Optimizing platelet count in animals

Boule

Introduction

Blood sampling from a patient who is scared can be very hard. To add a communication barrier to this is of course not ideal, but this is what veterinarians face every day.

Besides the well-being of, and the difficulty to sample from the patient, the stress can also result in biological effects on blood cell populations. Such a stress-related change can, for example, be platelet aggregation.

This article summarizes tips and pointers on how to improve the platelet count in animals using automated veterinary hematology systems.

Platelets, not your typical cells

A common issue when analyzing platelets is the merging of the PLT peak with the RBC peak, obscuring the valley in between these peaks. This issue can have several reasons, such as variability in PLT size, activation and aggregation of platelets, and the presence of RBC fragments or microcytic RBCs.

Platelet size and variability differs between animal species, for instance, cats have larger PLTs with a greater variability in size (MPV 11–18.1 fL) as compared to dogs, pigs, and humans (MPV 7.6–8.3 fL) (1). For cats, it is therefore a greater risk that the natural variation in size in between PLTs and RBCs overlaps.

Aggregated platelets

Aggregation of PLTs is a major source of errors in the PLT count. The aggregation is a result of the reactive nature of PLTs, which can be stimulated to aggregate by a variety of factors such as adenosine diphosphate (ADP) and serotonin (excreted from the PLTs themselves), adrenalin, vasopressin, collagen, and thrombin (1, 2). One theory for why PLTs from some animals, such as cats, are more prone to aggregation than others is their larger size (therefore containing and releasing more ADP and serotonin) and their sensitivity to stress-related substances such as adrenalin and vasopressin (1).

If there is a high degree of PLT aggregation in the sample, this will lead to less single PLTs and therefor a false low count, also called pseudo-thrombocytopenia.

Pseudo-thrombocytopenia can also be EDTA-induced. This is an *in vitro* phenomenon caused by anti-PLT antibodies from the patient binding to EDTA-activated epitopes on the PLT (3).

PLT count

Aggregated platelets pose a problem both for automated and manual PLT count. For impedance technology, where the different cell types are discriminated by size, PLT aggregates can have the same size as RBCs and therefore causing the two peaks to merge. If this happens, an instrument with a floating discriminator will not be able to find the minima between the peaks and the count is instead based on the beforehand set fallback discriminator of the hematology system, much like a fixed discriminator. It is therefore of great importance to understand the variability of PLTs and how these are distributed in the histogram so that the desired discriminator can be set accordingly (Fig 1). Furthermore, severe aggregation can also give effects on the WBC count, as the PLT aggregates could potentially be mis-interpreted as lymphocytes (2).

