LARGE PLATELET FRACTION (2023)

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Introduction:

Platelets or thrombocytes are small anucleate disc-shaped cell fragments with a diameter of 2 to 4 µm and a volume of 2 and 20 fL.
They play an important role in primary hemostasis, inflammation or innate immunity and originate from the cytoplasm of Megakaryocytes (MKs), one of the largest (50-100 µm) and rarest cells in the bone marrow (0.01% of nucleated cells). Through a Thrombopoietin (TPO)-driven process, MKs undergo a 5-day maturation where they become polyploid (128n)[1] and accumulate proteins and membrane.

Then, through a cytoskeletal-driven process, they extend into proplatelets in the blood stream and are converted into preplatelets. Fission of preplatelets generates platelets that survive 7 to 10 days in the blood stream.[1] The average platelet count is between 150 and 410 x 10^9/L.

Platelet associated parameters:

The HORIBA Medical Yumizen H2500 hematology analyzer provides 8 different parameters to screen the platelet population (Figure 1).

Parameters	Normal Ranges							
	PLT		MPV		PLT-O		PCT*	
Reference Limits	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper
Male	160	362	7.4	11.5	148.1	339.9	0.1	0.3
Female	172	411	7.6	11.8	152	374	0.16	0.37
Bibliographic Data[6]	150	400	7.4	12	150	400	0.15	0.40

Parameters	Normal Ranges							
	PDW*		P-LCC*		P-LCR*		LPF*	
Reference Limits	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper
Male	11.4	19.1	?	?	?	?	?	?
Female	11.8	19.6	?	?	?	?	?	?
Bibliographic Data[6]	11	20	?	?	?	?	?	?

Figure 1: Table of normal ranges for each Platelet-associated parameter based on a HORIBA Medical study.

These normal ranges were assessed by a study of 240 blood samples from a French population analyzed in duplicate on one Yumizen H1500 and two Yumizen H2500s following CLSI C28-A3.[2] Male and female sampling was performed using EDTA anticoagulant. These normal ranges may vary per sample population and/or geographical location. HORIBA Medical strongly recommends that each laboratory establishes its own normal ranges based upon its local population.

Blood platelet concentration (**PLT**) is the basic parameter of platelets in CBC and can indicate thrombocytopenia or thrombocytosis. **MPV**, or Mean Platelet Volume, is derived from the platelet distribution curve and measures the mean volume of the platelet population. **PLT-O** or Optical Platelet measures the concentration of platelets using impedance and optical extinction when impedance alone fails to do so. **PCT** or Plateletcrit measures the total platelet mass and can indicate quantitative abnormalities. **PDW** or Platelet Distribution Width measures the volume variability of platelets and can indicate platelet heterogeneity. **P-LCR/P-LCC** or Platelet Large Cell Ratio/Count quantifies every platelet with a volume larger than 12fL. These may be large platelets, platelet aggregates or microcytes.[3] LPF or Large Platelet Fraction measures the large platelet population using impedance and platelet optical extinction technology (PLT-Ox). Optical extinction allows correct separation between Red Blood Cells (RBC) and PLT curves when RBC/PLT interferences are observed due to the presence of giant platelets, fragmented RBC, microcytes or schistocytes. (Figure 2).

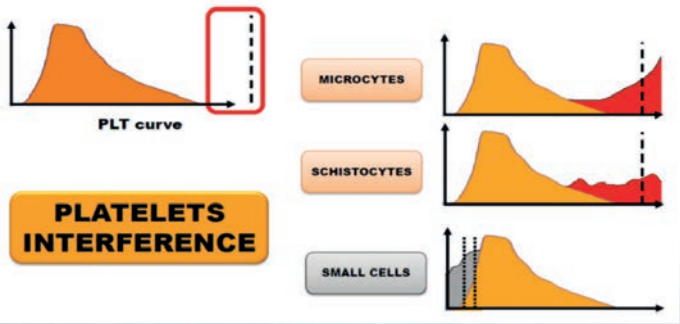


Figure 2: RBC/PLT interference

Large Platelet Fraction (LPF):

