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The LaboratoryMatters

Laboratory Medicine Newsletter for clinicians, pathologists & clinical laboratory technologists.

A CREST Laboratories Initiative.

All About Platelets

This issue highlights:

- Function of platelets
- Platelet disorders
- Platelet counts: Methods and methods
- Platelet transfusion
- Quiz

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ALL ABOUT PLATELETS

From the Director's Desk

Welcome to this edition of "LaboratoryMatters"!

We at "LaboratoryMatters" continue our efforts to keep our readers - pathologists, medical technologists, clinicians and others abreast with the contributions of clinical laboratory to diagnosis. This edition focuses on **Platelets**, those smallest, yet most notorious elements of all blood cells!

In this edition we have a Quiz to make the read more exciting and there are prizes for the first three correct entries.

Keep reading, keep learning and keep sharing

Until the next edition of LaboratoryMatters

Dr. Rani R
Director, CREST Laboratories

Platelets are anucleate particles about 2-4 μ in diameter. Accurate quantification of platelets in sample of blood is important both in health and disease.

While platelet counts form a part of all Complete blood count reports, this reportable parameter is often the subject to great variation and inaccuracy. It usually is the bane of every hematology laboratory.

Platelets are derived from the hemopoietic stem cell in the bone marrow in a process called thrombopoiesisand the progenitor cell is the megakaryoblast. The megakaryoblast undergoes nuclear division without cytoplasmic division giving rise to multinucleate forms of megakaryocytes with abundant cytoplasm. Platelets arise from the fragmentation of megakaryocytes in the bone marrow and contain no nuclear material. The nucleus is finally extruded and phagocytosed. This process is regulated by Thrombopoietin.

Platelets are released into the peripheral circulation in a day or two.

Normal life span of platelets is 5-10 days

Normal platelet count is age-specific.

The adult male and female has a platelet count of 150000 to 400000 platelets /μL of blood

In the pediatric age group, the platelet count varies between 200000 to 600000 /μLof blood.

Function of platelets:

Platelets along with the vascular endothelium and the coagulation factors form part of the hemostatic system.

When the vascular endothelium is breached, the platelets orchestrate a series of events that result in the formation of a primary hemostatic plug. The platelets contain two

types of granules;alpha and dense granules.The alpha granules contain hemostatic proteins such as fibrinogen, vWf, and growth factors (eg, platelet-derived growth factor). The dense granules contain proaggregatory factors such as adenosine 5'-diphosphate (ADP), calcium, and 5-hydroxytryptamine (serotonin).

Following endothelial damage, the platelets are involved in three processes that finally result in the formation of a primary hemostatic plug.

They are:

Adhesion: the platelets adhere to the exposed sub-endothelial collagen via von Willebrand factor and Glycoprotein Ib

