LARGE PLATELET FRACTION (2024)

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Introduction:

Platelets or thrombocytes are small anucleate discoid shape cell fragments with a diameter between 2 to 4 µm and a volume between 2 and 20 fL.
They play an important role in primary hemostasis, inflammation, or innate immunity. They originate from the cytoplasm of Megakaryocytes (MKs), one of the largest (50-100 µm) and rarest cells in the bonne narrow (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.

Through a cytoskeletal-driven process they then extend into proplatelets in the blood stream which are converted into preplatelets. Fission of preplatelets generates platelets, which 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 Yumizen H2500 hematology analyzer from HORIBA Evolutive Laboratory Organization (HELO 2.0) 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	44*	140*	18*	50*	1*	12*
Female	11.8	19.6	44*	140*	18*	50*	1*	12*
Bibliographic data[6]	11	20	*	*	*	*	*	*

Figure 1: Table of normal ranges for each Platelet-associated parameter based on HORIBA studies.
* Means Research Only Parameters (RUO). HORIBA, Clinical Performance Report (CPR). DEV-CPR-2023-0009.

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 carried out using EDTA anticoagulant. The normal ranges given vary depending on the sample populations and/or geographical location.
HORIBA highly recommends that each laboratory establishes its own normal ranges based upon its local population.

Expected values in an aff ected population:
HORIBA Yumizen H1500 and Yumizen H2500 can be used to help the physician identify patients with hematology parameters outside the established reference ranges by collecting data reflecting the patient's hematological or immunological status at a certain point in time.
In conjunction with other diagnostic information and with the attending physician's evaluation of the patient's condition, the data contributes to establishing a diagnosis and a clinical course of treatment. When a result is outside the reference range for the tested population, this may indicate a possible clinical significance.
In addition, flags alert the operator about abnormal cell populations during the results validation process.

Medical and diagnostic value of platelet parameters:

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-Ox or Optical Platelet measures the concentration of platelets using impedance and optical extinction when impedance technology can't distinguish between large platelets and interference from microcytic or fragmented red blood cells. PCT or Plateletcrit measures the to-tal platelet volume and can indicate quantitative platelet anomalies. **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 quantifi es every platelet with a volume larger than 12fL which could be large platelets, platelet ag-gregates or microcytes.[3] **LPF** or Large Platelet Fraction measures the large platelet population using impedance and platelet optical extinction technology (PLT-Ox). Optical extinction enables a correct separation between RBC and PLT curves when RBC/PLT interferences are observed due to the presence of giant platelets, fragmented RBC,