Platelets are involved in many different biological processes, so it is not surprising that circulating platelets are not equal. Platelets mainly differ in density and size. The normal range for platelet size is 5 to 10 fL. Therefore, the large platelet fraction corresponds to the platelet population with a size greater than 12 fL. This population mainly comprises large young platelets, also called Reticulated Platelets (RP) or Immature Platelets (IP). RP are newly released, larger and more reactive.[4] Like reticulocyte for RBC, RPs act as real-time biomarkers of platelet synthesis, also known as thrombopoiesis. RPs contain RNA strands which are used for protein synthesis. However, these strands do not come from MKs.[4]

RPs remain in the blood stream for 24 to 36 hours, after which, the RNA content and volume decreases.[4] The density and size of platelets may appear to be indicators of platelet age. However, according to some studies, platelet density may reflect platelet age, better than size.[5] RNA content related to platelet density is an even better age indicator.

Nevertheless, the majority of RPs are large platelets. The study states that “the majority of RP are large, with about 90% being confined to a 50% subpopulation of large platelets”.[6] A positive correlation between RP percentage and platelet size indices (MPV, P-LCR) was observed.

Large platelet fraction is mainly composed of RPs but also of large platelets that are not reticulated. These platelets are large but not young, as size is not a good marker for platelet age.

This population is less active in terms of protein synthesis. However, like RPs, this population has an increased hemostatic potential due to a higher contact region (15.8 fL).5 In some diseases such as cardiovascular disease, an increase in the large platelet fraction population, including functionally active platelets such as RPs, is associated with a poorer prognosis.[7] Thus, the screening of the Large Platelet Fraction can be useful for prognosis and management of various disorders. LPF screening can also be useful to assess the need for platelet transfusion.

The Yumizen Hematology Analyzer LPF parameter

The Yumizen H2500 by HORIBA Medical enables screening of the entire Large Platelet Fraction. This new Large Platelet Fraction (LPF) parameter provides laboratories with information that helps diagnosis within minutes, without changing their current HORIBA Medical equipment.

Before LPF, several parameters were used to screen the size of the platelet population and quantify large platelets, including MPV.

The MPV remains stable in healthy individuals so a variation can indicate a disease process such as immune thrombocytopenia, where MPV is increased.[5] PDW, P-LCC and P-LCR were also used. However, the latter three parameters do not specifically quantify large platelets.

The P-LCC/P-LCR count, in addition to large platelets, may include microcytes, schistocytes and platelet aggregates, giving an inaccurate large platelet result. The LPF parameter provides accurate and reliable results thanks to a combination of impedance and optical extinction technology. Impedance alone is good, but not accurate enough for platelet count because of debris, microcytes or platelet aggregates.

Optical extinction combined with impedance defines a unique signature for each cell, making it more accurate than impedance alone.

The Platelet Optical Extinction (PLT-Ox) mode on the Yumizen analyzer can combine the impedance and light extinction (absorbency) measuring methods, offering extremely reliable results (Figure 3).