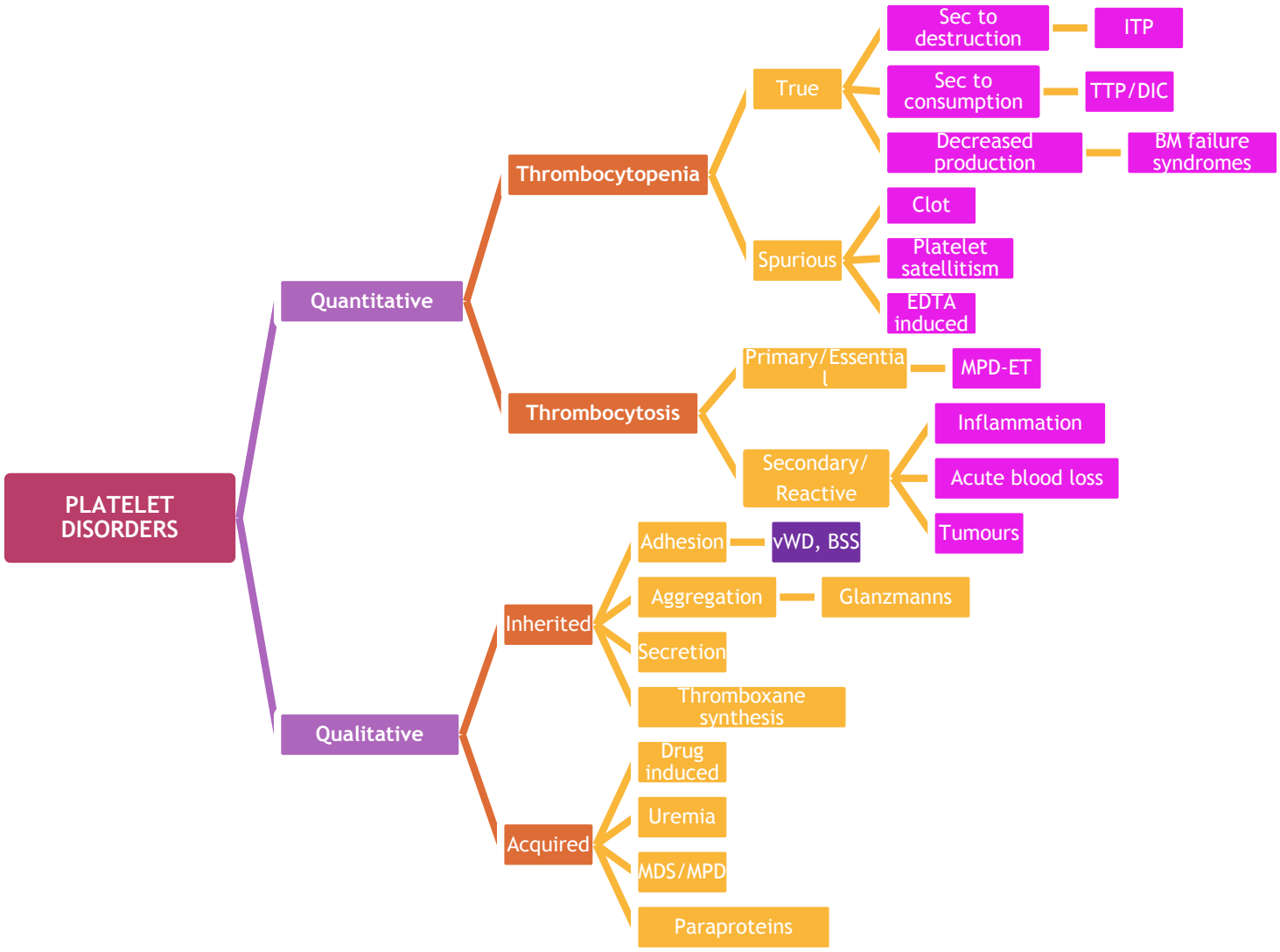
Activation: this causes a conformational change in shape of the platelets that exposes the binding sites on the receptors seen on the surface.

Aggregation: The activation of platelets causes platelet to platelet interaction and further platelet recruitment mediated by Glycoprotein IIb/IIIa complex and fibrinogen

Release: Activated platelets release granule contents and mediate thromboxane synthesis.

Platelets have a minor role to play in secondary hemostasis. Where the fibrin polymers formed as a result of activation of the coagulation cascade further strengthen the hemostatic plug resulting in the formation of a stable clot.

Platelet disorders



Laboratory Investigations to diagnose platelet disorders

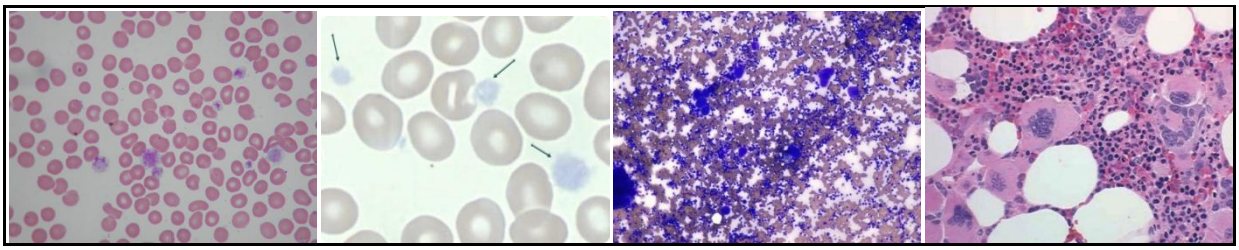
- Complete blood count
- Peripheral blood film examination
- Bone marrow examination (if required)
- Platelet aggregation studies to rule out inherited qualitative disorders

An accurate platelet count along with a detailed examination of the peripheral blood film by a trained pathologist is more than adequate to both diagnose and monitor platelet disorders. An insight into the etiology of platelets as also the morphology may be discerned by careful examination of the platelet histograms obtained from the cell counters.

