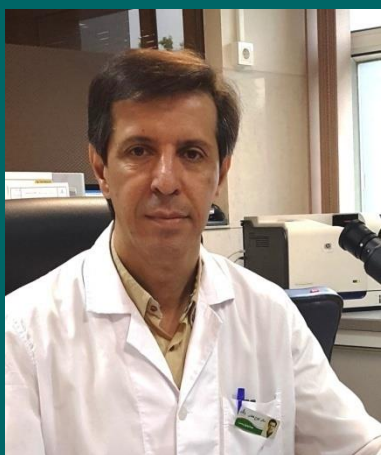


INTERPRETATION OF GRAPHS AND RESULTS OF ADVANCED HEMATOLOGY ANALYSERS PART-1



23th, Nov. 2022

Time: 9:00-13:00

Dr. Toraj Hemmati, CLS PhD
Hematology and Transfusion Medicine Specialist,
Emam Hosein Hospital

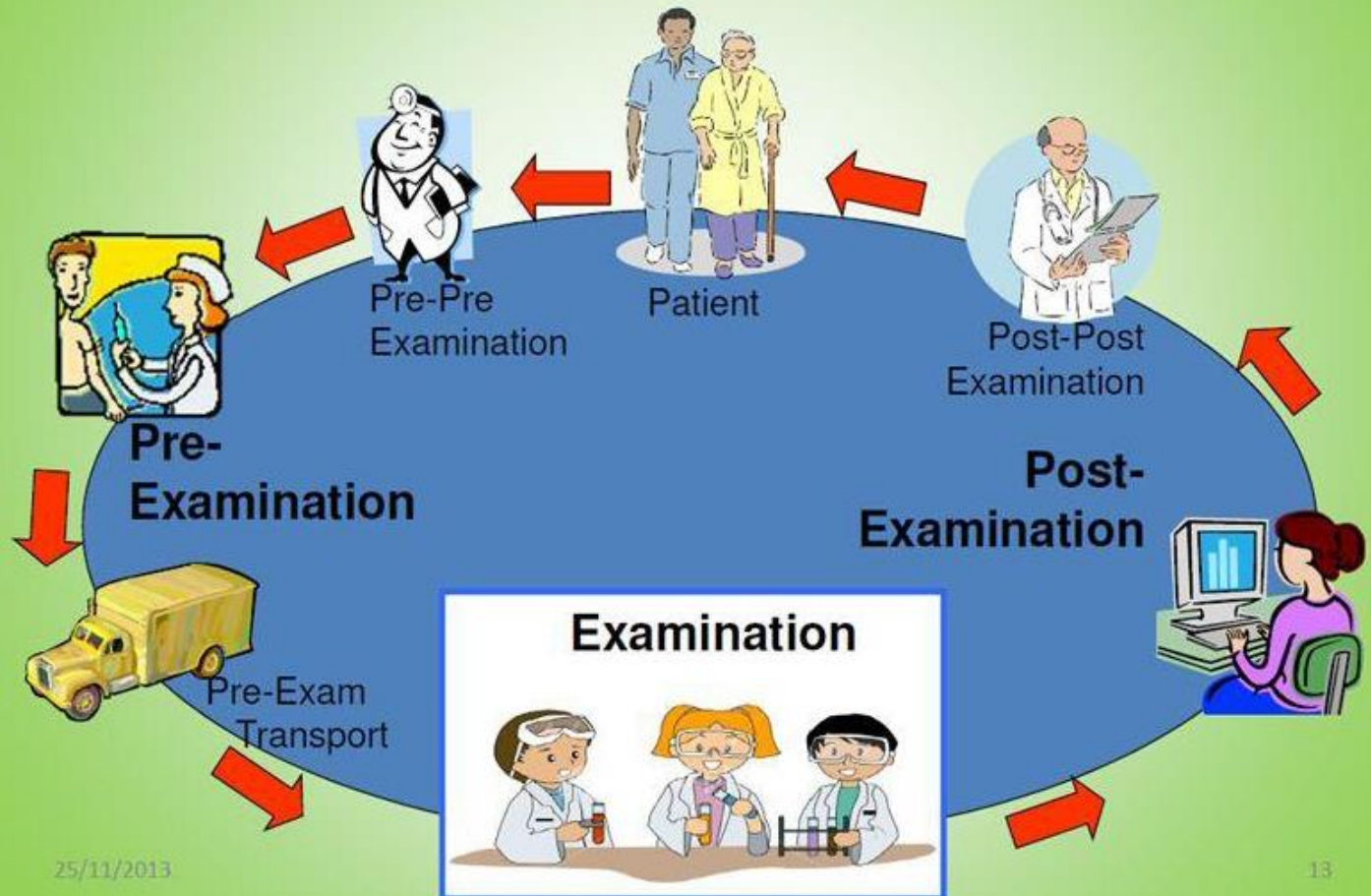
Factors affecting the outcome of a test

Analytical (8-15%)

Postanalytical (15-25%)

Preanalytical (60-70%)

The Laboratory Cycle



1 HISTOGRAM GRAPH IS WORTH 1000 NUMBER

BENIE CONSTANTINO,
CML HEALTHCARE INC., ONTARIO, CANADA

What looks like a CBC in lab??

Name: abolfazl poor

Patient ID: 4546

Sample No.: 4546

Birth 69(Age)

Date : 2019/06/03

Time : 08:03:39

Sex :

Ward :

Dr. :

Parameters

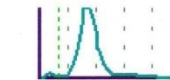
WBC 12.94 + [10³/uL]
 RBC 3.34 - [10⁶/uL]
 HGB 9.1 - [g/dL]
 HCT 28.3 - [%]
 MCV 84.7 [fL]
 MCH 27.2 [pg]
 MCHC 32.2 [g/dL]
 RDW-SD 48.6 [fL]
 RDW-CV 16.3 + [%]

PLT 362 [10³/uL]
 PDW 12.4 [fL]
 MPV 10.1 [fL]
 P-LCR 26.3 [%]
 PCT 0.37 + [%]

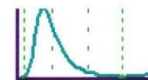
NEUT 9.62 * [10³/uL] 74.2 * [%]
 LYMPH 2.00 [10³/uL] 15.5 - [%]
 MONO 1.29 + [10³/uL] 10.0 + [%]
 EO 0.02 * [10³/uL] 0.2 * [%]
 BASO 0.01 [10³/uL] 0.1 [%]

Normal Ranges RBC

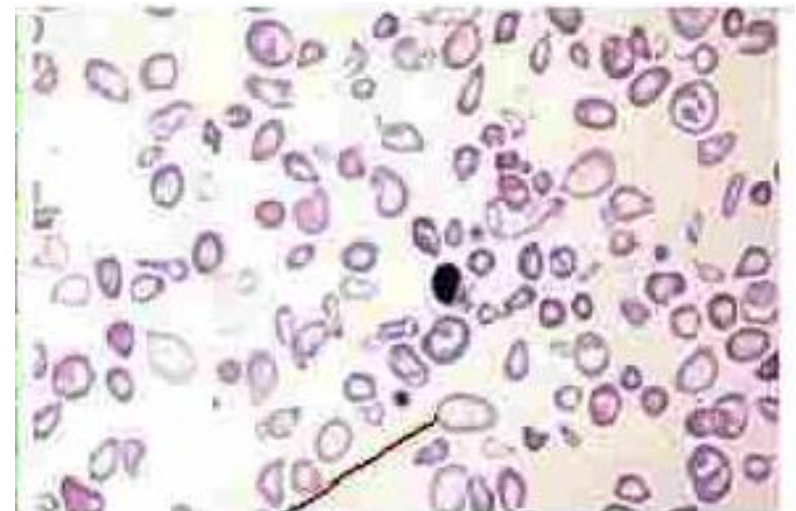
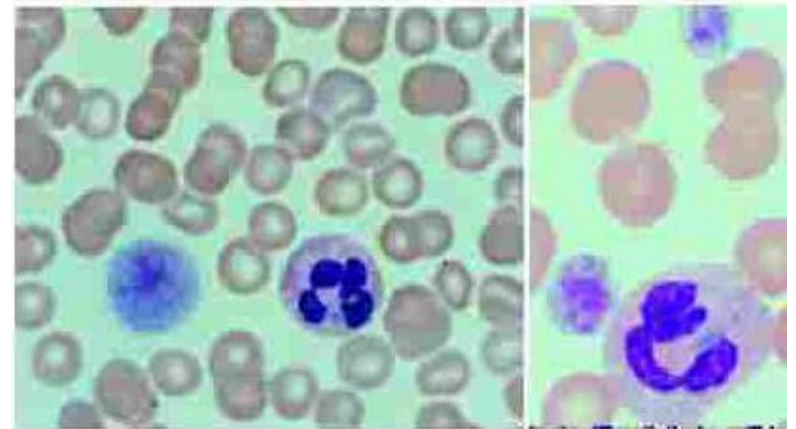
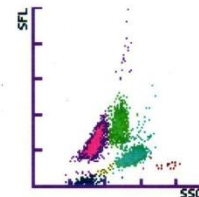
(4.50 - 11.00)
 (4.00 - 5.50)
 (12.5 - 17.5)
 (38.0 - 53.0)
 (80.0 - 97.0)
 (26.0 - 34.0)
 (31.0 - 36.0)
 (37.0 - 54.0)
 (11.0 - 14.5)
 (150 - 450)
 (9.0 - 14.0)
 (9.0 - 12.0)
 (13.0 - 43.0)
 (0.17 - 0.35)



PLT



DIFF



WBC IP Message(s)

RBC IP Message(s)

PLT IP Message(s)

Monocytosis
 Leukocytosis

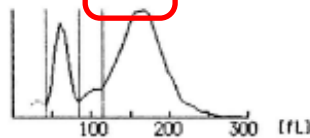
Anisocytosis
 Anemia

Left Shift?

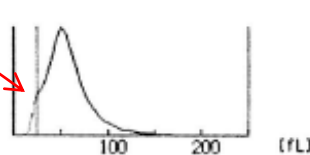
11/22/2022

What looks like a CBC in lab??

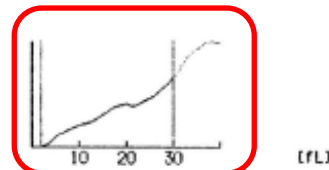
WBC		$5.8 \times 10^3 / \mu\text{L}$
RBC	RL*	$5.65 \times 10^6 / \mu\text{L}$
HGB	-	8.4g/dL
HCT	RL*	32.5%
MCV	RL*	57.5fL
MCH	RL*	14.9pg
MCHC	RL*	25.8g/dL
PLT	PLI	$1884 \times 10^3 / \mu\text{L}$



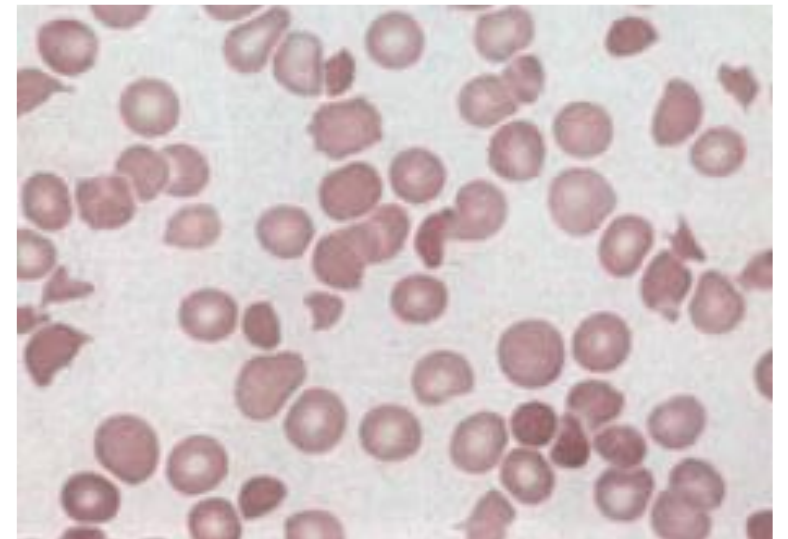
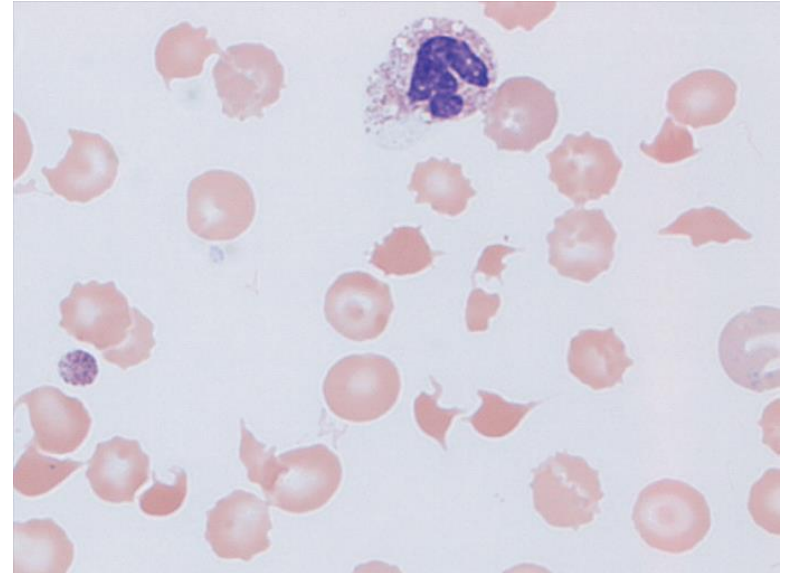
LVM%	21.0%
MXD%	7.2%
NEUT%	71.8%
LVM#	$1.2 \times 10^3 / \mu\text{L}$
MXD#	$0.4 \times 10^3 / \mu\text{L}$
NEUT#	$4.2 \times 10^3 / \mu\text{L}$



RDW RL* 32.3%



PDW	DW	---	-fL
MPV	PU	---	-fL
P-LCR	PU	---	-%



New Emerging Hematology Parameters

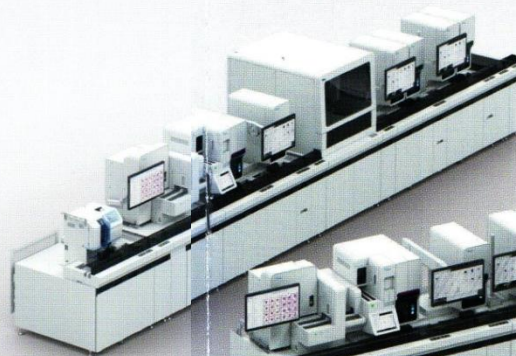
- CHCM
- CHr
- PCT
- P-LCR
- P-LCC
- MPX
- WBC-P
- IG
- IMG
- LFR
- MFR
- HFR
- IRF
- LUC
- PLT-O
- HDW
- IPF
- Ret-He
- ANC/AGC
- PLT-F

Tailoring your hematology

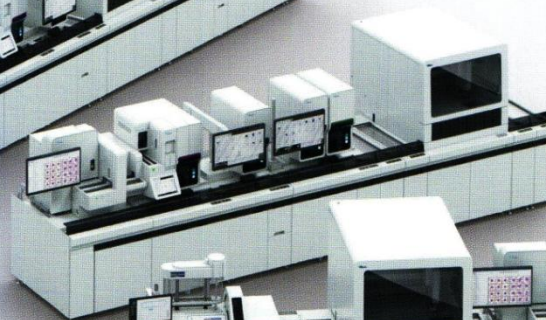
Productivity



XN-1000



XN-9100 Maximum Workload



XN-9100 Workload Balance



XN-9100 Sorting & Archiving



XN-3100

XN-9100 Compact Integration

XN-1000 Pure



XN-1500



XN-2000



* DI-60 is a registered product of CellaVision AB - www.cellavision.com
**TOSOH HLC-723 G11 Analyser is a registered product of Tosoh Corporation (Japan) - www.tosoh.com
***Interriner XN is a registered product of RR Mechatronics (The Netherlands) - www.rrmechatronics.com

Types of hematology analyzers & their development process

از دیدگاه تکاملی آنالیزهای هماتولوژی را می‌توان به سه دسته و پنج نسل تقسیم نمود:

- ۱- **سل‌کانترهای نیمه اتوماتیک** مثل سل کانتر CBCs که در این دستگاه مرحله رقیق سازی در خارج از دستگاه و به روش دستی انجام گرفته و سپس برای شمارش و آنالیز نهایی به دستگاه داده می‌شود.
 - ۲- **سل‌کانترهای اتوماتیک**: این دستگاه‌ها بین ۸ تا ۱۸ پارامتر هماتولوژی را اندازه‌گیری و محاسبه نموده و قادر هستند شمارش افتراقی سه قسمتی لکوسیت ها را انجام دهند که از این جهت به آنها 3Part هم گفته می‌شود. بیشتر سل کانترهای موجود در بازار ایران را این دستگاه‌ها تشکیل می‌دهند که از جمله آنها می‌توان سیستم‌های سری K, Helena, Hycell, کولتر S-Plus, Mindray و غیره را نام برد.
 - ۳- **سل‌کانترهای تمام اتوماتیک**: این دستگاه‌ها قادرند تا ۳۲ پارامتر هماتولوژی را اندازه‌گیری و محاسبه نمایند. همچنین امکان شمارش افتراقی پنج تا هفت قسمتی لکوسیت ها را نیز فراهم می‌آورند. از جمله این سل کانترها می‌توان تکنیکون‌های سری H1 و Adiva 120, سیستم‌های سری XE-2100 و XT-2000i, کولترهای LH750 و GEN-S و نهایتاً سل کانترهای Abbott-Cell Dyn اشاره نمود.
- سل کانترهای هماتولوژی شمارش تام و افتراقی سلول‌های خونی را براساس ویژگی‌هایی نظیر اندازه، تراکم، غلظت و حجم سلولی، تراکم و لوبولاریتی هسته، خواص بیوشیمیایی و آنزیماتیک سیتوپلاسم، رسانایی الکتریکی سلول، وضعیت گرانول‌ها و پراکنش نور تابشی به آنها انجام می‌دهند. از نظر اصول کار و روش ارزیابی سلول‌ها، دو روش عمده در سل کانترهای هماتولوژی وجود دارد [۱۱]:
- الف- تغییر در هدایت الکتریکی (امپدانس) همراه یا بدون کاپاسیتانس (یا رادیوفرکانس)
 - ب- روش‌های اپتیکال یا نوری که خود به سه دسته MAPSS (مثل Cell Tac و Cell Dyne), PANDA (مثل H1-3 و Advia), Fluorescence (مثل سیستم‌های میندری) و LARC (مثل سیستم کوباس و کورنینگ) تقسیم می‌شوند.

Kinds of hematology analyzer we have in the hematology Lab.



球計数装置 KX-21

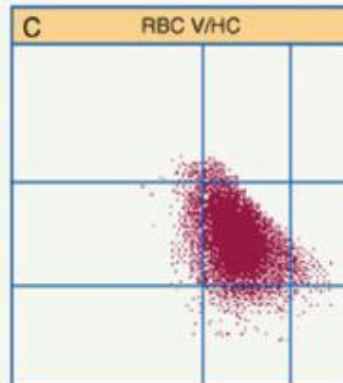
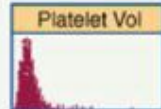
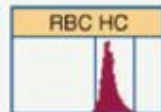
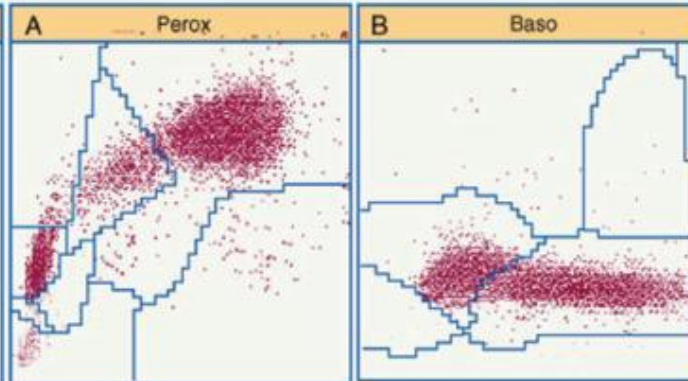
An example of a CBC result from the advanced Advia 120R cell counter

Header - WB	
SID: 4004	
Time & Date: 5/25/05 10:20:38AM	
Selectivity: CBC\DIFF\RETIC	
Sample Type: PATIENT	
Sex & Age:	
System: 30810242	
Sequence: 613	
Species: Human	
FOR LABORATORY USE ONLY	

Routine CBC		
WBC:	8.36	$\times 10^3$ CELLS/ μ L
RBC:	4.30	$\times 10^6$ CELLS/ μ L
HGB:	12.5	g/dL
HCT:	38.0	%
MCV:	88.4	fL
MCH:	29.1	pg
MCHC:	32.9	g/dL
CHCM:	32.7	g/dL
CH:	28.7	pg
RDW:	14.2	%
HDW:	2.32	g/dL
PLT:	332	$\times 10^3$ cells/ μ L
MPV:	7.4	fL

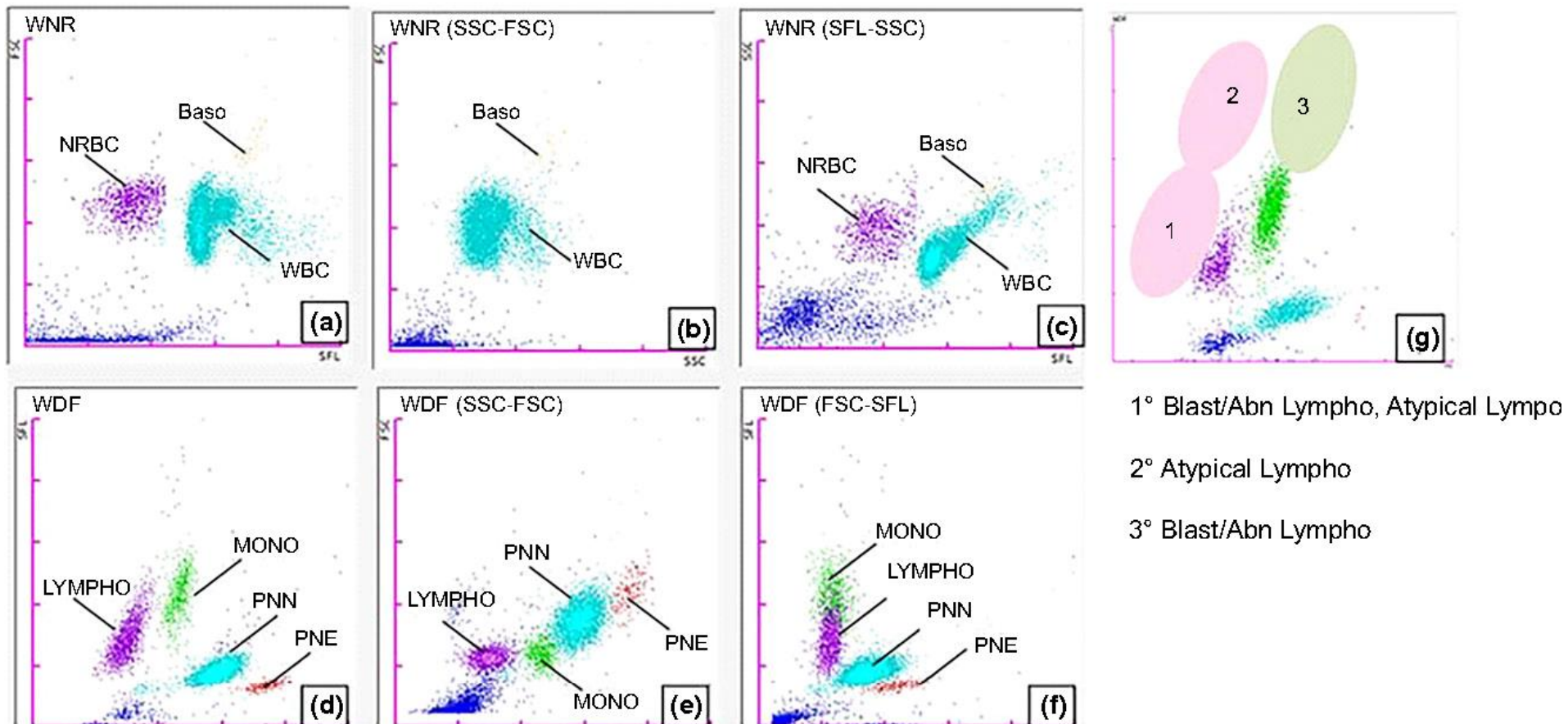
Routine WBC Differential		
	%	$\times 10^3$ cells/ μ L
WBC:		8.36
Neut:	69.3	5.79
Lymph:	21.7	1.81
Mono:	6.5	0.55
EOS:	0.9	0.08
Baso:	0.3	0.03
LUC:	1.2	0.10
LI:		1.96
MPXI:		-4.3
WBCP:	11/22/2022	8.88

