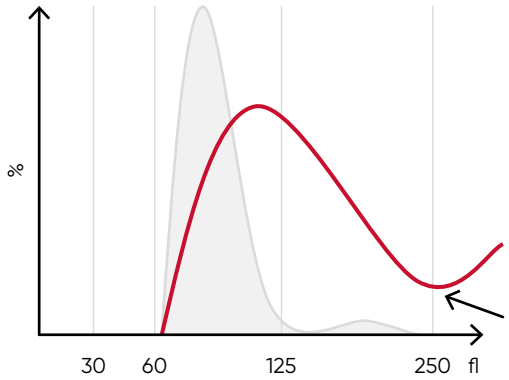


Fig 8. (A) Possible causes of an RBC histogram showing an abnormal high at lower discriminator can be RBC or WBC fragments, large (giant) PLTs, microcytic RBC, or PLT clumps. Action: check för PLT clumps on blood smear. Microscopic images showing (B) PLT clumps, (C) RBC fragments, (D) giant PLTs, and (E) microcytic RBCs.

(A)



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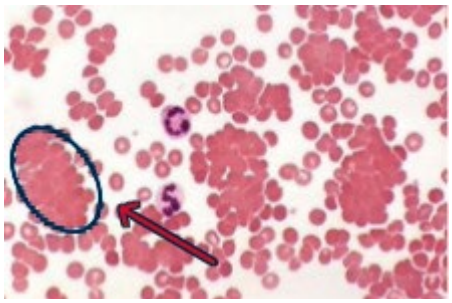

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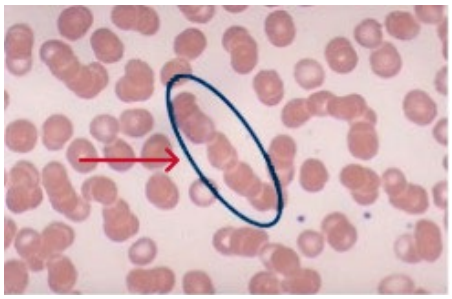

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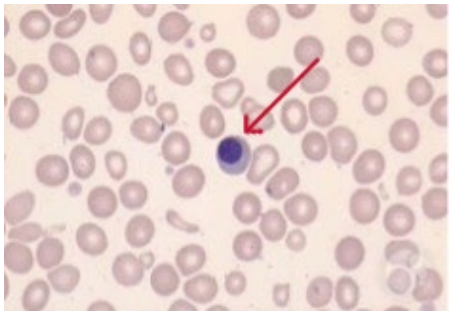

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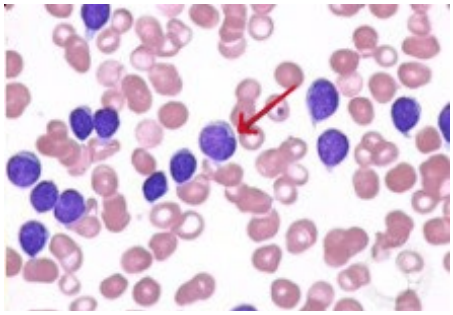

Fig 9. (A) Possible causes of an RBC histogram showing an abnormal high at upper discriminator can be RBC agglutination, cold agglutination, nucleated RBCs, or small LYMs, for example, due to chronic lymphocytic leukemia (CLL). Rule out cold agglutination by warming the sample at 37°C for 15 minutes and re-analyze. Action: look for agglutination on blood smear. Microscopic images showing (B) cold agglutination, (C) RBC agglutination, (D) nucleated RBCs, and (E) Small LYMs.

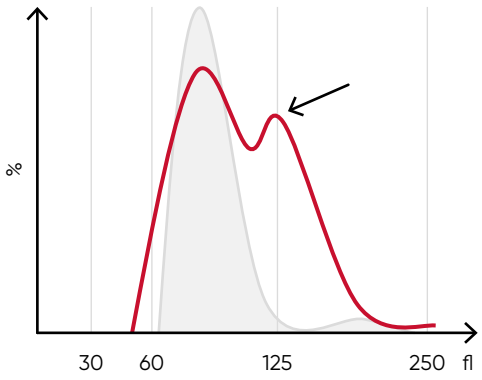

Fig 10. Dimorphic RBC population with significant anisocytosis with multiple RBC peaks of varying sizes can indicate iron deficiency in recovery, dual-deficiency anemia (iron and vitamin B12/folic acid), or recent blood transfusion. A large RDW is likely to be flagged. Check the smear for a dimorphic RBC population.