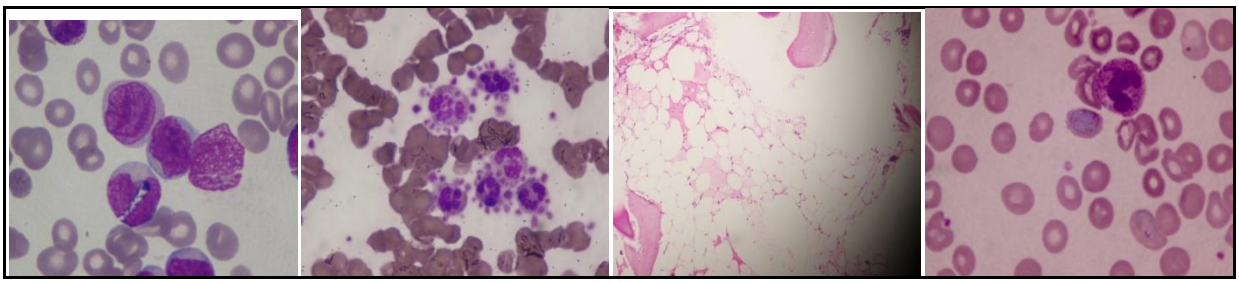
Peripheral blood film examination

- Helps to differentiate true from spurious thrombocytopenia
- Morphology of platelets may be discerned-small ,large, agranular, granular
- Presence of platelet clumps
- Presence of megakaryocyte fragments
- Associated pathology like red cell fragments, microcytes, malarial parasite, leukemia, myeloproliferative disorder, reactive lymphocytosis secondary to viral infection and the like that will give a clue to the cause of platelet disorder.

Bone marrow examination may be needed in cases where thrombocytopenia persists despite adequate treatment, aplastic anemia, myelophthisis, or other hematological malignancies.



a	b	c	d



e	f	g	h

Legend: Fig a: Bernard Soulier syndrome, Fig b: Gray platelet syndrome, Fig c: Immune mediated thrombocytopenia-bone marrow, Fig d: Essential thrombocythemia-

bonemarrow biopsy, Fig e: Acute Myeloid Leukemia, Fig f: Platelet satellitism, Fig g: Aplastic anemia-bone marrow biopsy, Fig h: Plasmodium vivax

Platelet function studies are performed on light transmission aggregometers using agonists like ristocetin, ADP, Collagen and arachidonic acid. Interpretation of these tests requires a trained pathologist.

Platelet counts: Methods and methods

Manual platelet count: One of the oldest methods, still in use, requested for by many clinicians is the counting chamber method using the Neubauer chamber and a platelet diluting fluid like 1% ammonium oxalate.

The disadvantages are the method is highly subjective and is prone to inter-observer variation; dust particles and other contaminants may be counted as platelets. The procedure is also time-consuming and needs a skilled technician to provide a fairly accurate platelet count.

Slide method: This is the most common procedure used in clinical laboratories. Here the assessment of platelets is done on a stained peripheral blood film. Platelets are identified in the ideal zone of the smear and platelets distributed in 10 oil immersion fields are counted. The average of this is then multiplied by 15000 to give the platelet count.

The advantages are that it is a rapid method and a smear review will provide information both on platelet morphology and any other associated pathology.

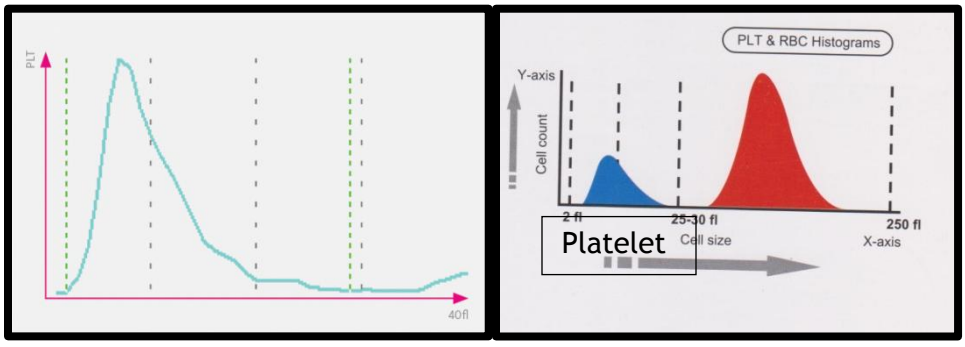
The disadvantages are that this procedure requires a well- made and stained blood film. It is prone to inter-observer variation and subjectivity. If the blood film is improperly made, the platelet distribution will be patchy and uneven; hence the counts will be inaccurate.