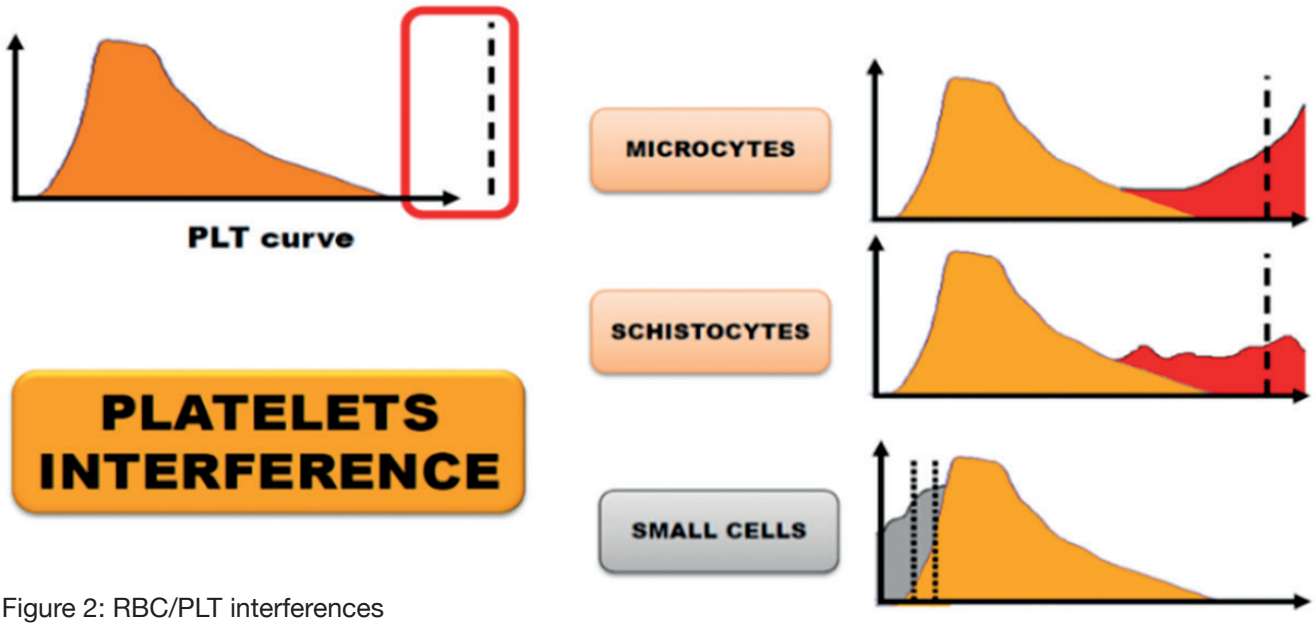

Figure 2: RBC/PLT interferences

Large Platelet Fraction (LPF): a new hematological marker for regenerating Thrombopoiesis

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, Large Platelet Fraction (LPF) corresponds to the platelet population larger than 20 fL. This population is mainly composed of large young platelets, also called reticulated platelets (RP) or Immature Platelets (IP).
The Young Platelets/Large Platelets/RP are newly released, larger, and more reactive.[4] Like reticulocytes for RBC, large young platelets act as real-time biomarkers of platelet synthesis, also known as thrombopoiesis. Large young platelets contain RNA strands which are used for protein synthesis. However, these strands do not come from MKs[4].

Before LPF, several parameters were used to screen the size of the platelet population and quantify large platelets, including MPV. MPV remains stable in healthy individuals so a variation may indicate a disease process, such as in immune thrombocytopenia where MPV increases.[5] PDW, P-LCC and P-LCR were also used. However, these three parameters don't specifically quantify large platelets. The P-LCC/P-LCR quantifications, in addition to large platelets, may include microcytes, schistocytes and platelet aggregates, yielding an inaccurate large platelet count.

Young Platelets or Large Platelets, also called Reticulated Platelets (RP), remain between 24 to 36 hours in the blood stream[8]. After this, 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[5,8]. RNA content related to platelet density is therefore a better age indicator. Nevertheless, the majority of Young Platelets or RPs are large platelets and the study indicates that "the majority of reticulated platelets are larger, 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 (LPF) is mainly composed of young platelets/RPs, but also large platelets that are not reticulated. These platelets are large but not necessarily young, as size is not a good marker for platelet age.
This population is less active in terms of protein synthesis. However, as for RPs, this population presents a higher hemostatic potential due to a higher contact region (15.8 fL)[5]. In some diseases such as cardiovascular disease, the increase of the large platelet fraction population including functionally active platelets like RPs, is associated with a poorer prognosis.[7] Thus, the screening of the Large Platelet Fraction can be useful for the prognosis and management of various disorders. LPF screening can also be useful to assess the need for platelet transfusion.

LPF parameter on the Yumizen Hematology Analyzer:

The HORIBA Yumizen H2500 provides a way to screen the entire large platelet fraction.
The new Large Platelet Fraction (LPF) parameter provides laboratories with information that enables diagnosis within minutes, without changing their current HORIBA equipment.

Large platelet count using the LPF parameter provides accurate and reliable results thanks to the combination of both 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 is a function of volume and optical index. The optical index of platelets is lower than the RBC index, so for the same volume, optical extinction enables discrimination between both cell types, providing greater accuracy than impedance alone.

The Platelet Optical Extinction (PLT-Ox) mode on the Yumizen analyzer gives two measuring methods, impedance and light extinction (absorbency), which are combined to obtain the most reliable result (Figure 3).

