White paper

# Veterinary hematology analysis: understanding histograms and scatterplots

Hematology analysis provides a cost-efficient tool for animal health screenings and disease investigations. A complete blood count (CBC) can give an 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 and scatterplots 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 tissues and organs and remove carbon dioxide that is carried back to the lungs and exhaled. In anemia investigations, the level of reticulocytes (immature RBCs) in circulation is sometimes also determined.

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 a bacterial infection. 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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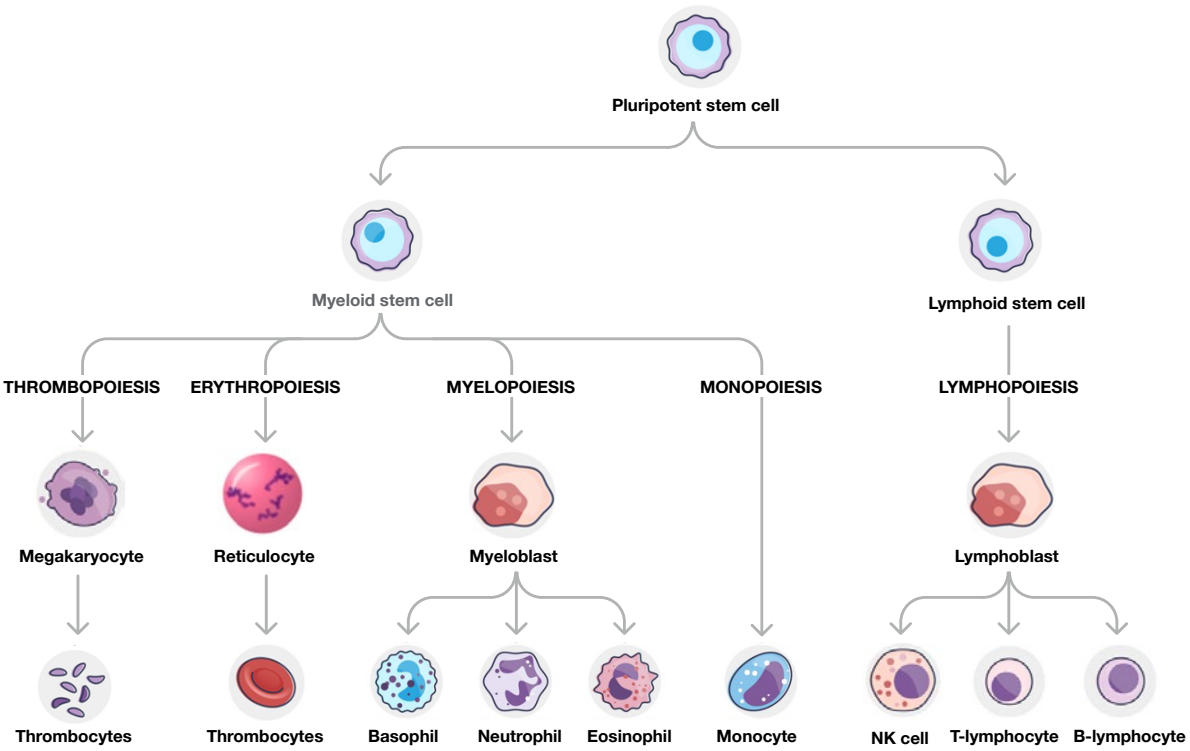



**Fig 1.** Overview of the hematopoietic blood cell production process, which is growth factor-dependent and strictly regulated to maintain steady-state blood levels. Abnormally high or low levels are associated with different types of disease conditions.

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 hemolytic reagent lyses the RBCs to release HGB and maintain the morphology of the WBCs to facilitate the counting of these cells. The results are presented as parameter values as well as in histograms and scatterplots, which are graphical representations of the numerical data of the different cell populations.

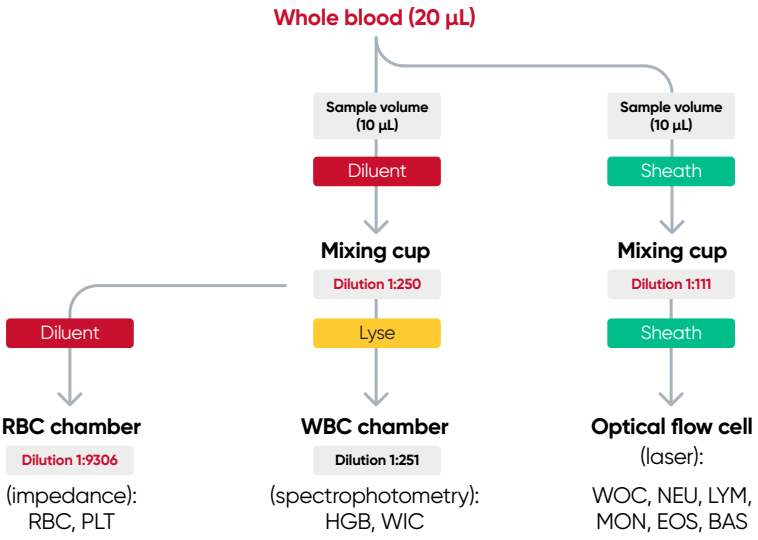



**Fig 2.** Measurement principle of H50V automated veterinary hematology system.

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). A normal histogram starts and ends on the baseline (Fig 4).

