## How Impedance Technology Works

The VETSCAN® HM5 is an automated hematology analyzer that uses impedance technology to electrically classify cell types based on size. Larger cells generate larger electrical pulses, while smaller cells generate smaller pulses. Clumps of smaller platelets may generate a larger electrical signal and in turn may be counted as a larger cell, such as a white blood cell or red blood cell.[1]

Platelet counts may be underestimated when platelet clumps (aggregates) are present in the sample.[1] **Platelet counts reported by any method represent the minimum number of free, individual platelets that are circulating and may report a lower platelet value due to clumping, especially in cats.**[2]

Histograms are graphical representations of the blood cell populations and are displayed on the results screen of the HM5. Inspection of platelet histograms can help avoid errors in impedance platelet counts that can occur due to clumping.[3]

Platelet Count Measurements

Cells

Electrical Signals

a   b   c   d
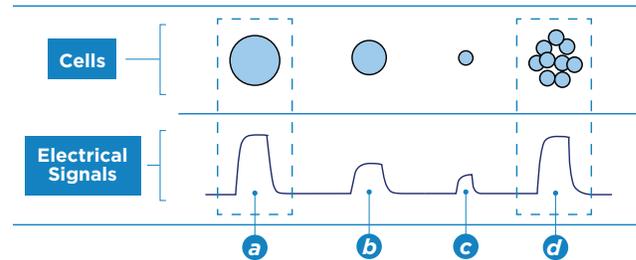
Measuring cell sizes for sizes based on voltage for:

a *Large cell*

b *Medium cell*

c *Small cell*

d *Clump of platelets*

Preventing Platelet Clumping

The most effective techniques to minimize platelet clumping include:

- Use the largest vein and needle appropriate for blood collection.[4]
- Do not hold off the vein for more than a few seconds before venipuncture.
- Perform a fast, atraumatic venipuncture, if possible. Do not redirect the needle after insertion.
- Ensure a proper ratio of anticoagulant to blood, by filling the tube according to the manufacturer's guidelines.
- Immediately mix the tube thoroughly after dispensing blood into it (invert 10-15x). [5]
- Run the sample immediately after collection.[6]
- Re-invert the tube just prior to analysis.
- Always check the tube for clots prior to running the sample.

Proper mixing by inversion.

Invert EDTA tubes 10-15 times immediately after adding the blood, and just prior to running a sample.

Ensure that the blood moves from one end of the tube to the other during each inversion.

Low Platelet Counts

When platelets are below the reference range on the HM5, this may either be due to the artifact of clumped platelets in the sample, patient or breed specific platelet variations (macrothrombocytopenia), or pathologic thrombocytopenia (disease). If the platelet count is below the reference interval a manual blood film should be evaluated.[7] Persistent mild thrombocytopenia (100,000 to 175,000 platelets/µL) is not specific for a particular disease.[8] Platelets < 50,000/µL may be at risk of surgical or trauma-induced hemorrhage and very severe thrombocytopenia (<20,000 plt/µL) is at risk of spontaneous hemorrhage and likely associated with immune mediated disease.[9]

Performing a Manual Platelet Count

Platelet numbers can be estimated using a blood smear. Examine the monolayer on 100x oil immersion objective. Calculate the average number of platelets/hpf in at least 10 fields. Multiply the average number of platelets by 15,000 to get a platelet estimate/µL. If platelet clumping is suspected, check the feathered edge to visualize platelet clumps. An exact measurement or estimate will not be possible in these cases.[10]

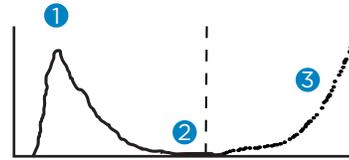# Platelet (PLT) Histogram Case Studies

Feline PLT Histogram

PLT Histogram, Normal[11, 12]

❶ The left side will slope upward to a peak, ❷ then trend downward toward the right side. Note the single, broad PLT peak. ❸ The PLT peak may not always reach the bottom of the graph on the right side, as shown here. ❹ The RBC graph begins to the right of the discriminator bar (dashed line).
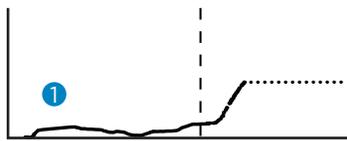
PLT Histogram, Typical Low[11, 12]

❶ In cases of severe thrombocytopenia, there will be no peak or curve. The curve will just hover over the X-axis.

PLT Histogram, Clumped[11]

❶ The broad PLT peak will decrease or disappear. ❷ The PLT curve will slope upward toward the discriminator line, never returning toward the X-axis. This indicates aggregations of clumped (larger) platelets. In more severe cases, a "W" flag will indicate severe aggregation of cells. In such cases, draw a new sample and/or verify adequate platelets with a blood smear.
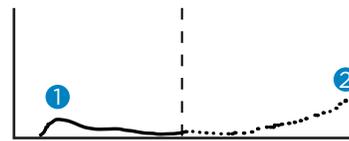
Canine PLT Histogram

PLT Histogram, Normal[11, 12]

❶ A normal canine PLT histogram has a single peak that sharply rises on the left to a form a sharp peak, then descends on the right side. ❷ Canine PLT graphs reach the X-axis on the right, if no platelet clumping is present. ❸ The RBC graph begins to the right of the discriminator bar (dashed line).
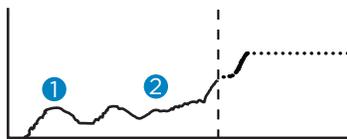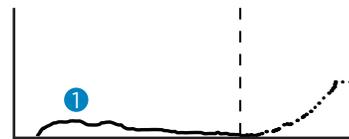
PLT Histogram, Typical Low[11, 12]

❶ The PLT peak in this histogram from a dog with immune mediated thrombocytopenia displays a small peak with limited area under the curve due to low relative cell number. ❷ The PLT peak is well separated from the RBC peak.

PLT Histogram, Clumped[11]

❶ The PLT peak will be broader than it is high, and can appear similar to thrombocytopenia peaks (above). However, clumped PLT may result in low PLT counts with a long tail in the granulocyte histogram. In severe cases, there may be increases in LYM and WBC counts, or a warning "L" flay may also be displayed. In such cases, draw a new sample and/or verify adequate platelets with a blood smear.

In addition to using the histograms, platelet count should be verified by a manual blood smear whenever platelets are below the reference range.

Contact Technical Support for additional information:

⊘ 800 822 2947   @ dxsupport@zoetis.com

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