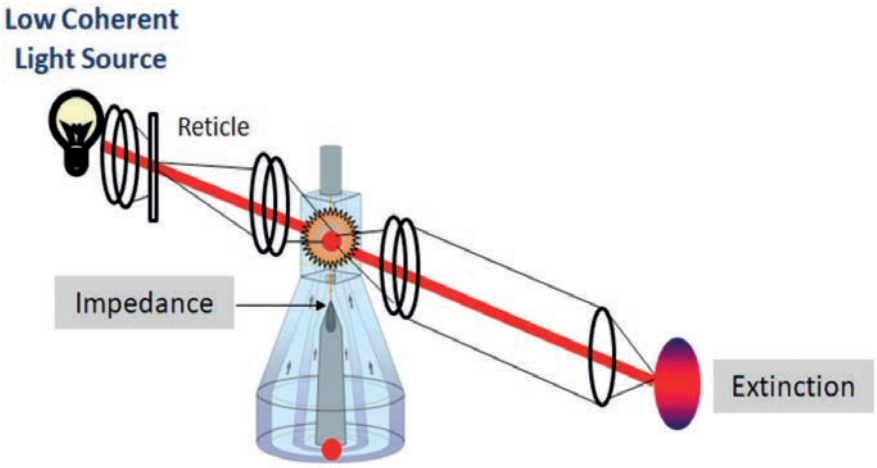

Figure 3: HORIBA PLT Ox measuring method using an incoherent light source.

The HORIBA measurement technology for platelet extinction is made using an incoherent light source (extended source).
The high numeric aperture of the light beam allows 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 generating a perfectly parallel beam for a vision of the cell from one dimension, measurements made using an incoherent light source are less vulnerable to cell anisotropy and its position or orientation in the beam, giving information on the intrinsic characteristics of the cell (Figure 4).

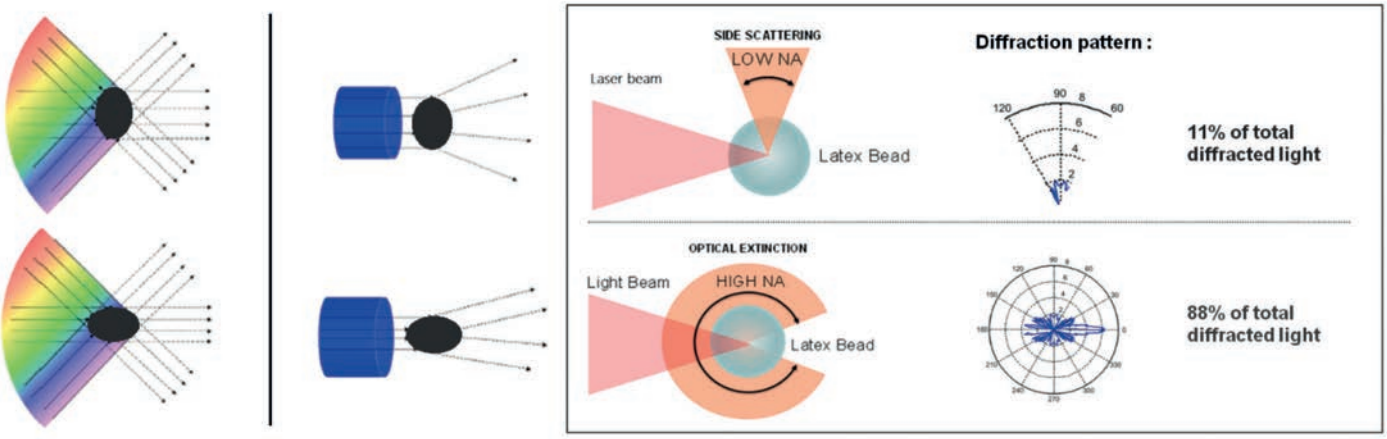

Figure 4: Spectral light scattering (left) compared to laser generated scattering (right). Laser and incoherent light beam technologies and their corresponding numerical apertures (NA) and diff raction patterns.

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 passage position and orientation at the measuring point, and needs to be centered on the axis.

Otherwise, the light response may be distorted and will give different results, as demonstrated in Figure 5. The example of erythrocytes, which are perfectly anisotropic, show that the results would be completely dissimilar if they are not spherized.

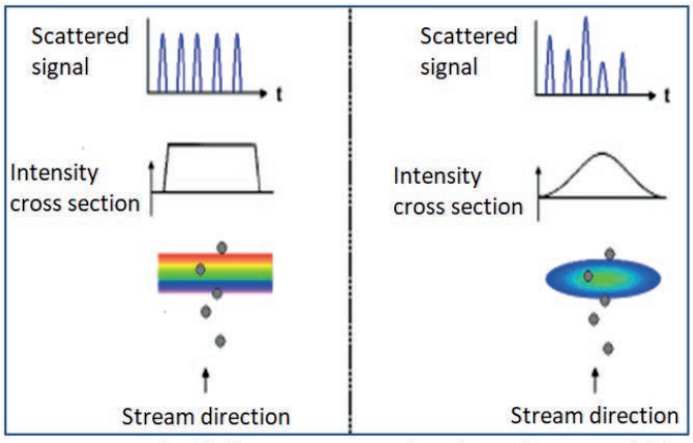

Figure 5: Spectral light (left) scattering compared to laser generated scattering (right).