Morphology Flags
MICRO:
MACRO:
HYPO:
HYPER:
ANISO:
HC VAR:
LEFT SHIFT:
BLASTS:
ATYPS:
NRBC:
IG:
LARGE PLT:
PLT CLUMPS:
MPO DEF:
RBC FRAG:
RBC GHOSTS:



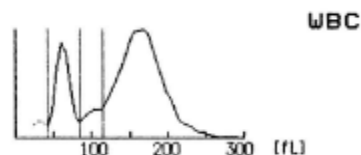
Morphology Flags
MICRO:
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ANISO:
HC VAR:
LEFT SHIFT:
BLASTS:
ATYPS:
NRBC:
IG:
LARGE PLT:
PLT CLUMPS:
MPO DEF:
RBC FRAG:
RBC GHOSTS:

An example of a CBC result from the advanced Sysmex XN 1000 cell counter

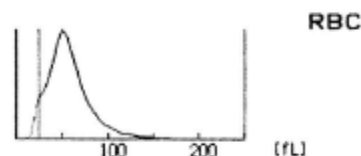


Report of an advanced cell counter vs partial diff one

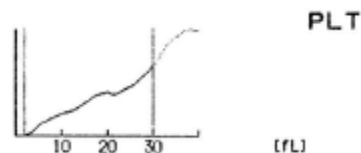
WBC $5.8 \times 10^9 / \mu\text{L}$
 RBC RL* $5.65 \times 10^6 / \mu\text{L}$
 HGB - 8.4 g/dL
 HCT RL* 32.5%
 MCV RL* 57.5 fL
 MCH RL* 14.9 pg
 MCHC RL* 25.8 g/dL
 PLT PU! $1884 \times 10^9 / \mu\text{L}$



LYM% 21.0%
 MXD% 7.2%
 NEUT% 71.8%
 LYM# $1.2 \times 10^9 / \mu\text{L}$
 MXD# $0.4 \times 10^9 / \mu\text{L}$
 NEUT# $4.2 \times 10^9 / \mu\text{L}$

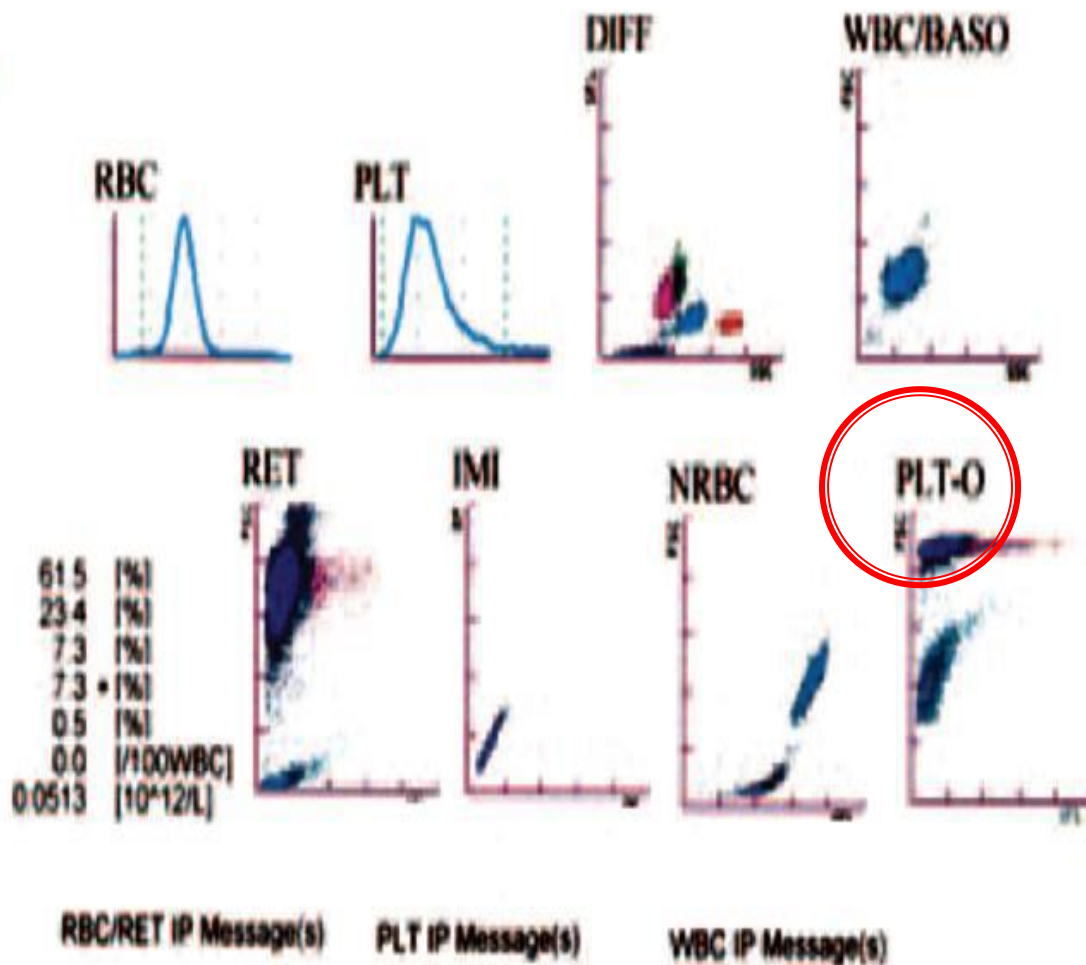


RDW RL* 32.3%



PDW DW ---, -fL
 MPV PU ---, -fL
 P-LCR PU ---, -%

WBC 7.51 [$10^9/\text{L}$]
 RBC 4.01 [$10^{12}/\text{L}$]
 HGB 13.2 [g/dL]
 HCT 39.6 [%]
 MCV 98.8 [fL]
 MCH 32.9 [pg]
 MCHC 33.3 [g/dL]
 PLT 178 [$10^9/\text{L}$]
 RDW-SD 51.9 [fL]
 RDW-CV 14.4 [%]
 PDW 15.8 [fL]
 MPV 12.9 [fL]
 P-LCR 49.3 [%]
 PCT 0.23 [%]
 NEUT 4.61 [$10^9/\text{L}$]
 LYMPH 1.76 [$10^9/\text{L}$]
 MONO 0.55 [$10^9/\text{L}$]
 EO 0.55 [$10^9/\text{L}$]
 BASO 0.04 [$10^9/\text{L}$]
 NRBC 0.00 [$10^9/\text{L}$]
 RET 1.28 [%]
 IRF 7.5 [%]
 LFR 92.5 [%]
 MFR 7.1 [%]
 HFR 0.4 [%]



شکل ۲۴-۱: نمونه‌ای از یک گزارش نهایی سیستم XE-2100

Advanced Clinical Hematology Parameters

What are these *****NEW***** tests?

White Blood Cells

IG (Immature Granulocyte)

Advanced
Clinical
Parameters

Red Blood Cells

Retic-Comprehensive

- 1) Retic
- 2) IRF (Immature Retic Fraction)
- 3) RET-He** (Reticulocyte Hemoglobin Equivalent)

NEW!

AUTOMATED!

Platelets

IPF (Immature Platelet Fraction)

Hematology is closer to the clinical setting than is thought.

IG

ITR and Neonatal Infection

- Infection / sepsis

**Advanced
Clinical
Parameters**

RET-He

- Anemia Management
- Improved Iron / EPO utilization
- Improved transfusion practice
- Prevent readmission

IPF

- Mechanism of Thrombocytopenia
- Decrease unnecessary bone marrow biopsies
- Improve transfusion practices

ITR: immature neutrophils to total neutrophils ratio

Hematology is closer to the clinical setting than is thought.

Blood component safety

The Blood Bank mode

- measures residual white and red blood cells in blood components.
- determines RBC and platelet concentrations in blood products.

The parameters

- HGB (haemoglobin)
- RET-He (reticulocyte haemoglobin equivalent)

help to evaluate the donor's blood status.

Donor safety

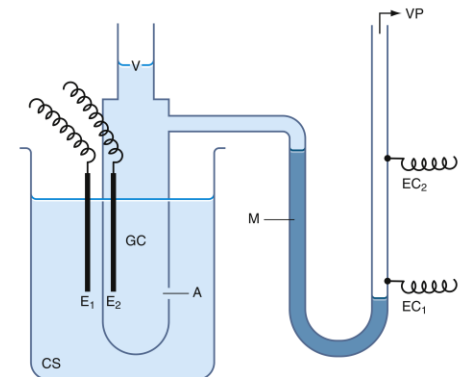
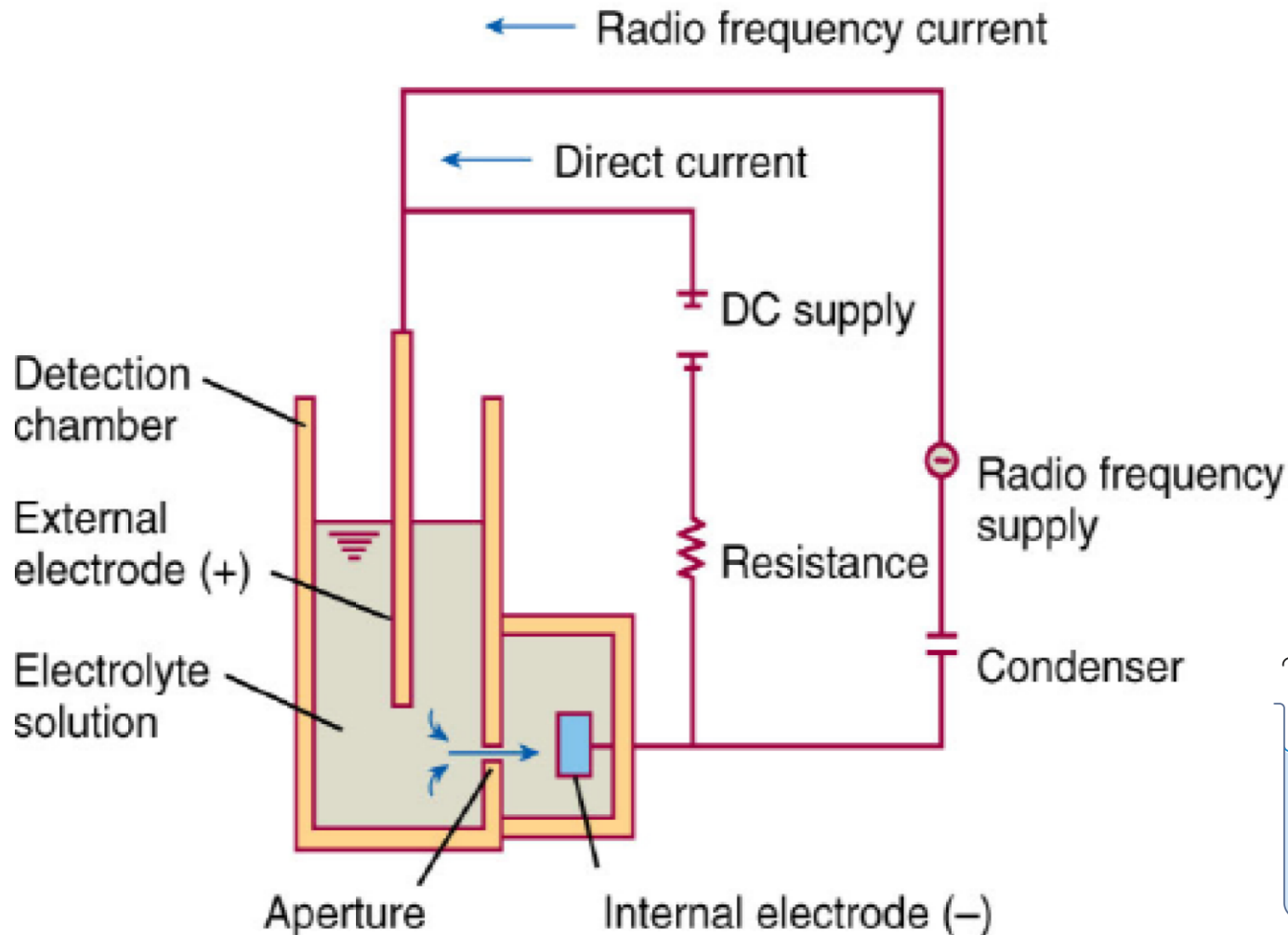


Patient care

The advanced clinical parameters

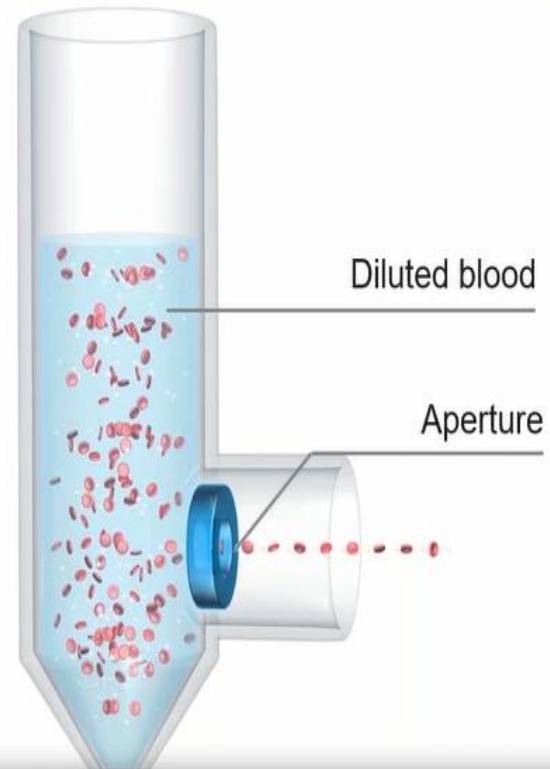
- RET-He
 - IPF (immature platelet)
- are used for monitoring the patients.

Mechanism of cell counting, cell volume determination and histogram formation



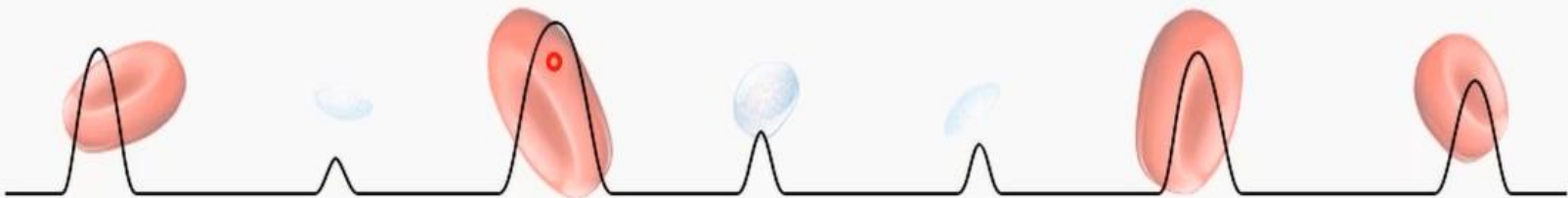
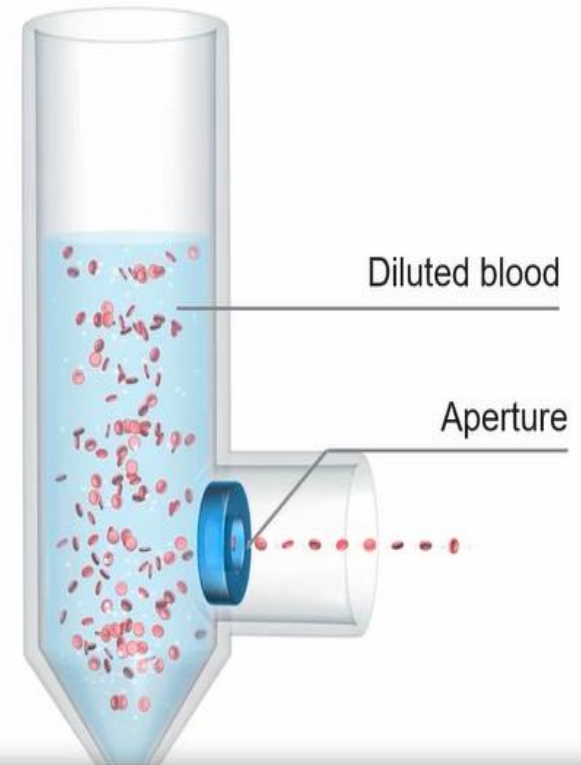
Volumetric Counting: DC detection method

- The cell-counting detector consists of an electrostatic field, limited by an aperture.
- When blood cells pass through the aperture, an electrolyte volume equivalent to that of the blood cell is displaced. This causes changes in resistance, which are detected as a measurable variable.



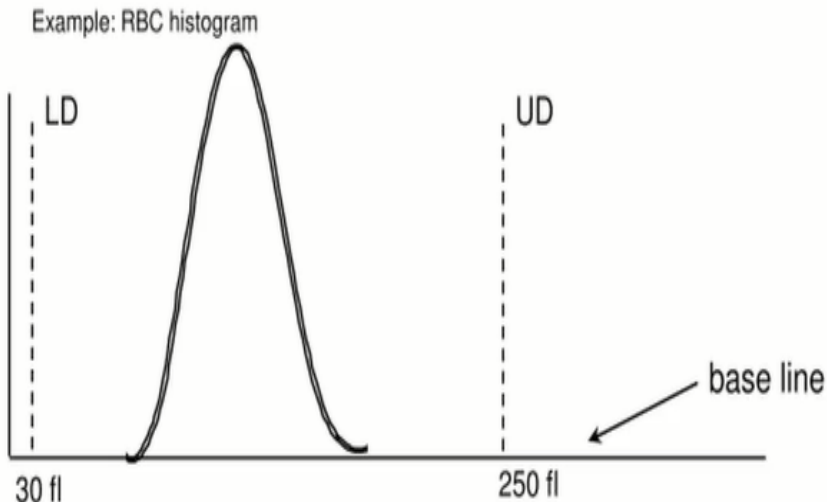
Volumetric Counting: DC detection method

- The cell-counting detector consists of an electrostatic field, limited by an aperture.
- When blood cells pass through the aperture, an electrolyte volume equivalent to that of the blood cell is displaced. This causes changes in resistance, which are detected as a measurable variable.



What is a Histogram & How is formed

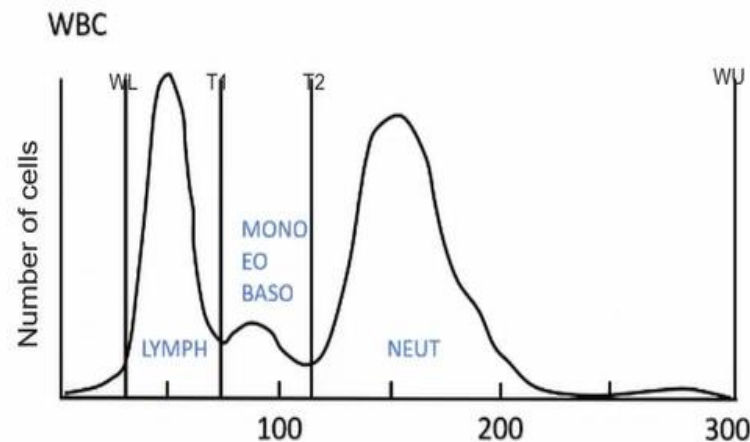
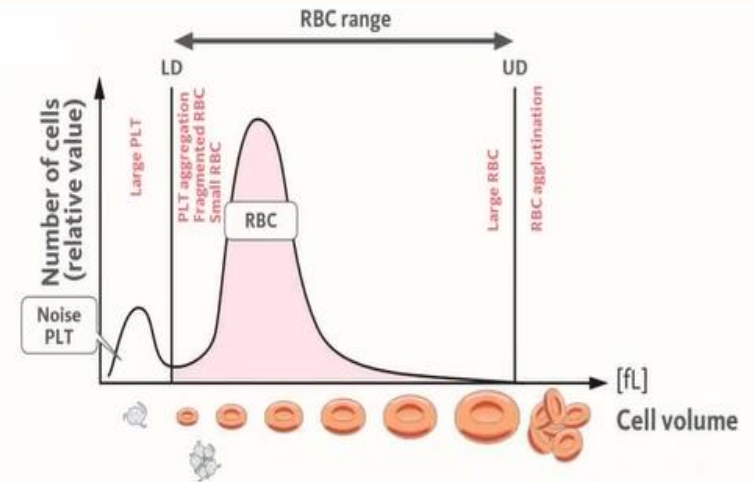
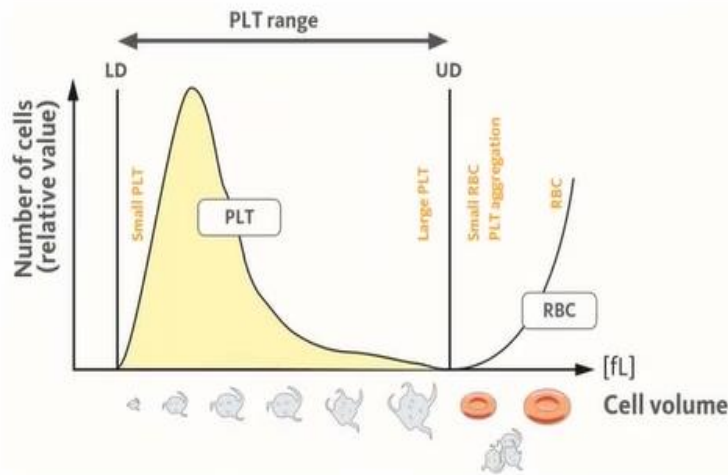
LD: lower discriminator
UD: upper discriminator



Histogram curves

- » Start and end at the baseline
- » Curve between upper and lower discriminator
- » PLT, RBC and WBC histograms

RBC, PLT and WBC Histograms



گلبول های قرمز و پلاکت در یک کانال شنارش می شوند و منحنی واحدی را ایجاد می کنند که برای سهولت بررسی بصورت ۲ منحنی توسط دستگاه ارائه می شوند.

