



Managing 5 part CBC Services

Dr Kunal Sehgal, M.D.
Consultant
Hematology Laboratory
Department of Lab Medicine
PD Hinduja National Hospital and MRC

drkunalsehgal@gmail.com

Automated Cell Counters

3 part vs. 5 part

What is the Diff?

3 part vs. 5 part

Cell Counter	3 part	5 part
Differential count	Neutrophils, lymphocytes, mixed	Neutrophils, lymphocytes, monocytes, eosinophils and basophils
Peripheral Smear	Is a must	PS can be made based on validated flag rules
Ease of use and maintenance	Easy with minimum no of reagents and processes	Requires skilled staff adequately trained for operation and maintenance
Cost	Cheap	Relatively Expensive

3 part vs. 5 part

Cell Counter	3 part	5 part
Principle of operation	Impedance based	Impedance based Fluorescence Flowcytometry (Sysmex –XE,XN series, Abott Cell Dyn) Volume Conductivity Scatter (Beckman coulter – LH series) Peroxidase staining (Seimens Advia)
Additional parameters	-	<ul style="list-style-type: none"> •Reticulocyte Count •NRBC •Immature platelet fraction •Immature granulocytes •Additional Scattergrams and flags

Ease of Use

	3 part	5 part
Machine initiation and Software	Easy automated process	Multiple steps. Requires technology savvy personnel.
No of reagents	2 to 3	5-15. Inventory management is critical
End user maintenance	Simple	Complicated and requires training

Cost per test

- 3 part counter
 - Cost of 2-3 reagents per Cycle
 - Sleep mode and startup –shutdown cost
 - Controls
 - Taxes

Approximately Rs. 20-30 per (CBC+Diff) test
all inclusive

Details required for Costing

- Average no. of Samples per day for 25 days a week or 300 days a year
- Startup shutdown cycles per year- 300-365

Sr. No.	Reagent	Pack size (ml)	Price / pack (Rs.) [#]	CBC +NRBC	CBC+WDF+NRBC	CBC+WDF+RET+NRBC
				Cycles / pack	Cycles / pack	Cycles / pack
1	<i>Cell Pack DCL</i>	20000	3500	714	606	488
2	<i>Sulfolyser</i>	3000	20000	6000	6000	6000
3	<i>Lysercell WNR</i>	8000	33000	5333	5333	3200
4	<i>Fluorocell WNR</i>	164	43000	8200	8200	8200
5	<i>Lysercell WDF</i>	8000	32000	0	5333	5333
6	<i>Fluorocell WDF</i>	84	39750	0	4200	4200
7	<i>Cellpack DFL</i>	3000	16000	0	0	2000
8	<i>Fluorocell Ret</i>	24	38000	0	0	1200
9	<i>Fluorocell PLT</i>	24	38000	0	0	0
10	<i>Cellclean</i>	50	7030	0	0	0

- Derive Cost per Cycle Cost per Cycle (CBC +NRBC) **19.66**
- Derive cost per test Cost per Cycle (CBC+ WDF +NRBC) **36.00**
- Add cost of Controls Cost per Cycle (CBC+WDF+RET +NRBC) **81.20**
- Cost for repeats and wastage

Cost per test

5 part counter

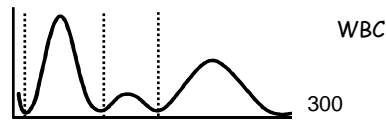
- Cost of reagents per Cycle – Complicated process
- Start up and shutdown costs – significant addition to costs and is volume dependent
- Controls – expensive, short expiry , cost is sample volume dependent
- Taxes
- Hidden costs – Always account for Dead volume, background checks, repeats, wastage, EQAS, etc
- Re evaluation of costs after six months - 'Consumption based Costing' is a must

Reagent Rental vs. Outright purchase vs. Partial Reagent Rental

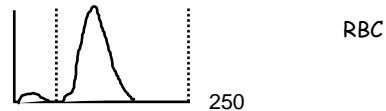
Approximately Rs. 50-70 per test (CBC+Diff) all inclusive

3 part Analyser

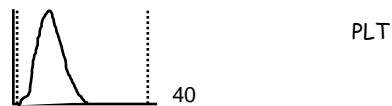
NO. 4
 Date: 9/10/95 15:11
 MODE: Whole Blood
 WBC 5,8 x 10³/μl
 RBC 4,84 x10⁶/μl
 HGB 13,7 g/dl
 HCT 42,0 %
 MCV 86,8 fl
 MCH 28,3 pg
 MCHC 32,6 g/dl
 PLT 257 x10³/μl



LYMPH% 31,2 %
 MXD% 6,8 %
 NEUT% 62,0 %
 LYMPH# 1,8 x10³/μl
 MXD# 0,4 x10³/μl
 NEUT# 3,6 x10³/μl



RDW-SD 40,0 fl



PDW 13,1 fl
 MPV 10,4 fl
 P-LCR 28,1 %

3 part Diff 18 parameters

Leucocyte Histogram

- Lymphocytes in % and absolute
- Eo, Mono, Baso in % and absolute
- Neutrophils in % and absolute

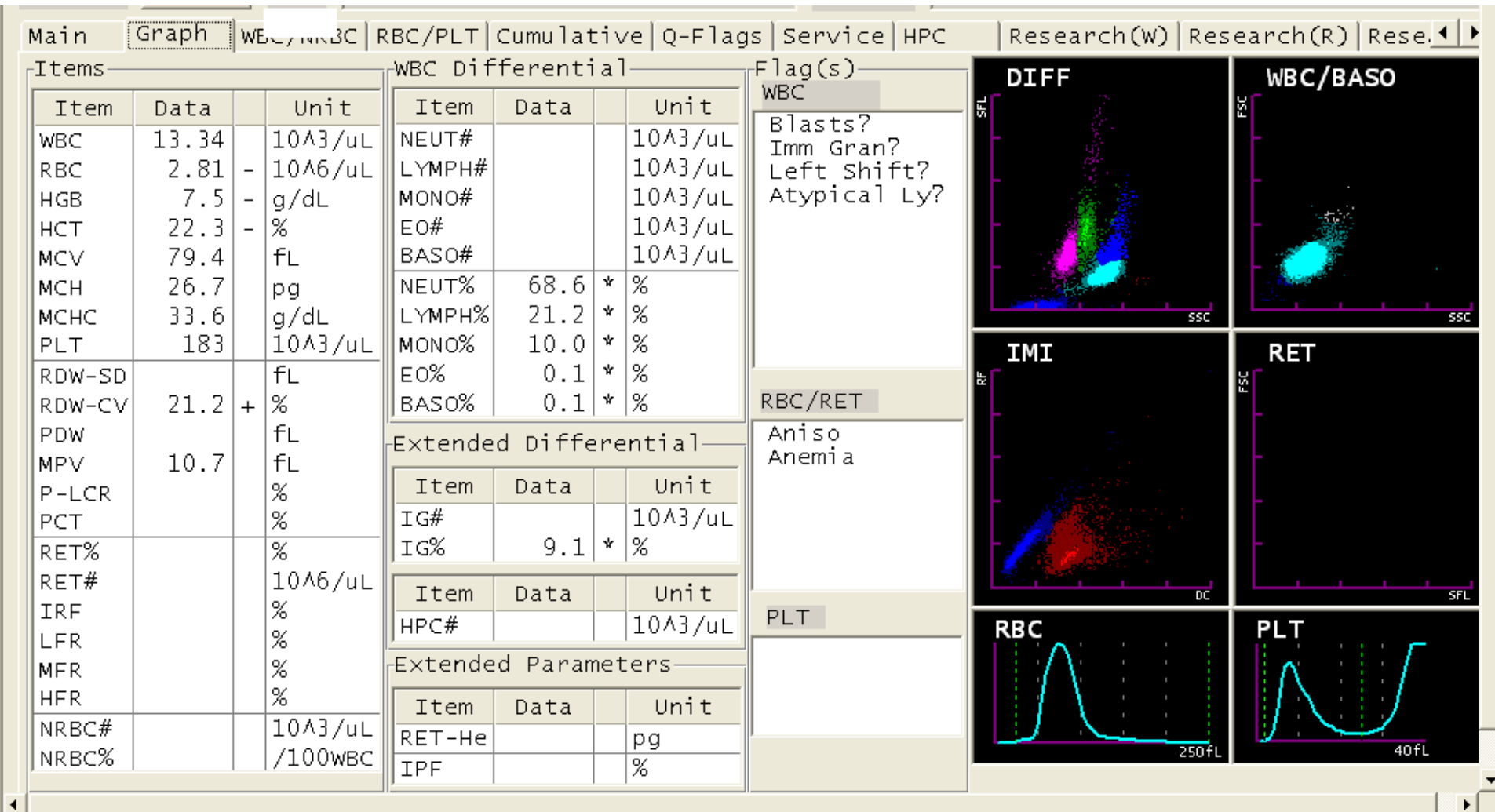
RBC - Histogram

- RBC Distribution Curve

Platelet Histogram

- PLT Distribution Curve
- Mean PLT Volume
- Share of bigger PLT

5 part Analyser



Message

Principles of 5 part instruments

Impedance and Optical Light Scatter combined with

Volume Conductivity Scatter
(Beckman coulter – LH series)

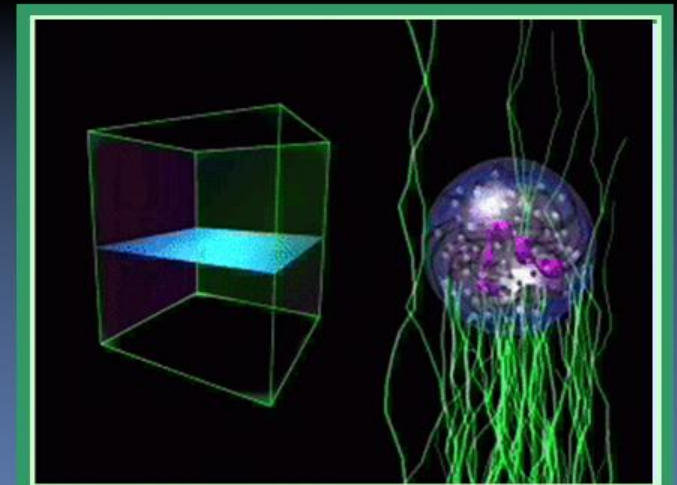
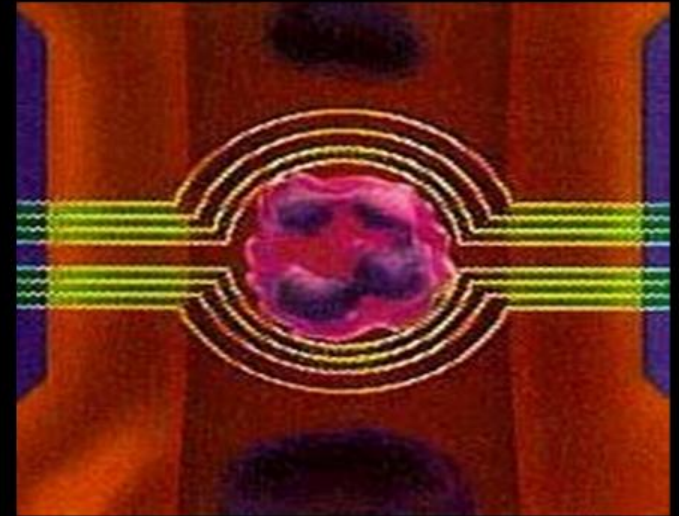
Peroxidase staining
(Seimens Advia)

Fluorescence Flowcytometry
(Sysmex –XE,XN series)

Beckman Coulter

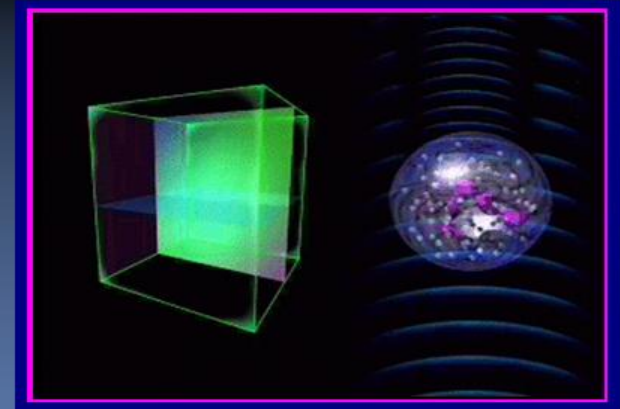
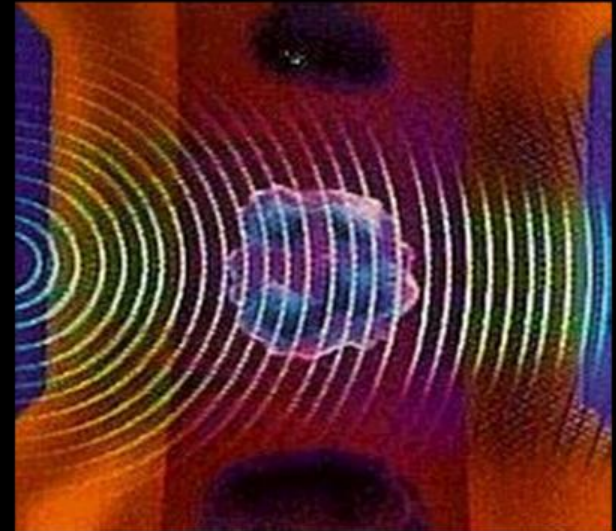
VOLUME MEASUREMENT

VCS utilises the Coulter Principle of counting and sizing to measure the volume of the cell by using Direct Current (DC) across the two electrode in a flow cell.



CONDUCTIVITY MEASUREMENT

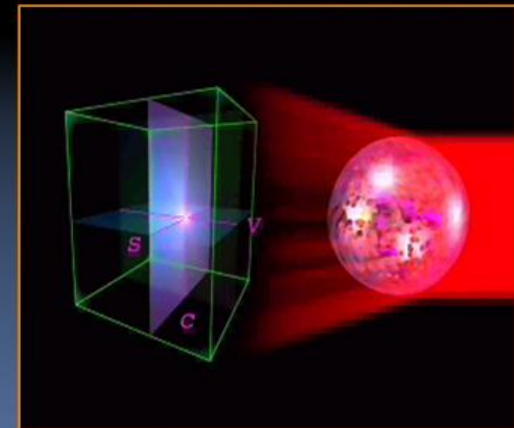
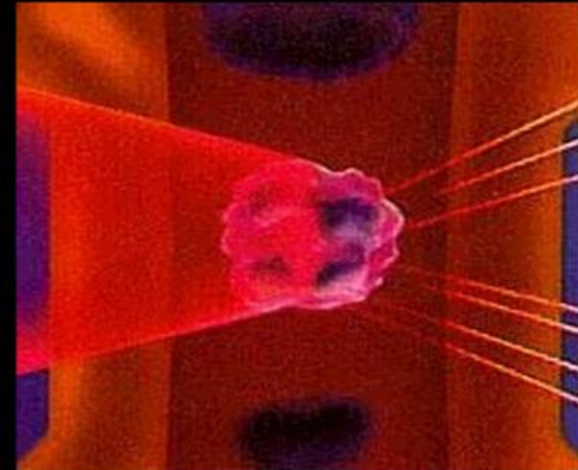
Cell exposed to RF, the RF energy penetrates into cell and reveal information about its size and internal structure.