In a three-angle scatterplot, small angle signal represents the cell volume information, the high and super-wide angle signals represent the cell complexity (Fig 5).

The histograms and scatterplots constitute important parts of the results and should be carefully studied, as they provide valuable and important insights about the sample processed by the analyzer.

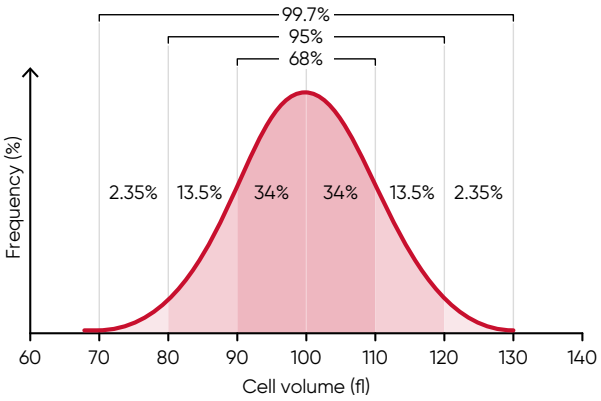



**Fig 3.** Cell variation in size within an individual population is called intra-individual biological variation. This variability has certain limits and is called Gaussian (or normal) distribution.

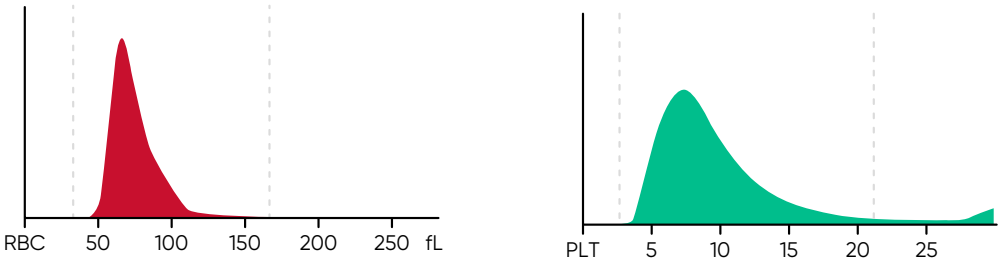



**Fig 4.** In H50V, results for red blood cell and platelet counts are visualized in histograms.

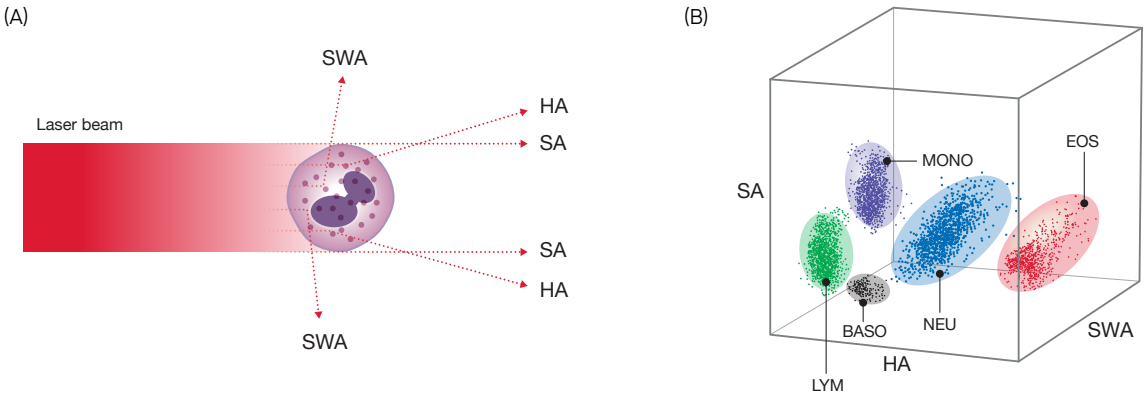



**Fig 5.** (A) Three-angle laser-scatter method, where the small angle (SA) signal represents the cell volume information, the high angle (HA) signal represents the cell nucleus information, and the super-wide angle (SWA) signal represents the cell nucleus and cytoplasm information. (B) In laser-based WBC differentiation, cell distribution is displayed in a 3D diagram, with the small, high, and super-wide angle light scatter signals on the axes (scatterplot).

## The RBC histogram

From the RBC histogram, the MCV and RDW values are calculated from the area under the curve (Fig 6). MCV is the average RBC volume, while RDW provides information about the degree of variation in RBC size (Fig 7). Examples of erythrocyte abnormalities are shown in Figures 8 to 11.