Automated hematology analyzers:

Hematology analyzers use various methods

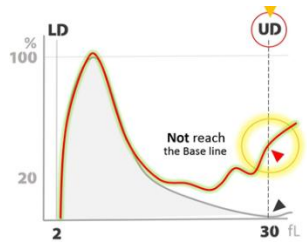
Technology	Principle	Mode	Advantages
Impedance	Measures change in electrical resistance proportional to cell volume and count. Upper thresholds vary between 25-35 fL	Primary	Large platelets with volumes > 40fL are omitted
Optical 1	Dual angle optical analysis, 90° polarized sidescatter vs. 7°intermediate angle scatter. Measures complexity and light intensity.	Primary	Larger platelets are measured
Optical 2	Measures optical light scatter. Uses cell size and density(refractive index) -2-dimensional platelet count	Primary	Measures true platelets and eliminates interferences
Optical 3	Polymethine fluorescent dye stains the RNA/DNA of reticulated cells and platelet membrane and granules	Secondary	Can be used as a reflex test in certain cell counters
Optical 4	Oxazine, an RNA fluorescent dye measures size, structure and DNA/RNA content	Secondary	Can be used as a reflex test in certain cell counters
Immuno	Off-line preparation of Monoclonal CD61 conjugated with FITC provides an ImmunoPlt assay	Secondary	Can be used as a reflex test in certain cell counters

Platelet histogram



Despite numerous technologies available for platelet counts on a hematology cell counter, platelet counts remain the bane of all laboratorians. This is mainly due to the platelet interfering factors that interfere with accurate counts especially at the high discriminators

Interferences with platelet counts:



Red cell factors	White cell factors	Platelet factors	Others
Microcytes	White cell fragments	Large platelets	Lipids
Fragments		Clumped platelets	Parasites
Acanthocytes			Microclots
Red cell ghosts			

When the curve of the platelet histogram fails to touch the baseline at the upper discriminator, the causes of interference to a reliable platelet count are platelet clumps, large platelets, microcytes all of which have a volume >30fL. The interference at the lower discriminator is usually not seen.

However with improving technologies, cellcounters that employ methods that especially use the size and the density/refractive index as parameters to identify platelets do result in fairly accurate and reliable platelet counts.

Monitoring of platelet counts:

All cases of thrombocytopenia require constant monitoring of platelet counts.

This can be done by 3 methods:

- By serial platelet counts
- Review of peripheral blood film
- Immature platelet fraction measurement

Immature platelet is defined as the young or reticulated platelet that is pinched off from the megakaryocytes. These develop into mature platelets in 1-2 days.

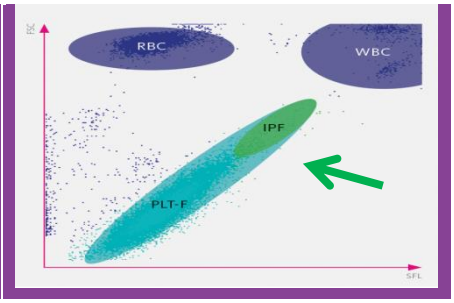
The Immature Platelet Fraction (IPF) is the percentage of these reticulated platelets with an increased RNA content found in the peripheral blood. They are released by the bone marrow in conditions of stress.

The normal IPF as measured by Fluorescence flow cytometry is between 1-6.1%.

Clinical utility of IPF: In patients with thrombocytopenia who show good bone marrow response the IPF% is high, despite thrombocytopenia. In cases of bone marrow failure or hypoplasia, the IPF% is low denoting poor megakaryocytic response to thrombocytopenia. It is also useful to monitor patients with thrombocytopenia due to dengue or other viral infections, post chemotherapy, post bone marrow transplant etc.