SCATTER MEASUREMENT

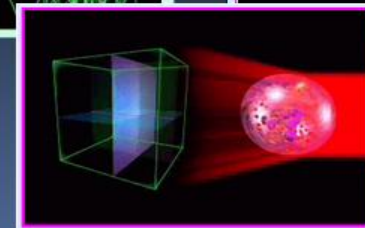
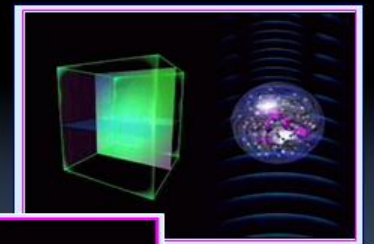
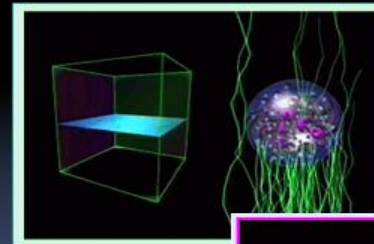
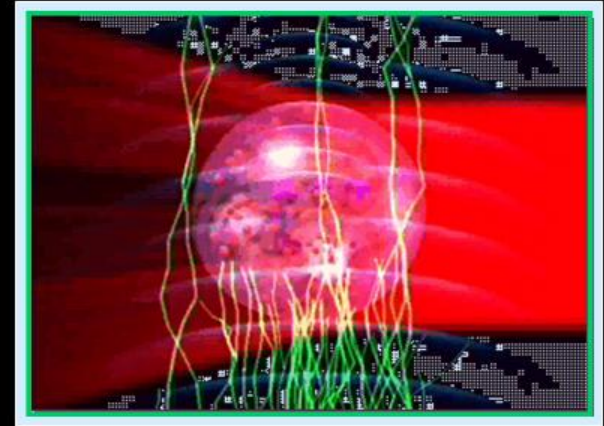
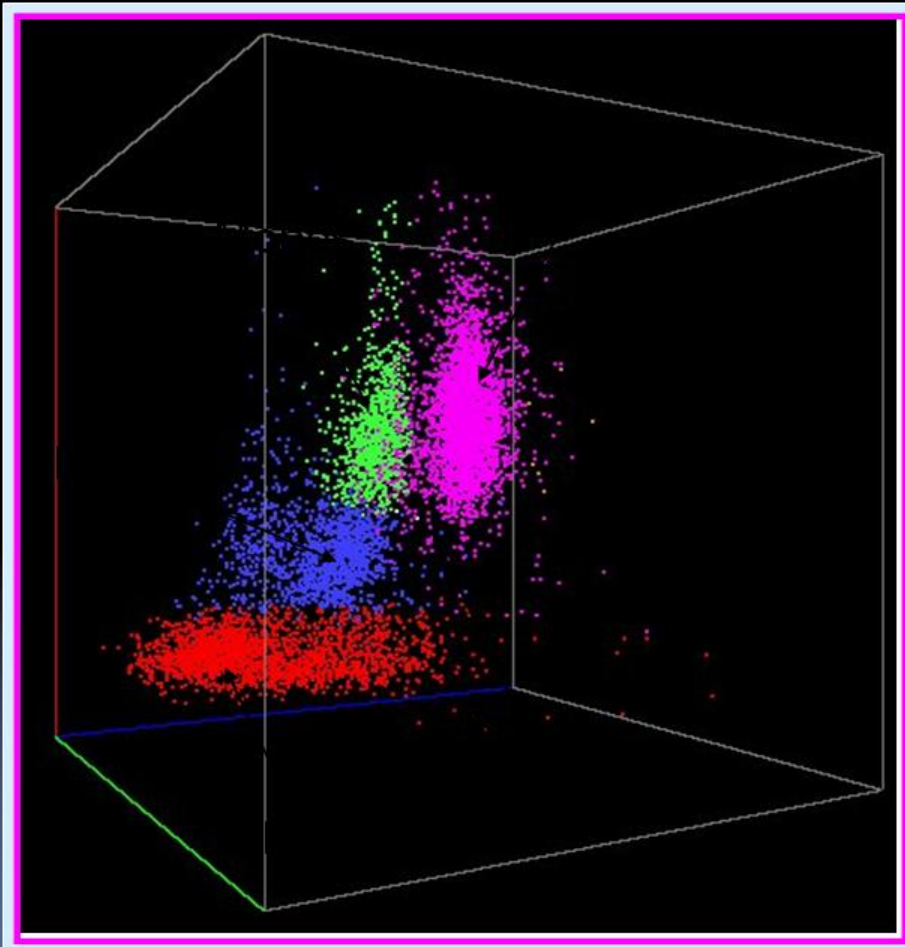
As cells pass in single stream (flow cell) they are struck by laser strike which gets scattered.

The light scatter at angles between 10 and 70 deg is used by VCS instruments.

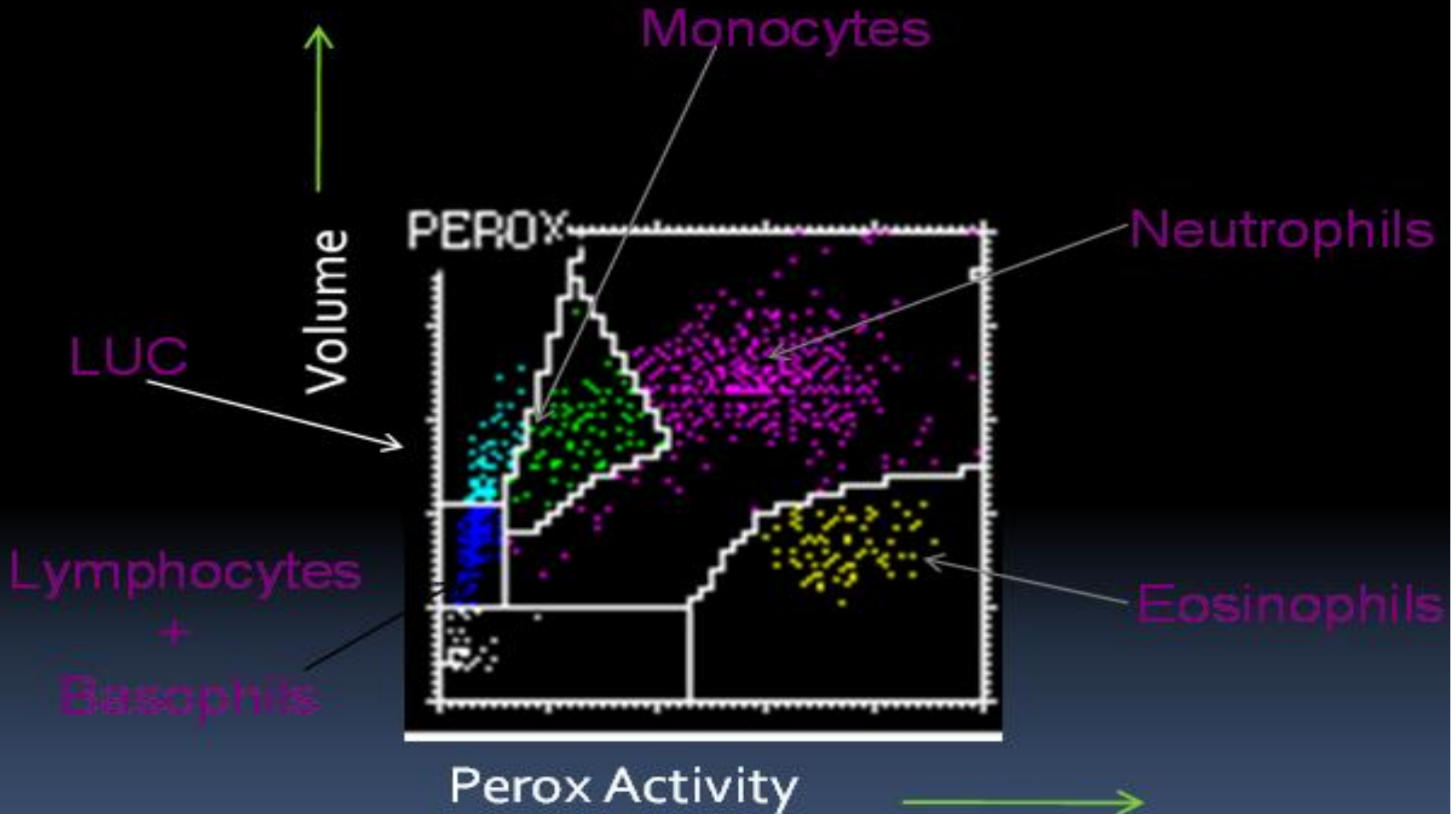


The scattered light gives information about cell surface and granularity

3D Data Analysis



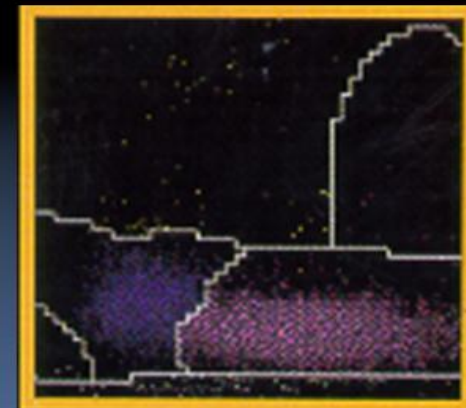
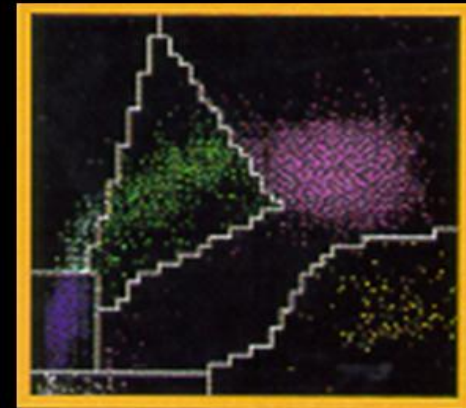
ADVIA TECHNOLOGY



ADVIA TECHNOLOGY

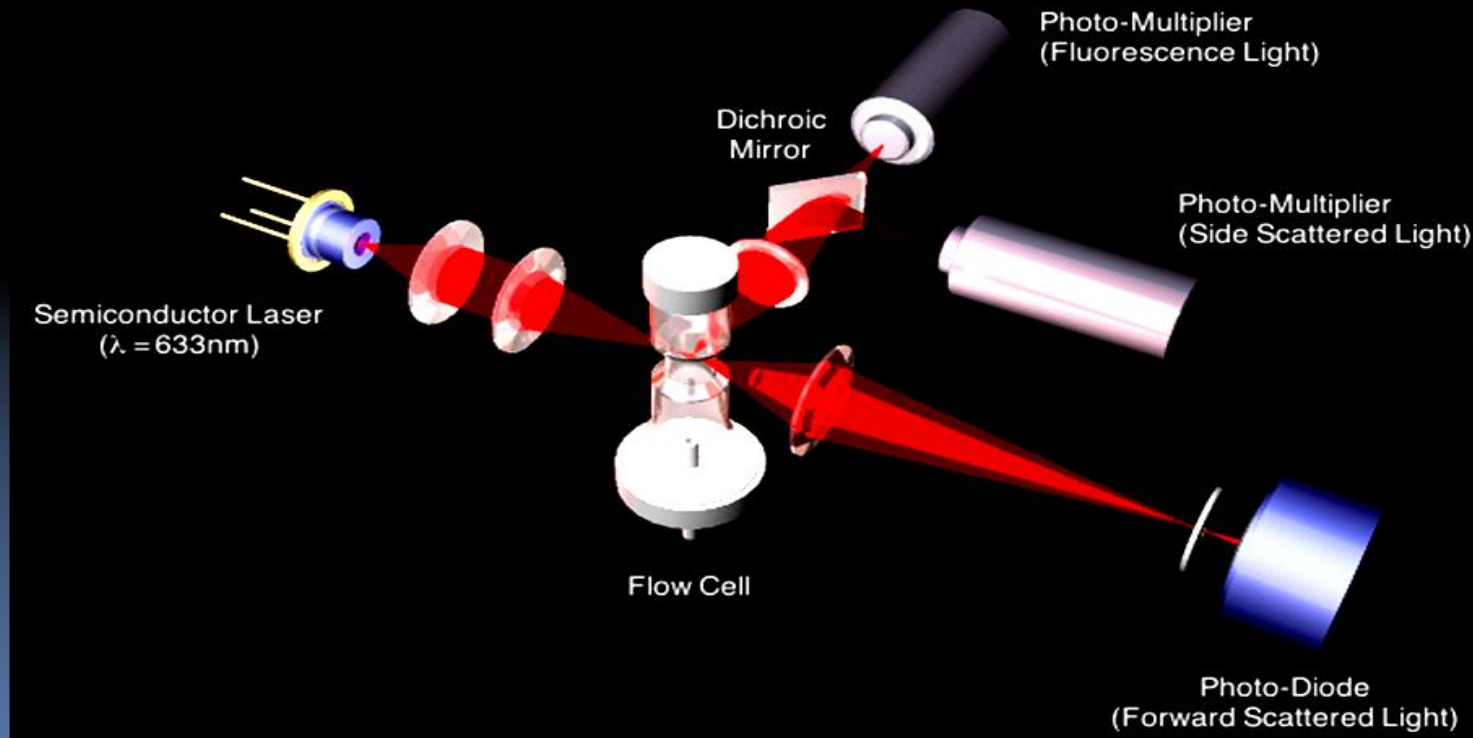
The ADVIA WBC differential is calculated from a 3 step process.