The PLT histogram

From the PLT histogram, the MPV and PDW values are calculated from the area under the curve (Fig 11). MPV is the average PLT volume, while PDW provides information on the degree of variation in PLT size. Examples of thrombocyte abnormalities are shown in Figures 12 to 15.

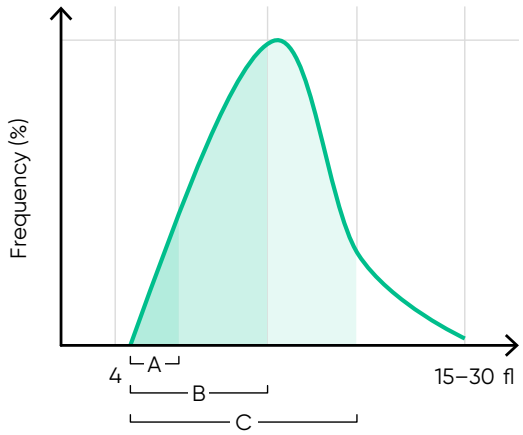

Fig 11. In Exigo H400, MCV is calculated as the sum of the size of all counted RBC pulses divided by the number of RBC pulses, RDW absolute value in fL (RDWa) is calculated as f(BAUC - AAUC), and RDW% is calculated as f((CAUC - AAUC)/ BAUC), where f is the normalization factor.

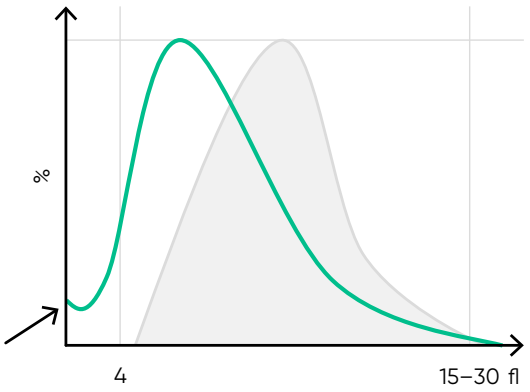

Fig 12. Possible causes of a PLT histogram showing an abnormal high at the lower discriminator can be a high background count, PLT aggregation, RBC fragments, or a bacterial contamination of the reagent. Action: check background count, run auto-rinse if required, and reanalyze the sample. Look for respective findings on blood smear. If this abnormality happens in many samples, check reagent for contamination. If the abnormality is due to PLT aggregation, an abnormal high at the upper discriminator is also shown.

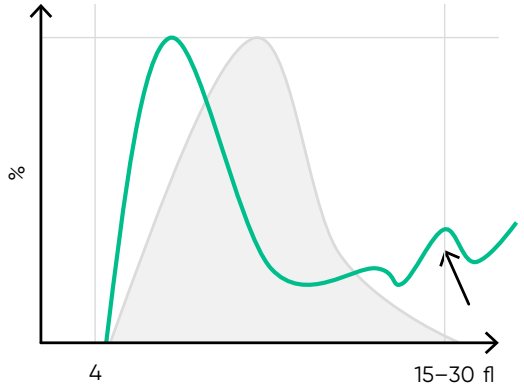

Fig 13. Possible causes of a PLT histogram showing an abnormal high at the upper discriminator can be giant PLTs, PLT clumps, microcytic RBCs, or RBC fragments. Action: look for respective findings on blood smear. If PLT satellitism is observed on smear, this can be an indication of EDTA-incompatibility. Re-collect using citrate as anticoagulant and reanalyze the PLT count.

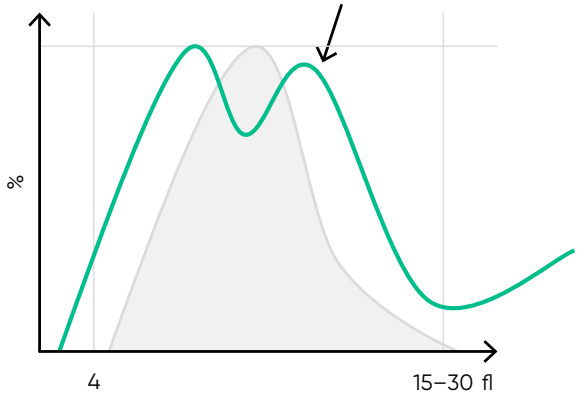

Fig 14. Multiple PLT peaks (dimorphic population) can be a reflection of recovery from chemotherapy, PLT clumping, or PLT anisocytosis, and can affect the PDW, P-LCR, or MPV values.