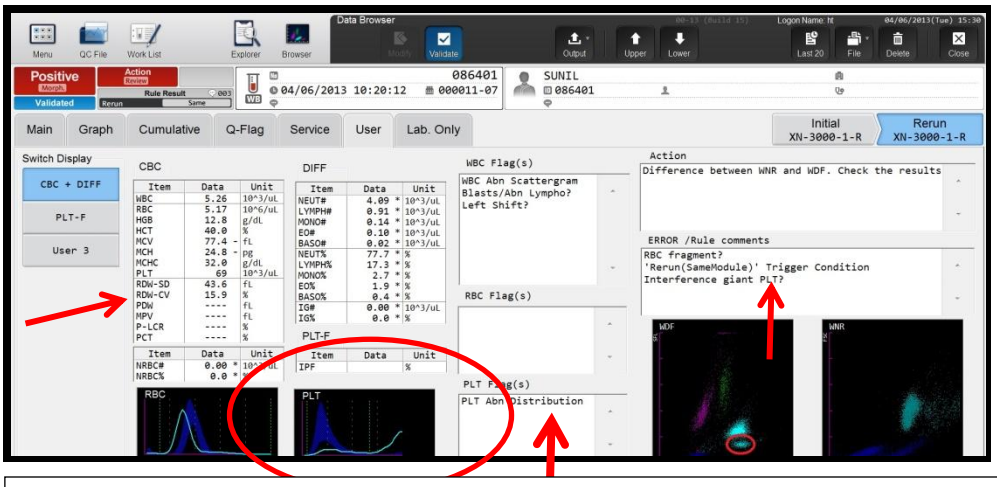
IPF% may also serve as a guide for the need for platelet transfusions. A high IPF% in a thrombocytopenic patient with no bleeds may not require platelet transfusions whereas one with a low IPF will require more stringent monitoring for clinical bleeds and may also warrant platelet transfusion.

Usually, IPF increases 1 or 2 days earlier than the total platelet count

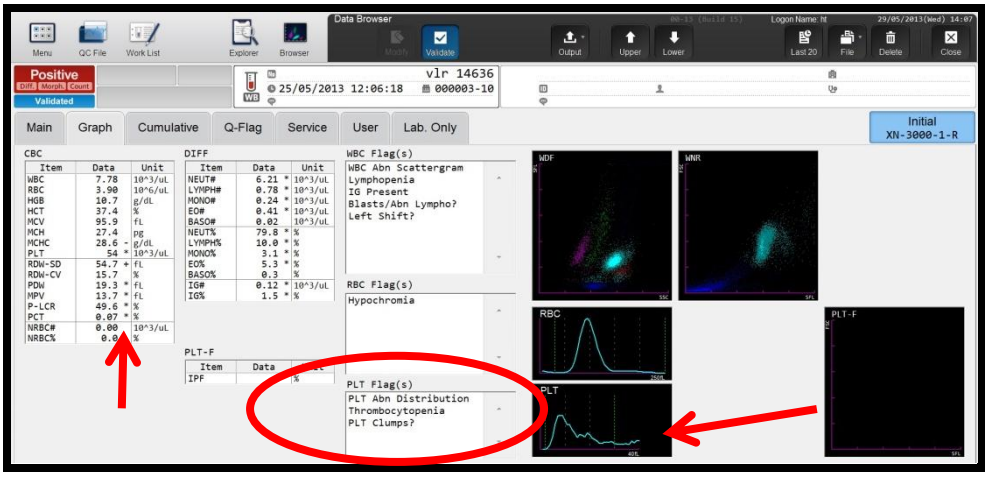
Increased IPF	Decreased IPF	
Increased platelet destruction/consumption IPF is an early predictor of platelet recovery following HPC transplantation.	Depressed bone marrow due to aplastic anemia, chemotherapy, BMT	