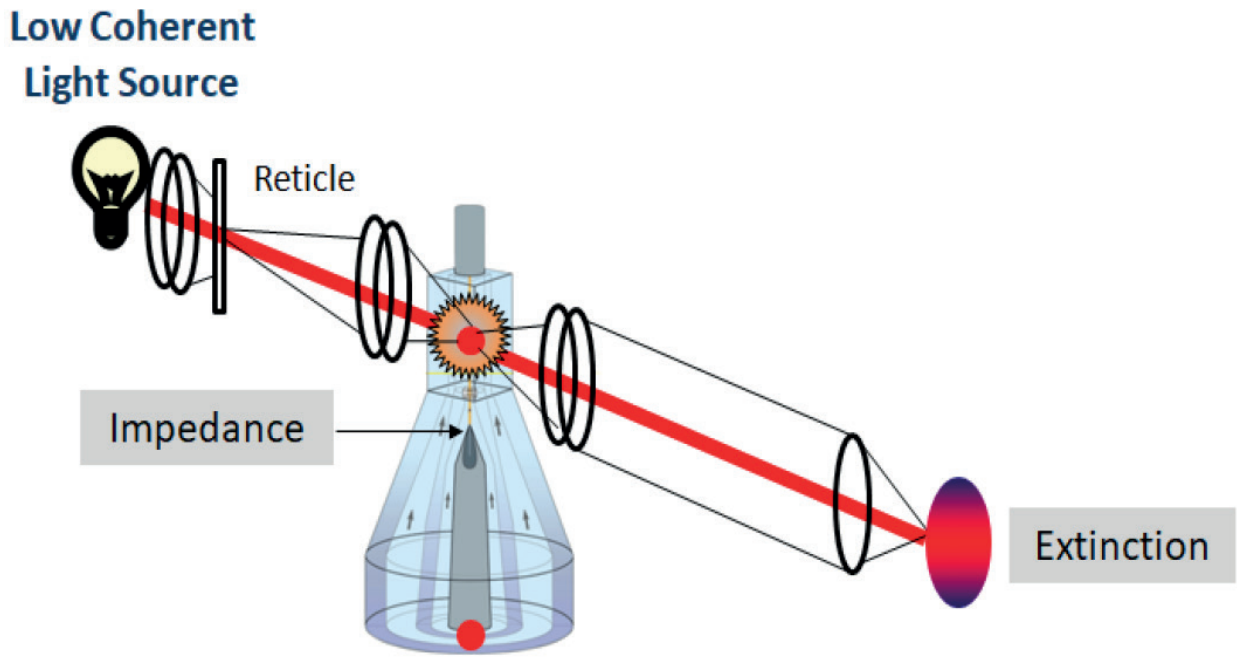


Figure 3: HORIBA Medical PLT-Ox measuring method using a low coherence light source.

The HORIBA measurement technology for platelet extinction uses a low coherence light source (extended source). The high numeric aperture of the light beam enables observation of cells from different angles, revealing both the morphology of the different cell compartments and intra-cytoplasmic spectroscopic characteristics. Contrary to a laser monochromatic light, which generates a perfectly parallel beam and consequently allows a vision of the cell from one dimension, the measurement made using a low coherence light source is less vulnerable to cell anisotropy and its position or orientation in the beam. It therefore provides detailed information on the intrinsic characteristics of the cell (Figure 4).