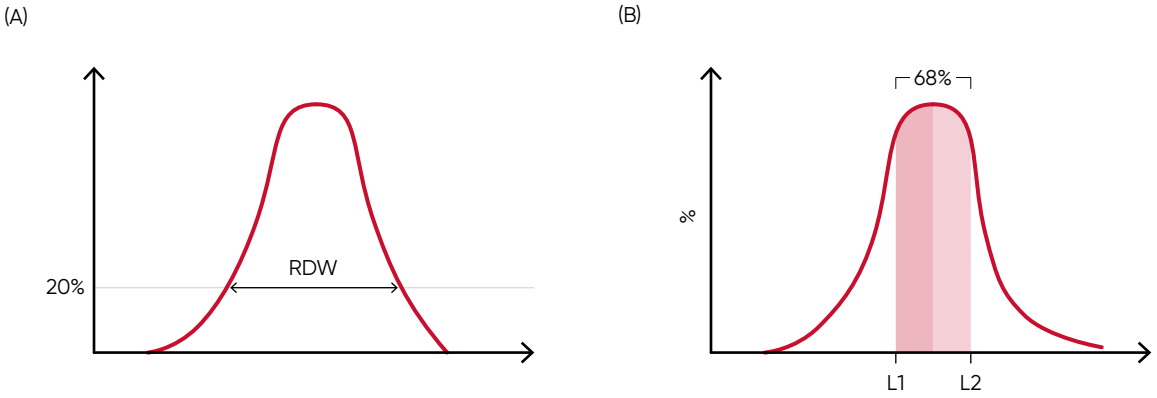



**Fig 6.** MCV is calculated as the sum of the size of all counted RBC pulses divided by the number of RBC pulses. (A) RDW-SD is the standard deviation of the average RBC size obtained by drawing an arbitrary line at a height of 20% of the y-axis. (B) RDW-CV is calculated as 100 × (L2 - L1)/(L2 + L1).

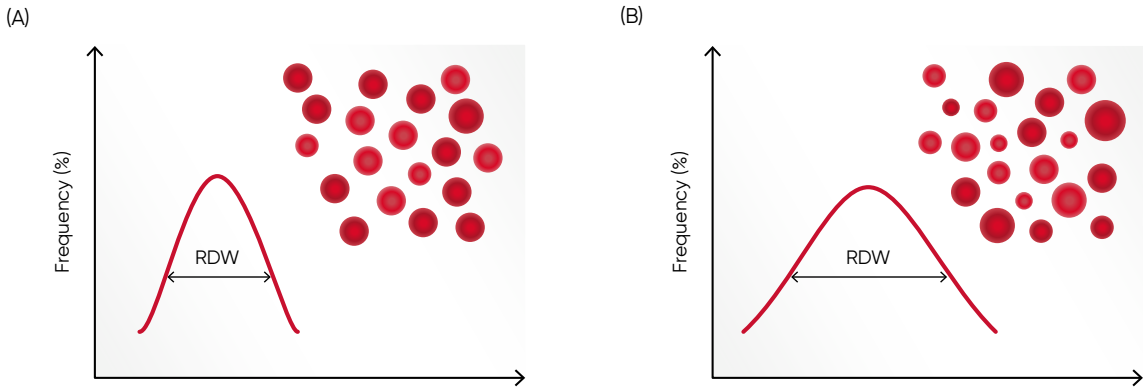



**Fig 7.** (A) Moderate anisocytosis. (B) Pronounced anisocytosis (RDW > 20%).

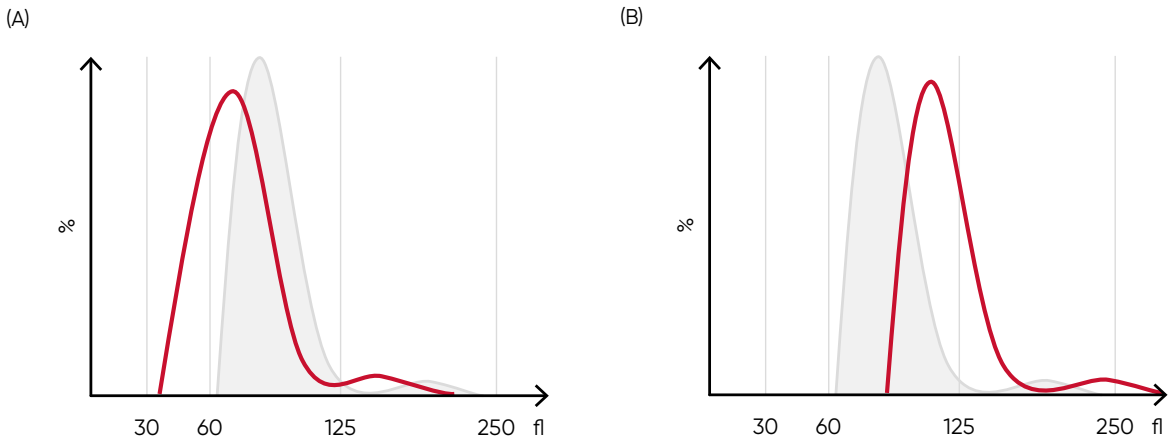
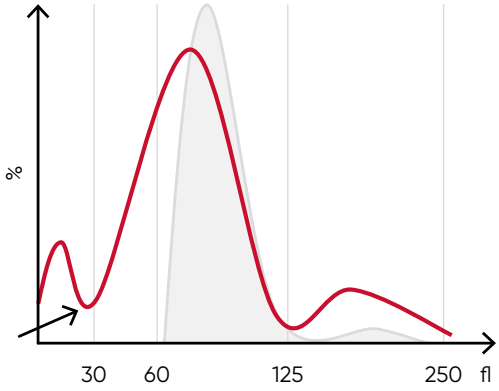



**Fig 8.** (A) A shift of the RBC histogram to the left gives an indication of microcytic RBCs (smaller than normal). (B) A shift of the RBC histogram to the right gives an indication of macrocytic RBCs (larger than normal).