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

The PLT-Ox 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.

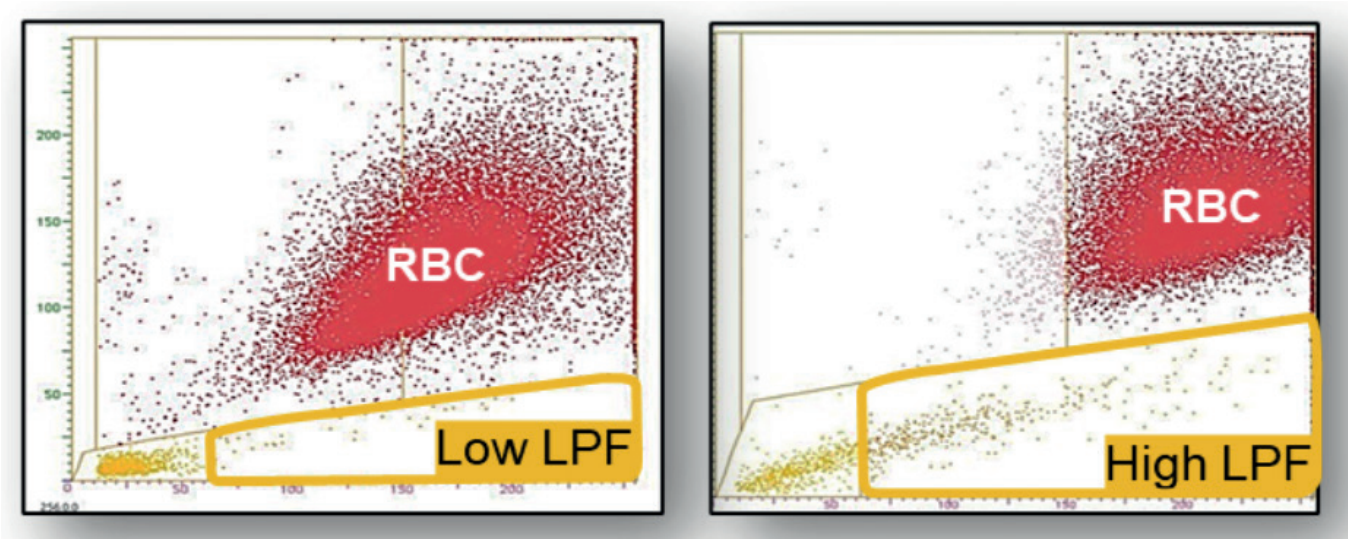

Figure 6: Optical Platelet (PLT-O) scattergrams, Low LPF (left) Vs High LPF (right) from a HORIBA Yumizen H2500.

What is the clinical significance of LPF?

The large platelet fraction parameter associated with impedance and platelet optical extinction technology allows a full coverage of the large platelet population when compared to RNA fluorescent dye-based technology.

The study[6] by V. Bodrova et al. illustrates the large platelet population and reticulated population distribution (Figure 7)