- Cells are stained by peroxidase reagent and analyzed for size and peroxidase stain intensity.
- Cell specific lysis reagents are used to separate basophils from all other white cells.
- Basos are subtracted from the lymph/baso cluster in the perox channel to calculate the lymphs.



Sysmex X-class analyzers- Fluorescence flow cytometry

Optical System



Fluorescence flow cytometry- (light scatter and fluorescent dyes)

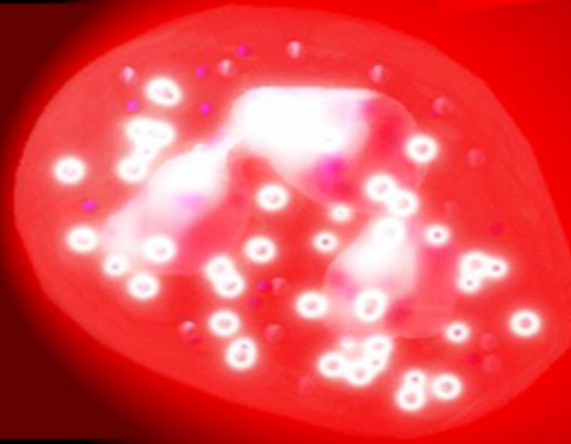
Laser Flowcytometry

Side Fluorescence Light :
RNA/DNA Information

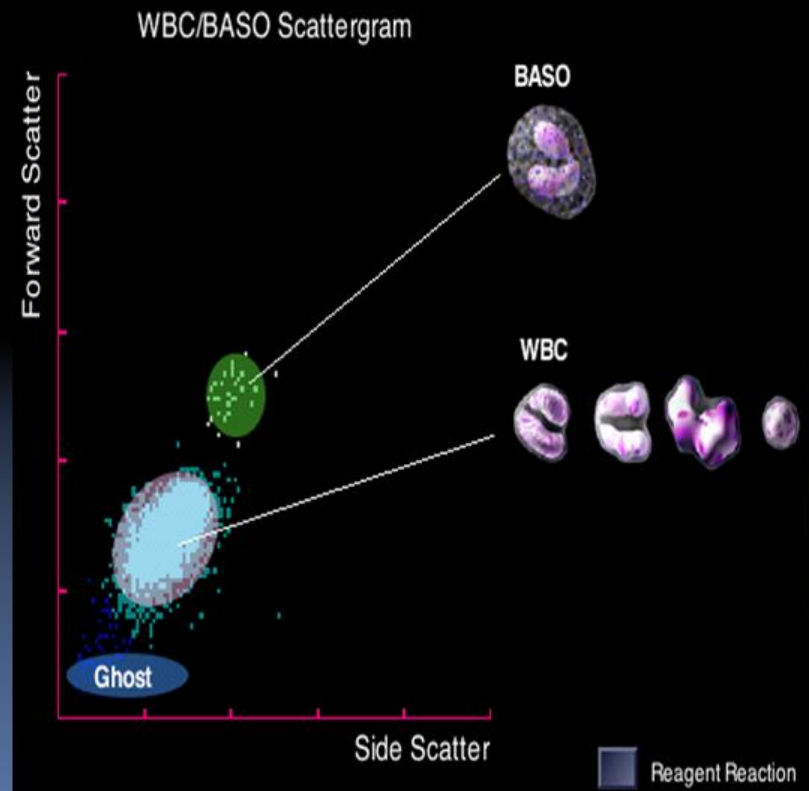
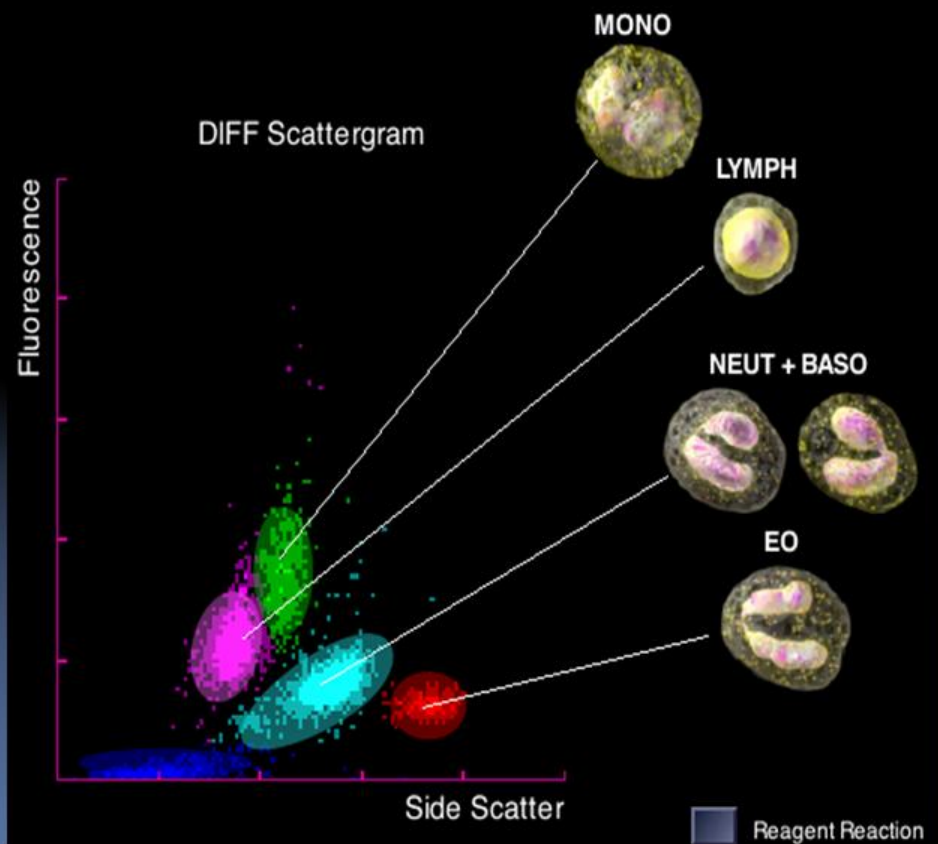
Side Scattered Light :
Intenal Cell Structure

Forward Scattered Light :
Cell Volume Information

Laser Beam
($\lambda = 633\text{nm}$)



Differential- SFL vs SSc (diff channel) FSc vs SSc (baso channel)



Basic parameters on a CBC Analyzer

- Basic hematological parameters
 - Hb, Hct, RBC Count
 - WBC with Differential (3 part/ 5 part)
 - Platelets
- Derived parameters
 - RBC:** MCV, MCH, MCHC, RDW
 - PLT:** MPV, PDW, P-LCR
- Histograms / Scattergrams

Additional parameters

- Principle of measurement
- Clinical relevance and uses
- Limitations



Novel parameter Am J Clin Pathol 2008;130:104-116	Machines	Clinical uses	Limitations
Immature reticulocyte fraction	Sapphire; Pentra 120 DX; LH 750; ADVIA 2120, XE 2100	Classification of anemias; monitoring the efficacy of therapy in nutritional anemia; early identification of marrow regeneration (after bone marrow transplantation or chemotherapy);	Not standardized; reference intervals method-dependent; higher sensitivity in fluorescence-based analyzers
Reticulated platelets	XE 2100	Differential diagnosis for causes of thrombocytopenia	Reduced availability; Lab ranges need to be derived
Immature granulocytes	XE 2100	Diagnosis of bacterial infections	Reduced availability
Nucleated RBCs	Sapphire, Pentra120 DX, LH 750, ADVIA 2120, XE 2100.	Diagnosis of hematologic diseases; prognostic factor in patients from surgery department or undergoing stem cell transplantation; evaluation of the efficacy of transfusion therapy in thalassemic syndromes	Higher performance On fluorescence Based methods
RBC fragments	ADVIA 2120, XE 2100	Diagnosis and monitoring of microangiopathies	Reduced availability; not standardized;
CHr, Ret He	ADVIA 2120, XE 2100	Diagnosis of iron-deficient erythropoiesis	Reduced availability
Hematopoietic Progenitor Cell mode	XE 2100	Surrogate for CD34 stem cell quantitation	Reduced availability, high imprecision

RETICULOCYTE MODE PARAMETERS

Test	Result	Units	Status	Reference Range
Reticulocyte Count	0.17	%	L	0.20 - 2.50
RPI (Reticulocyte Production Index)	0.10			

Comments : RPI=Corrected Reticulocyte Count / Reticulocyte Maturation Time in days.
RPI should be used only for adult anemic patients. RPI>2 indicates significantly increased hematopoiesis whereas RPI<2 indicates reduced response in an anemic patient.

IRF (Immature Reticulocyte Fraction)	1.20	%	L	2.00 - 16.50
--	-------------	---	---	--------------

Comments : IRF gives an idea about the least mature erythrocytes which contain the most RNA. In many clinical situations the IRF increases before the total reticulocyte count and can be used to monitor BM response.

Ret Hc (Reticulocyte Hb equivalent)	20.80	pg	L	28.70 - 34.10
RBC Hc (RBC Hb equivalent)	26.30	pg		26.00 - 30.40

Comments : Ret Hc provides an indirect measure of functional iron over the last 3-4 days. Ret Hc and RBC Hc are reduced in patients with Functional Iron Deficiency (FID). FID occurs when reticuloendothelial stores are normal to high but iron is not delivered for erythropoiesis (eg. Chronic renal dialysis, chronic inflammation, cancer patients).

IPF (Immature Platelet Fraction)	6.40	%	H	0.70 - 4.30
---	-------------	---	---	-------------

Comments : IPF is raised in patients with peripheral consumption/destruction of platelets (eg ITP, TTP) & is normal or low in patients with BM failure. IPF can be used for predicting platelet count recovery post chemotherapy or stem cell transplant.

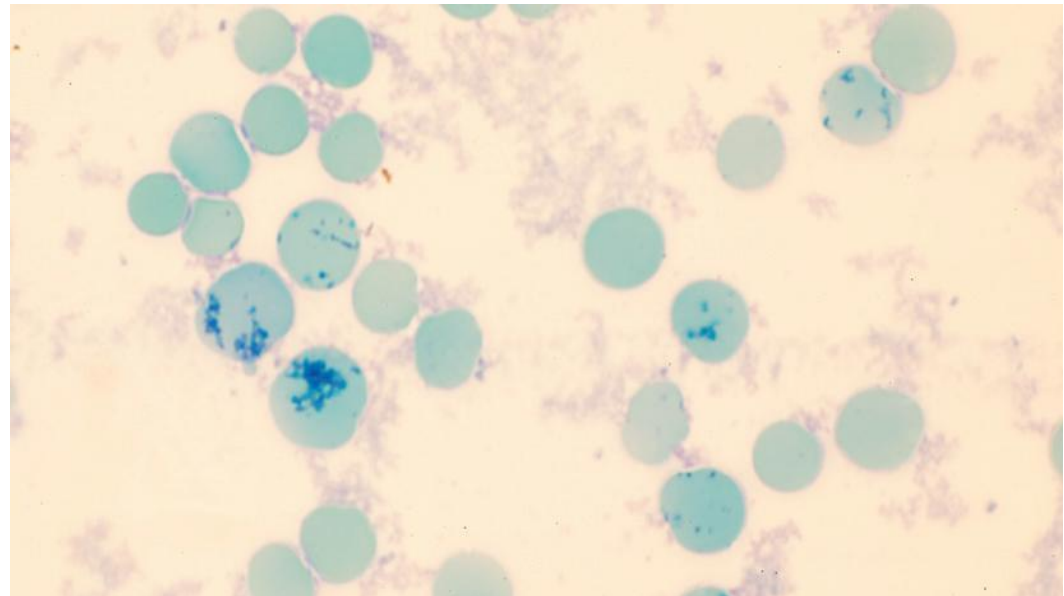
Comments : Ref.-C.Briggs.Int.Jnl>lab.hematol.2009,31,277-97

**End of Report **

Reticulocyte
Count
New Report
Format

Manual Reticulocyte Count

- Tedious
- Labour Intensive
- Subjective
- Very High CVs



Automated Reticulocyte Count

PROS

- Rapid
- Reproducible
- Reliable
- Research parameters

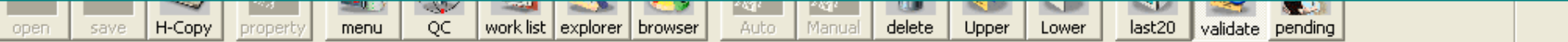
CONS

- Expensive
- Different machines use different dyes and techniques
- Standardisation is difficult
- Reference ranges to be established by every lab

Interpretation of Retic Count

High Retic Count	Low Retic Count
Blood Loss	Nutritional Deficiency- IDA, B12 deficiency
Hemolysis	Aplastic Anemia
Response to therapy	Post Chemo-radiation
Repopulating BM	BM infiltration- benign or malignant disorders

Normal Sample



Name: _____ Comment: _____

Main | Graph | WBC/NRBC | RBC/PLT | Cumulative | Q-Flags | Service | HPC | Research(W) | **Research(R)** | Rese. <

Item	Data	Unit
RBC	5.55	+ 10 ⁶ /uL
RBC-O	5.54	10 ⁶ /uL
HGB	16.3	g/dL
HCT	45.3	%
MCV	81.6	- fL
MCH	29.4	pg
MCHC	36.0	g/dL
RDW-CV	13.5	%
PLT	214	10 ³ /uL
PLT-I	214	10 ³ /uL
PLT-O	240	10 ³ /uL
MPV	11.4	fL
P-LCR	37.2	%
RET#	0.0561	10 ⁶ /uL
RET%	1.01	%
IRF	2.5	%
LFR	97.5	%
MFR	2.3	%
HFR	0.2	%
FRC%	0.06	%
IPF#	6.0	10 ³ /uL
H-IPF	0.6	%
IPF	2.8	%

Extended RET

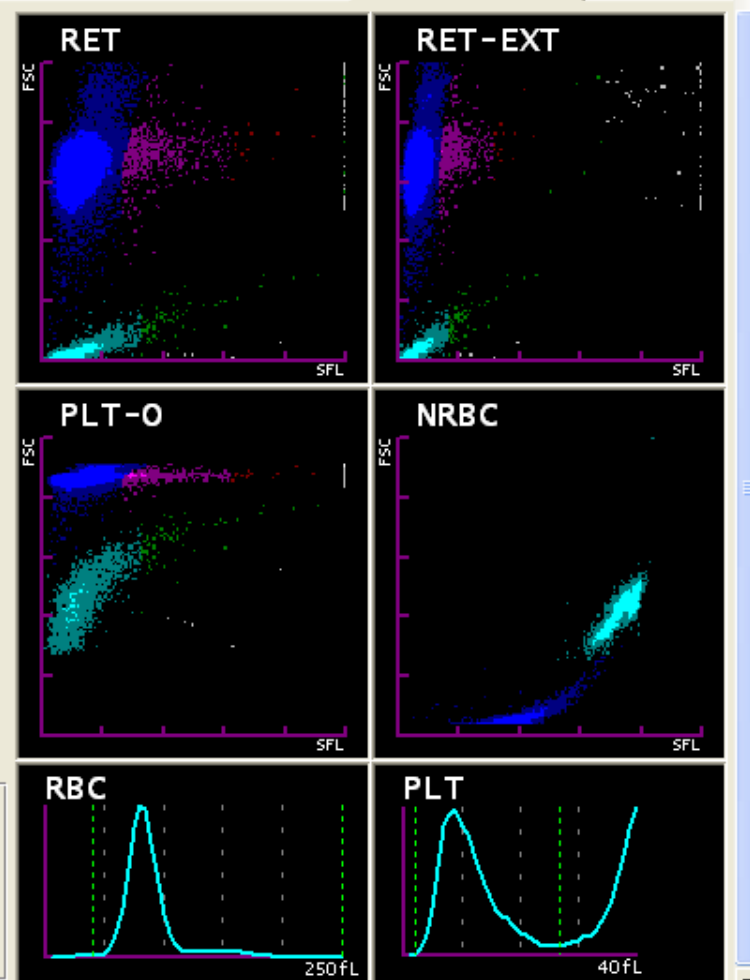
Item	Data	Unit
RET-He	33.1	pg
RBC-He	29.2	pg
D-He	3.9	pg
RET-Y	176.8	ch
RBC-Y	164.3	ch
TRF-Y	187.1	ch
RPI	1.0	
RET%	1.01	%

Items

Item	Data	Unit
NRBC#	0.00	10 ³ /uL
NRBC%	0.0	/100WBC

Flag(s)

RBC/RET PLT



17/M Anemia- Hb-5.3, Retic-18.13%,

Pat. ID

Sex

Dr.