Analysers counting channels

■ **کانال ۱:** شمارش RBC و PLT

■ Debris & Bacteria & Noise=0-2 fL

■ PLT=2-30 fL

■ RBC=25-200 fL

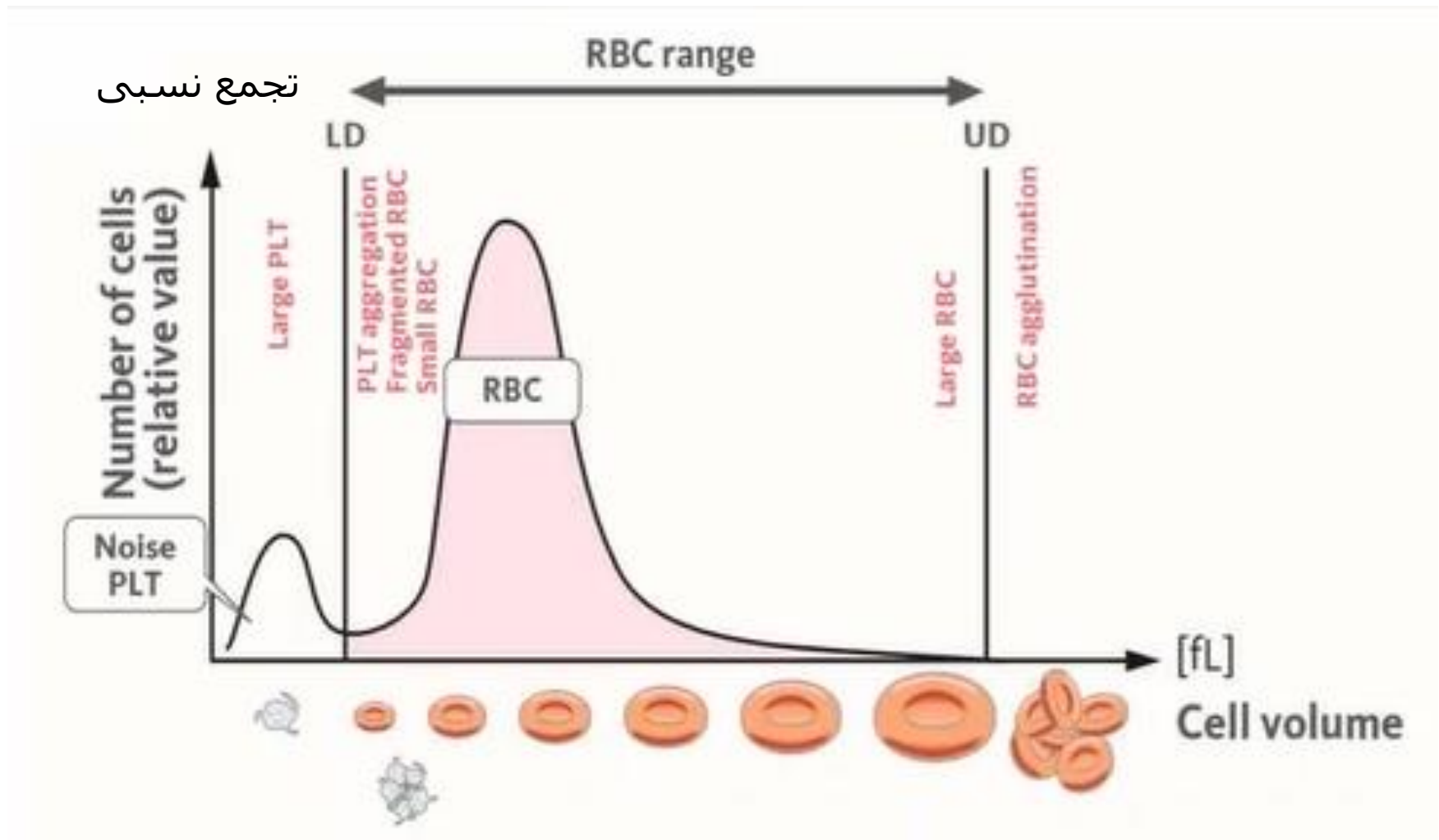
■ **کانال ۲:** شمارش WBC و Diff و اندازه گیری Hgb

■ Small cell(Lymph)=35-90 fL

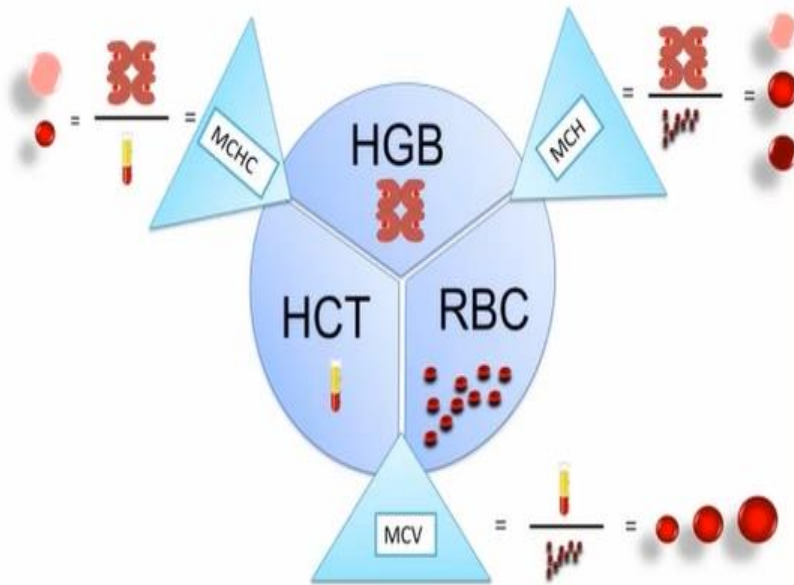
■ Mid cell=90-160 fL

■ Large cell=160-300 fL

RBC Histograms



Calculation of RBC Indices



Parameter	Function / meaning	Calculation
MCV (mean corpuscular volume)	Size or volume of one average RBC	$= \text{HCT} / \text{RBC}$
MCH (mean corpuscular haemoglobin)	Content of HGB in one average RBC	$= \text{HGB} / \text{RBC}$
MCHC (mean corpuscular haemoglobin concentration)	Average concentration of HGB in RBC	$= \text{HGB} / \text{HCT}$

Wintrobe indices

$$\text{MCV} = (\text{HCT}/\text{RBC}) \times 10$$

$$\text{MCH} = (\text{HGB}/\text{RBC}) \times 10$$

$$\text{MCHC} = (\text{HGB}/\text{HCT}) \times 100$$

Rutzky Rules

(Rule of 3)

Rule #1	$Hb \times 3 = Hct \pm 2$
Rule #2	$RBC \times 3.3 = Hgb \pm 1.5$
Rule #3	$RBC \times 9 = Hct \pm 3$
Rule #4	$MCH \times 3 = MCV \pm 3$

قوانین روتزکی فقط در افراد سالم ، طبیعی و بعضی موارد در افراد کم خون از نوع نورموسیت-
نورموکروم صدق می کند و برای کنترل اولیه نتایج و پیشگیری از خطا و اشتباهات دفتری
کاربرد دارد.

WINTROBE INDICES AND

THE RULES OF 3 (Rutzky Rules)

$$\text{MCV} = (\text{HCT}/\text{RBC}) \times 10$$

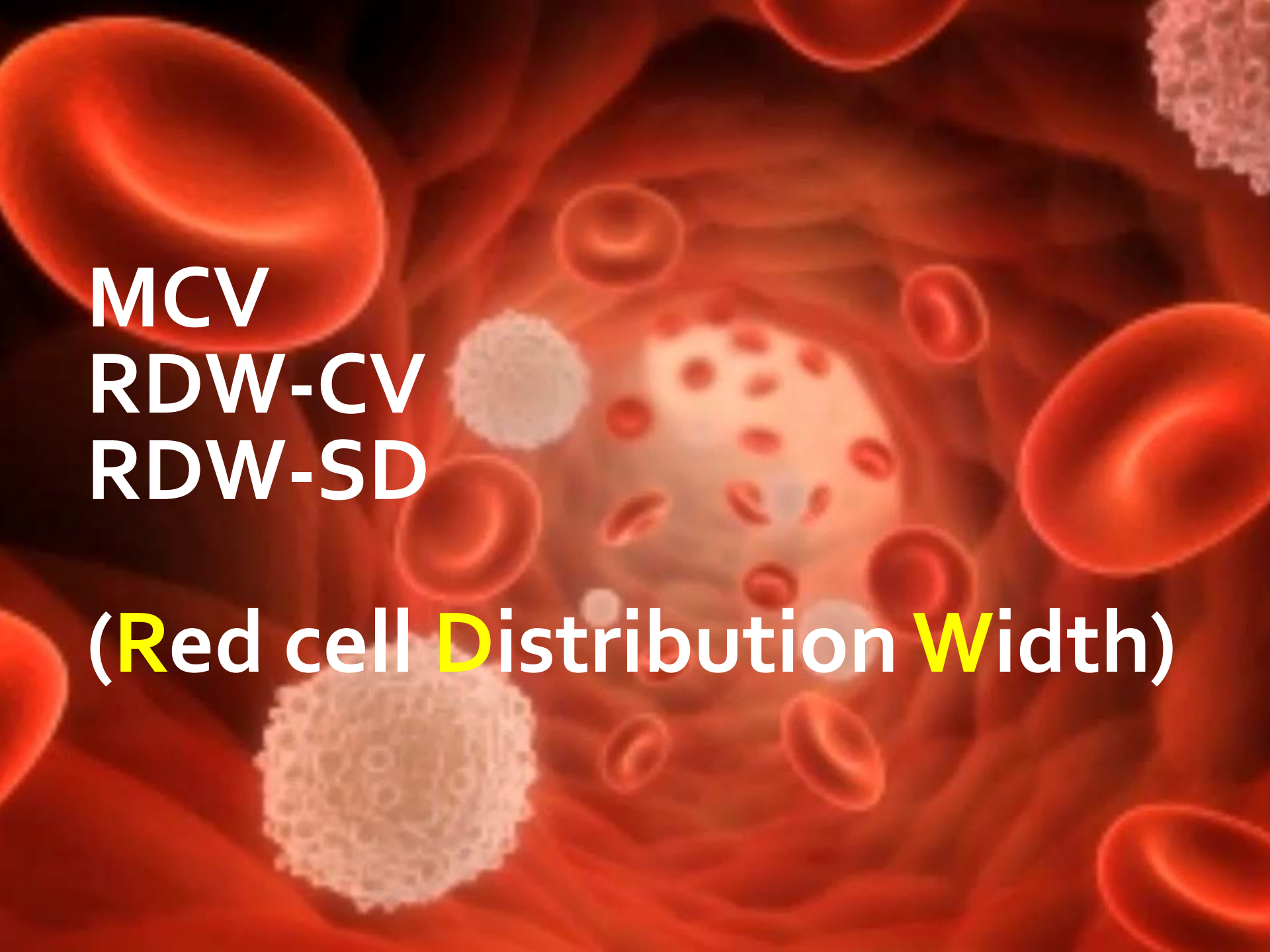
$$\text{RBC} \times 9 = \text{HCT} \quad \pm 3\%$$

$$\text{MCH} = (\text{HGB}/\text{RBC}) \times 10$$

$$\text{RBC} \times 3.3 = \text{HGB} \quad \pm 1.5 \text{ gm/dl}$$

$$\text{MCHC} = (\text{HGB}/\text{HCT}) \times 100$$

$$\text{HGB} \times 3 = \text{HCT} \quad \pm 3\%$$

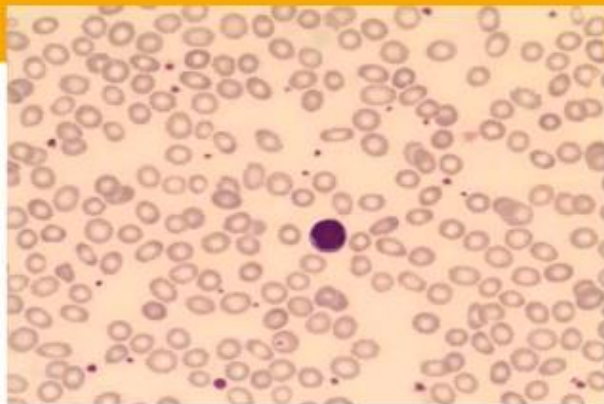
A microscopic view of blood cells, showing several red blood cells (erythrocytes) as biconcave discs and a few white blood cells (leukocytes) with visible nuclei. The background is a warm, reddish-orange color.

MCV
RDW-CV
RDW-SD

(Red cell Distribution Width)

MCV

- Definition :MEAN CELL VOLUME
- 80-94 FL
- <80 :MICROCYTE
- NEW BORN : 104-120 FL,,,less than 94 is considered microcytic of newborn
- <10 Y : 70 + AGE

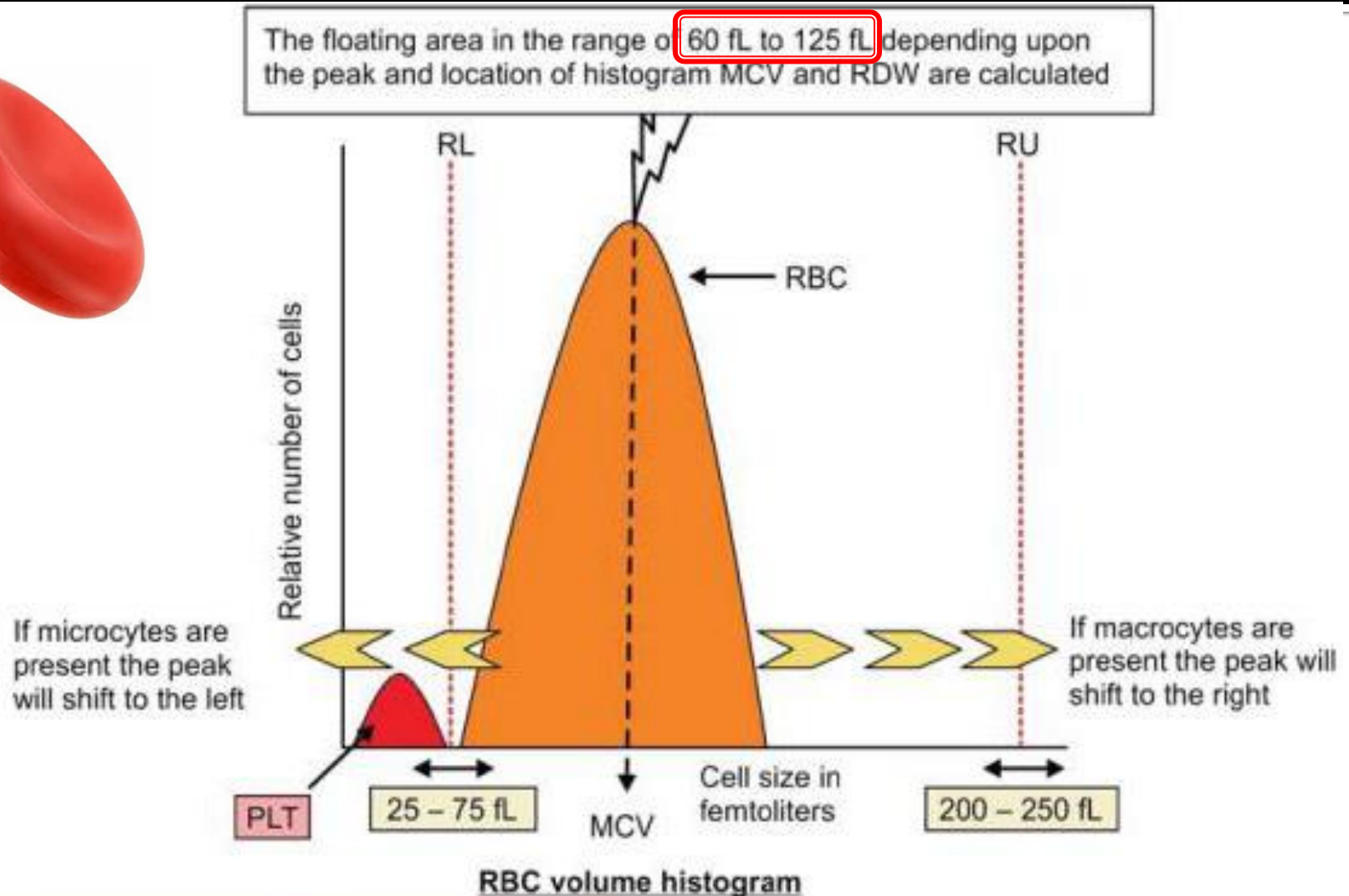


MCV & Age

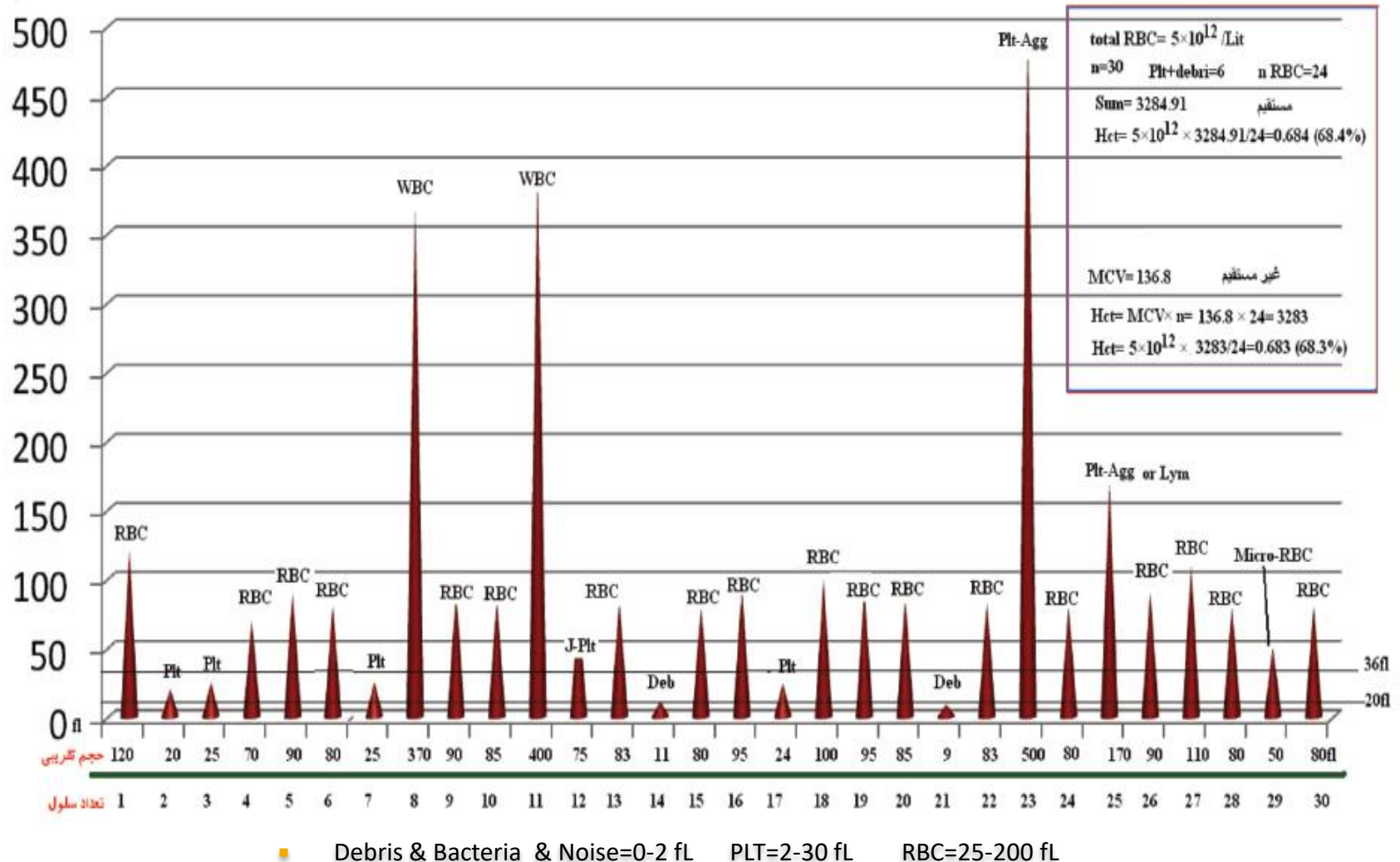
Age	Lowest Normal Hb (g/dL)	Normal Red Blood Cell Size Mean Corpuscular Volume (fL)	Fetal Hb (%)
Birth	14.0	100–130	55–90
1 mo	12.0	90–110	50–80
2 mo	10.5	80–100	30–55
3–6 mo	10.5	75–90	5–25
6 mo–1 yr	11.0	70–85	<5
1–4 yr	11.0	70–85	<2
4 yr–puberty	11.5	75–90	<2
Adult female	12.0	80–95	<2
Adult male	14.0	80–95	<2

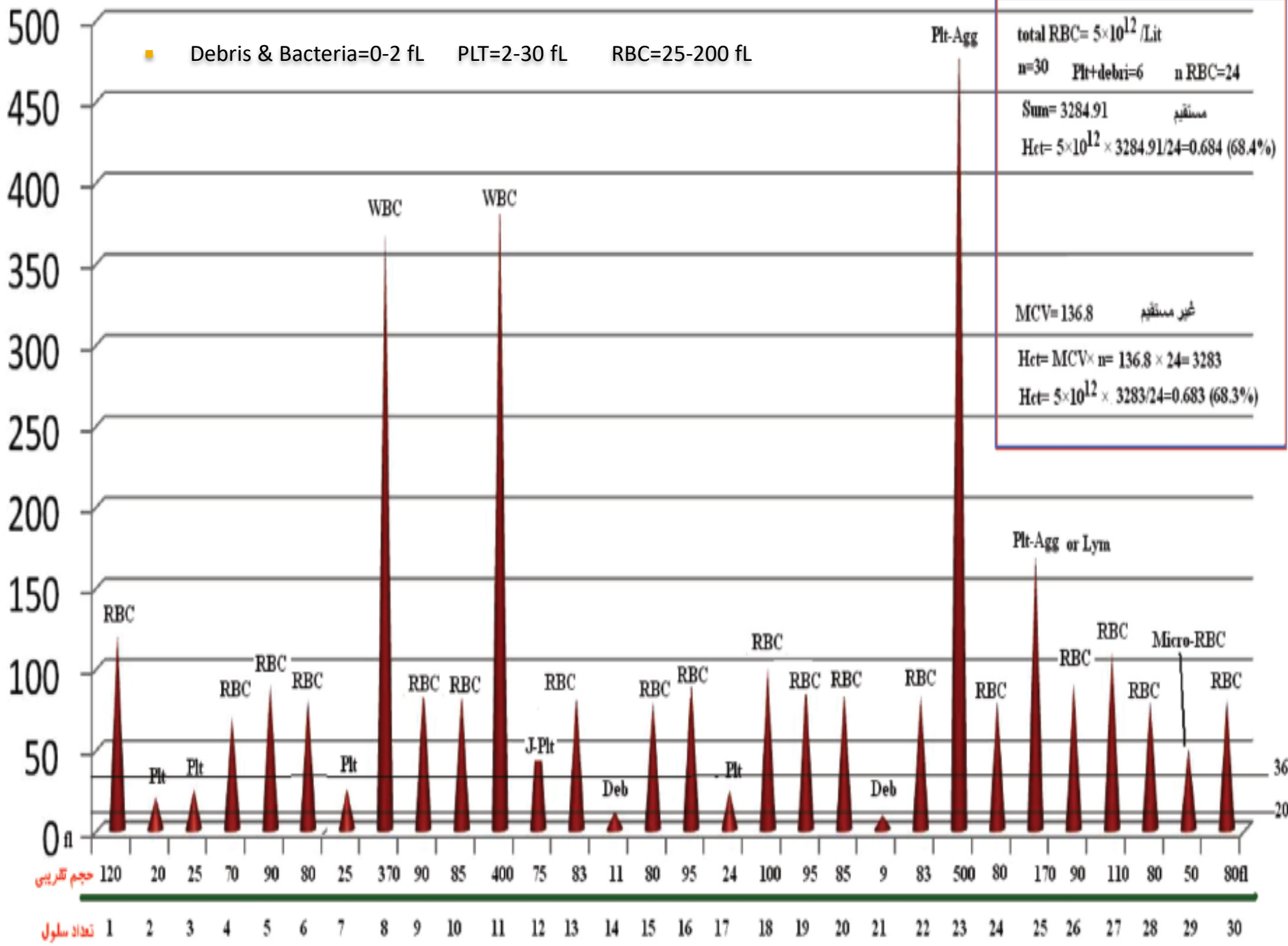
Hb > 22 g/dl is considered **neonatal polycythemia** and is dangerous because it slows down brain blood supply