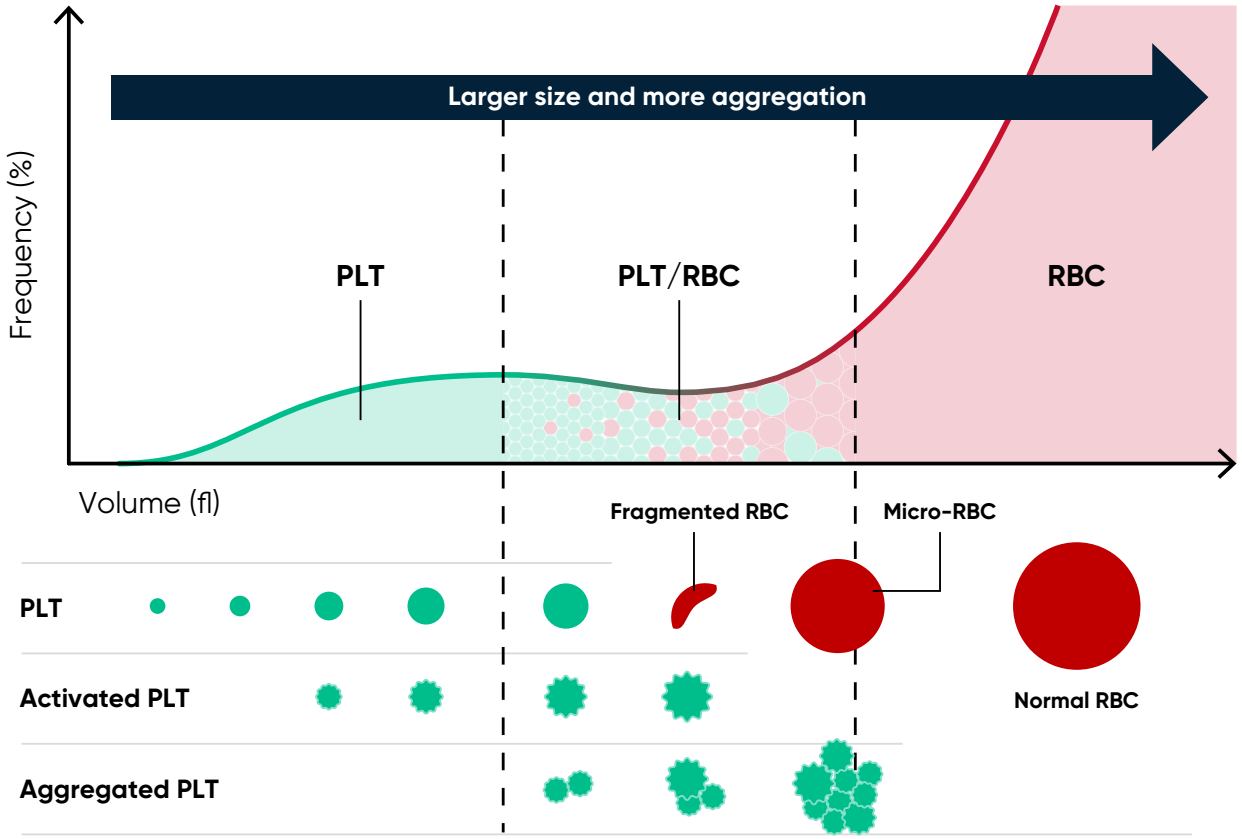


Fig 1. Graphical illustration describing the distribution of different cell types and sizes in the histogram.

Since automated count using optical technology do not only discriminate by size, the two populations will not merge in the same way. However, even these systems have a difficulty reading PLT aggregates as these have a different complexity than single PLTs and therefore a different scatter (1, 2). As the scatter of PLT aggregates line up very close to the granulocytes (NEU, EOS, BASO), this may lead to a false elevation of these cells (4).

Since the aggregation results in an uneven distribution of PLTs, even manual count is affected. However, the manual smear should always be performed to confirm the presence of aggregates, especially if the PLT count from the automatic hematology system is unexpectedly low.

System optimization

MPA-functionality

Compared to venous blood sampling, capillary blood sampling provides a less stressful experience for the animal and can therefore minimize stress-related effects on the blood cell populations – an important enabler for a reliable veterinary cell count. Capillary sampling also decreases the sample volume needed for analysis, which is especially important for small or dehydrated animals.

The micro-pipette adapter (MPA) featured on the Exigo H400 enables a complete blood count (CBC) from one drop of blood collected in a capillary sample tube – a functionality well suited for smaller animals (Fig 2).

Fig 2. The MPA capillary sample feature of the Exigo H400 is especially suitable for smaller animals such as cats.

Aperture optimization

In general, small cells demand a higher sensitivity of the analyzer to be counted correctly. To allow accurate count of the smaller blood cells, the Boule veterinary systems are equipped with a narrower capillary aperture for the RBC and PLT count compared with human cell counters. The narrower aperture (Fig 3) allows for smaller corpuscles such as the PLTs and certain RBCs to be detected with a higher sensitivity compared to a standard instrument.

Flexible discriminators

Boule systems employ a floating discriminator to separate the PLTs from the RBCs. The floating discriminator automatically finds the lowest point (valley) in between the two peaks and thereby gives an optimized separation. Should a valley not be found, the discriminator is set to a fallback mode which is a default setting in the systems. The Exigo H400 further offers the possibility to adjust the limits of the discriminator and the fallback.* This enables the laboratory to evaluate and set their own discriminator for PLT/RBC populations in different profiles, thereby making an informed decision on what to count as PLTs and what to count as RBCs.