Time 03:32

Name

Comment

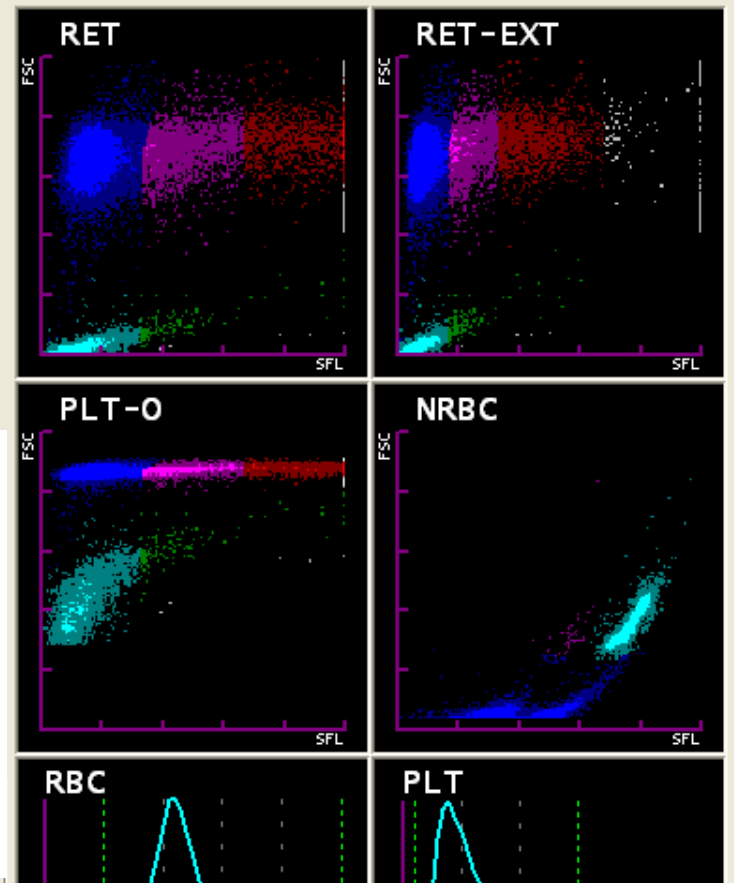
Main | Graph | WBC/NRBC | RBC/PLT | Cumulative | Q-Flags | Service | HPC | Research(w) | Research(R) | Rese. ◀ ▶

Item	Data		Unit
RBC	1.68	-	10 ¹⁶ /uL
RBC-O	1.67		10 ¹⁶ /uL
HGB	5.3	-	g/dL
HCT	18.9	-	%
MCV	112.5	+	fL
MCH	31.5		pg
MCHC	28.0	-	g/dL
RDW-SD	72.3	+	fL
RDW-CV	18.3	+	%
PLT	266		10 ¹³ /uL
PLT-I	266		10 ¹³ /uL
PLT-O	260		10 ¹³ /uL
PDW	12.6		fL
MPV	10.7		fL
P-LCR	30.6		%
PCT	0.29		%
RET#	0.3046		10 ¹⁶ /uL
RET%	18.13		%
IRF	33.8		%
LFR	66.2		%
MFR	17.8		%

Item	Data	Unit
RET-He	33.2	pg
RBC-He	30.0	pg
D-He	3.2	pg
RET-Y	177.4	ch
RBC-Y	166.9	ch
TRF-Y	183.1	ch
RPI	3.3	
RET%	18.13	%

Normal Ranges at
PDHNNH

Reticulocyte %
Range- 0.39%-1.85%
Literature – 0.2 - 2.5%



Hb- 8.7 , Retic – 0.2%

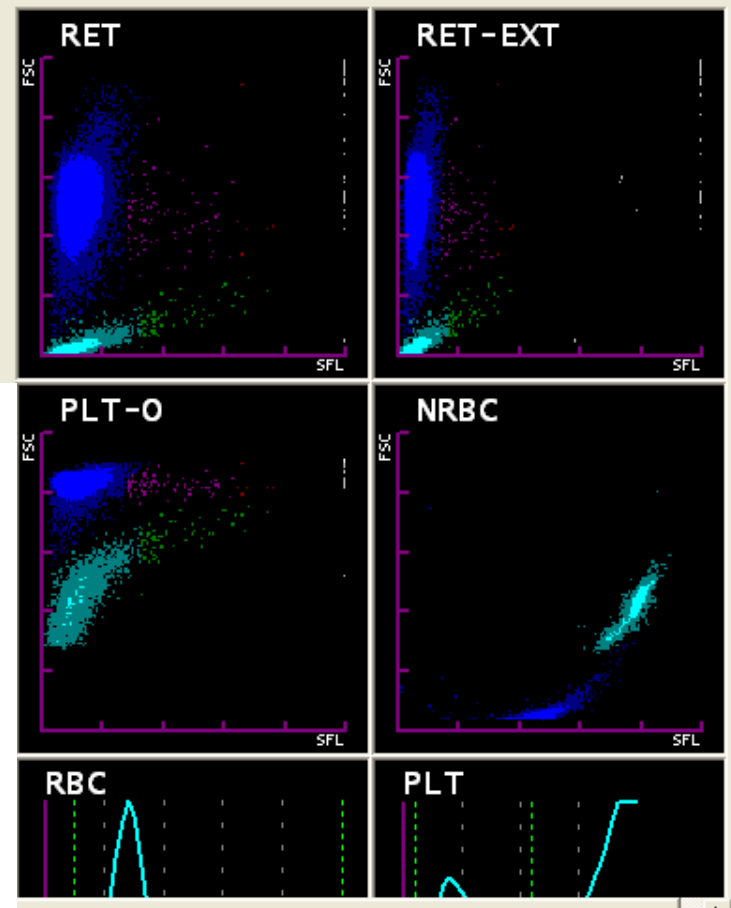
Positive Sample No. 052151 Birth Ward Date 25/11/2012
 Pat. ID RET Sex Dr. Time 06:01
 Name Comment

Item	Data	Unit
RBC	4.03	10 ⁶ /uL
RBC-O	3.92	10 ⁶ /uL
HGB	8.7	g/dL
HCT	28.1	%
MCV	69.7	fL
MCH	21.6	pg
MCHC	31.0	g/dL
RDW-SD	39.8	fL
RDW-CV	15.8	%
PLT	230	10 ³ /uL
PLT-I	230	10 ³ /uL
PLT-O	222	10 ³ /uL
PDW	13.0	fL
MPV	10.8	fL
P-LCR	32.8	%
PCT	0.25	%
RET#	0.0080	10 ⁶ /uL
RET%	0.22	%
IRF	0.8	%
LFR	93.2	%
MFR	6.8	%
UFR	0.0	%

Item	Data	Unit
RET-He	18.8	pg
RBC-He	21.4	pg
D-He	-2.6	pg
RET-Y	119.4	ch
RBC-Y	132.5	ch
IRF-Y	127.2	ch
RPI	0.1	

Normal Ranges at PDHNNH

Reticulocyte %
 Range- 0.39%-1.85%
 Literature – 0.2 - 2.5%



Hb- 5.8 , Retic – 4.73%

open save H-Copy property menu QC work list explorer browser Auto Manual delete Upper Lower last20 validate pending

Name Comment
Main Graph WBC/NRBC RBC/PLT Cumulative Q-Flags Service HPC Research(w) Research(R) Rese.

Items

Item	Data	Unit
RBC	1.70	10 ⁶ /uL
RBC-O	1.61	10 ⁶ /uL
HGB	5.8	g/dL
HCT	18.4	%
MCV	108.2	fL
MCH	34.1	pg
MCHC	31.5	g/dL
RDW-SD	93.5	fL
RDW-CV	24.5	%
PLT	110	10 ³ /uL
PLT-I	110	10 ³ /uL
PLT-O	105	10 ³ /uL
PDW	11.3	fL
MPV	10.3	fL
P-LCR	26.4	%
PCT	0.11	%
RET#	0.0804	10 ⁶ /uL
RET%	4.73	%
IRF	10.3	%
LFR	83.7	%
MFR	14.8	%
HFR	1.5	%

Extended RET

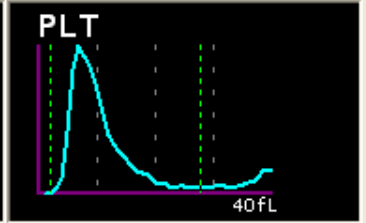
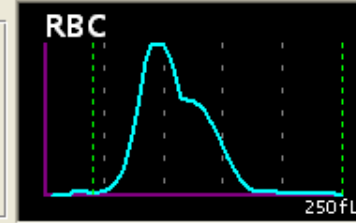
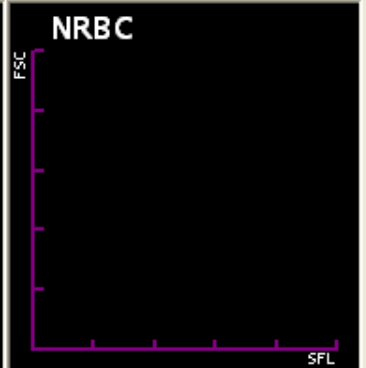
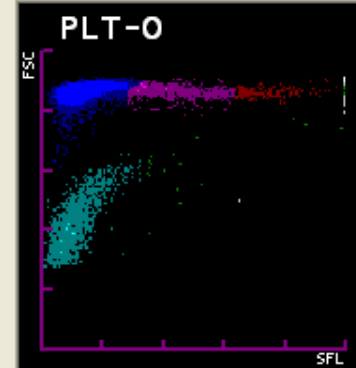
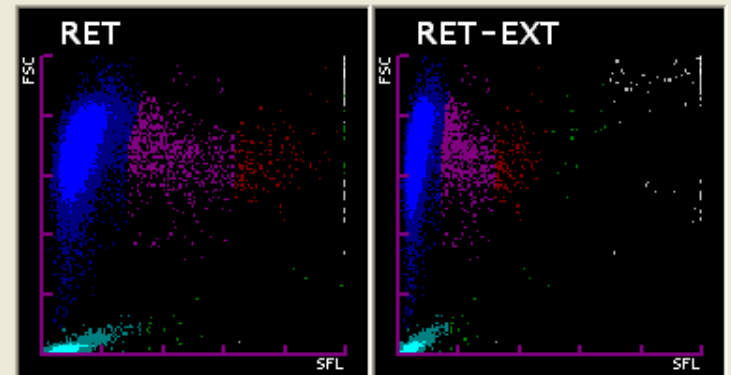
Item	Data	Unit
RET-He	31.0	pg
RBC-He	32.1	pg
D-He	-1.1	pg
RET-Y	170.4	ch
RBC-Y	173.7	ch
IRF-Y	165.0	ch
RPI	0.8	

Item	Data	Unit
FRC#	0.0145	10 ⁶ /uL
FRC%	0.85	%

Items

Item	Data	Unit
NRBC#		10 ³ /uL
NRBC%		/100WBC

Flag(s)
RBC/RET PLT
Aniso
Macro
Anemia



Reticulocyte Production Index-RPI

Reticulocyte Production Index is calculated as follows:

$$1. ReticIndex = ReticCount * \frac{Hematocrit}{NormalHematocrit}$$

A value of 45 is usually used as a normal hematocrit.