MCV & RDW in Histogram



MCV & RBC Histogram

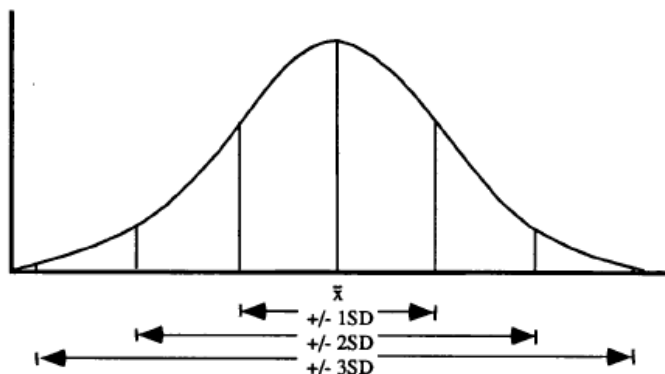




MCV & RDW in Histogram

دامنه یا پهنای انتشار مهم گلبول‌های قرمز (RDW):

حجم و اندازه گلبول‌های قرمز خون با هم برابر نبوده و هنگام گزارش نتایج نیز نمی‌توان حجم تک تک سلول‌ها را اعلام نمود، از این رو میانگین آنها را به صورت پارامتر MCV گزارش می‌کنند. اندازه سلول‌ها در شرایط طبیعی می‌تواند کمتر یا بیشتر از میانگین کل آنها باشد که در این صورت الگوی پراکندگی آنها از توزیع طبیعی زنگوله‌ای شکلی تبعیت می‌کند که به آن **نمودار گوسین (Gaussian Distribution)** گفته می‌شود. در این نمودار $\frac{68}{100}$ سلول‌ها در محدوده $\pm 1SD$ ، $\frac{95}{100}$ سلول‌ها در محدوده $\pm 2SD$ و $\frac{99}{100}$ سلول‌ها در محدوده $\pm 3SD$ قرار می‌گیرند که از نظر استانداردهای هماتولوژی محدوده $X \pm 2SD$ با ۵٪ احتمال خطا، به عنوان محدوده نرمال نتایج آزمایشات هماتولوژی مدنظر قرار گرفته و از خطای ۵ درصدی مذکور چشم‌پوشی می‌شود.



شکل ۵۴-۱: نمودار زنگوله‌ای شکل از توزیع طبیعی اریتروسیت‌ها در سه محدوده 1SD، 2SD و 3SD که از نظر استانداردهای هماتولوژی، ضریب اطمینان ۹۵٪ (معادل ضریب خطای ۵٪) با میزان خطای $\pm 2SD$ مطابقت دارد.

MCV در حقیقت یک میانگین بوده و لذا تغییرات و پراکندگی اعداد حول میانگین را نشان نمی‌دهد. به عبارتی، واحد میانگین، مقدار MCV سه سلول ۹۰، ۱۰۰ و ۱۱۰ فمتولیتري و مقدار MCV سه سلول ۹۹، ۱۰۰ و ۱۰۱ فمتولیتري را یکسان و برابر ۱۰۰ fl نشان می‌دهد ولی **انحراف معیار (SD)** سنجشی از **درجه پراکندگی** در یک جمعیت با پراکندگی نرمال (مثل جمعیت اریتروسیتی) است که در مثال فوق SD اولی ۱۰ و SD دومی ± 1 محاسبه می‌شود. همان‌طوری که مشاهده می‌شود، SD فقط مقدار پراکندگی را نشان می‌دهد و بزرگی آن را نشان نمی‌دهد. برای مثال $SD=2$ را در نظر بگیرید، عدد ۲ در برابر عدد ۱۰، رقمی بزرگ ولی در برابر عدد ۱۰۰، رقمی کوچک محسوب می‌شود، از این رو **برای بزرگی نسبی تغییرات فوق از پارامتر ضریب تغییرات (CV)** استفاده می‌شود که درصد SD از مقدار میانگین را نشان می‌دهد، مثلاً $CV=5$ یعنی اینکه، تغییرات SD از نظر بزرگی، حدود ۵٪ از مقدار میانگین را تشکیل می‌دهد.

RDW : Why we need to

- MCV

- RDW-CV

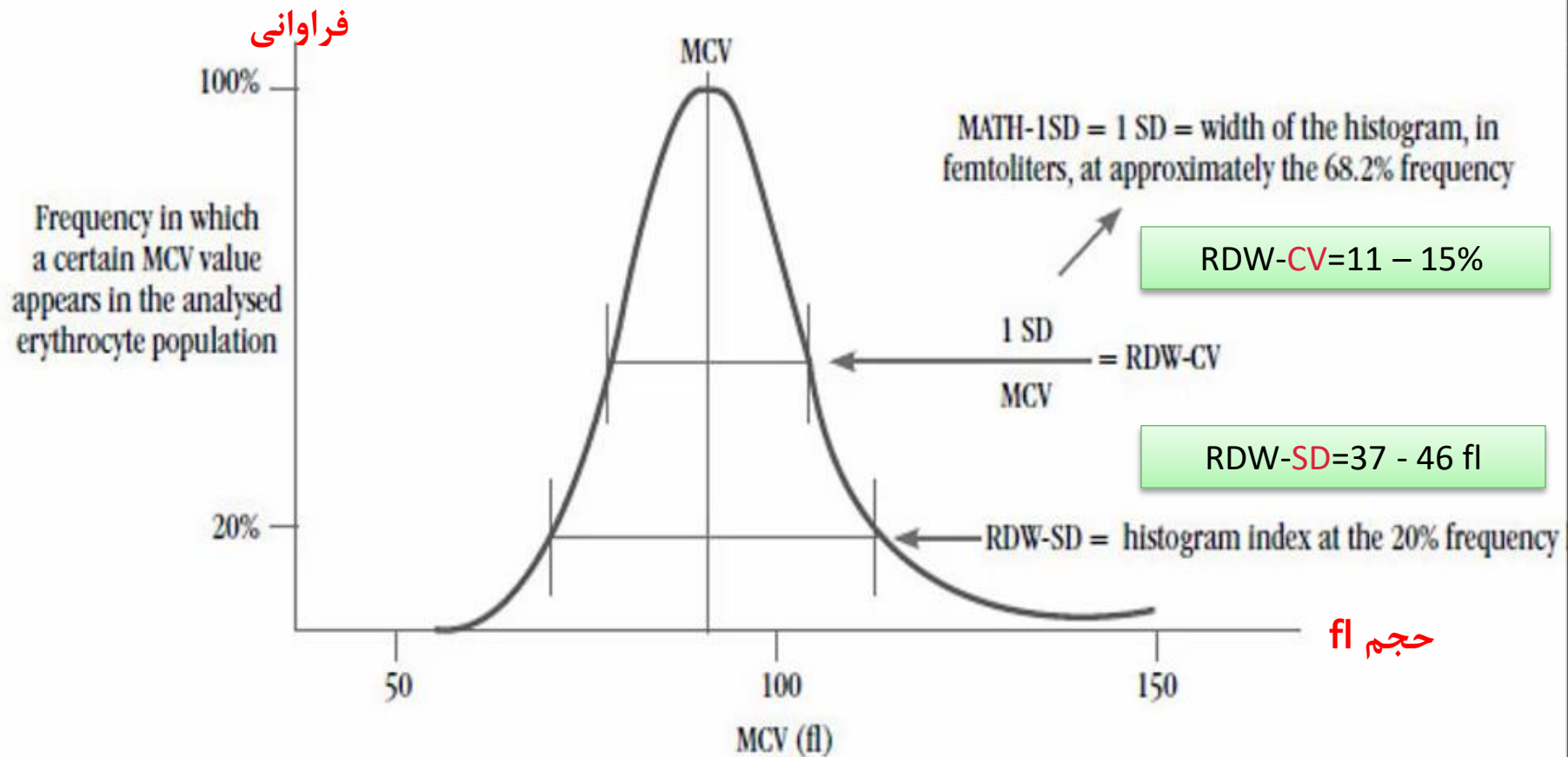
- RDW-SD

Red Cell Distribution Width

دامنه یا پهنای انتشار حجم گلبول‌های قرمز (RDW)

- Anisocytosis

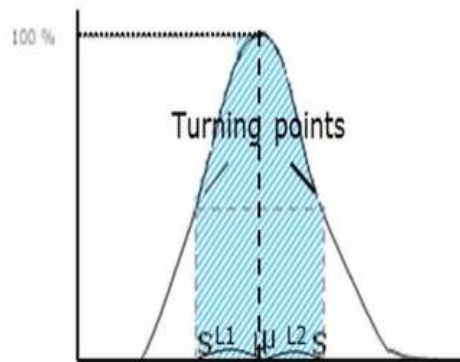
RDW (CV & SD)



شکل ۵۶-۱۰: در واقع RDW-SD به نوعی پهنای هیستوگرام RBC در ارتفاع یا فراوانی ۲۰٪ بوده و RDW-CV نسبت پهنای هیستوگرام RBC در فراوانی ۶۸٪ به مقدار MCV می باشد.

RBC Distribution Width-RDW

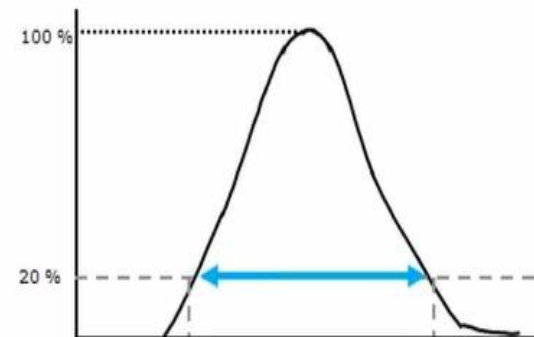
- RDW-CV: Red Blood Cell distribution width-Coefficient Variation



$$\text{RDW-CV} = \frac{L2-L1}{L2+L1} \times 1000$$

+/- single standard- deviation equivalent to 68.26 % of the distribution area.

- RDW-SD: Red Blood Cell distribution width-Standard Deviation



RDW-SD is calculated in 20 % of the total height of the distribution curve.

We need to MCV, both RDWs because:

- Considering that (**MCV**) only shows the **average** or mediocrity of a population if the **RDW** (SD & CV) is not taken into account, the **correct clinical interpretation** of the RBC volume distribution status is not obtained
- **RDW-SD** takes measurements in "fL" and basically measures the width of red cells size distribution histogram – it calculates the width at the **20% height level** of the histogram.
- **RDW-CV** is expressed in percentage (%) and is calculated from **MCV** and **1SD**
- The reference range for RDW is as follows:
 - RDW-SD 37-46 fL in adult RDW-SD 47-70 fL in neonates
 - RDW-CV 11-15% in adult RDW-CV 13.6-18.0% in neonates

نقص MCV در چیست؟ نقش RDW-SD چیست؟

نقص MCV در چیست؟ فرض کنید:

MCV=99, MCV=100, MCV=101..... $\text{Mean(MCV)}=100$ $\text{SD}\pm 1$

MCV=95, MCV=100, MCV=105..... $\text{Mean(MCV)}=100$ $\text{SD}\pm 5$

MCV=50, MCV=100, MCV=150..... $\text{Mean(MCV)}=100$ $\text{SD}\pm 50$

MCV=1, MCV=100, MCV=199.... $\text{Mean(MCV)}=100$ $\text{SD}\pm 99$

MCV چون میانگین را نشان می دهد بین این جمعیت ها نمی تواند فرقی قائل شود.

به کمک می آید **RDW-SD**

RDW-CV

5-10-15-----	mean(MCV)=10.....SD=5	CV= 50%
95-100-105-----	mean(MCV) 100.....SD=5	CV= 5%
995-1000-1005----	mean(MCV) 1000...SD=5	CV= 0.5%

آیا SD می تواند معیار کاملی باشد؟ خیر

پس ما به یک اندکس دیگر نیاز داریم تا نقص SD و MCV را پوشش دهد :

RDW-CV

Rules of using RDW & disadvantage of RDWs

• **ضعف RDW-SD** این است که زمانی که RBC افزایش می یابد ، RDW-SD بطور کاذب کاهش می یابد مانند موارد تالاسمی مینور

• **ضعف RDW-CV** این است که زمانی که MCV افزایش می یابد ، RDW-CV بصورت کاذب کاهش می یابد مانند آنمی های ماکروسیتیک و مگالوبلاستیک

$$SD = \sqrt{\frac{\sum (V - MCV)^2}{RBC}}$$

$$RDW-CV = (RDW-SD \times 0.28 \times 100) / MCV$$

قانون استفاده از RDW

RDW-CV در میکروسیتوز و نرموسیتوز معیار تشخیص آنیزو است

RDW-SD در ماکروسیتوز و مگالوسیتوز ($MCV > 100$) معیار آنیزو است

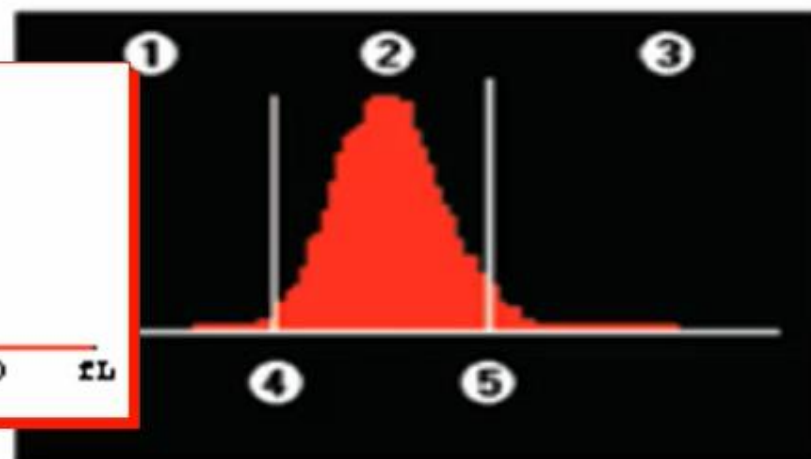
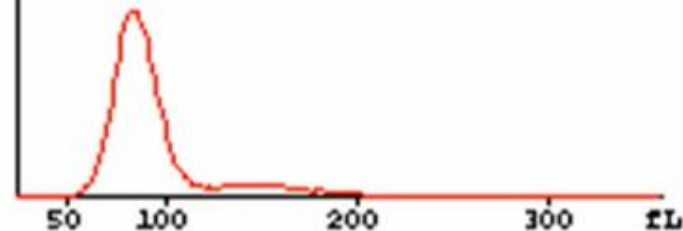
به عبارت دیگر یک جمعیت RBC با MCV بالا ممکن است در هیستوگرام بصورت پهن مشاهده شود اما RDW-CV نرمال باشد

در نقطه مقابل در جمعیت میکروسیت با MCV پائین، منحنی باریک (کوچک) اما RDW-CV افزایش یافته باشد.

RDW -Clinical applications

- *“RDW is likely a independent marker for chronic processes leading ultimately to a fatal outcome in patients and the general population.”*
- Elevated RDW has been associated with:
 - Decreased eGFR in CRF patients
 - Overall increased myocardial infarction rate in CHDs
 - Overall cancer mortality rate
 -

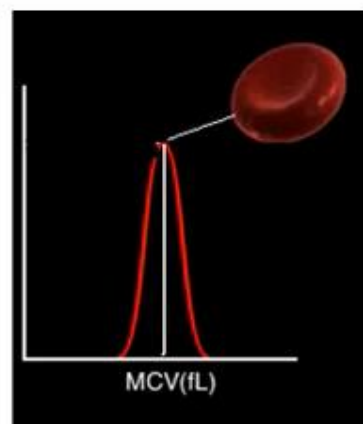
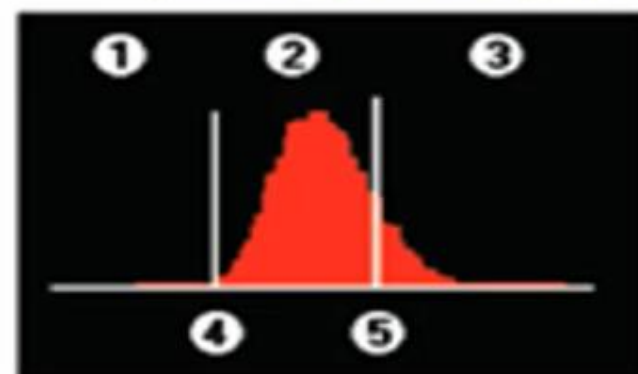
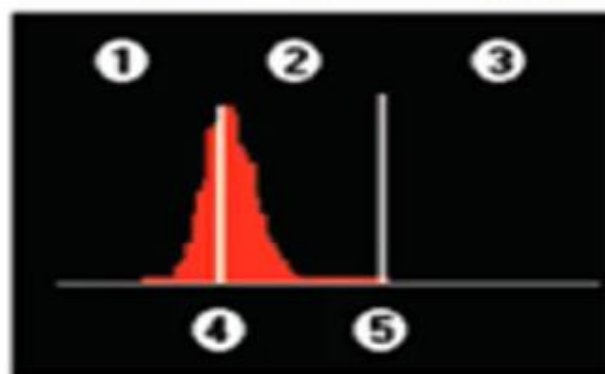
RBC Histogram



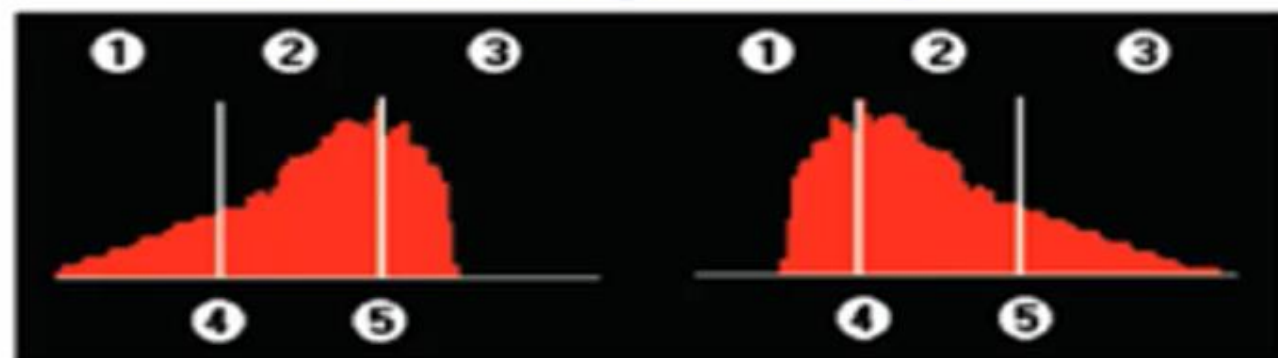
- 1 Microcytic region
- 2 Normocytic region
- 3 Macrocytic region
- 4 60 fL marker
- 5 120 fL marker

(microcytic sample)

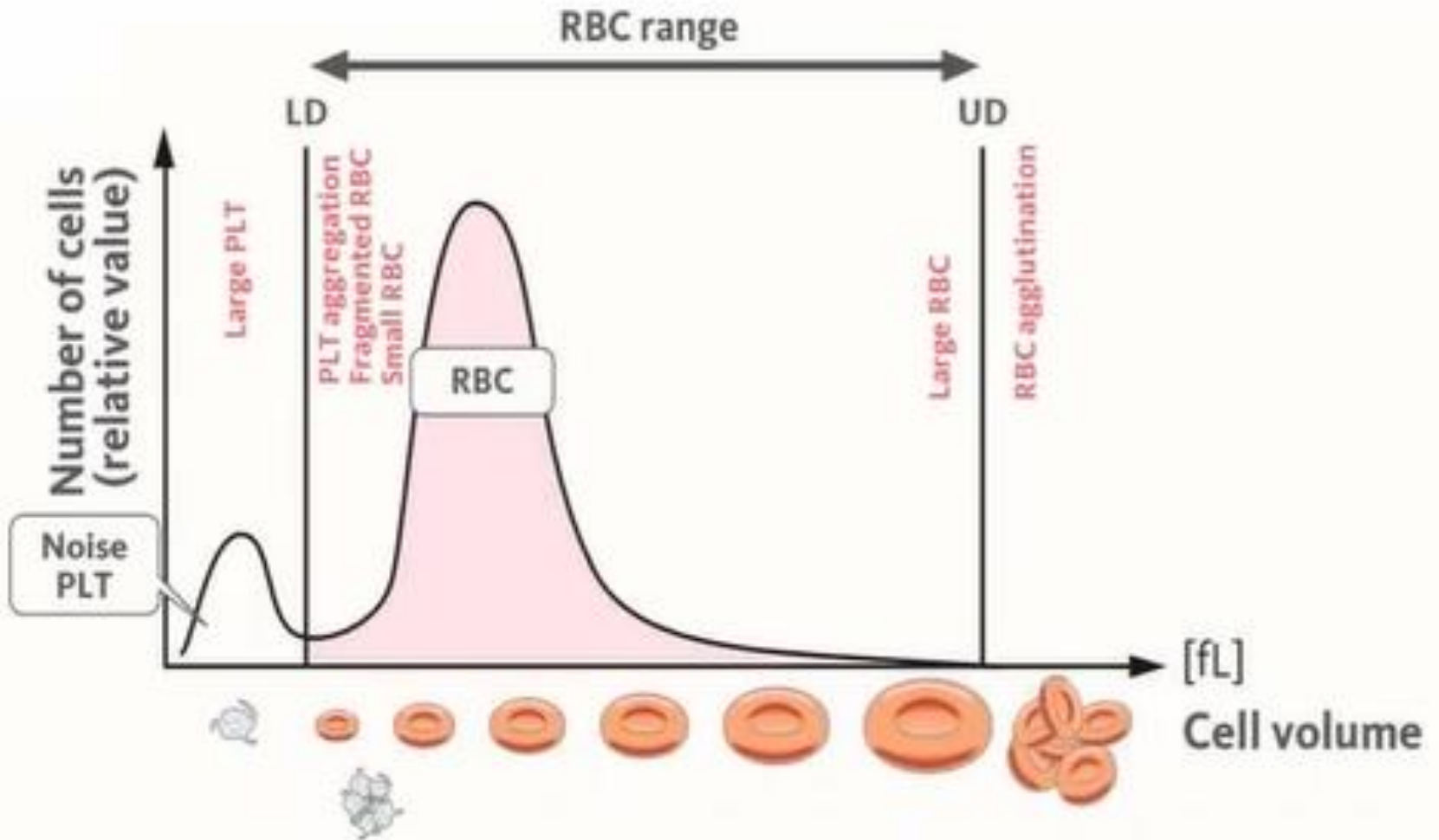
(macrocytic sample)