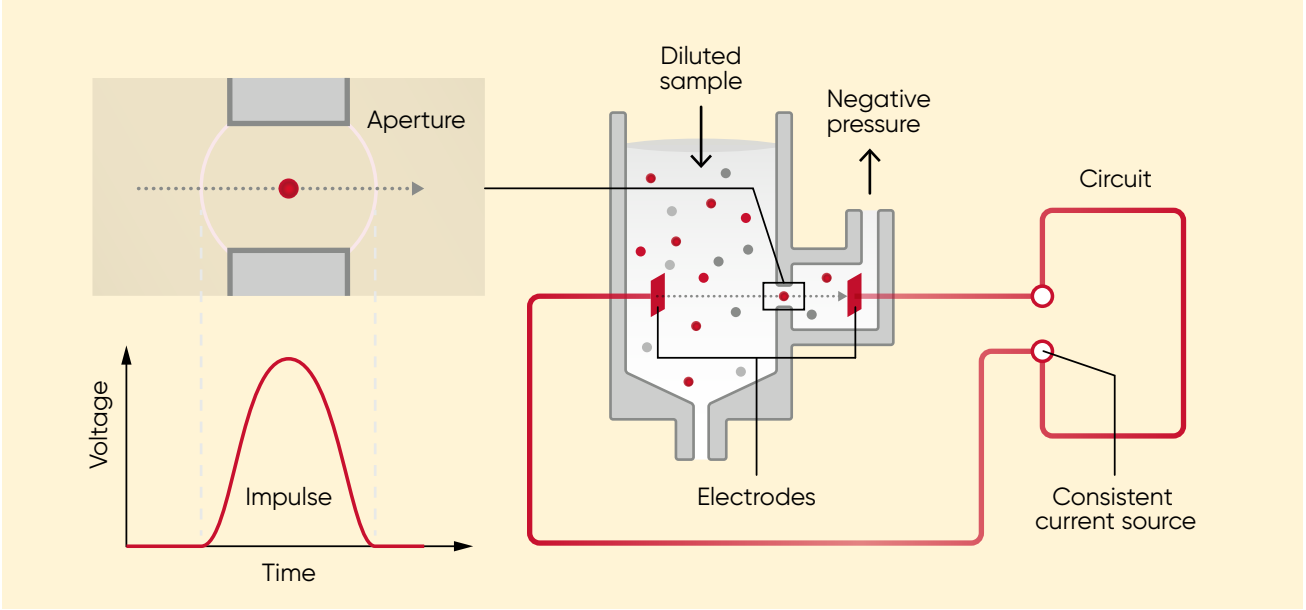
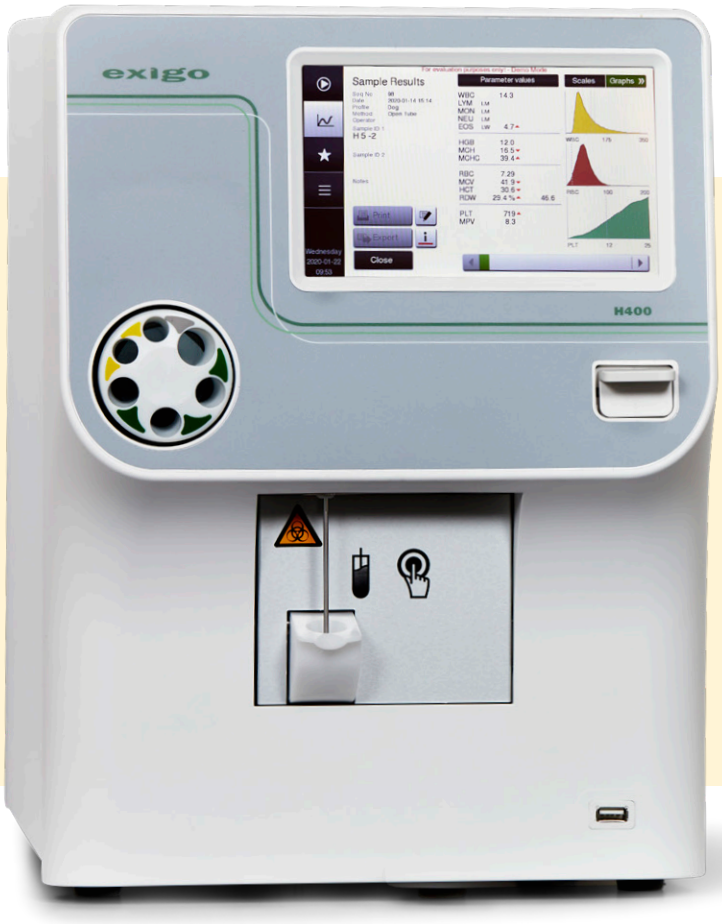


Fig 3. The Exigo H400 features a narrow capillary aperture of 60 µm, resulting in a sensitive system capable of counting the smaller blood cells. Each cell passing through the aperture causes a pulse in the electrical current. The number of generated pulses correlates with the number of cells, whereas the size of the pulse is related to the cell size.

*To adjust the discriminators in the Exigo H400, please contact your local service technician or log in to the "advanced set up menu" and follow the instructions in the manual.

Flagging

A sample with a very low PLT count should always be investigated with a manual smear to confirm true thrombocytopenia. Boule veterinary systems includes automatic flagging of samples when the PLT count is abnormally low, indicating that the sample should be investigated further in a blood smear.



Conclusion

To optimize the platelet count in animals, consider the following:

- MPA inlet for capillary blood sampling allows for a less stressful sampling method for the animal as well as a minimized sample volume, suitable for smaller animals.
- Narrow cell count aperture to increase the sensitivity when counting smaller corpuscles such as platelets.
- Floating and flexible discriminator settings, choose which cells to include.
- Flagging of suspected results.

Counting PLTs is inherently difficult due to the reactive nature of the cells. If the result is unexpectedly low, or aggregation is suspected, always confirm the findings in a manual smear.

References

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4. Interpreting IDEXX ProCyte Dx* Hematology Analyzer Dot Plots. © 2011 IDEXX Laboratories, Inc.

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