2. The next step is to correct for the longer life span of prematurely released reticulocytes

This relies on a table:

Hematocrit (%)	Retic survival(days) = maturation correction	PB	BM
36-45	1.0	1	3
26-35	1.5	1.5	2.5
16-25	2.0	2	2
15 and below	2.5	2.5	1.5

So, in a person whose reticulocyte count is 5%, hemoglobin 7.5 g/dL, hematocrit 25%,

$$RPI = \frac{ReticIndex}{MaturationCorrection} \rightarrow RPI = \frac{5 * \frac{25}{45}}{2} = 1.4$$

RPI Interpretation

- RPI is used for evaluation only in anemic patients
- RPI < 2 with anemia - Decreased production of reticulocytes and therefore red blood cells.
- RPI > 2 with anemia - loss of red blood cells (destruction, bleeding, etc) with increased compensatory production of reticulocytes

Normal Hb-16.3, Normal Platelet Count-214, Normal RPI-1, Normal IRF and IPF

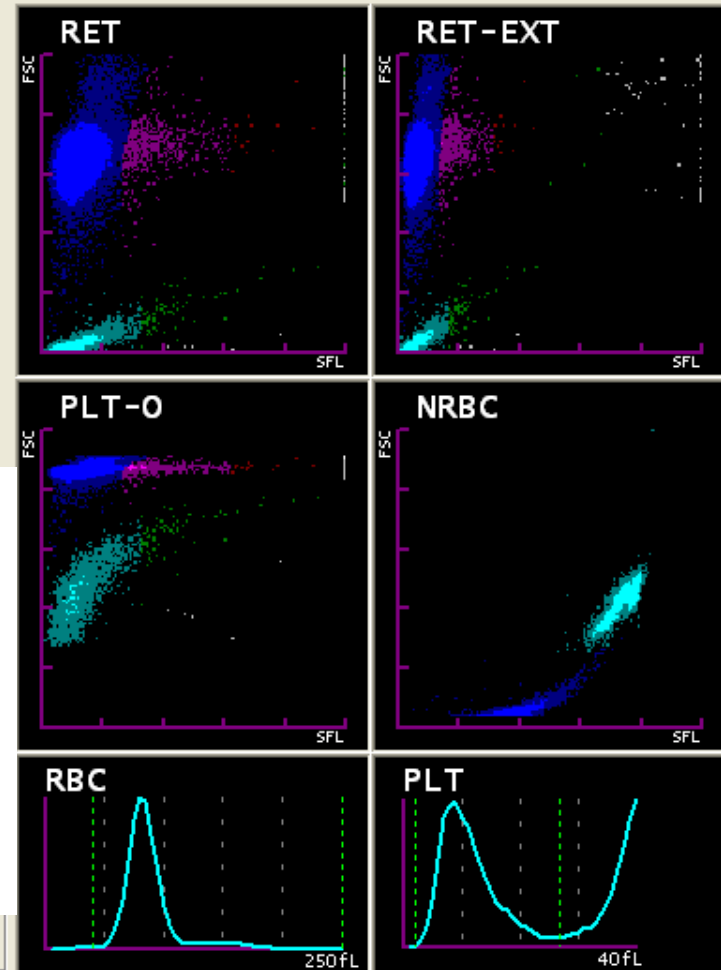
Main | Graph | WBC/NRBC | RBC/PLT | Cumulative | Q-Flags | Service | HPC | Research(W) | Research(R) | Rese. |

Item	Data	Unit
RBC	5.55 +	10 ⁶ /uL
RBC-O	5.54	10 ⁶ /uL
HGB	16.3	g/dL
HCT	45.3	%
MCV	81.6 -	fL
MCH	29.4	pg
MCHC	36.0	g/dL
RDW-CV	13.5	%
PLT	214	10 ³ /uL
PLT-I	214	10 ³ /uL
PLT-O	240	10 ³ /uL
MPV	11.4	fL
P-LCR	37.2	%
RET#	0.0561	10 ⁶ /uL
RET%	1.01	%
IRF	2.5	%
LFR	97.5	%
MFR	2.3	%
HFR	0.2	%
FRC%	0.06	%
IPF#	6.0	10 ³ /uL
H-IPF	0.6	%
IPF	2.8	%

-Extended RET-		
Item	Data	Unit
RET-He	33.1	pg
RBC-He	29.2	pg
D-He	3.9	pg
RET-Y	176.8	ch
RBC-Y	164.3	ch
TRF-Y	187.1	ch
RPI	1.0	%
RET%	1.01	%

Normal Ranges at
PDHNNH

Reticulocyte %
Range- 0.39%-1.85%
Literature – 0.2 - 2.5%



Hb- 5.8 , Retic – 4.73%

open save H-Copy property menu QC work list explorer browser Auto Manual delete Upper Lower last20 validate pending

Name Comment
Main Graph WBC/NRBC RBC/PLT Cumulative Q-Flags Service HPC Research(w) Research(R) Rese.

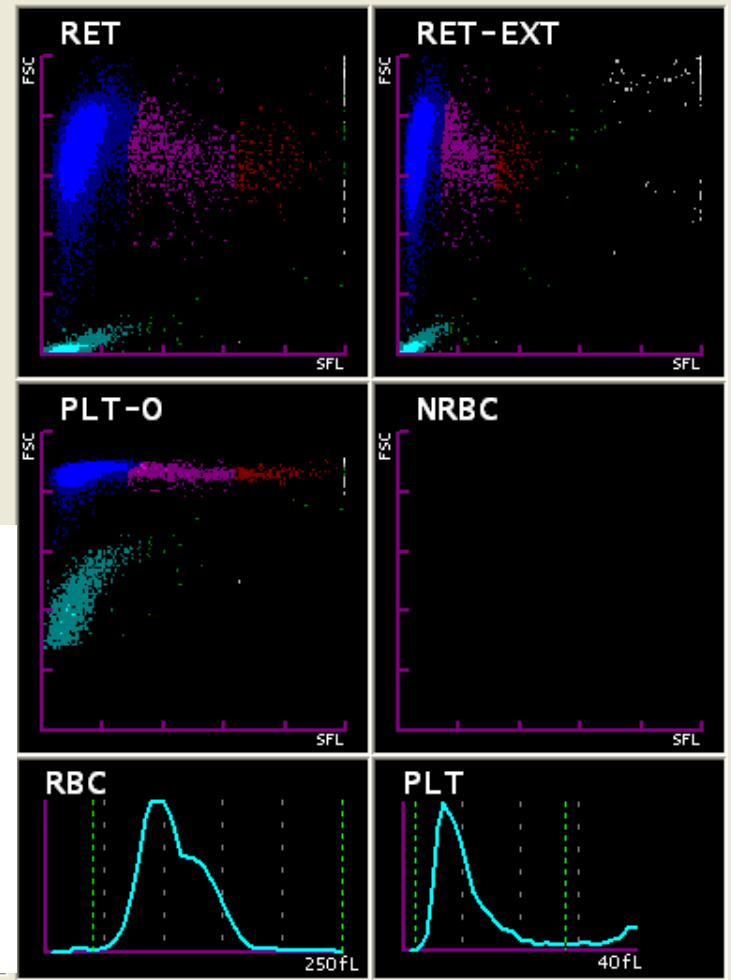
Item	Data	Unit
RBC	1.70	10 ⁶ /uL
RBC-O	1.61	10 ⁶ /uL
HGB	5.8	g/dL
HCT	18.4	%
MCV	108.2	fL
MCH	34.1	pg
MCHC	31.5	g/dL
RDW-SD	93.5	fL
RDW-CV	24.5	%
PLT	110	10 ³ /uL
PLT-I	110	10 ³ /uL
PLT-O	105	10 ³ /uL
PDW	11.3	fL
MPV	10.3	fL
P-LCR	26.4	%
PCT	0.11	%
RET#	0.0804	10 ⁶ /uL
RET%	4.73	%
IRF	16.3	%
LFR	83.7	%
MFR	14.8	%
HFR	1.5	%

Item	Data	Unit
RET-He	31.0	pg
RBC-He	32.1	pg
D-He	-1.1	pg
RET-Y	170.4	ch
RBC-Y	173.7	ch
IRF-Y	165.0	ch
RPI	0.8	

Item	Data	Unit
FRC#	0.0145	10 ⁶ /uL
FRC%	0.85	%

Normal Ranges at PDHNNH

Reticulocyte %
Range- 0.39%-1.85%
Literature – 0.2 - 2.5%



17/M Anemia- Hb-5.3, Retic-18.13%,

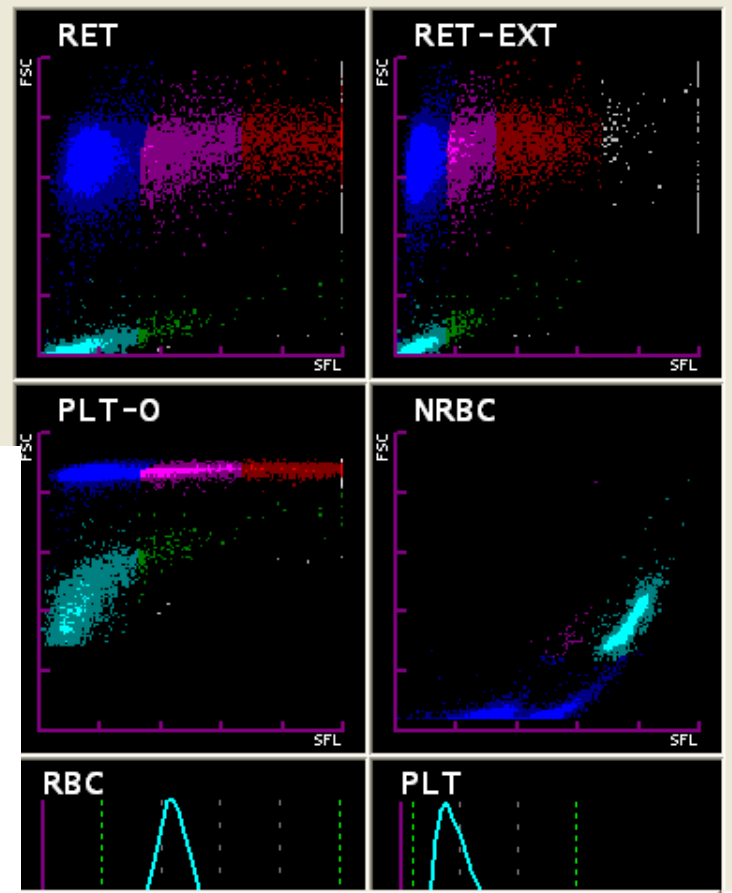
Pat. ID SAIRAJ 62033-35 Sex Dr. Time 03:32

Name Comment

Main Graph WBC/NRBC RBC/PLT Cumulative Q-Flags Service HPC Research(w) Research(R) Rese.

Item	Data		Unit
RBC	1.68	-	10 ¹⁶ /uL
RBC-O	1.67		10 ¹⁶ /uL
HGB	5.3	-	g/dL
HCT	18.9	-	%
MCV	112.5	+	fL
MCH	31.5		pg
MCHC	28.0	-	g/dL
RDW-SD	72.3	+	fL
RDW-CV	18.3	+	%
PLT	266		10 ¹³ /uL
PLT-I	266		10 ¹³ /uL
PLT-O	260		10 ¹³ /uL
PDW	12.6		fL
MPV	10.7		fL
P-LCR	30.6		%
PCT	0.29		%
RET#	0.3046		10 ¹⁶ /uL
RET%	18.13		%
IRF	33.8		%
LFR	66.2		%
MFR	17.8		%

Item	Data	Unit
RET-He	33.2	pg
RBC-He	30.0	pg
D-He	3.2	pg
RET-Y	177.4	ch
RBC-Y	166.9	ch
TRF-Y	183.1	ch
RPI	3.3	
RET%	18.13	%



Normal Ranges at PDHNNH

Reticulocyte %
Median- 0.92%
Range- 0.39%-1.85%

Heilmeyer Staging

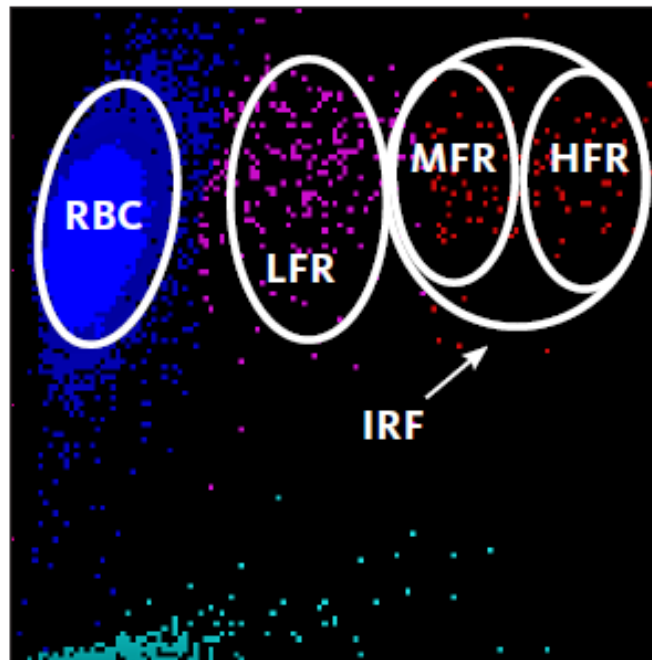
Maturation stages according to Heilmeyer	Morphological description	Quantification according to Seip (normal %)
Stage 0	Nucleolus	
Stage I	Reticulum consists of dense clots	< 0.1
Stage II	Loosely arranged reticulum	7.0
Stage III	Diffusely arranged reticulum	32.0
Stage IV	Some scattered granulae	61.0



Reticulocyte maturation

LFR	MFR	HFR
Low	Medium	High
Fluorescence	Fluorescence	Fluorescence
Reticulocytes	Reticulocytes	Reticulocytes
Little RNA	More RNA	High level of RNA
Mature reticulocytes	Semi-mature reticulocytes	Immature reticulocytes
Reference range: 86.5 - 98.5%	Reference range: 1.5 - 11.3%	Reference range: 0 - 1.4%

Tab. 6 Maturation stages of reticulocytes



IRF is the sum of MFR and HFR, i.e. the immature reticulocytes, and is referred to as the 'reticulocyte maturation index'.

$$\text{IRF} = \text{MFR} + \text{HFR}$$

Reference range

IRF:	f	1.1 - 15.9 %
	m	1.5 - 13.7 %

In-vitro stability of IRF

6 hours

Normal Range at PDHNNH

IRF	1.9 %-17.9%
LFR	81.5%-98.1%
MFR	1.9% -16.08%
HFR	0 % -3.39%

Normal Hb-16.3, Normal Platelet Count-214, Normal RPI-1, Normal IRF and IPF

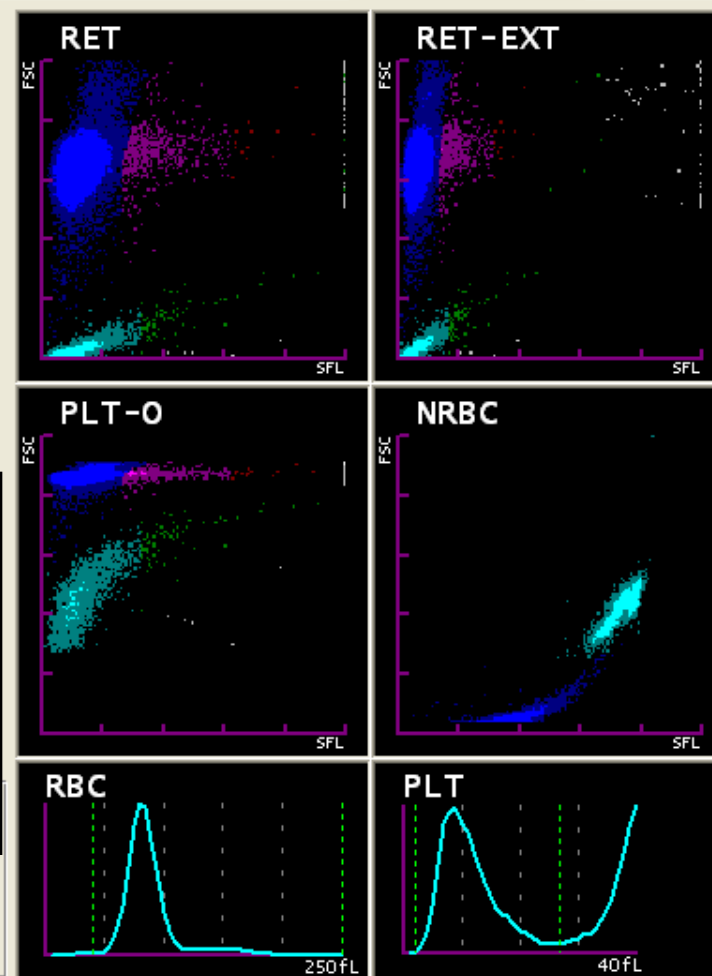
Main | Graph | WBC/NRBC | RBC/PLT | Cumulative | Q-Flags | Service | HPC | Research(W) | **Research(R)** | Rese. |