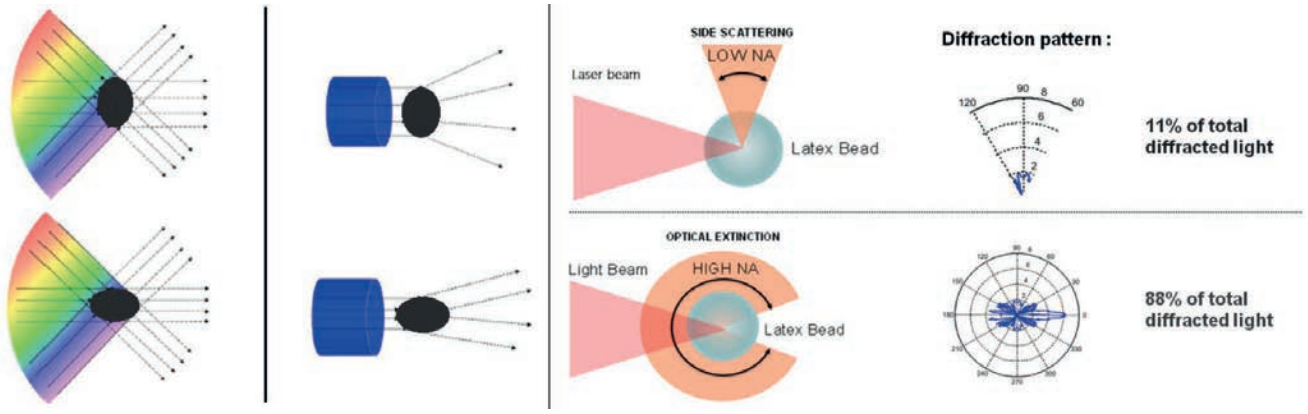
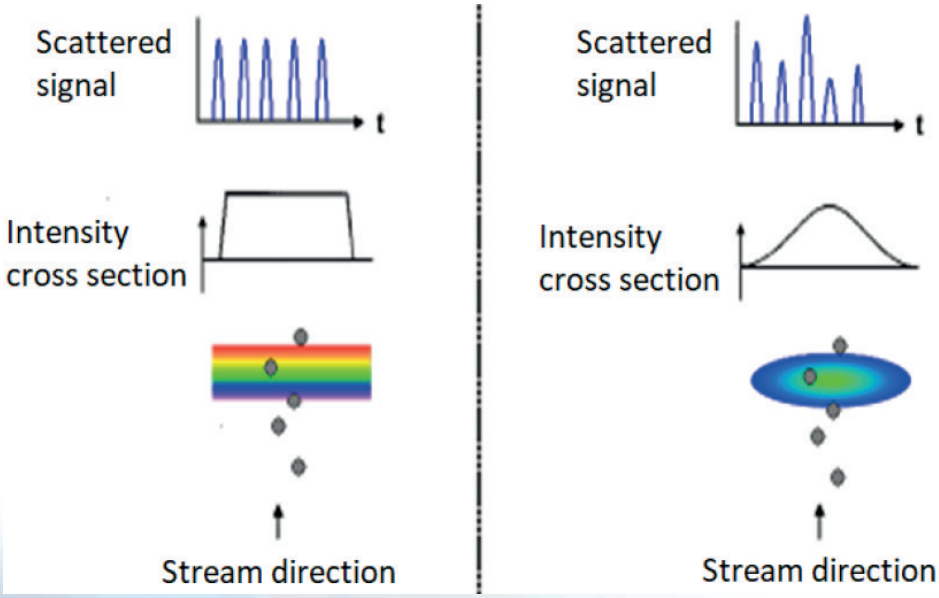


Figure 4: Spectral light scattering (left) compared to laser generated scattering (right). Laser and low coherence light beam technologies and their corresponding numerical apertures (NA) and diffraction patterns.

With a spectral light source such as laser monochromatic light, the signal related to the characteristic of the cell is highly dependent on the cell position and orientation at the measuring point, which needs to be centered on the axis.

If it is not centered, the light response may be distorted and give different results, as demonstrated in Figure 5. The example of erythrocytes, which are perfectly anisotropic, show that the results differ if they are not spherized.



PLT-Ox mode differentiates platelets from red blood cell (RBC) populations based on refractive index. Each cell is measured both in resistivity (volume) and absorbency (cytochemistry). From these measurements, a matrix is drawn up, with volumes on the X-Axis and optical absorbency on the Y-Axis (Figure 6).

The PLT-O parameter corresponds to the percentage of platelets relative to the RBC identified on the matrix, multiplied by the count of RBC identified in the measuring chamber. There is no need to calibrate this parameter since it relies on the calibration of the RBC.