(A)

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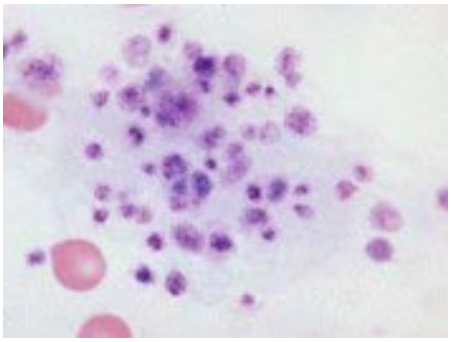

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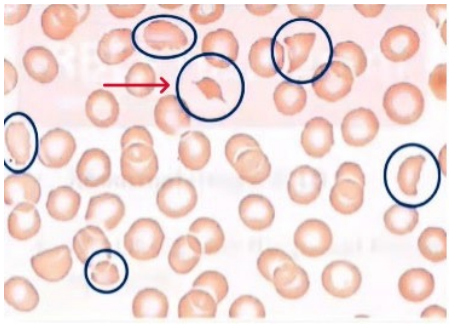

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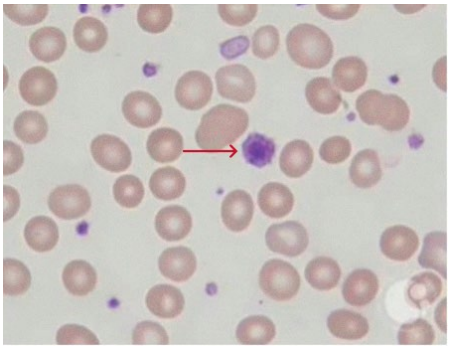

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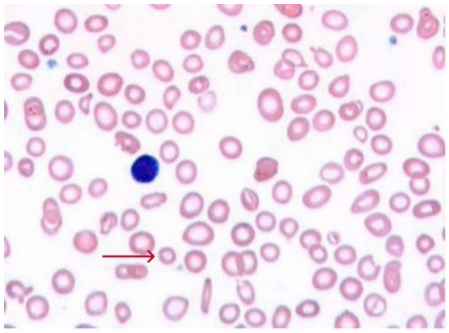
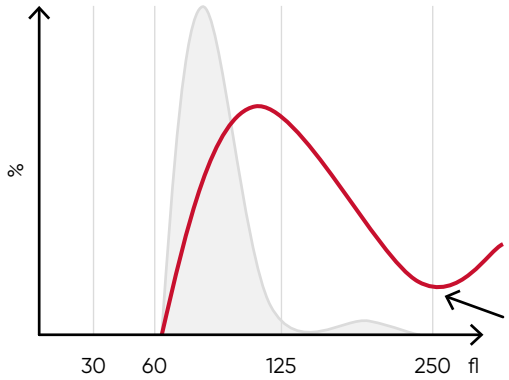


**Fig 9.** (A) Possible causes of an RBC histogram showing an abnormal high at lower discriminator can be RBC or WBC fragments, large (giant) PLTs, microcytic RBC, or PLT clumps. Action: check för PLT clumps on blood smear. Microscopic images showing (B) PLT clumps, (C) RBC fragments, (D) giant PLTs, and (E) microcytic RBCs.

(A)

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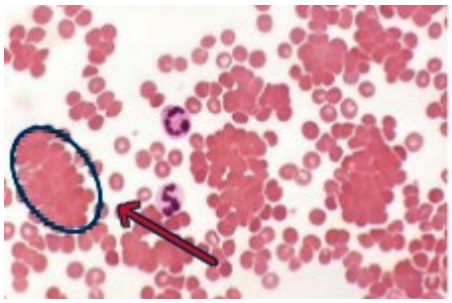

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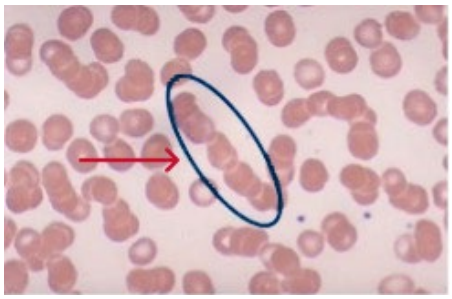

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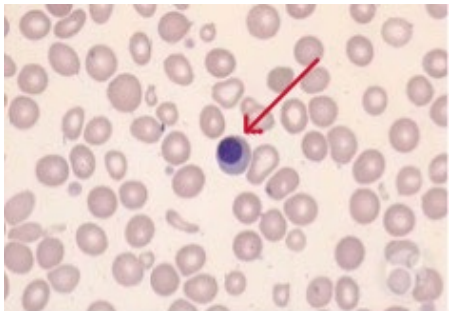

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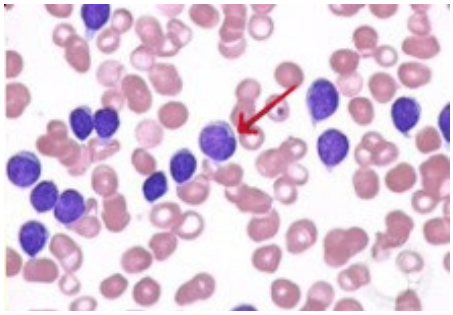


**Fig 10.** Possible causes of an RBC histogram showing an abnormal high at upper discriminator can be RBC agglutination, cold agglutination, nucleated RBCs, or small LYMs, for example, due to chronic lymphocytic leukemia (CLL). Rule out cold agglutination by warming the sample at 37°C for 15 minutes 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.