Item	Data	Unit
RBC	5.55 +	10 ⁶ /uL
RBC-O	5.54	10 ⁶ /uL
HGB	16.3	g/dL
HCT	45.3	%
MCV	81.6 -	fL
MCH	29.4	pg
MCHC	36.0	g/dL
RDW-CV	13.5	%
PLT	214	10 ³ /uL
PLT-I	214	10 ³ /uL
PLT-O	240	10 ³ /uL
MPV	11.4	fL
P-LCR	37.2	%
RET#	0.0561	10 ⁶ /uL
RET%	1.01	%
IRF	2.5	%
LFR	97.5	%
MFR	2.3	%
HFR	0.2	%
FRC%	0.06	%
IPF#	6.0	10 ³ /uL
H-IPF	0.6	%
IPF	2.8	%

Extended RET		
Item	Data	Unit
RET-He	33.1	pg
RBC-He	29.2	pg
D-He	3.9	pg
RET-Y	176.8	ch
RBC-Y	164.3	ch
IRE-Y	187.1	ch
RPI	1.0	%
RET%	1.01	%

Normal Range at PDHNNH

IRF	1.9 %-17.9%
LFR	81.5%-98.1%
MFR	1.9% -16.08%
HFR	0 % -3.39%



17/M Anemia- Hb-5.3, Retic-18.13%,

Pat. ID SAIRAJ 62033-35 Sex Dr. Time 03:32

Name Comment

Main Graph WBC/NRBC RBC/PLT Cumulative Q-Flags Service HPC Research(w) Research(R) Rese.

Item	Data	Unit
RBC	1.68	- 10 ¹⁶ /uL
RBC-O	1.67	10 ¹⁶ /uL
HGB	5.3	- g/dL
HCT	18.9	- %
MCV	112.5	+ fL
MCH	31.5	pg
MCHC	28.0	- g/dL
RDW-SD	72.3	+ fL
RDW-CV	18.3	+ %
PLT	266	10 ¹³ /uL
PLT-I	266	10 ¹³ /uL
PLT-O	260	10 ¹³ /uL
PDW	12.6	fL
MPV	10.7	fL
P-LCR	30.6	%
PCT	0.29	%
RET#	0.3046	10 ¹⁶ /uL
RET%	18.13	%
IRF	33.8	%
LFR	66.2	%
MFR	17.8	%

Item	Data	Unit
RET-He	33.2	pg
RBC-He	30.0	pg
D-He	3.2	pg
RET-Y	177.4	ch
RBC-Y	166.9	ch
TRF-Y	183.1	ch
RPI	3.3	
RET%	18.13	%

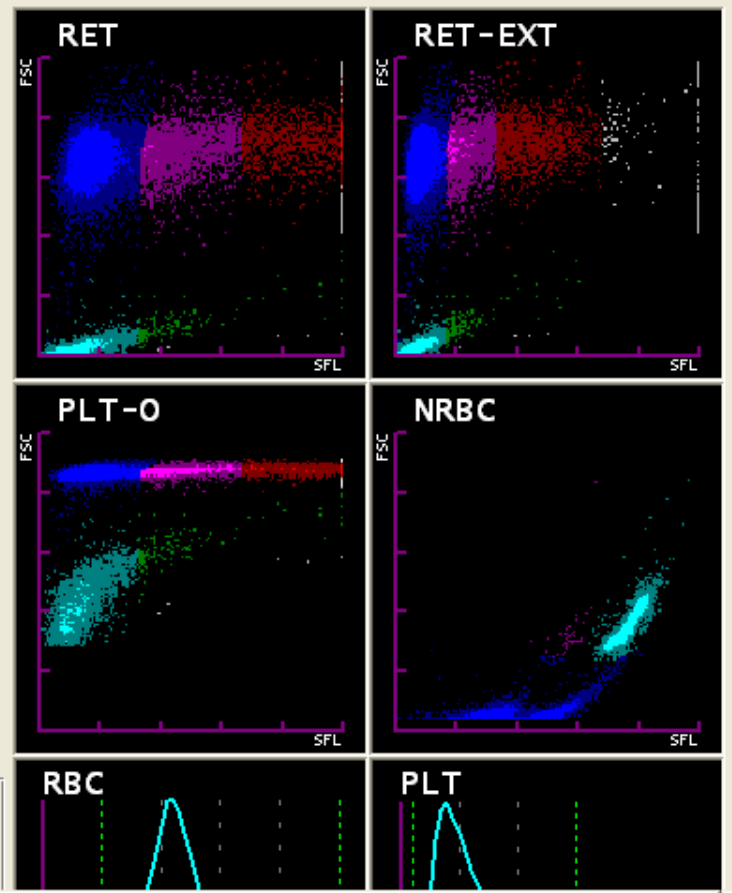
Normal Range at PDHNNH

IRF 1.9 %-17.9%

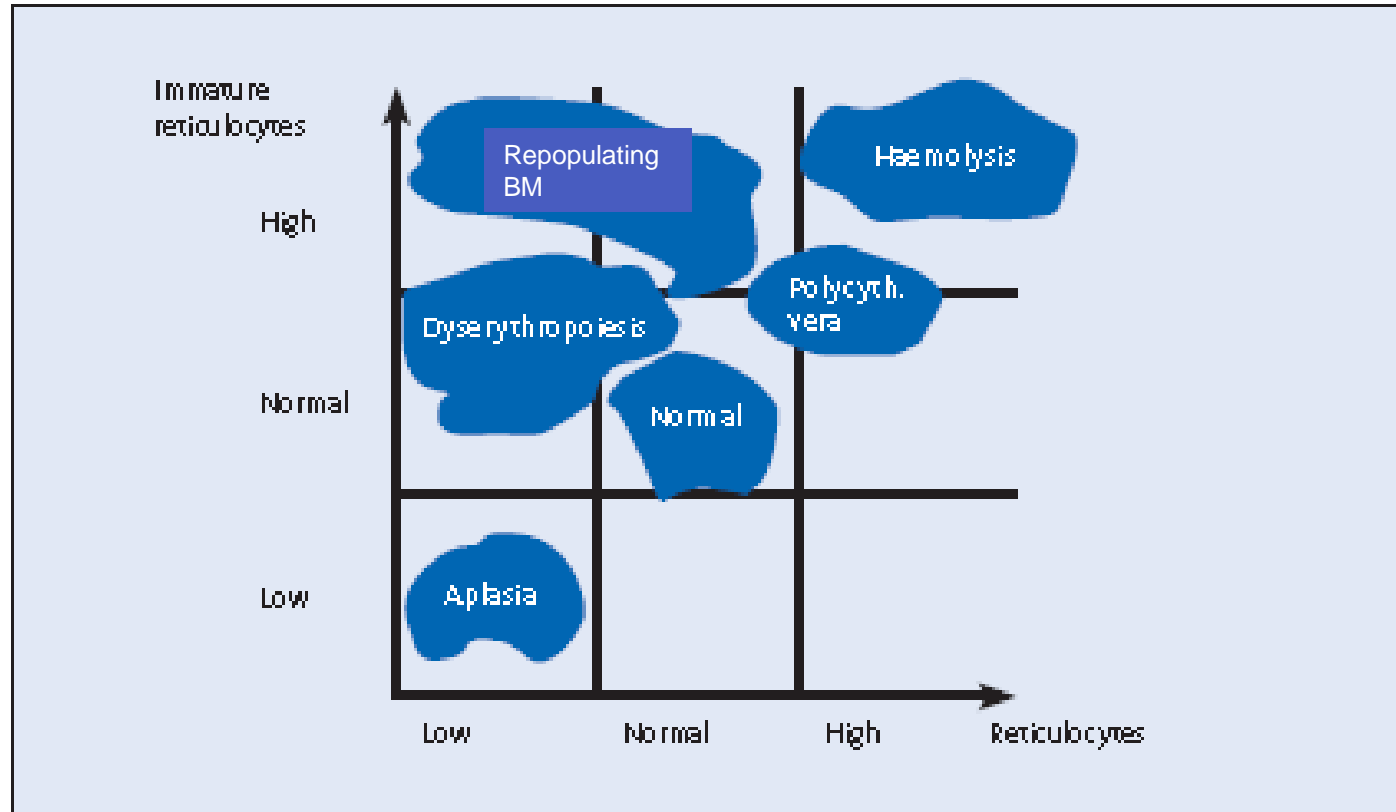
LFR 81.5%-98.1%

MFR 1.9% -16.08%

HFR 0 % -3.39%



Clinical Use of IRF



Indicators of haematopoietic recovery after bone marrow transplantation: the role of reticulocyte measurements.

[d'Onofrio G](#), [Tichelli A](#), [Foures C](#), [Theodorsen L](#).

Universita Cattolica, Roma, Italy.

Abstract

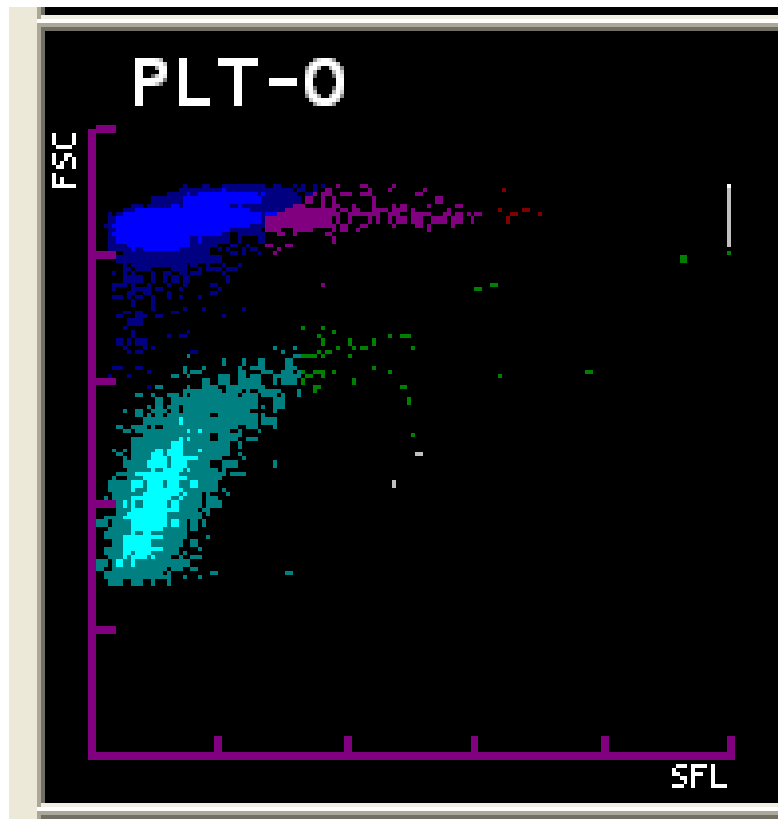
The aim of this project was to study **haematological recovery in patients** following different types of bone marrow transplantation (**BMT**).

Among 12 different variables, the parameters with the highest specificity or predictive value for monitoring recovery were the **absolute neutrophil count (ANC)** of $0.5 \times 10^9/l$, an **absolute reticulocyte count (RET)** above $20 \times 10^9/l$ **high fluorescent reticulocyte fraction (HFR)** above 5%.

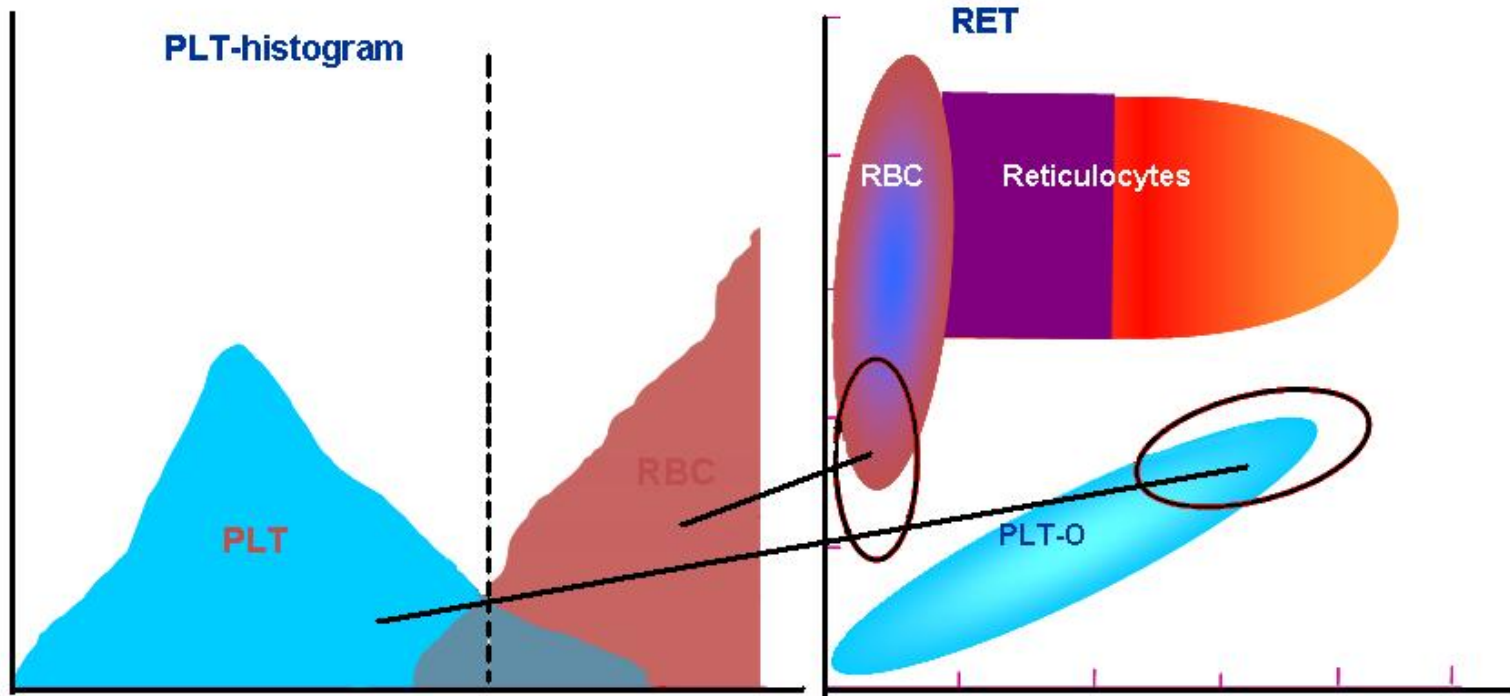
Among these variables, the **HFR fraction was the earliest and most sensitive index of engraftment in 79.1% of patients**, HFR recovery requiring a median time of 13 days after infusion, in comparison with a median period of 19 and 18 days, respectively, for RET and ANC ($P < 0.0001$).