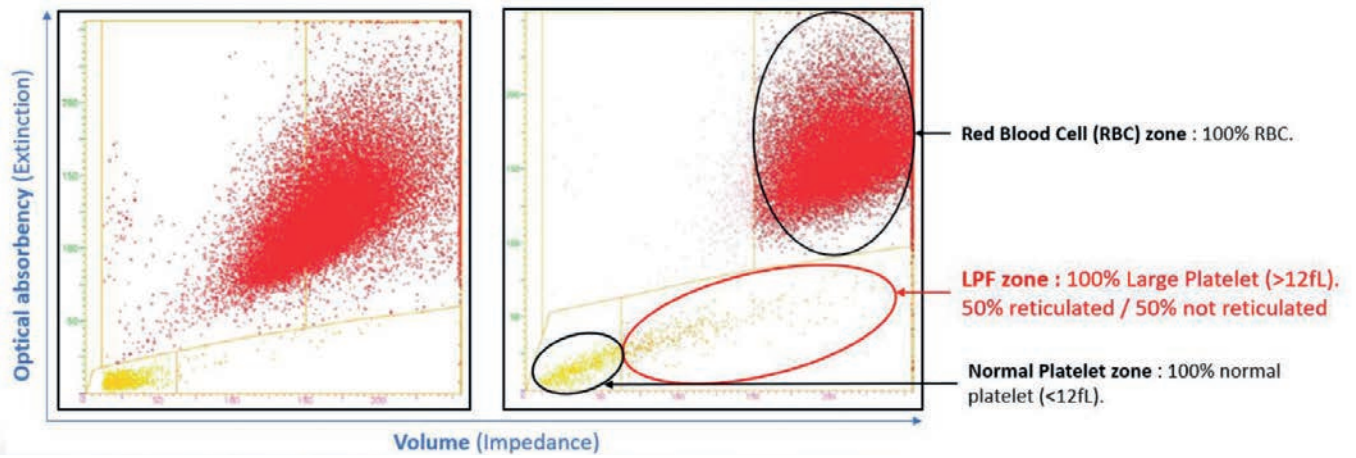


Figure 6: Optical Platelet (PLT-O) scattergrams, Low LPF (left) Vs High LPF (right) from HORIBA Yumizen H2500.

What is the clinical significance of LPF?

The Large Platelet Fraction (LPF) parameter combines impedance and platelet optical extinction technology enabling a full coverage of the large platelet population compared to RNA fluorescent dye-based technology. A study by V. Bodrova et al. detailed that: "In accordance with previous data, flow cytometry analysis in this study confirmed that the majority of Reticulated Platelets have greater size, about 90% of them being confined to a 50% subpopulation of large platelets."

This study6 presents the large platelet population and reticulated population distribution as follows: (Figure 7)