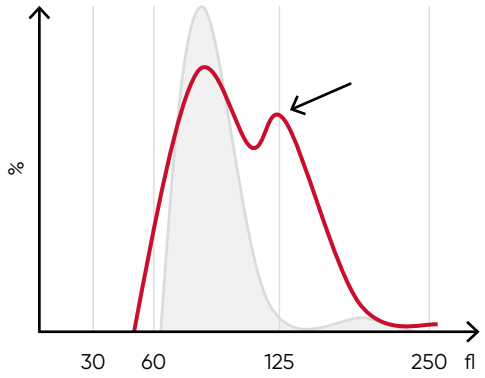



**Fig 11.** Dimorphic RBC population. A significant anisocytosis with multiple RBC peaks of varying sizes can indicate iron deficiency in recovery, dual-deficiency anemia (iron and vitamin B12/folic acid), or recent blood transfusion. Check manual blood smear for presence of a dimorphic RBC population.

## The PLT histogram

From the PLT histogram, the MPV and PDW values are calculated from the area under the curve (Fig 12). MPV is the average PLT volume, while PDW provides information on the degree of variation in PLT size. The PLT large cell ratio (P-LCR) is the amount of larger PLT particles in relation to the total number of PLT particles, and this value is used to calculate the PLT large cell concentration (P-LCC) (Fig 13). Examples of thrombocyte abnormalities are shown in Figures 14 to 17.

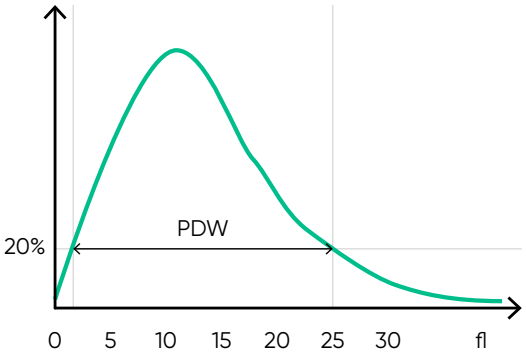



**Fig 12.** A normal platelet histogram shows a left-skewed distribution between 2 and 30 fL, with main part of the cells in the range of 2 to 15 fL. MPV is calculated as the sum of the size of all counted PLT pulses divided by the number of PLT pulses. PDW-SD is the geometric standard deviation of the average PLT size obtained by drawing an arbitrary line at a height of 20% of the y-axis.

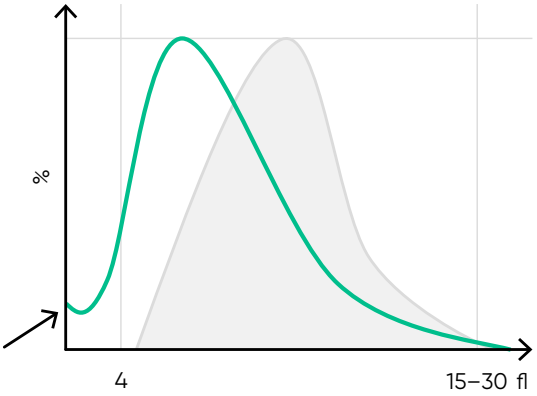



**Fig 14.** Possible causes of a PLT histogram showing an abnormal high at the lower discriminator can be a high background count, PLT aggregation, RBC fragments, or a bacterial contamination of the reagent. Action: check background count, run auto-rinse if required, and reanalyze the sample. Look for 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 upper discriminator is also shown.

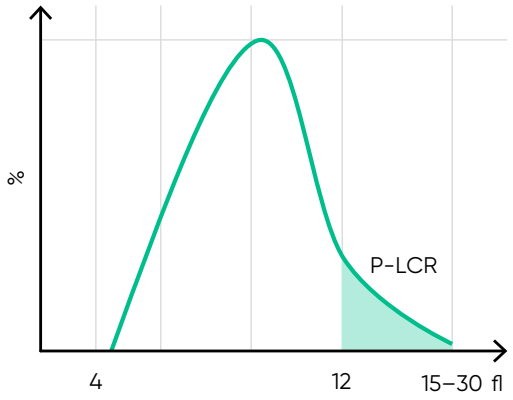



**Fig 13.** P-LCR is the counted number of larger PLT particles divided by the total counted PLT particles. The value is used to calculate P-LCC (= PLT × P-LCR). Interference with the P-LCR value can be due to the presence of PLT clumps, giant PLT, or microcytic RBCs in the sample.

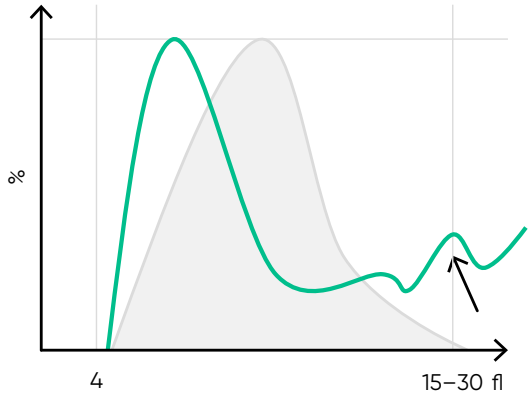



**Fig 15.** Possible causes of a PLT histogram showing an abnormal high at the upper discriminator can be giant PLTs, PLT clumps, microcytic RBCs, or RBC fragments. If PLT satellitism is observed on smear, this can be an indication of EDTA-incompatibility. Re-collect using citrate as anticoagulant and reanalyze the PLT count.