Platelet Parameters

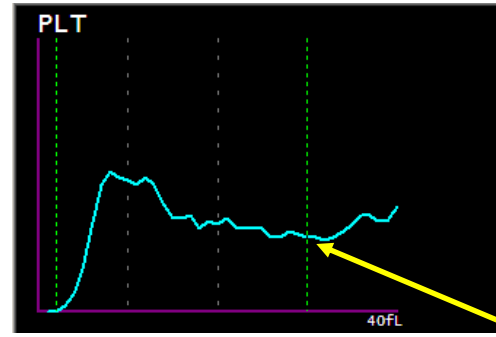
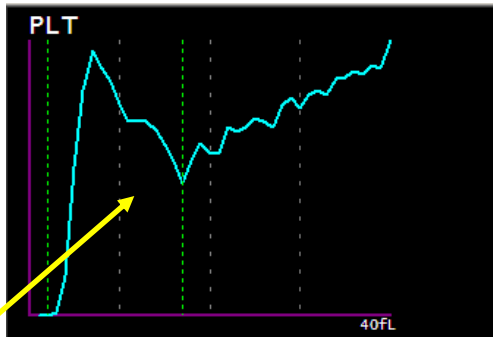
- Optical Platelet Count
- Immature Platelet fraction



Advantages of Optical Platelet Counting

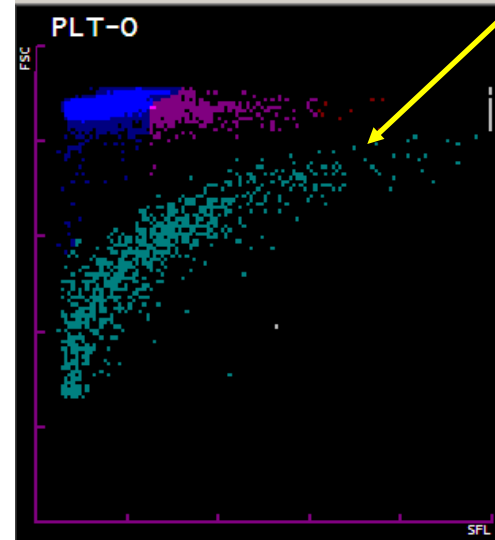
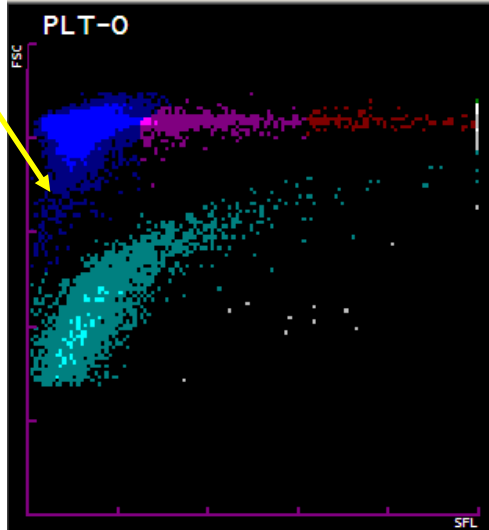


Optical Platelet Enumeration



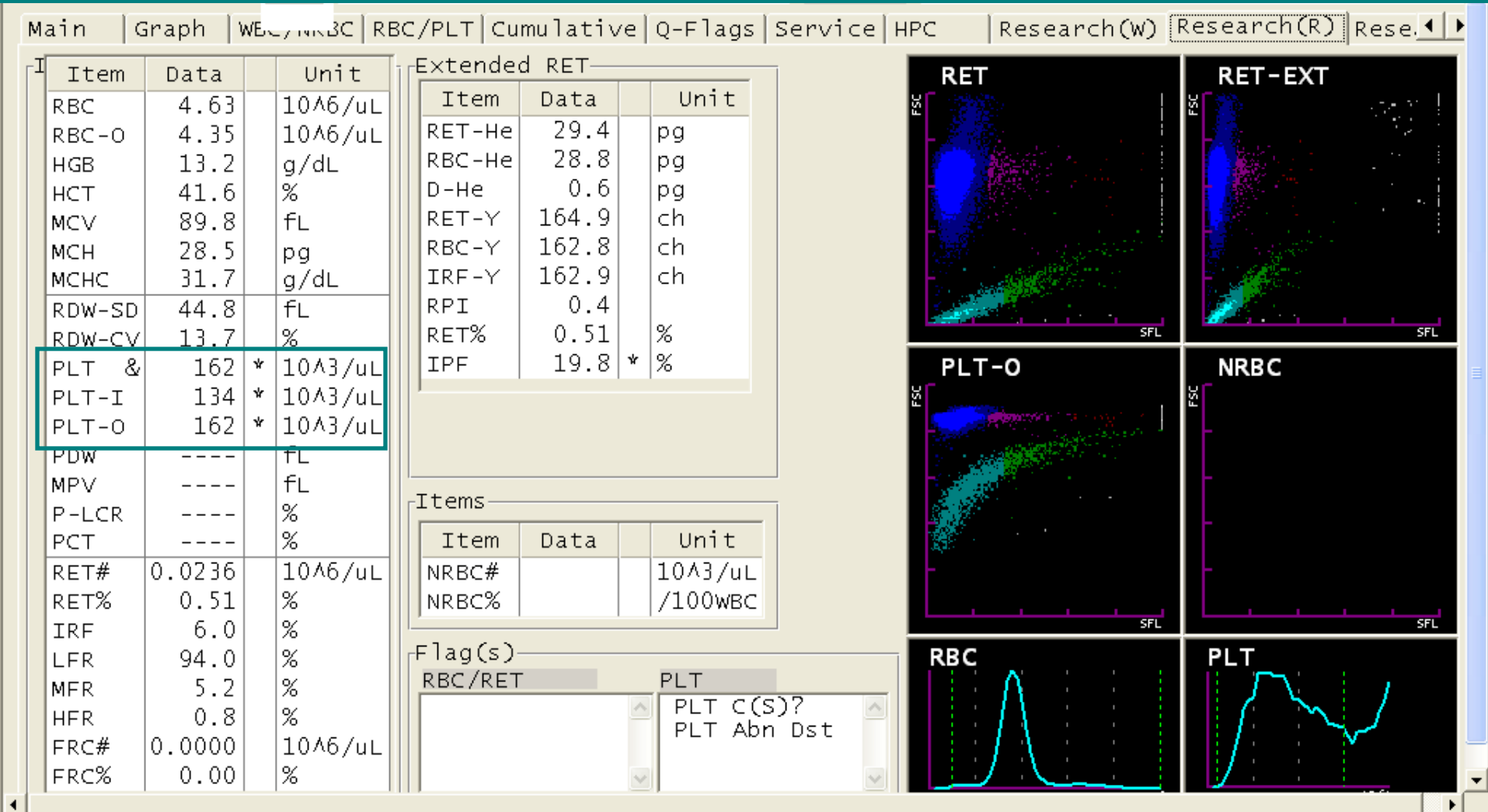
Microcytic RBC

Giant PLT



31/F, Blood Donor, East Indian Origin,

Normal Hb and WBC, Impedance Plt- 134, Platelet O –162, Morphologically- Many Giant platelets



Message

XE-2100-1

start

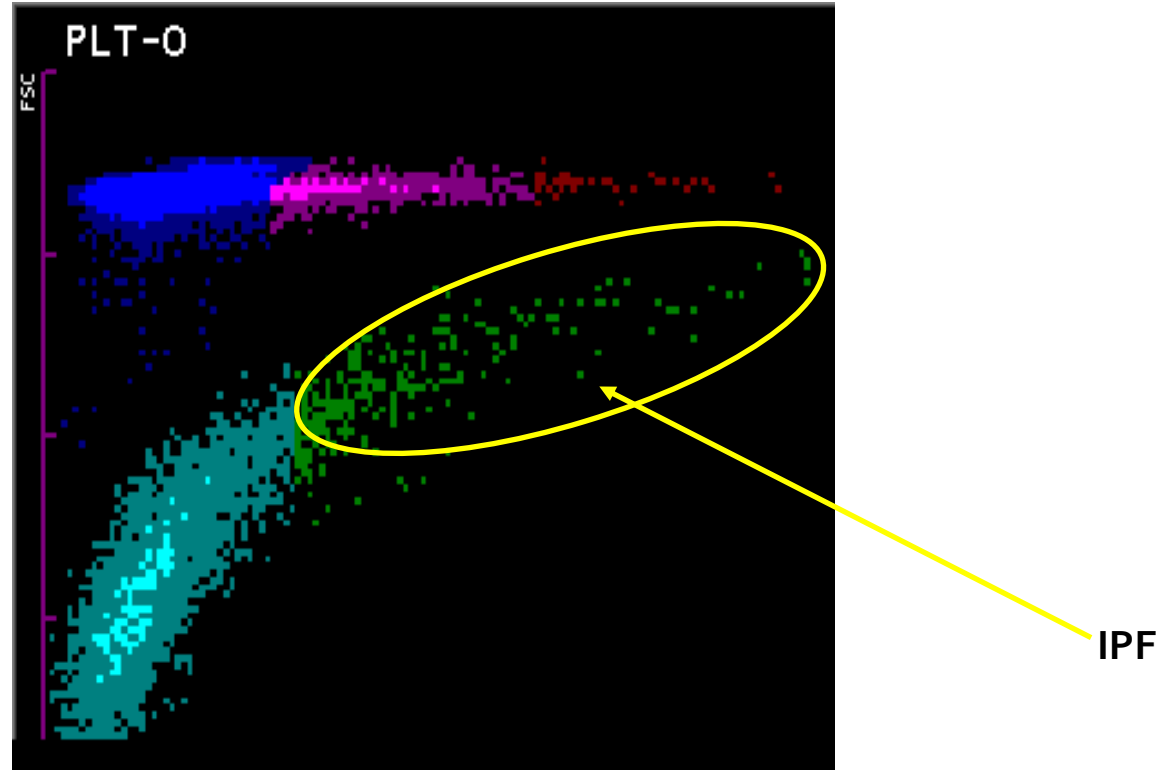
XE-2100 - [Data Brow...

KUNAL SEHGAL

PLT O OVER i ne INDI...

11:35 AM

Immature Platelet fraction



Immature PLT are identified by its **increase in fluorescence** (more RNA), **FSC is also higher**.

Immature Platelet fraction

1) Pathogenesis of low platelet count:

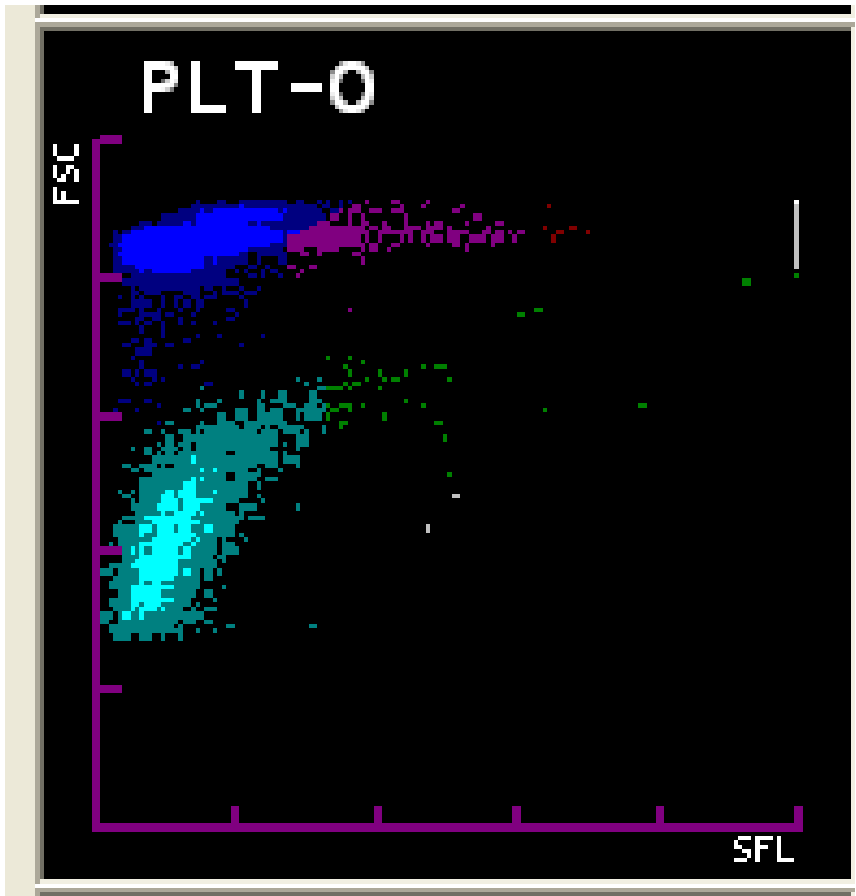
- Increased destruction or usage: Increased IPF
High IPF suggests an active bone marrow. (e.g., ITP)
- Decreased production: Reduced IPF
Low IPF suggests depressed bone marrow function

2) Bone Marrow regeneration :

IPF - First indicator of bone marrow regeneration

FDA approved

IPF - Normal Range



IPF – Various studies have shown
Normal range as
0.5 to 5.2%, 1.1 to 6.1%, 0.5 to 3.2 %