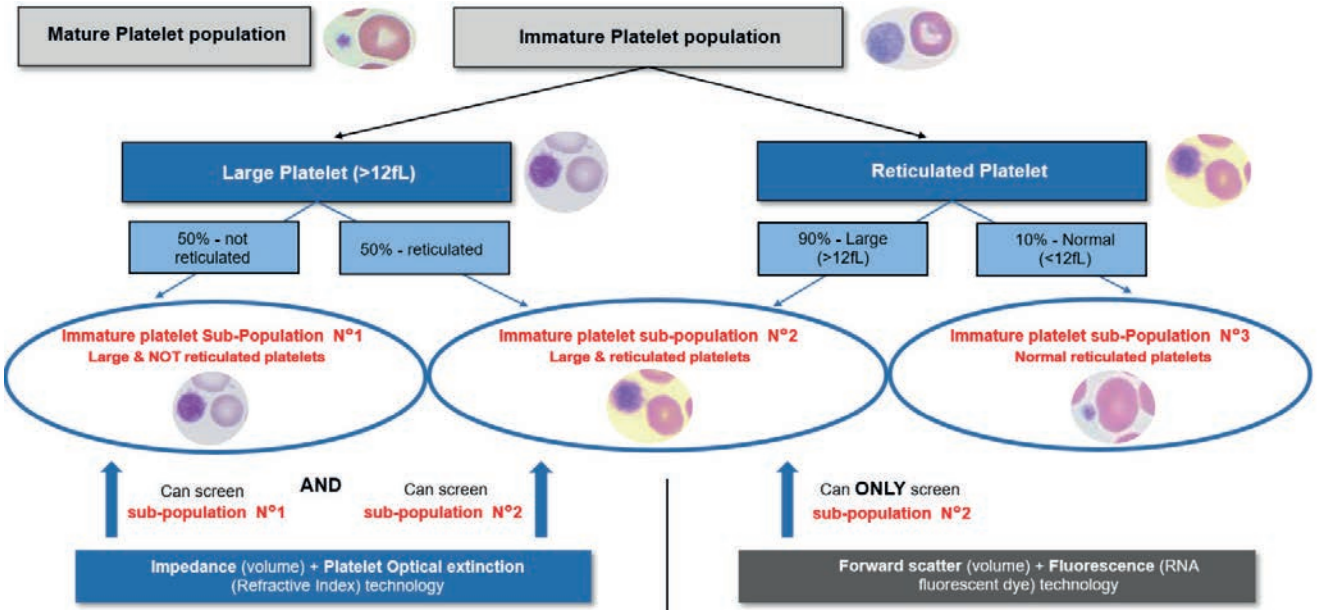

Figure 7: Visual representation of large and reticulated platelet distribution and the screening technology.

This visual representation clearly shows that HORIBA technology offers the best coverage of the Large Platelet population, making it an appropriate tool for screening large platelets and assisting a diagnosis.

A comparative clinical study was conducted between HORIBA Large Platelet Fraction (fluorescent free) associated technology and RNA fluorescent dye-based technology[4].

A total of 328 venous blood samples were selected randomly and after outlier exclusion, 272 venous blood samples were analyzed.

The criteria for sample selection 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, thus demonstrating a good correlation between these two technologies. The new LPF parameter can help laboratories with critical decision making and is comparable to this sample criteria selection.

But when can LPF screening be useful?

Platelet turnover is mainly regulated by TPO or by IL-1α upon inflammation. TPO and IL-1α can promote platelet synthesis and release. When 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] Transition to a high platelet functional activity may indicate increased risk of thrombotic events and is also observed in cardiovascular diseases.[5]

High Young Platelets/Large Platelets/RP levels are observed in Disseminated intravascular coagulation (DIC), thrombotic thrombocytopenic purpura (TTP), hemolytic uremic syndrome (HUS), immune thrombocytopenic purpura (ITP), and blood loss.

A low level of RP is observed with bone narrow suppression, bone narrow failure syndromes, nutritional deficiencies or drug induced myelosuppression.[7,8]

In acute cardiovascular syndrome (ACS), RP quantification helps with the prognosis (a high RP level is associated with poorer prognosis) and for monitoring anti-platelet treatment such as P2Y12 inhibitors.[7] Indeed, studies show that there is an excess proportion of RP within the platelet aggregates as RPs have higher thrombotic potential, such as recruiting thrombin.

In thrombocytopenia, Young Platelets/RP screening can be used to differentiate the type of thrombocytopenia.
A high level of Young Platelets/RP is associated with a peripheral destruction or acute blood loss type of thrombocytopenia whereas a low level of RP is associated with a bone marrow failure (BMF) type of thrombocytopenia.[4]

In reactive or myeloproliferative thrombocytosis, when an increase in Young Platelet/Large Platelet/RP is observed, there is a higher risk of thrombosis.[4,8] In Preeclampsia, an increased level of RP suggests an active thrombopoiesis in order to compensate for platelet consumption.[4] Young Platelet/Large Platelet level screening could be useful in cases of bone marrow/stem cell transplantation to predict platelet regeneration and could also be used to inform decisions on postponing prophylactic platelet transfusion in patients undergoing PBSC or receiving chemotherapy.[4].

CONCLUSION:

Screening of the large platelet population has a clinical interest.
Consequently, HORIBA with its HELO 2.0 version Yumizen H2500 Hematology analyzer offers the Large Platelet Fraction (LPF) parameter for better diagnostic and clinical management.

This parameter is based on accurate and reliable technology, combining both impedance and platelet optical extinction.

This technology offers the best coverage and screening of Young Large Platelet populations available on the market.

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