RBC Volume histogram (anisocytosis)



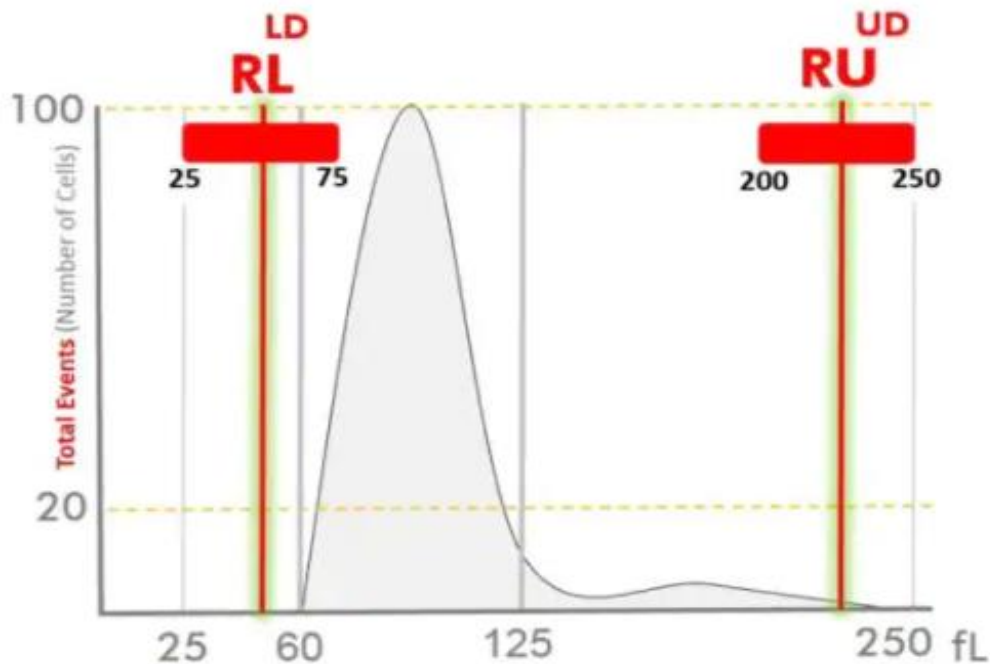
RBC Histograms



RBCs Histogram

Volume Histogram

Red Cell Discriminators



RBCs Histogram

Volume Histogram

The RBC histogram has **2 Flexible** discriminators that discriminate RBC curve from other curves.

RL (RBC Low Discriminator)

Fluctuates between **25** and **75** fL

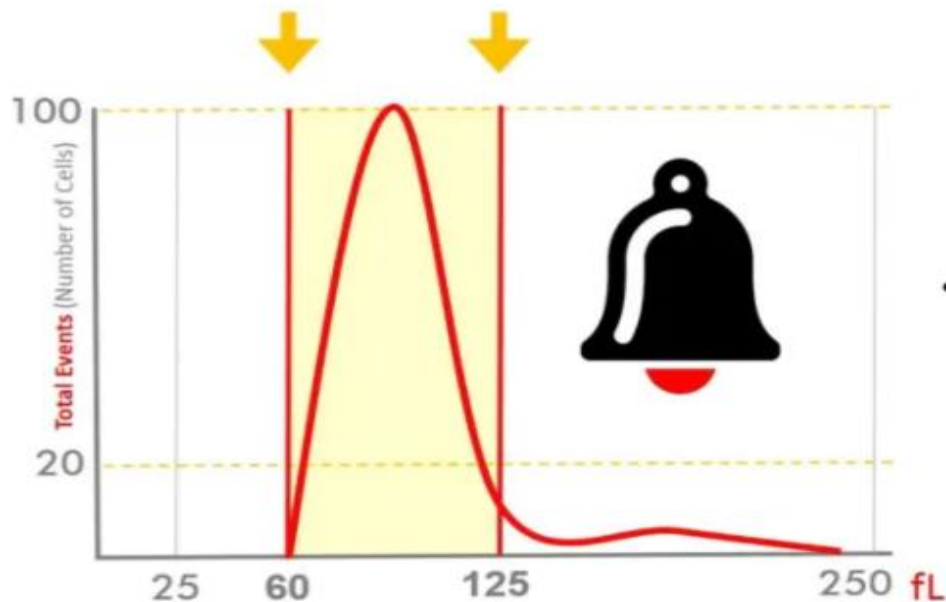
RU (RBC Upper Discriminator)

Fluctuates between **200** and **250** fL

RBCs Histogram

Volume Histogram

In a homogeneous cell population, the RBC curve assumes a symmetrical **bell-shaped** or **Gaussian** distribution.



RBCs Histogram

Volume Histogram

- The area of the peak is used to calculate the **MCV** and **RDW**. This area represents **60 fL** to **125 fL**.

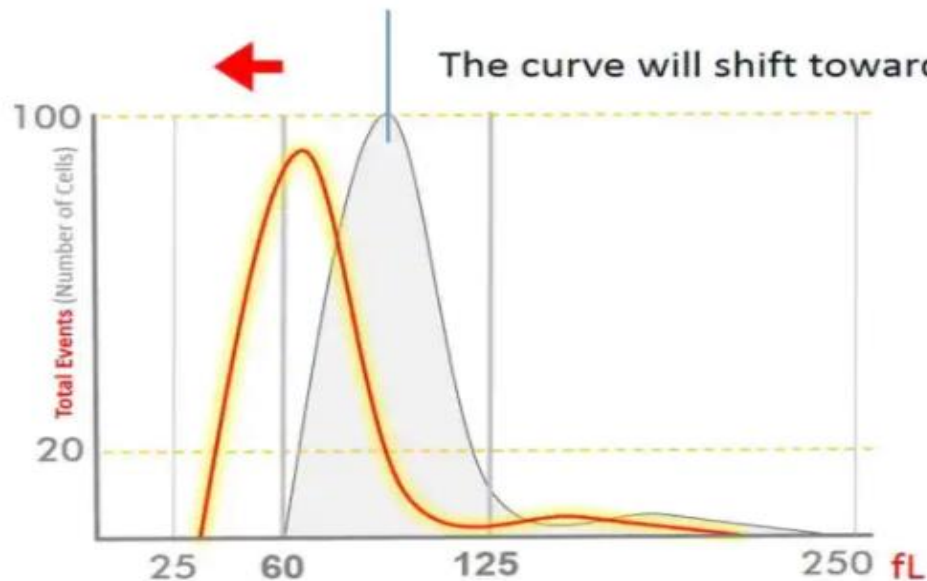
RBCs Histogram

Volume Histogram

If The RBCs are **smaller** than normal (= **Microcytic**)

RBCs Histogram

Volume Histogram



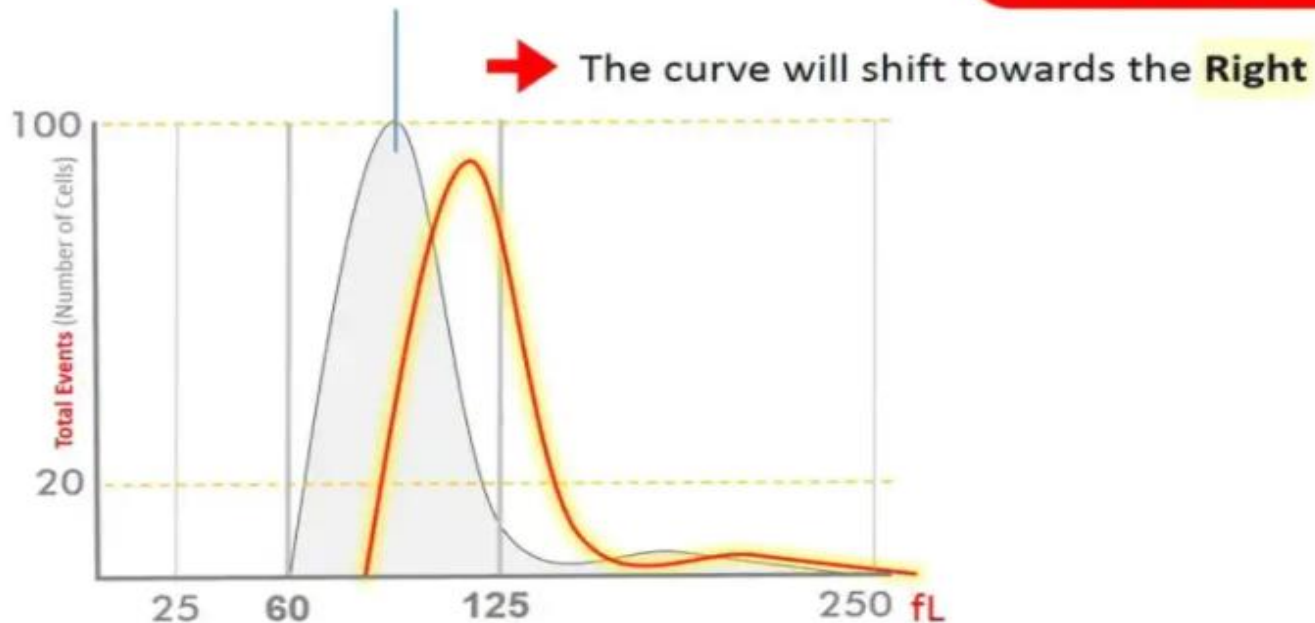
RBCs Histogram

Volume Histogram

If The RBCs are **larger** than normal (= **Macrocytic**)

RBCs Histogram

Volume Histogram

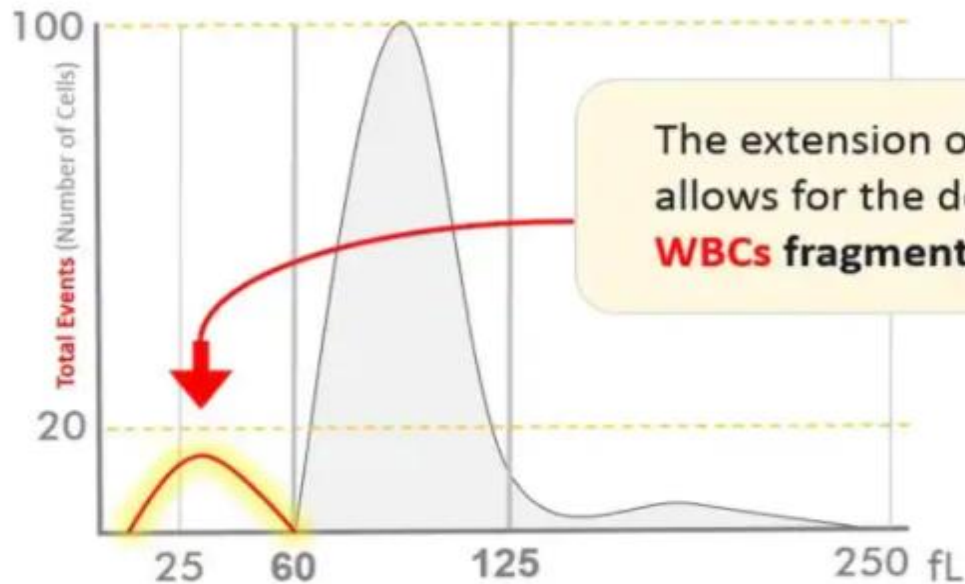


RBCs Histogram

Volume Histogram

RBCs Histogram

Volume Histogram



The extension of the lower end of the scale allows for the detection of **RBCs fragments**, **WBCs fragments** and **large platelets**.

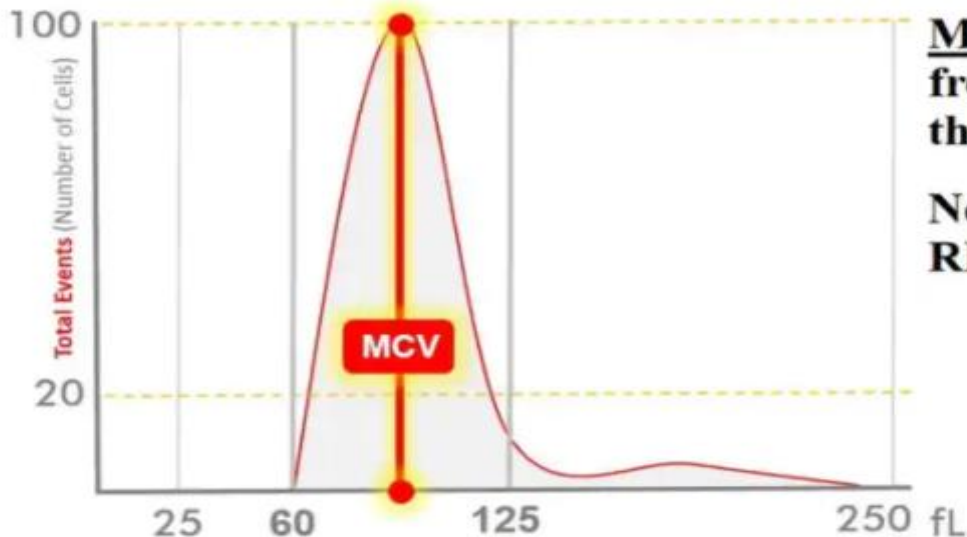
RBCs Histogram

Volume Histogram

RBCs Histogram

Volume Histogram

The **MCV** is calculated from the area **under** the peak



MCV is perpendicular line from **peak** of the curve to the **base**

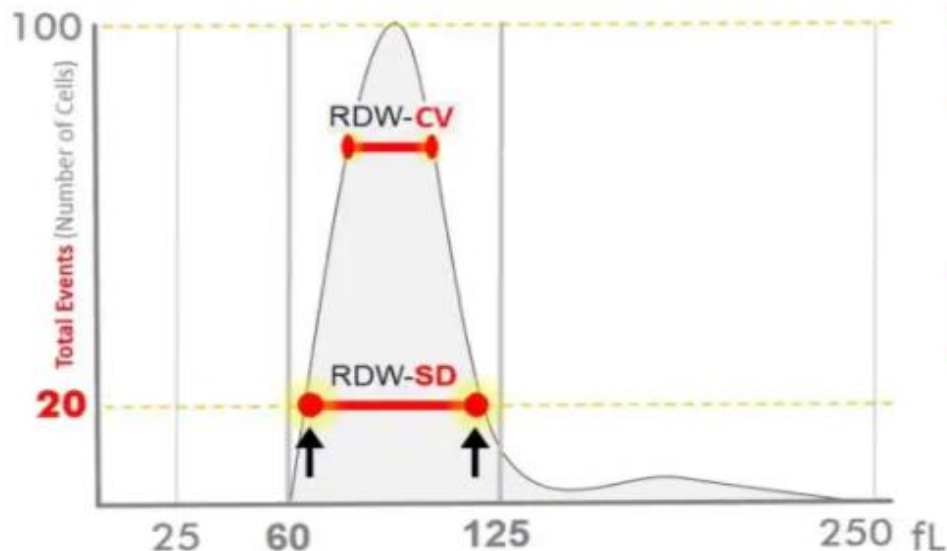
Normal RBC histogram → RBC located between 55-125 fL

RBCs Histogram

Volume Histogram

The Red cell Distribution Width **RDW**

An important parameter for measurement of degree of **variation** in RBCs **size**.



RBCs Histogram

Volume Histogram

RDW has **2** forms:

1 RDW-**CV** %

- **Coefficient** of variation percentage
- **Calculated** by $SD/MCV \times 100$
- The normal range is **11.5%** to **14.5%**

2 RDW-**SD** fL

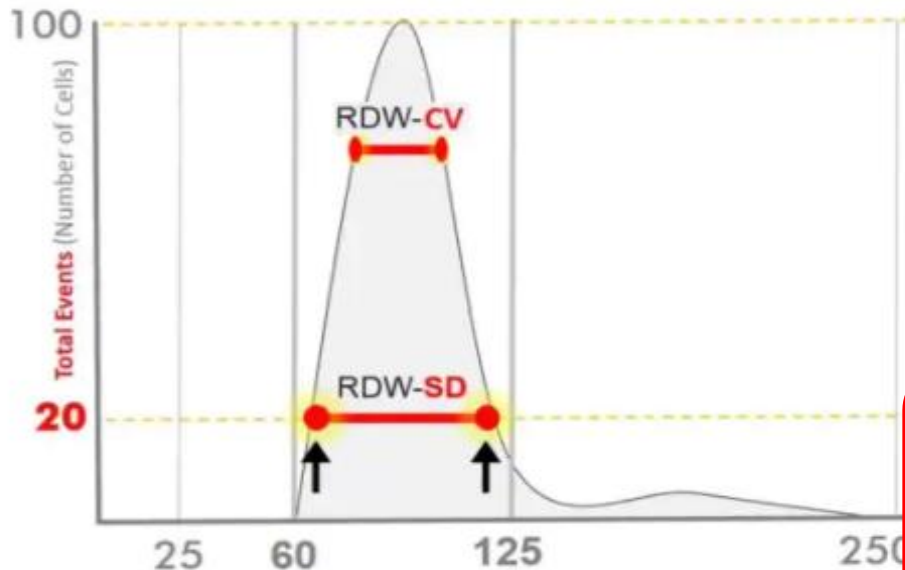
- **Standard Deviation**
- Obtained by drawing an arbitrary line at a **height** of **20%** on the y-axis in fL.
- The normal range is **35 – 45 fL**

RBCs Histogram

Volume Histogram

The Red cell Distribution Width **RDW**

An important parameter for measurement of degree of **variation** in RBCs **size**.



RBCs Histogram

Volume Histogram

RDW has **2** forms:

1 RDW-**CV** %

- **Coefficient** of variation percentage
- **Calculated** by $SD/MCV \times 100$
- The normal range is **11.5%** to **14.5%**

2 RDW-**SD** fL

To avoid interference in the calculations of RDW, the information **< 20%** of scale on RBCs histogram are excluded:

- Aperture artifacts
- RBC agglutinates, right
- Giant PLTs, left

Clinical application of MCV & RDW

Relationship of RDW and MCV

RBCs Histogram

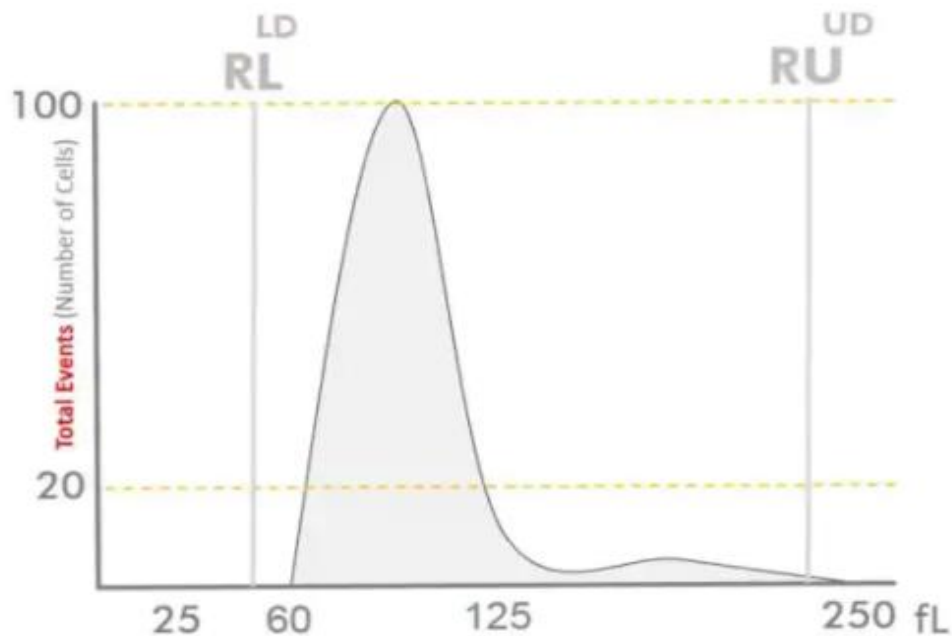
Volume Histogram

	Microcytic ↓ MCV	Normocytic Normal MCV	Macrocytic ↑ MCV
Normal RDW	Heterozygous α or β thalassemia Anemia of Chronic Disease	Anemia of Chronic Disease	Myelodysplasia Aplastic Anemia
↑ Increased RDW	Iron deficiency Sideroblastic anemia Hb H disease S - β thalassemia	Anemia of Chronic Disease Sideroblastic anemia Hb SS or SC diseases Mixed deficiencies Early IDA	Megaloblastic anemia Reticulocytosis

Children
teenagers

RBCs Histogram

Volume Histogram



RBCs Histogram

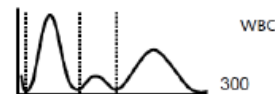
Volume Histogram



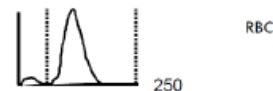
RBCs Histogram

Volume Histogram

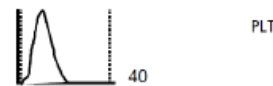
NO. 4
 DATE: 9/10/95 15:11
 MODE: WHOLE BLOOD
 WBC 5,8 x 10³/μl
 RBC 4,84 x 10³/μl
 HGB 13,7 g/dl
 HCT 42,0 %
 MCV 86,8 fl
 MCH 28,3 pg
 MCHC 32,6 g/dl
 PLT 257 x 10³/μl



LYMPH% 31,2 %
 MXD% 6,8 %
 NEUT% 62,0 %
 LYMPH# 1,8 x 10³/μl
 MXD# 0,4 x 10³/μl
 NEUT# 3,6 x 10³/μl



RDW-SD 40,0 fl



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

WL: Abnormal height at lower discriminator of WBC Histogram (LD)
WU: Abnormal height at upper discriminator of WBC Histogram (UD)
T1: Valley 1 not found
T2: Valley 2 not found
F1, F2, F3: Abnormal height at the points **T1** or **T2**; adjacent fractions are marked

RL: Abnormal height at lower discriminator of RBC Histogram (LD)
RU: Abnormal height at upper discriminator of RBC Histogram (UD)
MP: Multiple peaks: Distinguish ?? of two RBC Populations
DW: The distribution (RDW) can not be detected because the Histogram does not cross the 20 % limit twice.