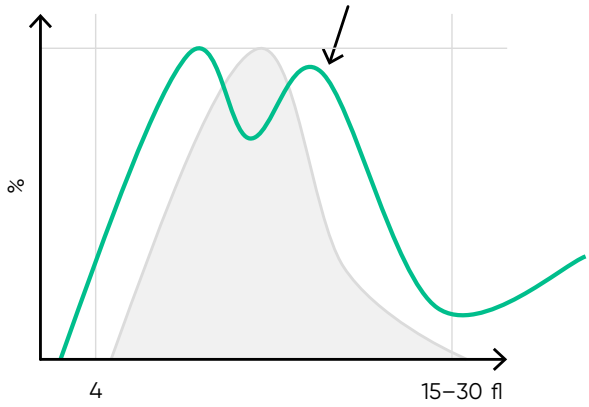



**Fig 16.** Multiple PLT peaks (dimorphic population) can be a reflection of recovery from chemotherapy, PLT clumping, or PLT anisocytosis, and can affect the PDW, P-LCR, or MPV values.

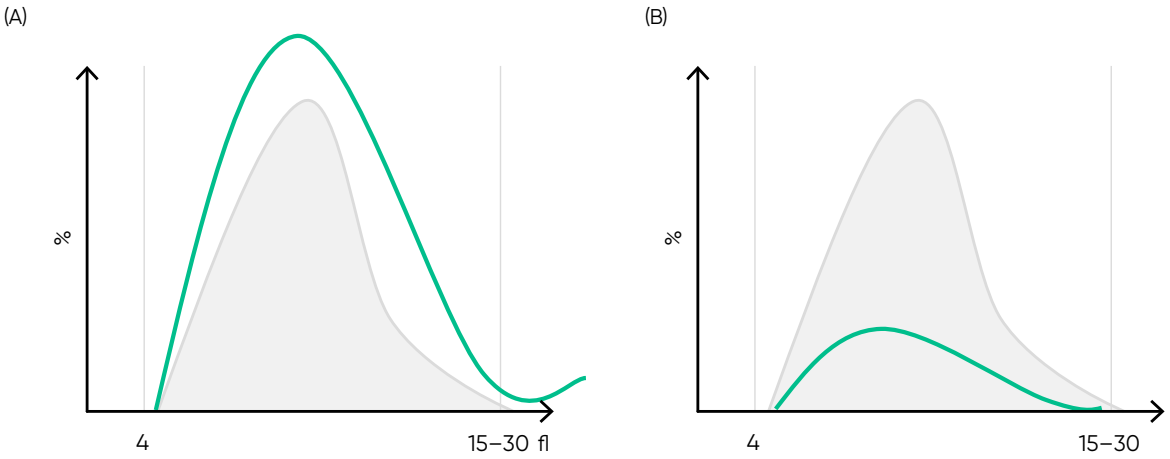



**Fig 17.** Effect of (A) thrombocytosis and (B) thrombocytopenia on the histogram.

## The WBC scatterplots

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 (2–15 fL) do not interfere with the results for the larger WBCs (Fig 18). Examples of leukocyte normal samples and abnormalities are shown in Figures 19 to 24.