Normal Ranges Derived at Hinduja
Hospital

- **IPF - 0.7- 4.3%**

Normal Hb-16.3, Normal Platelet Count-214, Normal RPI-1, Normal IRF and IPF

Main | Graph | WBC/NRBC | RBC/PLT | Cumulative | Q-Flags | Service | HPC | Research(W) | Research(R) | Rese. ◀ ▶

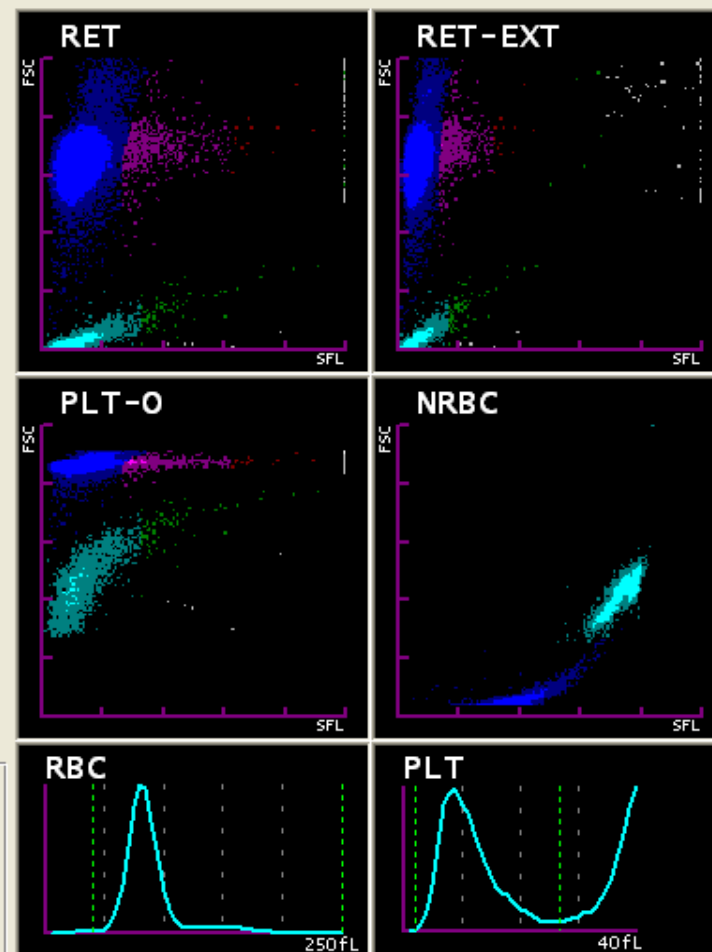
Item	Data	Unit
RBC	5.55 +	10 ⁶ /uL
RBC-O	5.54	10 ⁶ /uL
HGB	16.3	g/dL
HCT	45.3	%
MCV	81.6 -	fL
MCH	29.4	pg
MCHC	36.0	g/dL
RDW-CV	13.5	%
PLT	214	10 ³ /uL
PLT-I	214	10 ³ /uL
PLT-O	240	10 ³ /uL
MPV	11.4	fL
P-LCR	37.2	%
RET#	0.0561	10 ⁶ /uL
RET%	1.01	%
IRF	2.5	%
LFR	97.5	%
MFR	2.3	%
HFR	0.2	%
FRC%	0.06	%
IPF#	6.0	10 ³ /uL
H-IPF	0.6	%
IPF	2.8	%

Extended RET

Item	Data	Unit
RET-He	33.1	pg
RBC-He	29.2	pg
D-He	3.9	pg
RET-Y	176.8	ch
RBC-Y	164.3	ch
TRF-Y	187.1	ch
RPI	1.0	%
RET%	1.01	%

Normal Ranges Derived
at Hinduja Hospital

- **IPF - 0.7- 4.3%**



15/f with petechial rash and thrombocytopenia - 17×10^3 ,

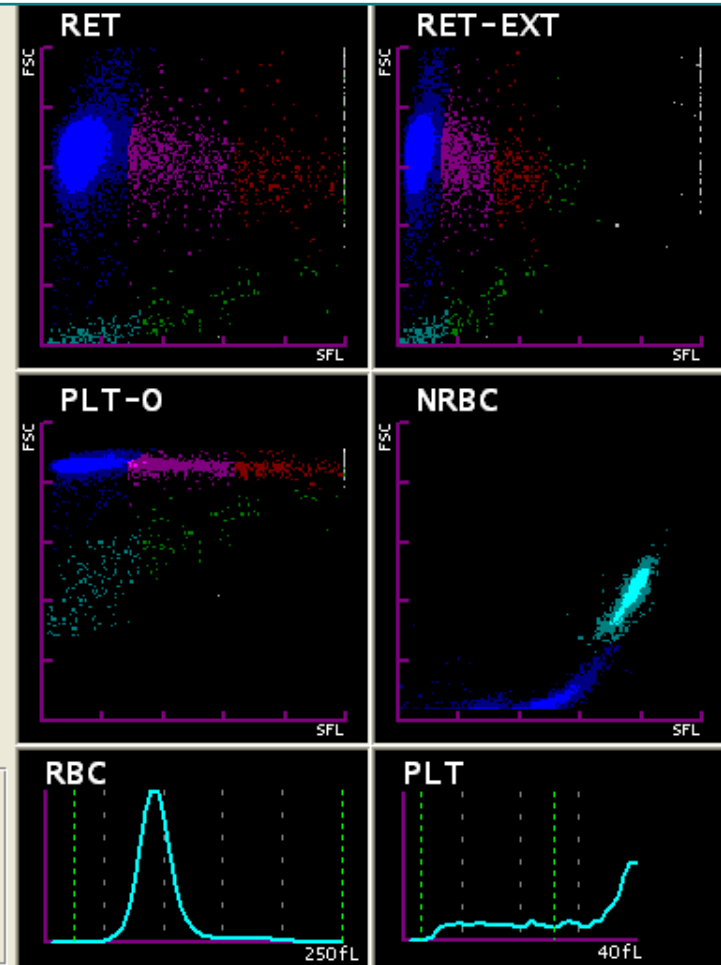
IPF-31.4%

Item	Data	Unit
RBC	3.11	$10^6/\mu\text{L}$
RBC-O	3.08	$10^6/\mu\text{L}$
HGB	9.2	g/dL
HCT	28.8	%
MCV	92.6	fL
MCH	29.6	pg
MCHC	31.9	g/dL
RDW-CV	14.5	%
PLT	17	$10^3/\mu\text{L}$
PLT-I	11	$10^3/\mu\text{L}$
PLT-O	17	$10^3/\mu\text{L}$
MPV	----	fL
P-LCR	----	%
RET#	0.0796	$10^6/\mu\text{L}$
RET%	2.56	%
IRF	21.4	%
LFR	78.6	%
MFR	17.4	%
HFR	4.0	%
FRC%	0.15	%
IPF#	5.3	$10^3/\mu\text{L}$
H-IPF	18.6	%
IPF	31.4	%

Item	Data	Unit
RET-He	27.0	pg
RBC-He	29.7	pg
D-He	-2.7	pg
RET-Y	156.1	ch
RBC-Y	165.8	ch
IRF-Y	147.3	ch
RPI	0.9	%
RET%	2.56	%

Normal Ranges Derived at Hinduja Hospital

- **IPF**
0.9-5.6%, mean-2.35%



Dengue NS1 Ag positive – KHAR HNH

Platelet - 27000, IPF – 26.1%

Graph WBC/NRBC RBC/PLT Cumulative Q-Flags Service HPC Research(W) Research(R) Research(P)

Items WBC Differential Flag(s) DIFF WBC/BASO

Item	Data	Unit
WBC	5.03	10 ³ /uL
RBC	2.10	10 ⁶ /uL
HGB	7.3	g/dL
HCT	22.8	%
MCV	108.6	fL
MCH	34.8	pg
MCHC	32.0	g/dL
PLT &	27	10 ³ /uL
RDW-SD	70.2	fL
RDW-CV	18.8	%
PDW	----	fL
MPV	----	fL
P-LCR	----	%
PCT	----	%
RET%	0.29	%
RET#	0.0061	10 ⁶ /uL
IRF	21.9	%
LFR	78.1	%
MFR	14.1	%
HFR	7.8	%
NRBC#		10 ³ /uL
NRBC%		/100WB

Parameters Measured

- Platelet count
- IPF

Within 6 hrs of receiving the sample

Item	Data	Unit
IG#		10 ³ /uL
IG%		%

Item	Data	Unit
HPC#		10 ³ /uL

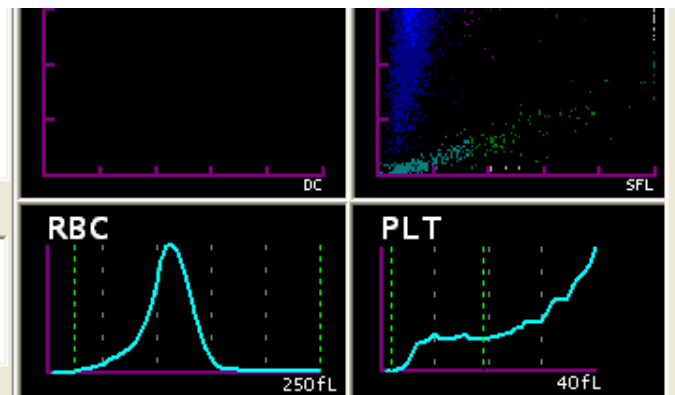
Extended Parameters

Item	Data	Unit
RET He	55.3	pg
IPF	26.1	%

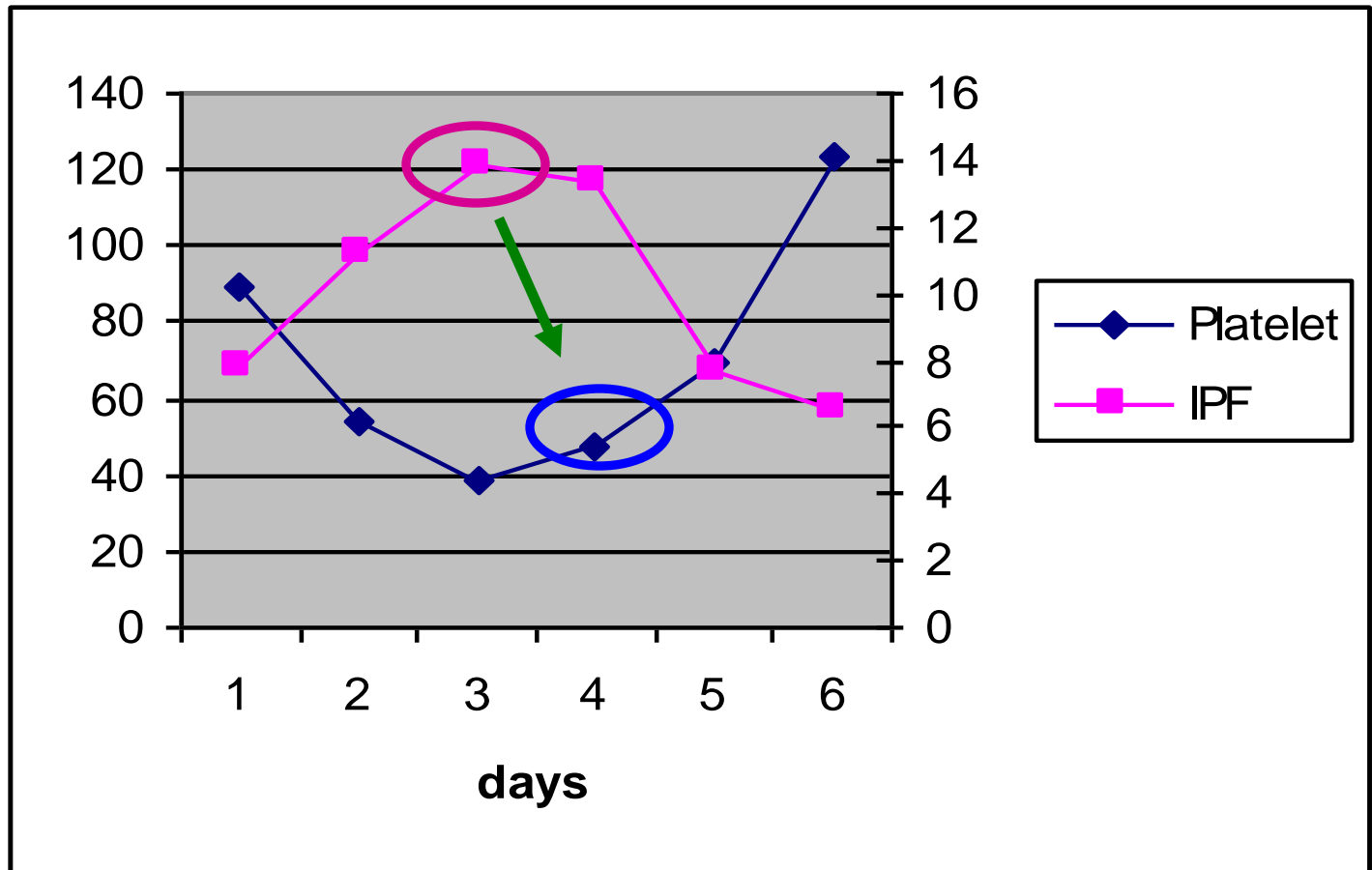
Macro Anemia

PLT

PLT Abn Dst
Thrombo-



DAYS	1	2	3	4	5	6
PLATELETS	89	54	39	47	70	123
IPF	7.8	11.2	13.9	13.3	7.7	6.5



IPF in Dengue

- IPF IS A POTENTIAL TOOL FOR PREDICTING PLATELET RECOVERY IN DENGUE PATIENTS HAVING THROMBOCYTOPENIA.
- A SINGLE VALUE OF $>10\%$ IS INDICATIVE OF PLATELET RECOVERY WITHIN 24-48 HRS

Summary

- Many new parameters have been added to the conventional CBC.
- Increased sensitivity and precision (e.g., IGs, nRBCs).
- Parameters like Ret-He do not have another comparable/ manual method, making such a parameter invaluable
- Improved Turn around Time

Summary

- IPF gives insight into the pathogenesis of thrombocytopenia. Can help avoid unnecessary BM examination and platelet transfusions.
- Newer parameters add a lot of extra information to the standard CBC, which may translate into better patient care.
- Novel Parameters still need to be standardised across instruments and labs should make their own normal ranges before using them



Thank You