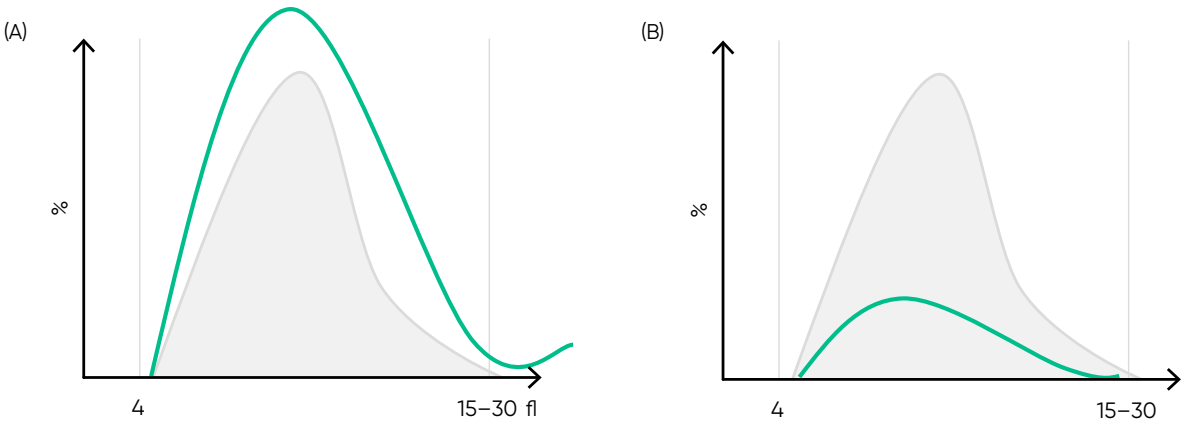

Fig 15. Effect of (A) thrombocytosis and (B) thrombocytopenia on the histogram.

The WBC histograms

The lyse reagent reduces the size of the cells to a specific volume for each cell type. As the RBCs are lysed by the hemolytic reagent, only PLTs and the WBCs are visible in the WBC channel. However, the small PLT particles (8–12 fL) do not interfere with the results for the larger WBCs (Fig 16). Examples of leukocyte abnormalities are shown in Figures 17 to 22.