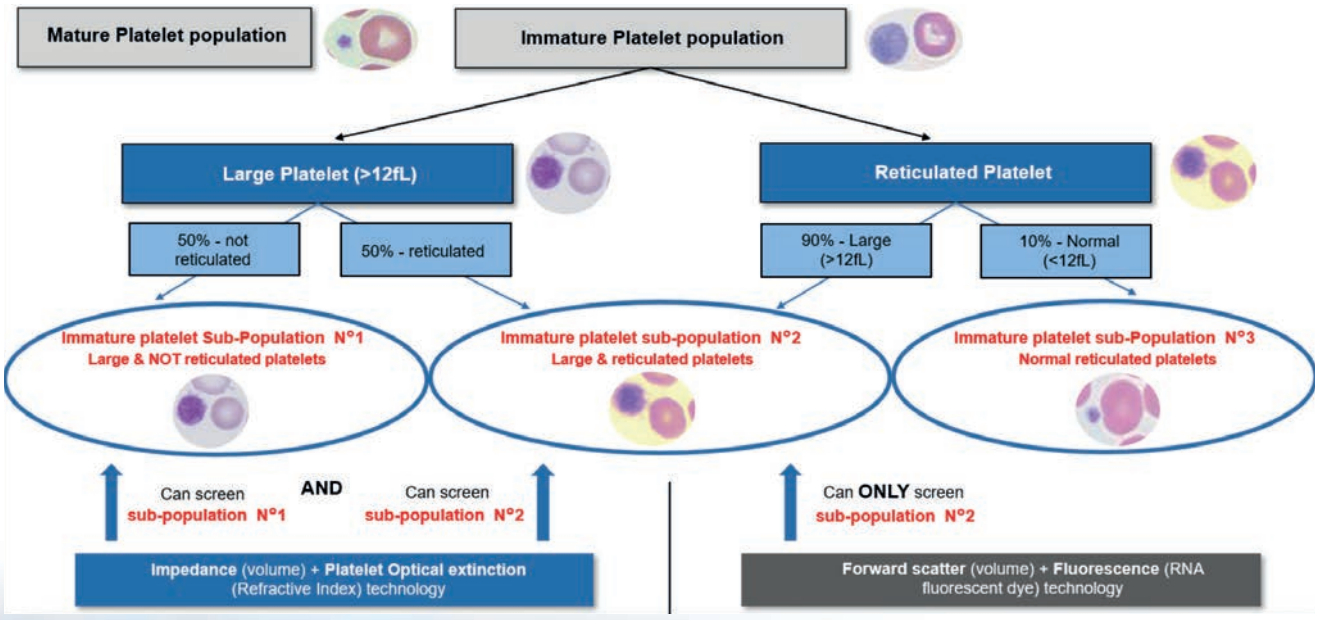


Figure 7: Visual representation of the large and reticulated platelet distribution and technology enabling screening.

This diagram clearly shows that HORIBA Medical's technology offers the best coverage of the Large Platelet population, making it a more appropriate tool to screen large platelets and support diagnosis.
A comparative clinical study was conducted between the HORIBA Medical LPF associated technology and RNA fluorescent dye-based technology. A total of 328 venous blood samples were selected randomly. After outlier exclusion, 272 were analyzed. Sample selection criteria were: one group with MPV>11.5 fL, one group with MPV<11.5 fL, schistocytes flag or MCV <60 fL. All samples were processed in parallel on both hematology analyzers, in duplicate and within 4 hours of phlebotomy.
A manual slide review was also performed following CLSI H20-2a guidelines.
All results were correlated and thus demonstrated a good correlation between these two technologies. The new LPF parameter can help laboratories in critical decision making and is compatible with this sample criteria selection.

But when is LPF screening useful?
Platelet turnover is mainly regulated by TPO or IL-1α upon inflammation. TPO and IL-1α can promote platelet synthesis and release. When the platelet demand is high, an increase in young large platelet or reticulated platelets is observed.[5]
Studies suggest that there is a positive correlation between RP percentage, platelet size and platelet aggregation.[6] Therefore, the transition to a high platelet functional activity might increase the risk of thrombotic events and is observed in cardiovascular diseases.[5]

High RP levels are observed in disseminated intravascular coagulation (DIC), thrombotic thrombocytopenic purpura (TTP), hemolytic uremic syndrome (HUS), immune thrombocytopenic purpura (ITP), and blood loss. On the other hand, low level of RP is observed with bone narrow suppression, bone narrow failure syndromes, nutritional deficiencies or drug induced myelosuppression.[7]
In acute cardiovascular syndrome (ACS) RP quantification helps with the prognosis (a high RP level is associated with a poorer prognosis) and for monitoring anti-platelet treatment such as P2Y12 inhibitors.[7] Studies show that there is an overproportion of RP within the platelet aggregates, as RPs have higher thrombotic potential, such as recruiting thrombin.
RP screening can be used to differentiate types of thrombocytopenia. High levels of RP are associated with a peripheral destruction or acute blood loss type of thrombocytopenia, whereas low levels of RP are associated with a bone marrow failure (BMF) type of thrombocytopenia.[4]
In reactive or myeloproliferative thrombocytosis, when an increase in RP is observed, there is a higher risk of thrombosis.[4] In Preeclampsia, an increased level of RP suggests an active thrombopoiesis in order to compensate for platelet consumption.[4] RP level screening could be useful in the case of bone marrow/stem cell transplantation as it could predict the platelet regeneration and could be used to postpone prophylactic platelet transfusion in patients undergoing PBSC or receiving chemotherapy.[4]

Conclusion:
Screening of the large platelet population is of clinical interest.
This is why HORIBA Medical's Yumizen H2500 hematology analyzer offers the Large Platelet Fraction parameter.

This parameter is based on accurate and reliable technology combining both impedance and platelet optical extinction to offer the best coverage and screening possibility of Young / Large Platelet populations compared to other currently available technologies.

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