PL: Abnormal height at lower discriminator of PLT Histogram (LD)
PU: Abnormal height at upper discriminator of PLT Histogram (UD)
MP: Multiple Peaks found
DW: The distribution (PDW) can not be detected because the Histogram does not cross the 20 % limit twice.

Flags of WBC

Flags of RBC

Flags of PLT

AG: The particle count equal to or less than the LD exceeds a prescribed range. Probable cause is platelet agglutination, which does not alter WBC count but may result in decreased platelet count. Therefore, this flag is added to the PLT parameter.

Flags, Marks and data display

Flags	Marks	Data display
WBC	WL*	6.2 x 10 ³ /μL
RBC	!	0.29 x 10 ⁶ /μL
HGB		10.8 g/dL
HCT	!	2.8 %
MCV	---,-	fL
MCH	+++ ,+	pg
MCHC	+++ ,+	g/dL
PLT	AG	0 x 10 ³ /μL

Marks	Meaning
!	Data outside the range of guaranteed linearity
+	Data above the upper limit set for normal assessment
-	Data above the lower limit set for normal assessment
*	Data of low reliability

Display	Meaning
+++ ,+	Data outside the display range
*** *	Impossible to calculate the value due to abnormality in measurement
---,- / ----	Value could not be calculated due to data error. The analysis was performed in pre-diluted mode and for this reason the particle distribution analysis parameters could not be calculated

RBC Histogram Error Flags

List of **RBC** error flags in order of priority

RL	Relative frequency for LOWER discriminator (LD) exceeds the range. May be caused by changes in red blood cell morphology, platelet clumps, or electrical noise.
RU	Relative frequency for UPPER discriminator (UD) exceeds the range. May be caused by electrical noise.
MP	Two or more peaks in the histogram.
DW	Particle distribution width error when the 20% frequency does not cross the histogram two times. The peak is taken as 100%.

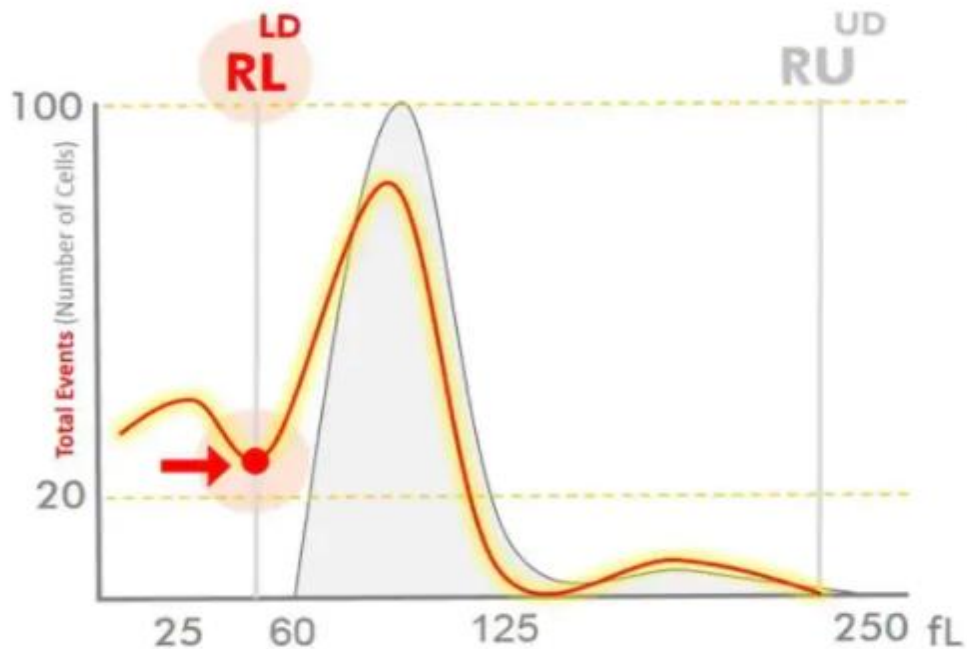
RBCs Histogram

Volume Histogram

Flag 1

RL Flag

LD Flag



RBCs Histogram

Volume Histogram

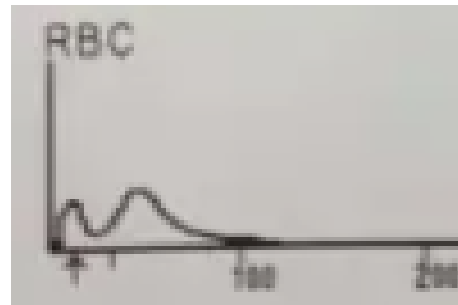
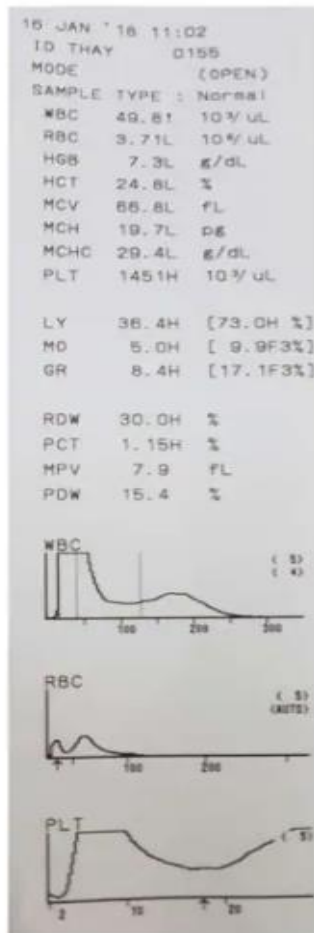
- **Abnormal Height at LD**
- The LD exceeds the preset height by **> 10 %**

Possible Causes :

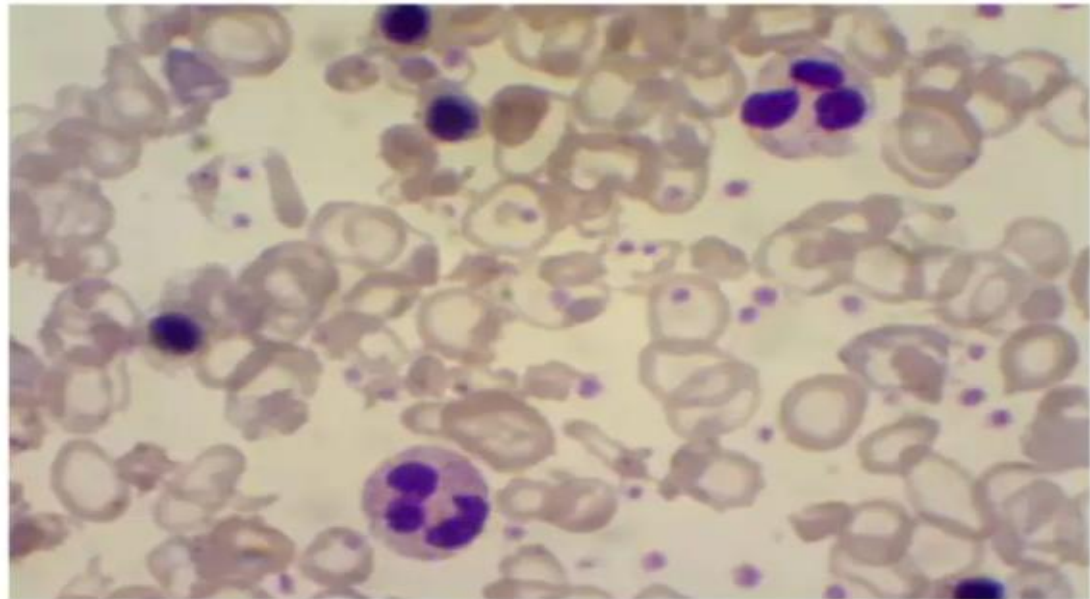
- **Platelet** Clumps
 - **RBCs** fragments
 - Giant **Platelets**
 - Micro **RBCs**
 - Noise
- RBC morphology

RBCs Histogram

Volume Histogram



Thalassemia major → WBC / RBC / PLT



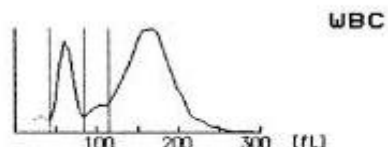
Abu Jad Caesar

RBCs Histogram

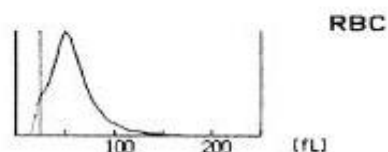
Volume Histogram

RL flag

WBC $5.8 \times 10^9 / \mu\text{L}$
 RBC RL* $5.65 \times 10^6 / \mu\text{L}$
 HGB - 8.4 g/dL
 HCT RL* 32.5%
 MCV RL* 57.5 fL
 MCH RL* 14.9 pg
 MCHC RL* 25.8 g/dL
 PLT PU! $1884 \times 10^9 / \mu\text{L}$



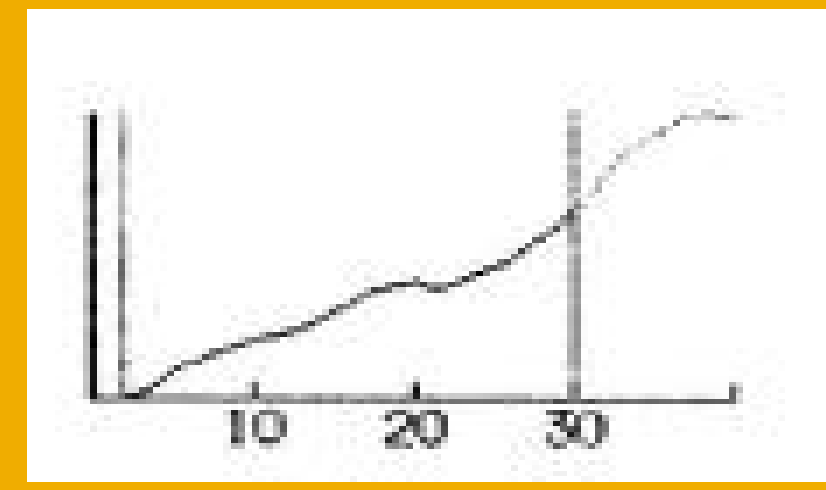
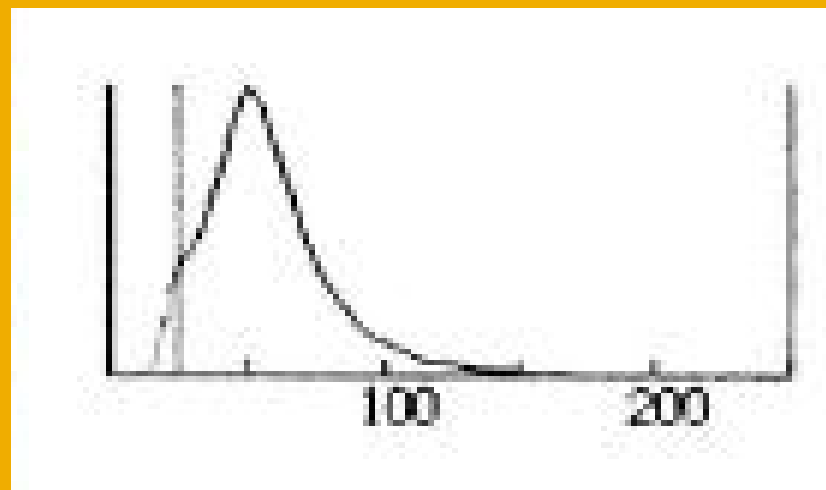
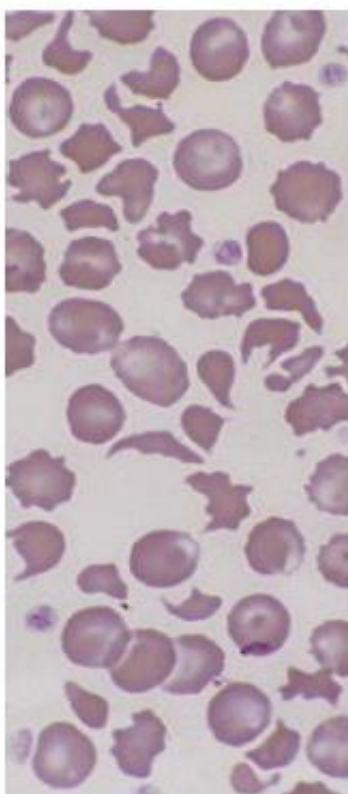
LYM% 21.0%
 MXD% 7.2%
 NEUT% 71.8%
 LYM# $1.2 \times 10^9 / \mu\text{L}$
 MXD# $0.4 \times 10^9 / \mu\text{L}$
 NEUT# $4.2 \times 10^9 / \mu\text{L}$



RDW RL* 32.3%



PDW DW ---. -fL
 MPV PU ---. -fL
 P-LCR PU ---. -%



شکل ۱۱-۱: (راست) CBC و گستره خون محیطی از اتریگاسیون پلاکتی (فلاگ WL, MP, PL) و (چپ) CBC و گستره خون محیطی از شیتوسیتوز شدید (فلاگ RL, PU و DW)

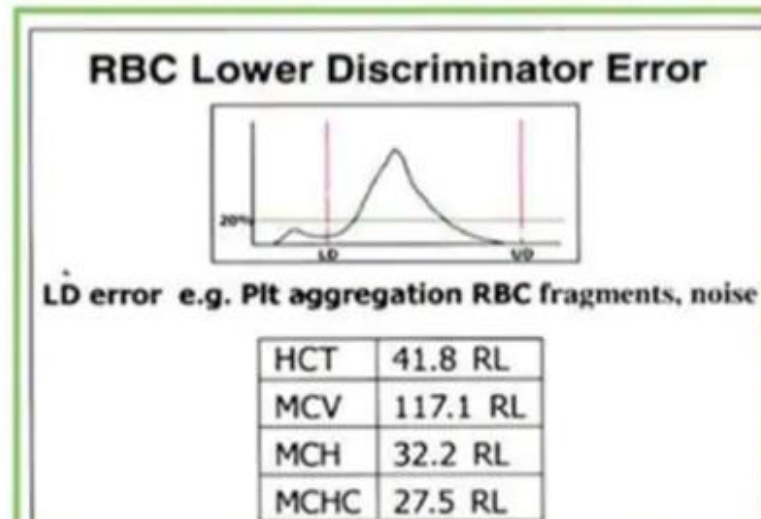
RBCs Histogram

Volume Histogram

Schistocytes & extreme microcytes

RL flag

- RBC count, HCT, MCV, MCH and MCHC show RL flag



- In case of fragmented RBC and extreme microerythrocytosis ➡ there is no clear separation in volume between platelets and erythrocytes.
- Due to high numbers of RBC ➡ platelet result might be false high and should be checked with alternative methods.

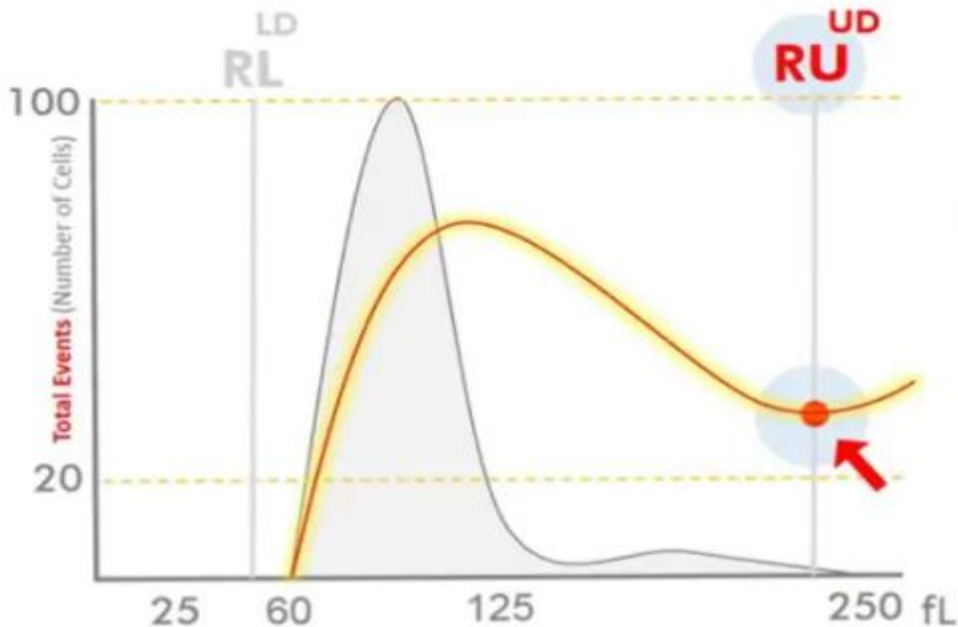
RBCs Histogram

Volume Histogram

Flag 2

RU Flag

UD Flag



RBCs Histogram

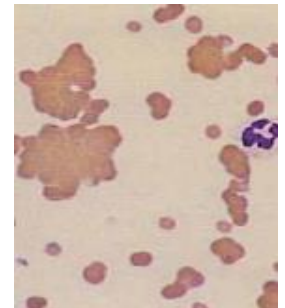
Volume Histogram

- **Abnormal Height at UD**

- The UD exceeds the preset height by **> 5 %**

Possible Causes :

- **RBCs Agglutination**
- **Cold Agglutinins**
Disappear when the samples are incubated at 37 °C
- **Nucleated RBCs**
- **CLL**
when small lymphoid cells are present in a very high number



Case 9

RBCs Agglutination

RBCs Histogram

Volume Histogram

Parameters

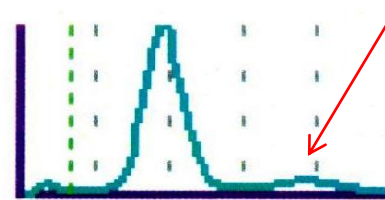
WBC	5.82	[10 ³ /uL]
RBC	3.05 *	[10 ⁶ /uL]
HGB	12.8 *	[g/dL]
HCT	30.9 *	[%]
MCV	101.3 *	[fL]
MCH	42.0 *	[pg]
MCHC	41.4 *	[g/dL]
RDW-SD	43.4	[fL]
RDW-CV	16.0 +	[%]

RU Flag
UD Flag

Anisocytosis

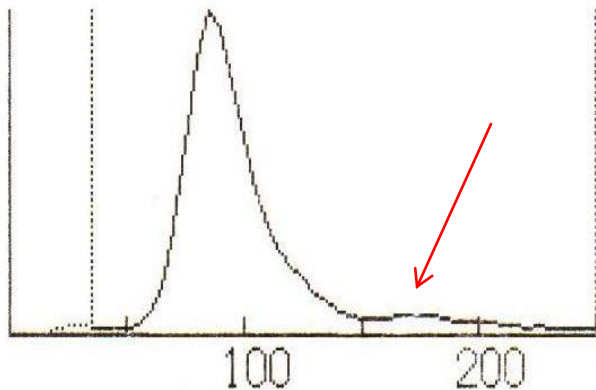
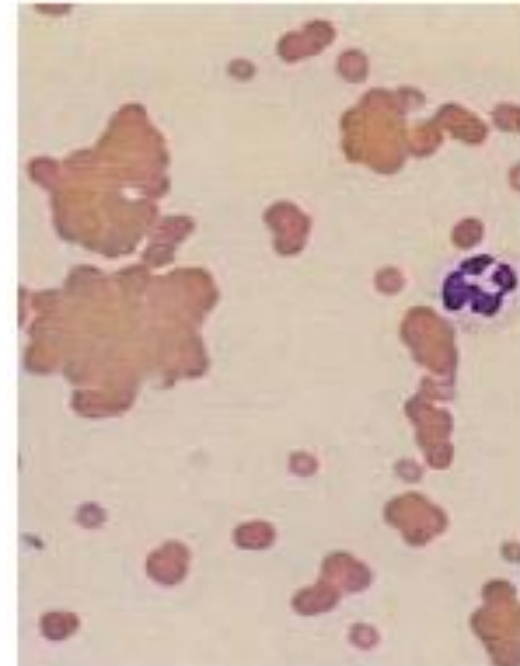
RBC Agglutination?
Turbidity/HGB
Interf?