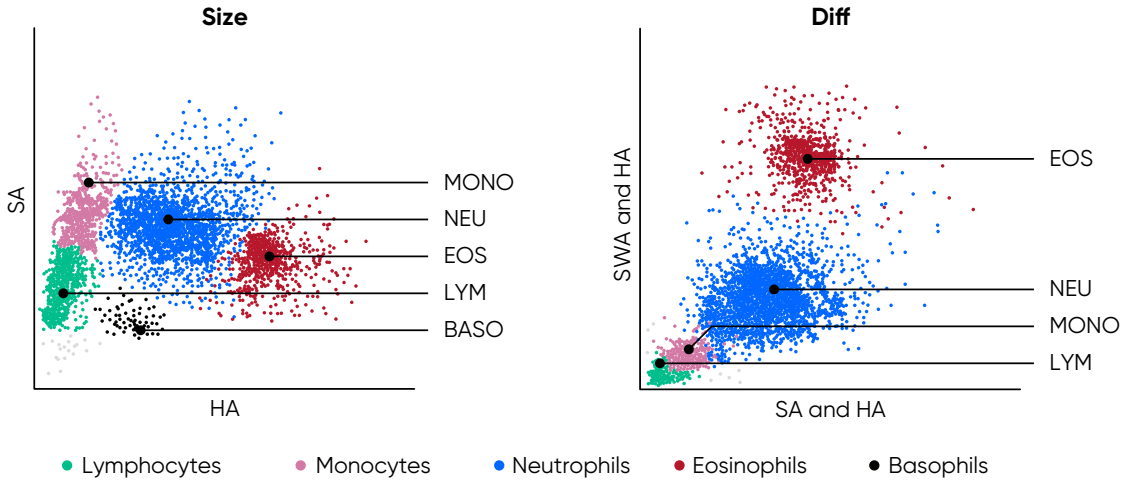



**Fig 18.** Scatterplots from a normal sample, showing WBC subpopulations.

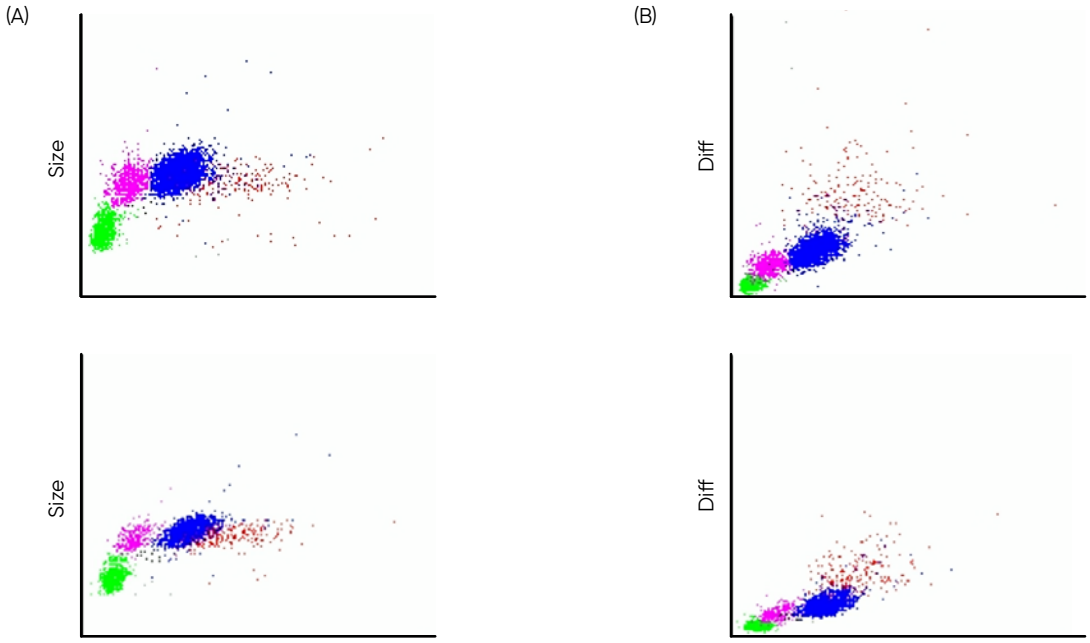



**Fig 19.** In normal (A) dog and (B) cat samples analyzed in H50V, NEU (blue), MON (pink), and LYM (green) are shown as distinct clusters, while EOS (red) constitute a slightly less dense cluster.

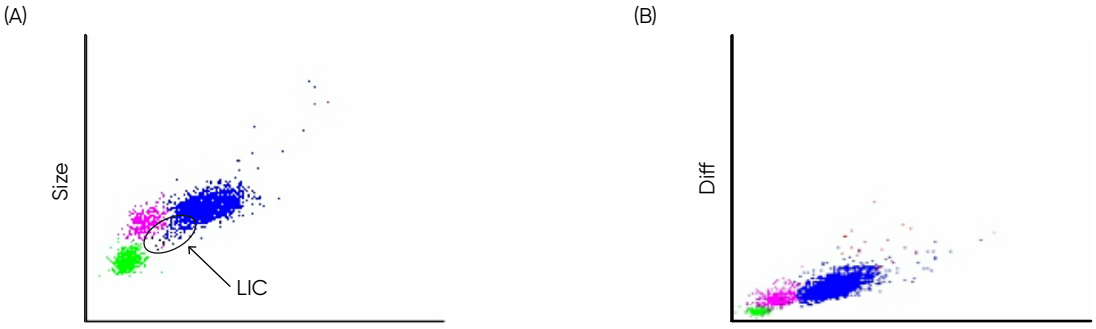



**Fig 20.** H50V scatterplots of an inflammatory sample from a canine patient. Possible cause of the stretch of the NEU cell cluster (blue) can be a presence of large immature cells (LIC).


**Fig 21.** H50V scatterplots of an inflammatory sample from a canine patient. A poor separation of the MON (pink) and NEU (blue) cell clusters can be caused by the presence of large immature cells (LIC), causing an overlap.

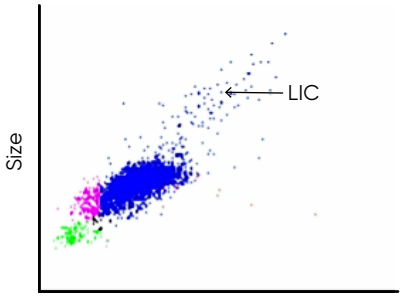



**Fig 22.** H50V scatterplots from a sample from a canine patient with severe leukocytosis due to confirmed uterus inflammation, with presence of rod-shaped neutrophils. The scatterplot shows poor separation of the MON (pink) and NEU (blue) cell clusters and the presence of large immature cells (LIC) visible in the upper right corner.

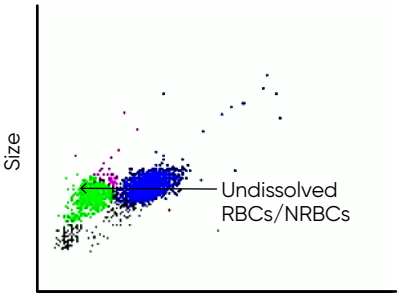



**Fig 23.** H50V scatterplot from a canine patient with a confirmed anemic profile. Ghost cells (gray) indicate unlyzed RBCs, reported as NRBCs in H50V. Action: confirm findings under the microscope.