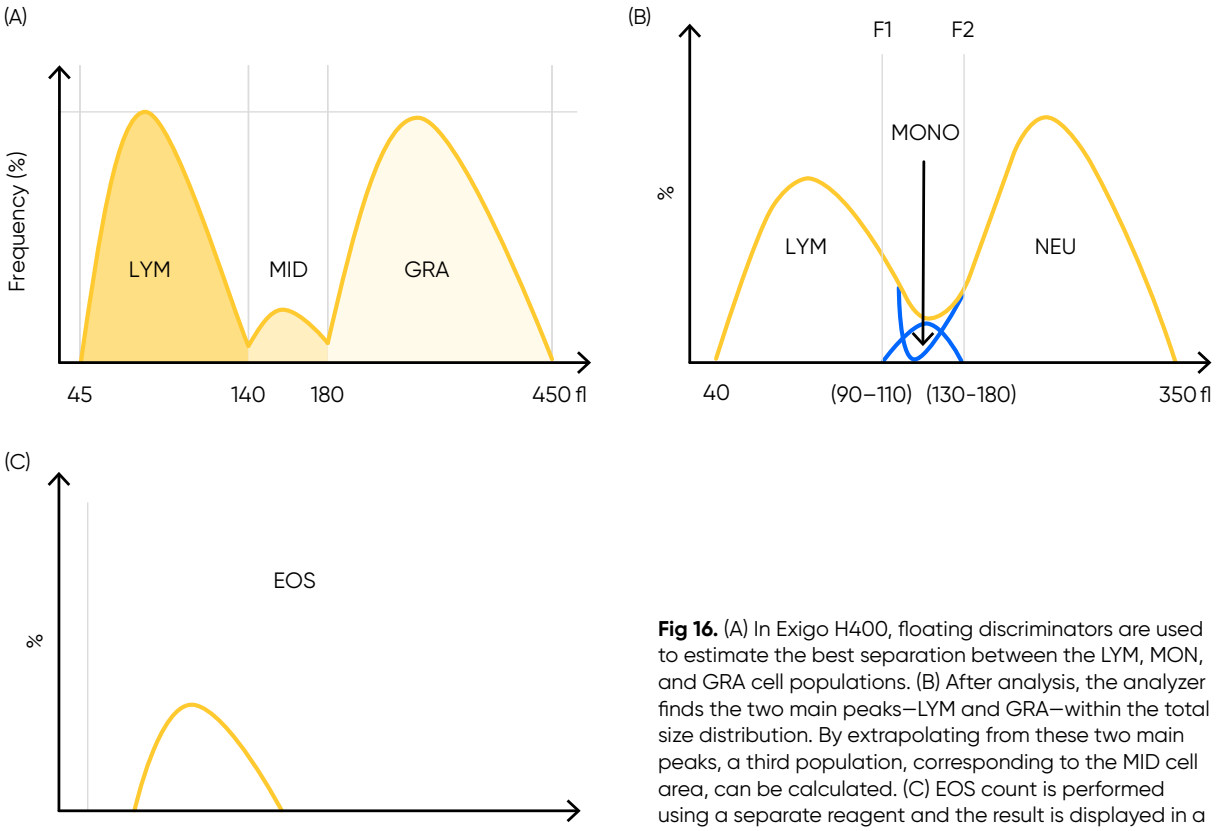

Fig 16. (A) In Exigo H400, floating discriminators are used to estimate the best separation between the LYM, MON, and GRA cell populations. (B) After analysis, the analyzer finds the two main peaks—LYM and GRA—within the total size distribution. By extrapolating from these two main peaks, a third population, corresponding to the MID cell area, can be calculated. (C) EOS count is performed using a separate reagent and the result is displayed in a separate histogram.

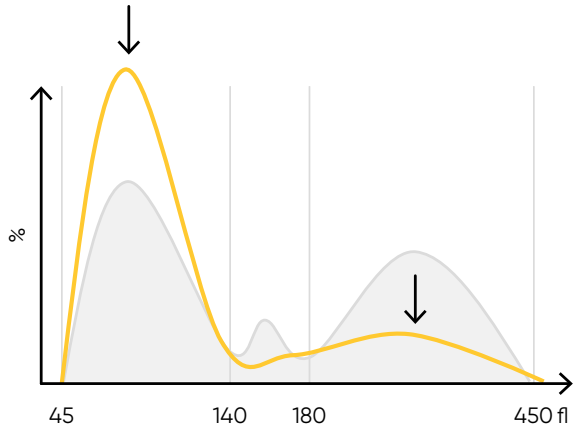

Fig 17. Sample results in case of lymphocytosis and neutropenia, which can be caused by a viral infection.

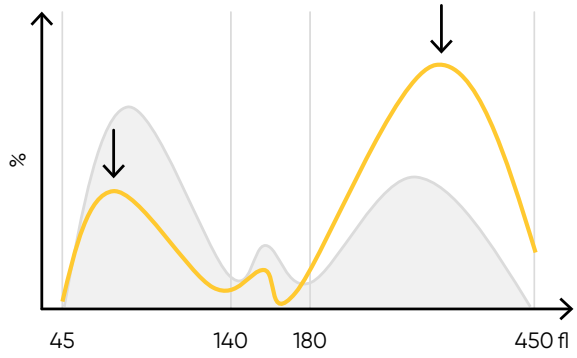

Fig 18. Sample results in case of lymphopenia and neutrophilia, which can be caused by a bacterial infection.