RBC



PLT

RBC



Cold Agglutination

Case 9

RBCs Agglutination

RBCs Histogram

Volume Histogram

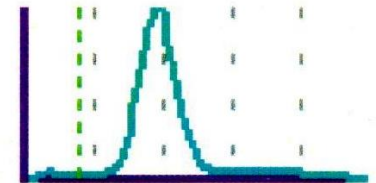
Parameters

WBC	5.82	[10 ³ /uL]
RBC	3.05 *	[10 ⁶ /uL]
HGB	12.8 *	[g/dL]
HCT	30.9 *	[%]
MCV	101.3 *	[fL]
MCH	42.0 *	[pg]
MCHC	41.4 *	[g/dL]
RDW-SD	43.4	[fL]
RDW-CV	16.0 +	[%]

RU Flag
UD Flag

RBC

RBC



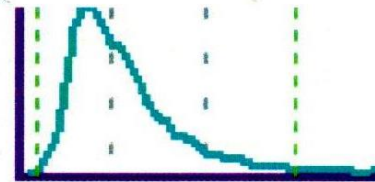
PLT

PLT

Parameters

WBC	6.21	[10 ³ /uL]
RBC	4.17	[10 ⁶ /uL]
HGB	12.6	[g/dL]
HCT	38.8	[%]
MCV	93.0 +	[fL]
MCH	30.2	[pg]
MCHC	32.5	[g/dL]
RDW-SD	43.8	[fL]
RDW-CV	13.2	[%]

After



Before



After

Cold Agglutination

Before & after 30-60 min incubation of blood in 37°C water bath

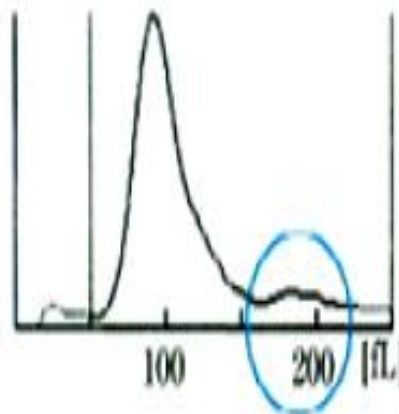
Case 9

RBCs Agglutination

RBCs Histogram

Volume Histogram

RBC-Histogram



Results

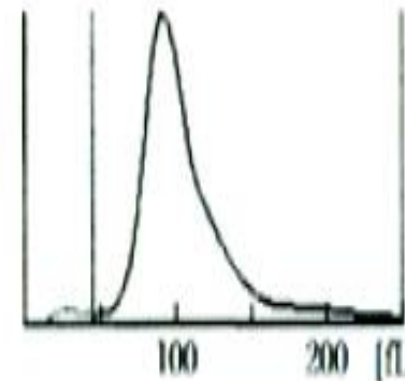
RBC	RU*	2.23x10 ¹² /L
HGB		14.4g/dl
HCT	RU*	24.9%
MCV	RU*	111.7fl
MCH	RU*	64.6pg
MCHC	RU*	57.8g/dl
RDW	*	25.4fl

RU Flag

UD Flag

Incubation 30 min

RBC-Histogram



Results

RBC	4.35x10 ¹² /L
HGB	14.5g/dl
HCT	43.5%
MCV	100.0fl
MCH	33.3pg
MCHC	33.3g/dl
RDW	14.7fl

$$\text{MCH} = \text{Hb} / \text{RBC}$$

$$\text{MCHC} = \text{Hb} / \text{HCT}$$

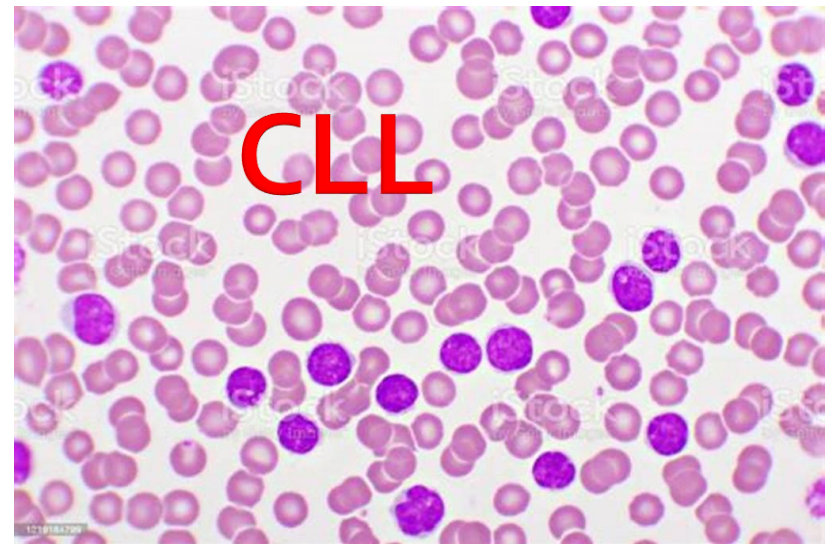
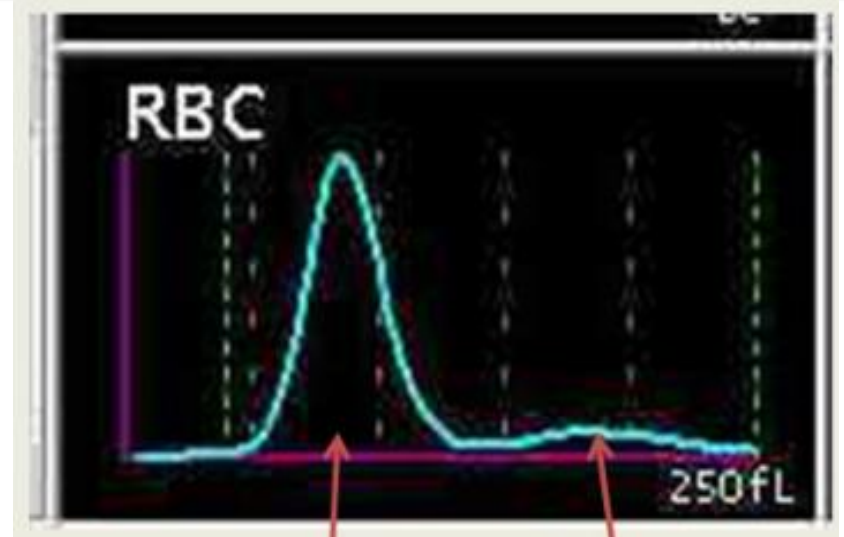
Flag 2**RU Flag**

UD Flag

RBCs Histogram

Volume Histogram

Item	Data		Unit	Flag(s)
WBC	489.53	@	$10^3/\mu\text{L}$	WBC
RBC	3.73	*	$10^6/\mu\text{L}$	Imm Gran? Abn Ly/L_B1? WBC Abn Scg
HGB	9.2		g/dL	
HCT	37.0	*	%	
MCV	99.2	*	fL	
MCH	24.7	*	pg	
MCHC	24.9	*	g/dL	
PLT	209		$10^3/\mu\text{L}$	
RDW-SD	----		fL	RBC/RET
RDW-CV	----		%	RBC Abn Dst Dimorph Pop
MPV	11.1		fL	
RET%	2.69		%	
RET#	10.03	*	$10^4/\mu\text{L}$	
IRF	0.131		Ratio	
NRBC#	0.00		$10^3/\mu\text{L}$	PLT
NRBC%	0.0		/100WBC	



VERY HIGH WBC – CLL

Graph | WBC/NRBC | RBC/PLT | Cumulative
Measurement Parameters

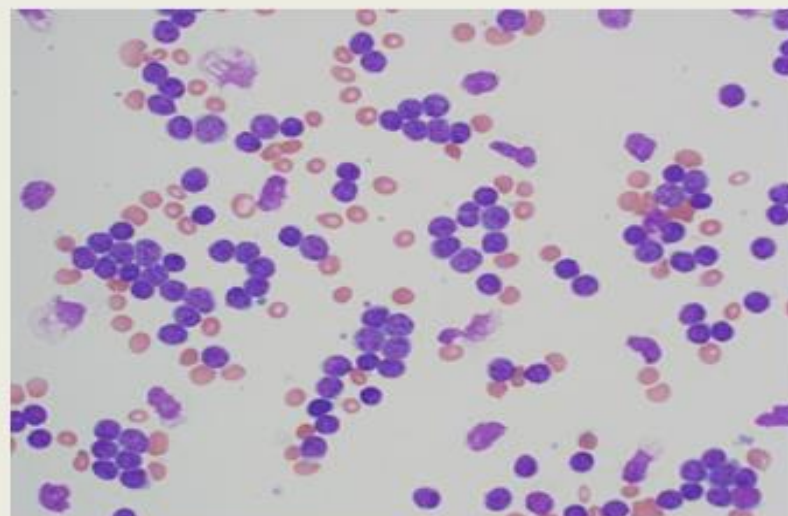
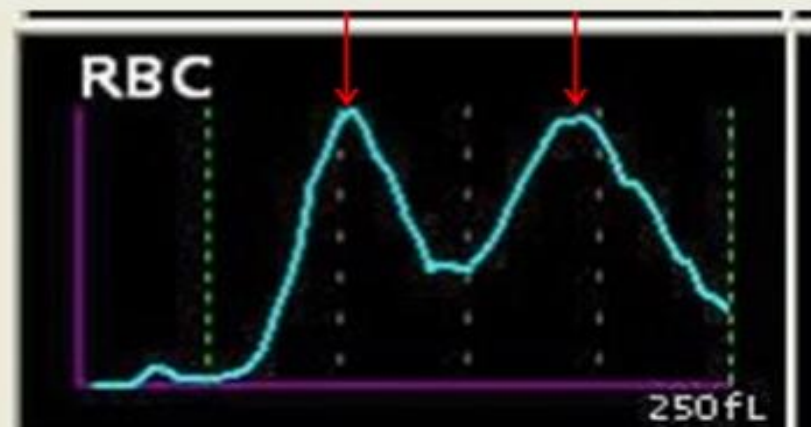
Item	Data	Unit
WBC	746.24	$10^3/\mu\text{L}$
RBC	1.37	$10^6/\mu\text{L}$
HGB	2.4	g/dL
HCT	21.4	%
MCV	156.2	fL
MCH	17.5	pg
MCHC	11.2	g/dL
PLT	95	$10^3/\mu\text{L}$
RDW-SD	----	fL
RDW-CV	----	%
MPV	10.2	fL
RET%	----	%
RET#	----	$10^4/\mu\text{L}$
IRF	----	Ratio
NRBC#	0.00	$10^3/\mu\text{L}$
NRBC%	0.0	/100WBC
Item	Data	Unit
RET-He	----	pg
IPF	----	%

Measure

Item
NEUT#
LYMPH#
MONO#
EO#
BASO#
NEUT%
LYMPH%
MONO%
EO%
BASO%

Item
IG#
IG%

Item
HPC#



Flag(WBC)

Flag(RBC/RET)

Imm Gran?
Atypical Ly?
Abn Ly/L_B1?

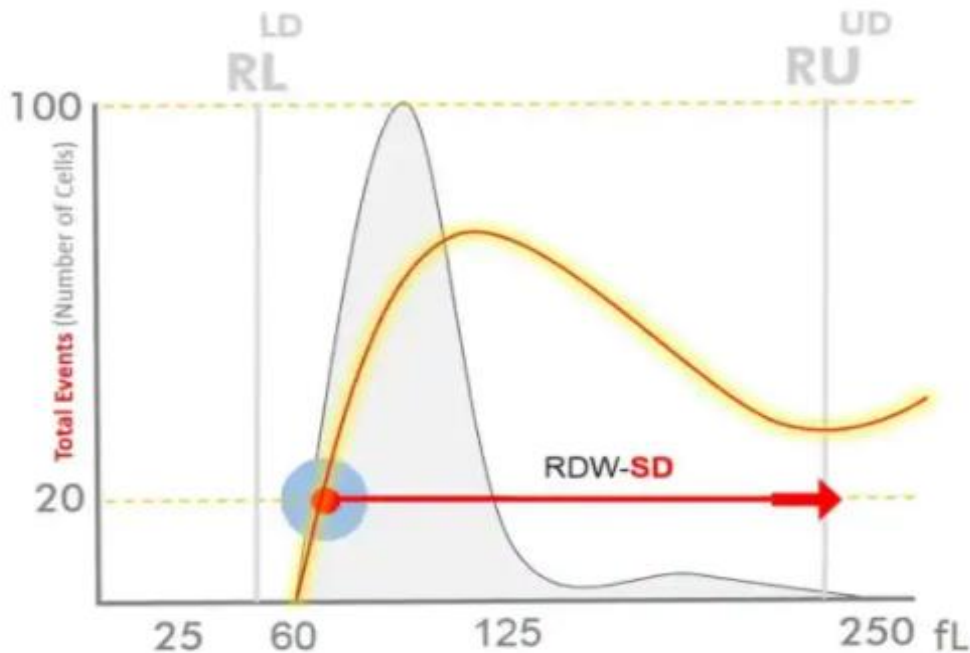
RBC Abn Dst
Dimorph Pop
Hypochromia

RBCs Histogram

Volume Histogram

Flag 3

RDW Flag



RBCs Histogram

Volume Histogram

- Abnormal RDW
- This flag is seen when the curve does not match the 20% line **twice**.

Possible Causes :

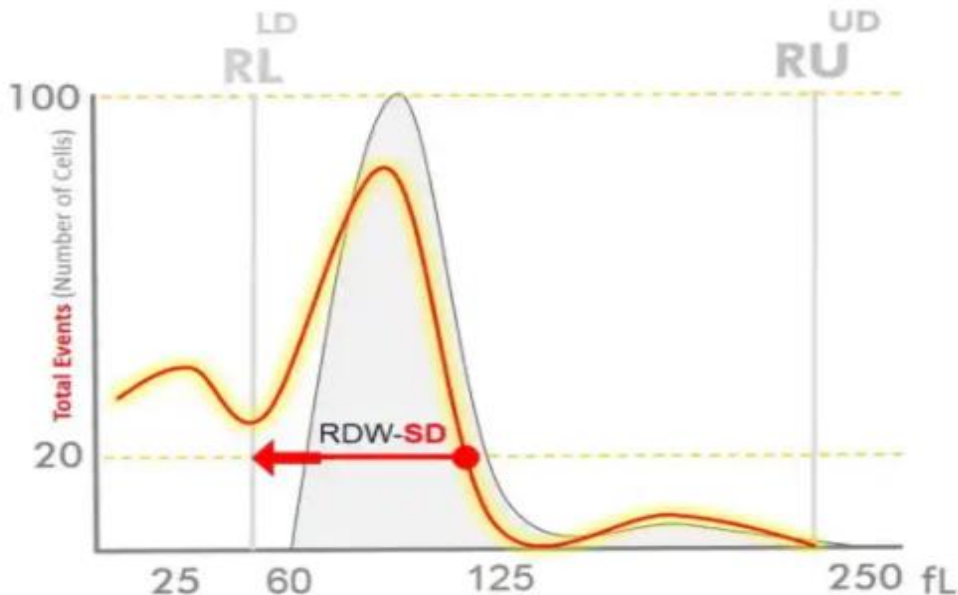
- The same as **RU** flag
 - **RBCs Agglutination**
 - **Cold Agglutinins**
Disappear when the samples are incubated at **37 °C**
 - **Nucleated RBCs**
 - **CLL**
when small lymphoid cells are present in a very high number

RBCs Histogram

Volume Histogram

Flag **3**

DW Flag



RBCs Histogram

Volume Histogram

- Abnormal RDW
- This flag is seen when the curve does not match the 20% line **twice**.

Possible Causes :

- The same as **RL** flag

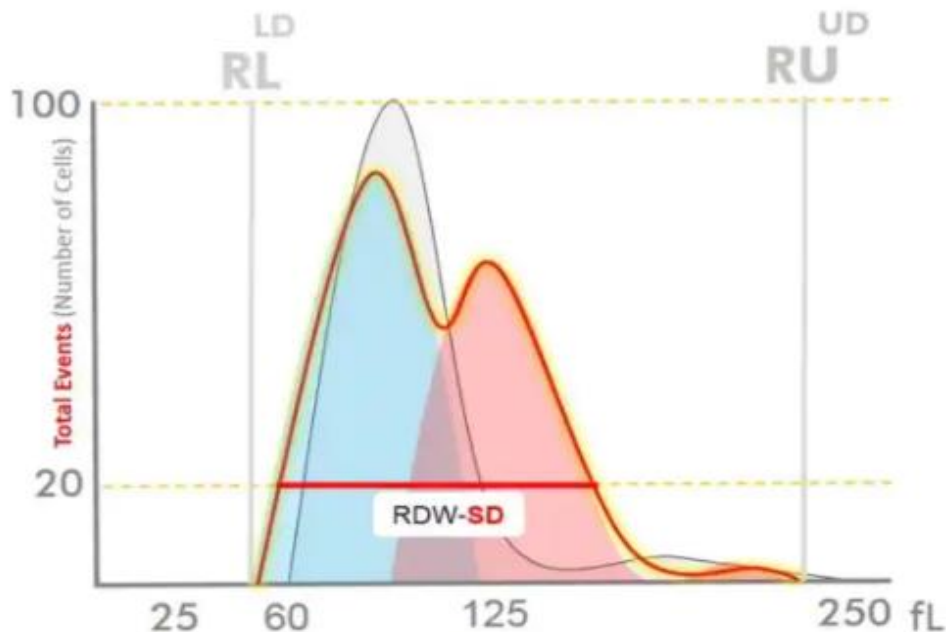
RBCs Histogram

Volume Histogram

Flag 4

MP Flag

Multiple Peaks



RBCs Histogram

Volume Histogram

- ↑ RDW (Anisocytosis)

- Possible causes

1. Iron deficiency in recovery
2. After blood transfusion
3. Dual deficiency anemia

(Iron and Vit.B₁₂/Folic)

Clinical Cases

RBCs Histogram

Volume Histogram



RBCs Histogram

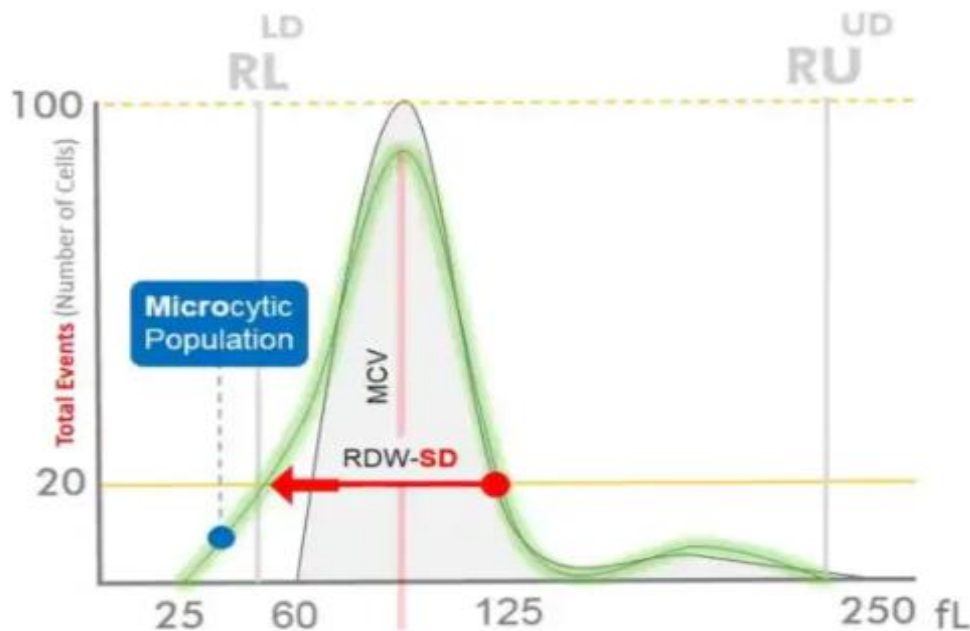
Volume Histogram

Case 1

Early Iron Deficiency

RBCs Histogram

Volume Histogram



The **Base** of the curve :

- **Wider** = RDW is $\uparrow$
(**Earliest** indicator)
- Extended toward **left** side $\leftarrow$
(**Microcytic** population)

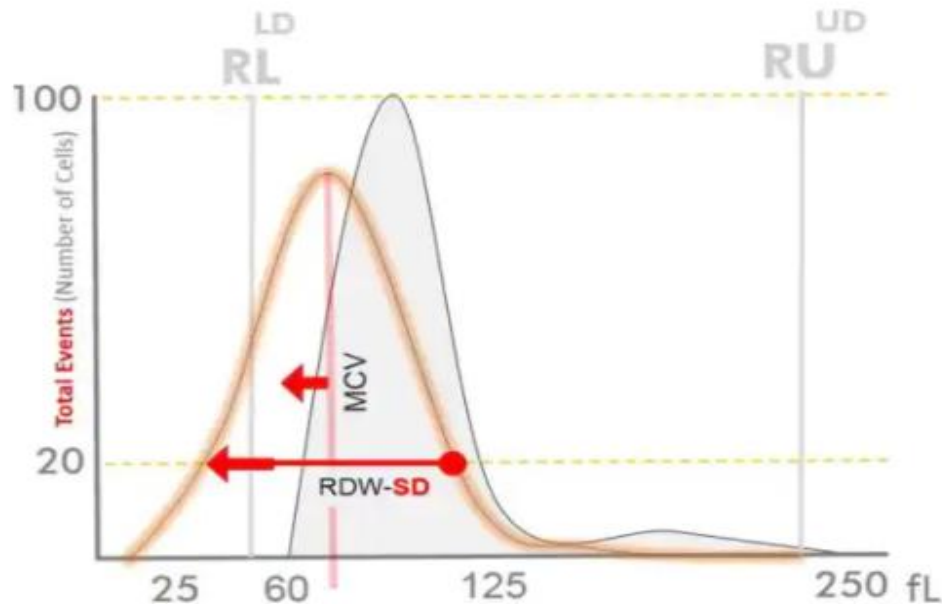
MCV still normal

RBCs Histogram

Volume Histogram

Case 2

Advanced Iron Deficiency



RBCs Histogram

Volume Histogram

The **entire** curve :

- More **Wider** = RDW is $\uparrow\uparrow$

RBCs **heterogeneity**

- Shifted to the **left** = MCV $\downarrow$

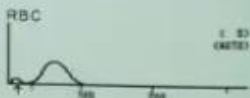
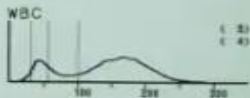
(**Microcytic** population)

RBCs Histogram

Volume Histogram

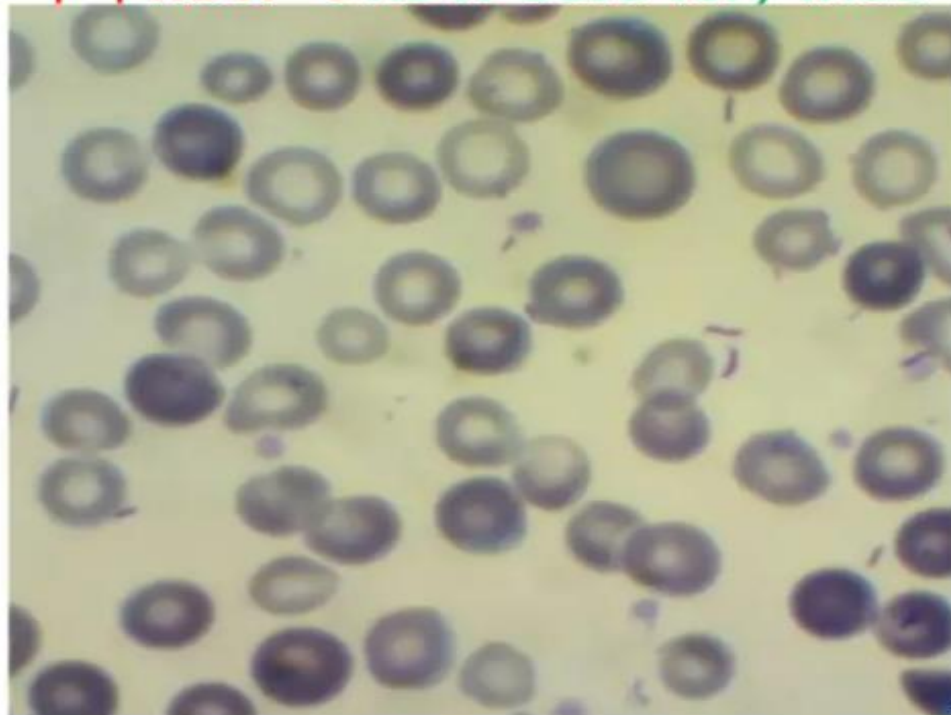
Advanced iron deficiency

27 DEC '17 10:20
 ID: SAEDA 31
 MODE (OPEN)
 SAMPLE TYPE: Normal
 WBC 8.5 10³/μL
 RBC 4.61 10⁶/μL
 HGB 10.4L g/dL
 HCT 33.8L %
 MCV 74.5L fL
 MCH 23.1L pg
 MCHC 31.0L g/dL
 PLT 434H 10³/μL
 LY 1.5 [18.1 %]
 MO 0.3 [3.3 %]
 GR 6.7 [78.6 %]
 RDW 20.9H %
 PCT 0.25 %
 MPV 5.8 fL
 PDW 16.7 %