Platelet parameters in cellcounters

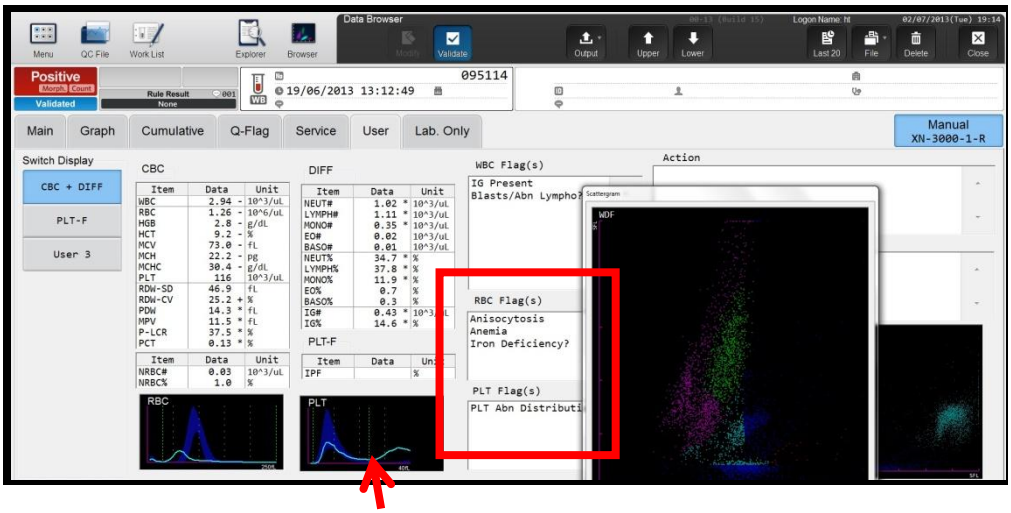
Parameter	Description	Normal range	Clinical application
MPV	Mean Platelet volume	7-11 fL	Denotes the platelet size and is useful to determine the cause of thrombocytopenia and monitor platelet counts
PDW	Platelet Distribution width	9-14 fL	Denotes heterogeneity of platelet size Increased in destructive causes of thrombocytopenia
LPLT	Large platelet- denotes the number of platelets >20fL present in sample	<4 *10^3/µL	Denotes immature platelets & constitutional macrothrombocytes
P-LCR	Platelet-large cell ratio Percentage of large platelets with volume >12fL	15-35%	Denotes immature platelets & constitutional macrothrombocytes as a percentage of total platelets
PLT-CLM	Platelet clumps	Presence of clumps > 300	Differentiates true and pseudo thrombocytopenia
MPC	Mean platelet component	27-28.2g/dL	Denotes platelet activation. Used as an indicator for Acute Coronary states and for monitoring of antiplatelet therapy
PCT	Plateletcrit	0.12-0.30%	Represents the total platelet mass Denotes quantitative measure of platelets



Platelet histogram showing interference by microcytic red cells and/or giant



Platelet histogram denoting presence of platelet clumps



Platelet transfusion:

Platelets available for transfusion are either Pooled random donor platelets (RDP) or Single Donor Platelet (SDP)

One unit of RDP is obtained by pooling together 5-6 units of platelets (15-20 ml each) from 5-6 different blood donors. The total volume is approximately 100-120 ml.

A unit of blood that is donated at a blood bank is split into 3 parts; packed red cells, platelets and fresh frozen plasma.

One unit of SDP is obtained from a single donor after connecting the donor to an apheresis machine. In this procedure, only platelets are collected and then red cells are returned to the donor as part of the procedure.

Either RDP or SDP may be transfused. However in certain situations where the patient becomes unresponsive to repeated transfusions of RDP, then a trial with SDP is indicated. In such cases, HLA matched platelets may have to be used. Usually, the platelets need not be blood group specific and transfusion may be done across blood groups. However caution needs to be exercised while transfusing Rh negative women of reproductive age group with Rh positive platelets so as to prevent alloimmunisation.

Indications for platelet transfusion:

- Prophylactic for surgery: Depending on the type of surgery and anticipated blood loss
- Therapeutic in case of active bleeds
- In specific cases like hematological malignancies or post-chemotherapy, platelets are transfused based on the clinical condition and not just the platelet counts
- In cases of infections like dengue, malaria, sepsis transfusion is done in cases of active bleeds
- In cases of chronic thrombocytopenia, platelet transfusions are not routinely practiced and may be ordered depending on the clinical scenario.

To determine the effectiveness of platelet transfusions, Platelet counts are monitored after 1, 4 and 24 hours post-transfusion to check for platelet increments.

Despite being the smallest component of blood, the platelets present numerous problems both to the laboratory and to the clinician.

Quiz:

1. Impedance counts fail to measure (a) red cells (b) fragments (c) large platelets (d) hemoglobin
2. The factors that interfere with measurement of accurate platelet counts are (a) microcytes, fragments and large platelets (b) microcytes, macrocytes and fragments (c) fragments, platelet clumps and lipid (d) macrocytes, WBC fragments, large platelets
3. IPF represents (a) platelets fragments (b) reticulated platelets with RNA content (c) immature platelets with DNA (d) platelets that are large in size
4. The following parameters denote platelet anisocytosis (a) MPV, MPC, PCT,LPLT (b) PDW, MPC, P-LCR, MPV (c) MPV, PDW, LPLT, PCT (d) P-LCR, MPC, PCT, LPLT

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   The first 3 right answers will win exciting gifts