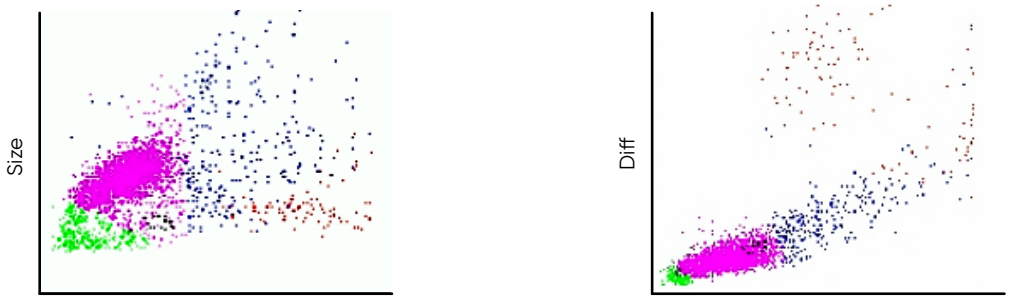



**Fig 24.** H50V scatterplots showing a sample result with high risk of WBC misclassification. The abnormal scatterplots show indistinct cell populations and do not clearly identify the NEU group. Possible causes include errors in sampling; consider recollecting a fresh blood sample. If the scatterplots remain abnormal, evaluate in blood smear under the microscope. If no abnormalities are observed in the blood smear, contact technical support.

# The reticulocyte scatterplot

Reticulocytes are reported in an absolute count as well as in a percentage of the RBC count and visualized in a scatterplot (Fig 25). The presence of reticulocytes in a sample can also be seen as a slight curvature on the right side of the RBC histogram (Fig 26).

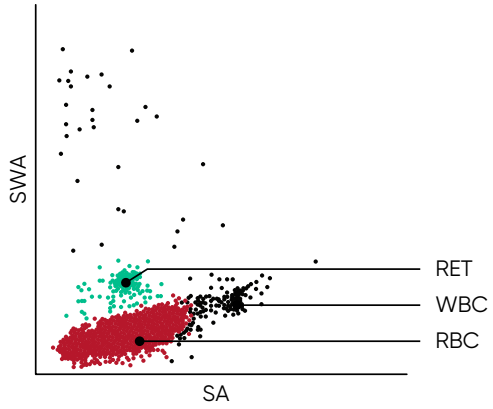
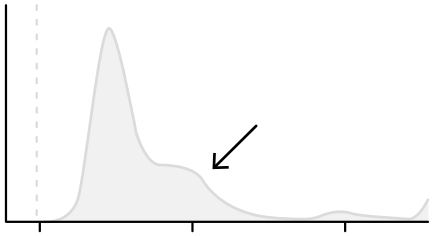



**Fig 25.** H50V reticulocyte scatterplot. Reticulocytes appear as a green cluster above red RBC group.

(A)



(B)

| Param. | Result | Unit |
|---|---|---|
| **RBC** | L 3.73 | 10^12/L |
| **HGB** | L 8.6 | g/dL |
| **HCT** | L 25.4 | % |
| **MCV** | 68.1 | fL |
| **MCH** | 23.0 | Pg |
| **MCHC** | 33.8 | g/dL |
| **RDW_CV** | 12.9 | % |
| **RETIC** | H 4.2 | % |
| **RETIC_ABS** | H 157 | 10^9/L |
| **IRF** | 21.36 | % |

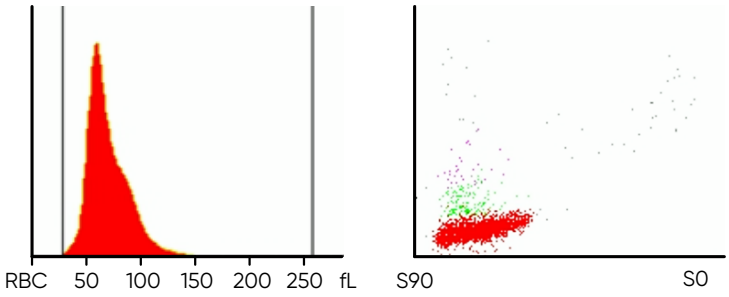



**Fig 26.** (A) An asymmetrical RBC histogram with an upward turn at the upper end. Larger immature reticulocytes in circulation can increase variation in red blood cell size and result in a small curvature of the RBC histogram. (B) H50V result list and scatterplots from a patient with confirmed regenerative anemia, showing low HCT, RBC, and HGB, slightly increased MCV, detected reticulocytes, and a slight curvature on the right side of the RBC histogram.

## Conclusion

As the test is cost-efficient and simple to use, a complete blood count is widely requested in veterinary diagnostics, both as initial patient assessments as well as in monitoring of disease progression and treatment efficacy. A modern hematology system reports a range of parameters as numerical data. As supplement, the analyzer provides histograms and scatterplots that visualize the counted cell populations in size distribution curves and event plots. Understanding these graphs can help verify the cell counts, serve as a quality control check, identify uncommon diseases, and indicate sample integrity.

## Disclaimer

H50V 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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