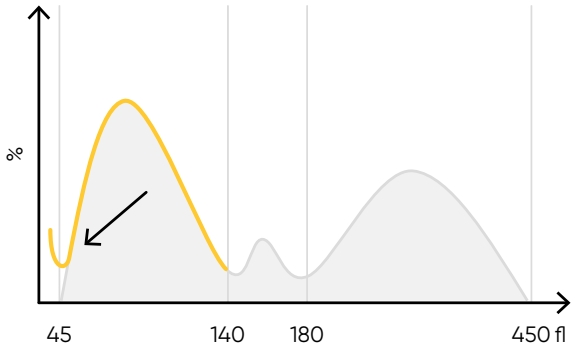

Fig 19. Possible causes of a WBC histogram showing an abnormal high at the lower discriminator can be PLT clumps, giant PLT, nucleated RBCs, lyse-resistant RBCs, or cryoglobulins.

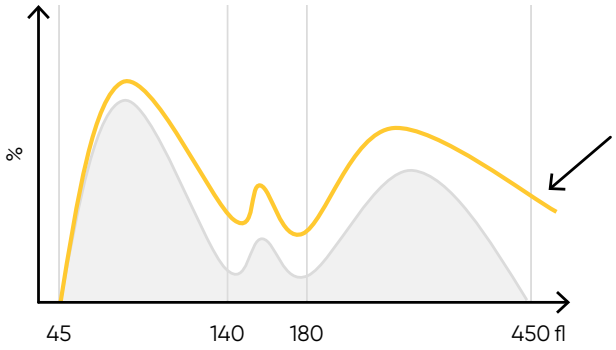

Fig 20 Possible causes of a WBC histogram showing an abnormal high at the upper discriminator can be severe neutrophilia or extreme leukocytosis (> 100 × 10^9 cells/L). Action: dilute sample, reanalyze WBC differential count, and check by manual microscopy.

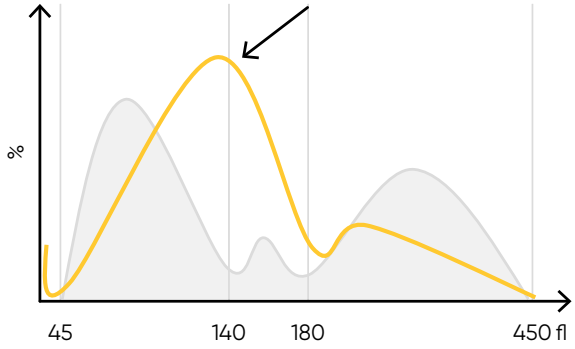

Fig 21. Possible causes of a WBC population with no plateau found at the discriminator can be the presence of blast cells, eosinophilia, basophilia, plasma cells, or abnormal/variant LYMs. Action: blood smear analysis is recommended.

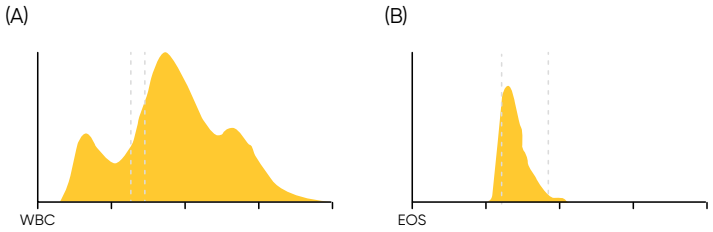

Fig 22. (A) A left shift of the granulocyte peak could be an indication of eosinophilia. The recommended action is to run a WBC differential count including an EOS count or to check blood smear for elevated eosinophils. (B) Eosinophil histogram from Exigo H400 showing elevated EOS counts.

Conclusion

Due to its cost-efficiency and simplicity, a complete blood count is widely requested in veterinary diagnostics, both for initial patient assessments and for monitoring of disease progression and treatment efficacy. Modern hematology systems report a range of parameters as numerical data. Additionally, the analyzer provides histograms that visualize the size distribution of the counted cell populations. Understanding these histograms can help verify cell counts, serve as a quality control check, identify uncommon diseases, and assess sample integrity.

Disclaimer

Exigo H400 from Boule Diagnostics is an automated hematology analyzer for in vitro veterinary diagnostic use under laboratory conditions. Boule products do not make diagnoses on patients. Boule products (systems, software, and hardware) are intended for professional use in medical practices, hospitals, and clinical laboratories to support medical diagnoses. This data, in conjunction with other diagnostic information and the evaluation of the patient's condition, can be used by a trained clinician to establish a patient's diagnosis and to define a clinical treatment.

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