↑
 Normal curve

↑↑RDW → RDW base very wide

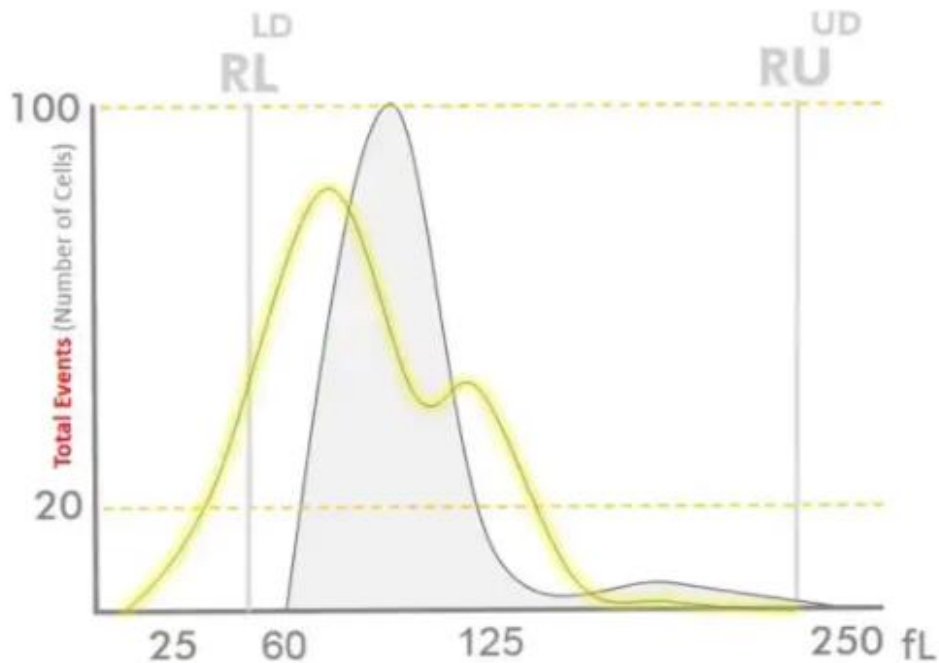


RBCs Histogram

Volume Histogram

Case 3

Recovery from Iron Deficiency



RBCs Histogram

Volume Histogram

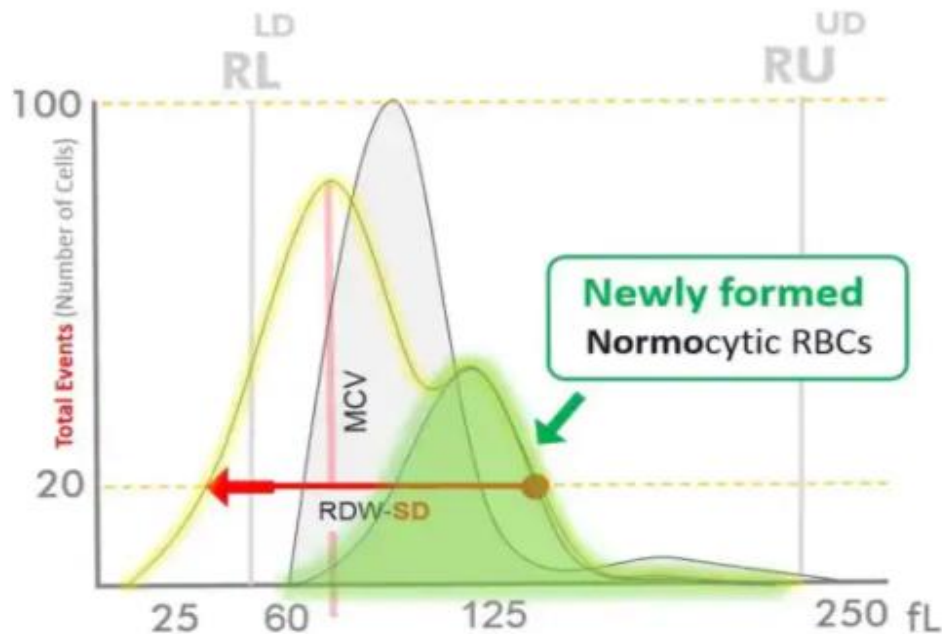
- RBCs count is ↑↑
- MCV is not normal yet
- 2 Populations could be easily distinguished :

RBCs Histogram

Volume Histogram

Case 3

Recovery from Iron Deficiency



RBCs Histogram

Volume Histogram

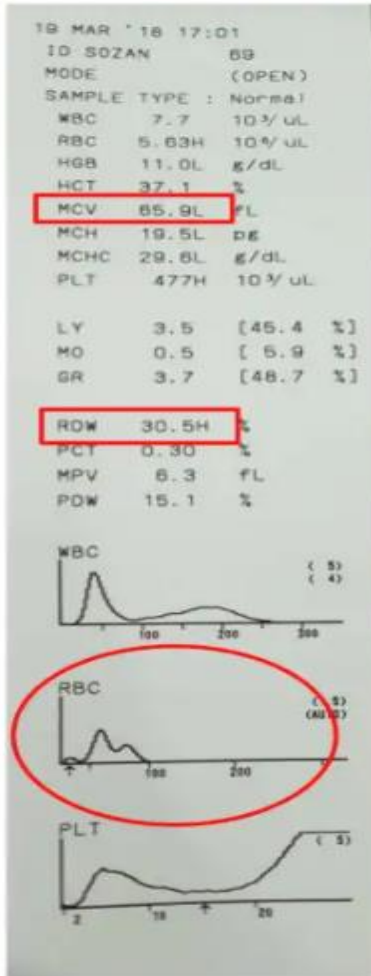
- RBCs count is ↑↑
- MCV is not normal yet
- 2 Populations could be easily distinguished :

The **pre-existing** microcytic RBCs

The **newly formed** normocytic RBCs

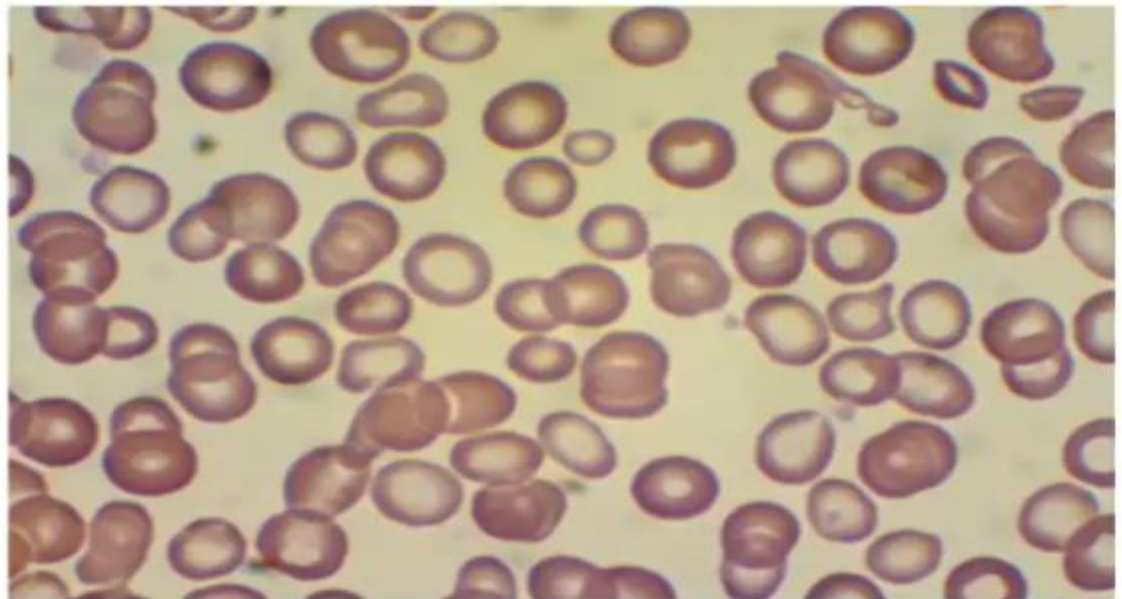
RBCs Histogram

Volume Histogram



After iron therapy-**Early** recovery

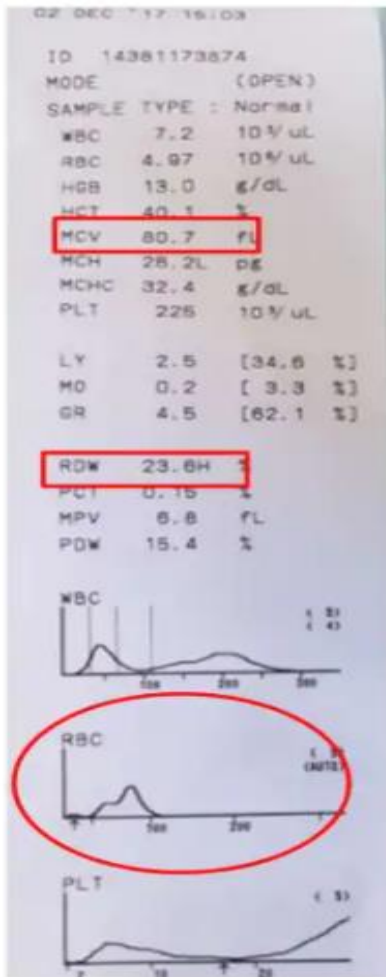
↑↑RDW → RDW base very wide (MP)



Abu Jad Caesar

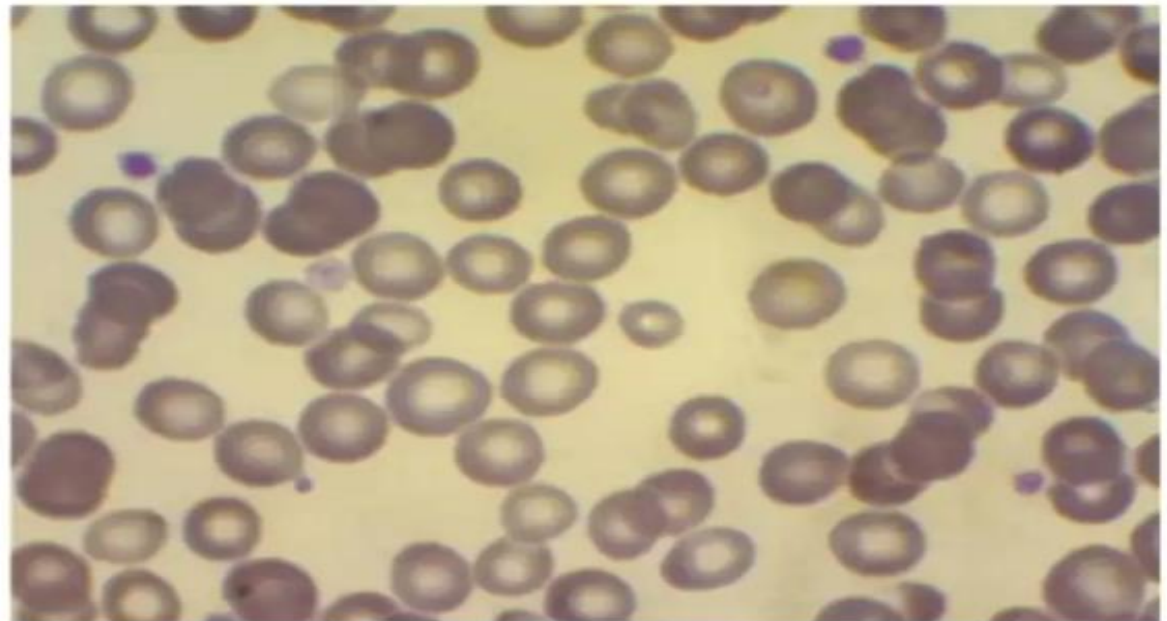
RBCs Histogram

Volume Histogram



After iron therapy-**Advanced** recovery

↑↑RDW → RDW base very wide (MP)



Abu Jad Caesar

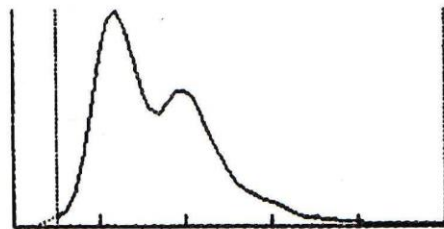
RBCs Histogram

Volume Histogram

Iron Deficiency Anemia being Treated

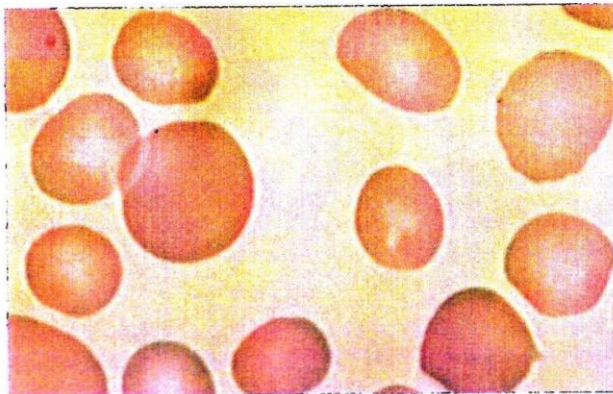
2nd Week of Treatment

<RBC Histogram>



<measurement data>

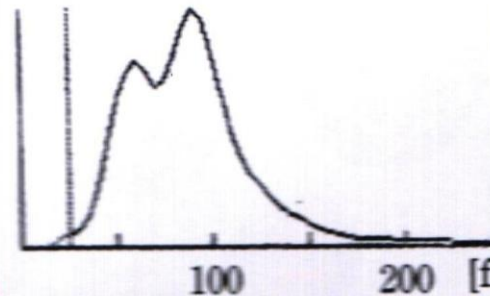
RBC	$4.37 \times 10^{12}/L$
HGB	104g/L
HCT	0.353
MCV	— 80.8fL
MCH	— 23.8pg
MCHC	— 295g/L
RDW	MP* 0.357



($\times 1000$)

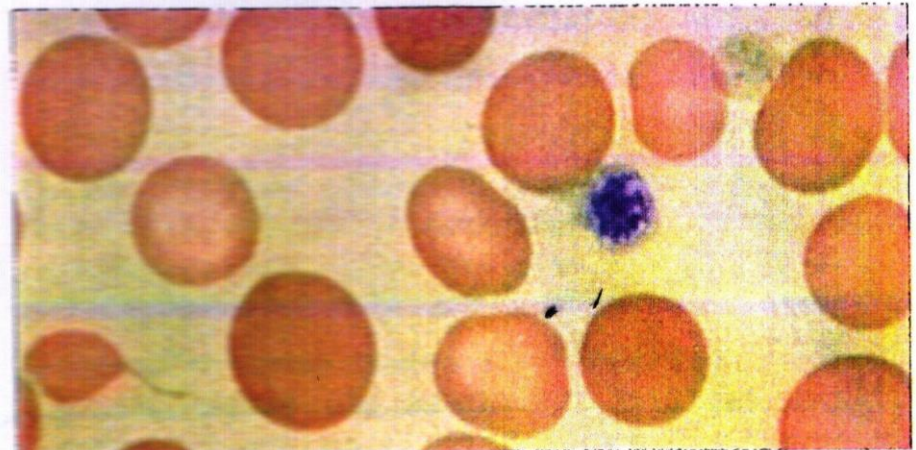
4th Week of Treatment

<RBC Histogram>



<measurement data>

RBC	$5.14 \times 10^{12}/L$
HGB	132g/L
HCT	0.425
MCV	— 82.7fL
MCH	— 25.7pg
MCHC	311g/L
RDW	MP* 0.319

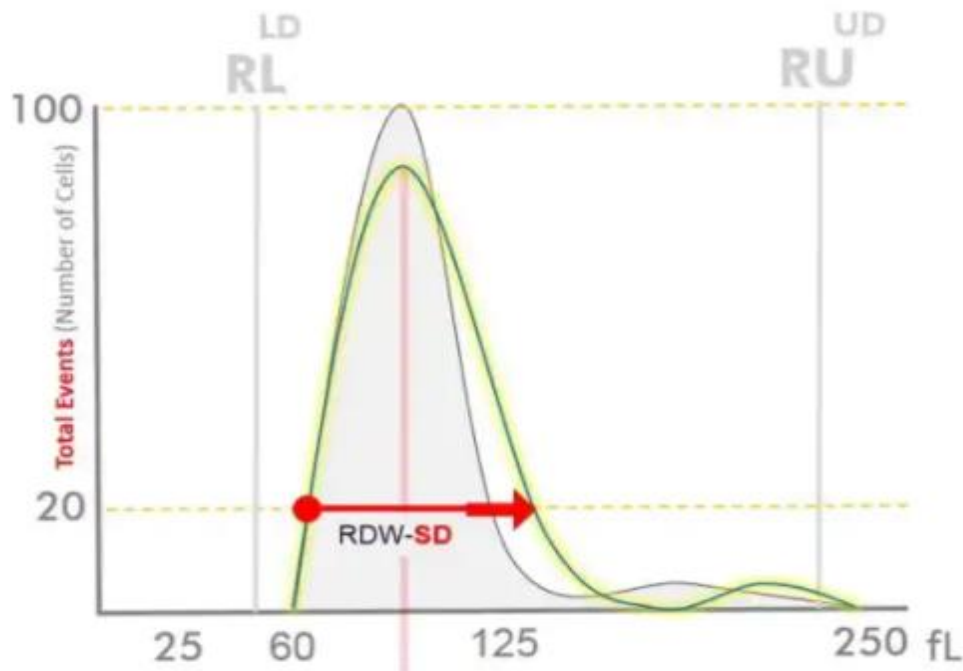


RBCs Histogram

Volume Histogram

Case 4

Early Megaloblastic Anemia



RBCs Histogram

Volume Histogram

- **MCV is still Normal**

but **RBCs** count and **Hgb** are slightly ↓

The **Base** of the curve :

- **Wider** = RDW is ↑

(**Earliest** indicator)

- Extended toward **Right** side

(**Macrocytic** population)

RBCs Histogram

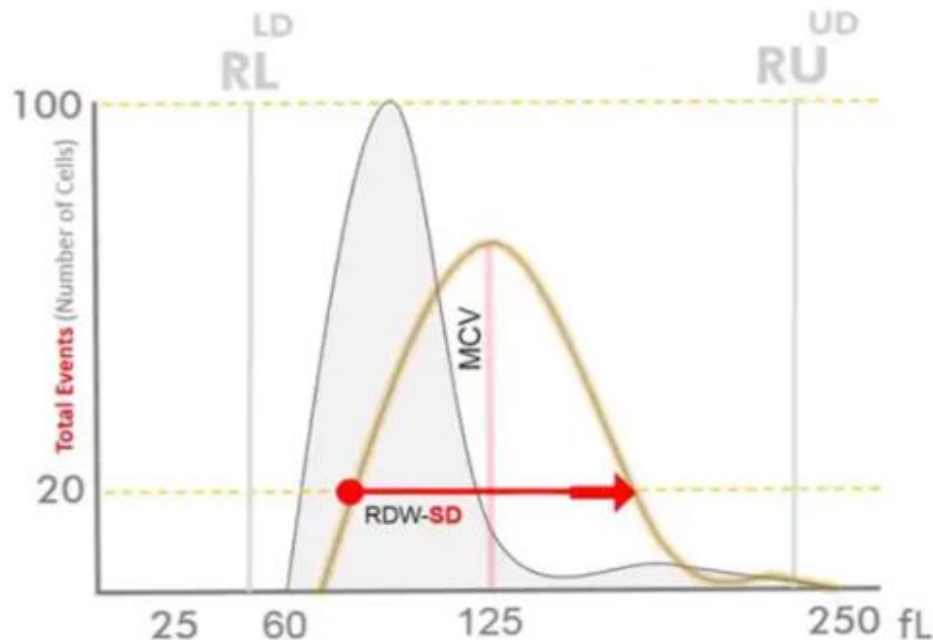
Volume Histogram

Case 5

Advanced Megaloblastic Anemia

RBCs Histogram

Volume Histogram



- RBCs count ↓
- Hb ↓

The **entire** curve :

- More **Wider** = RDW is ↑↑

RBCs **heterogeneity**

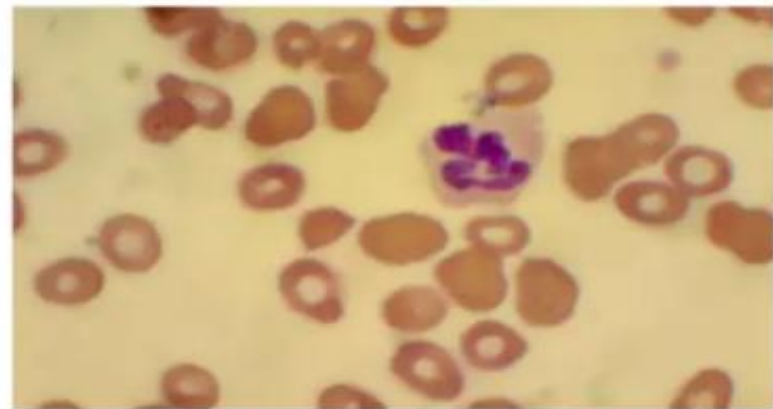
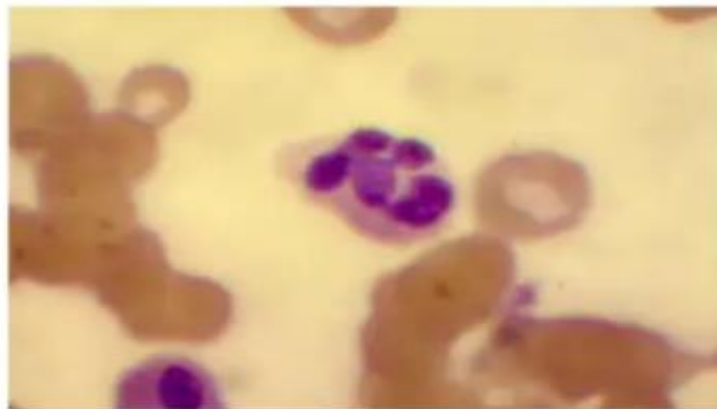
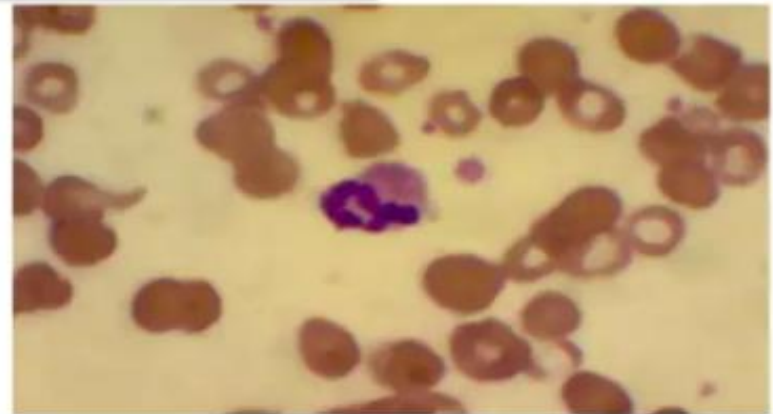
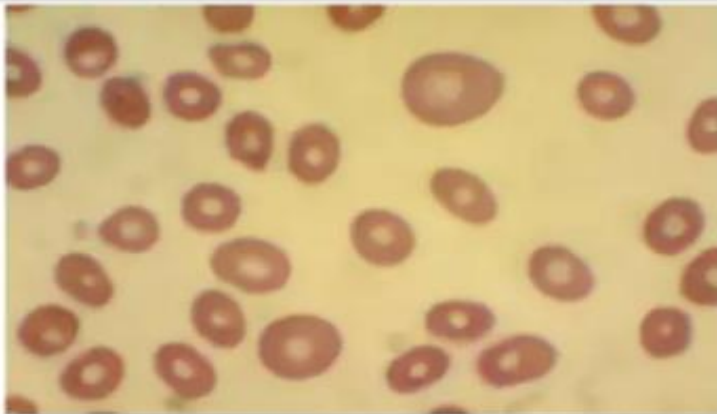
- Shifted to the **Right** = MCV ↑

Macrocytic population

RBCs Histogram

Volume Histogram

Macro-ovalocytes and neutrophil hypersegmentation

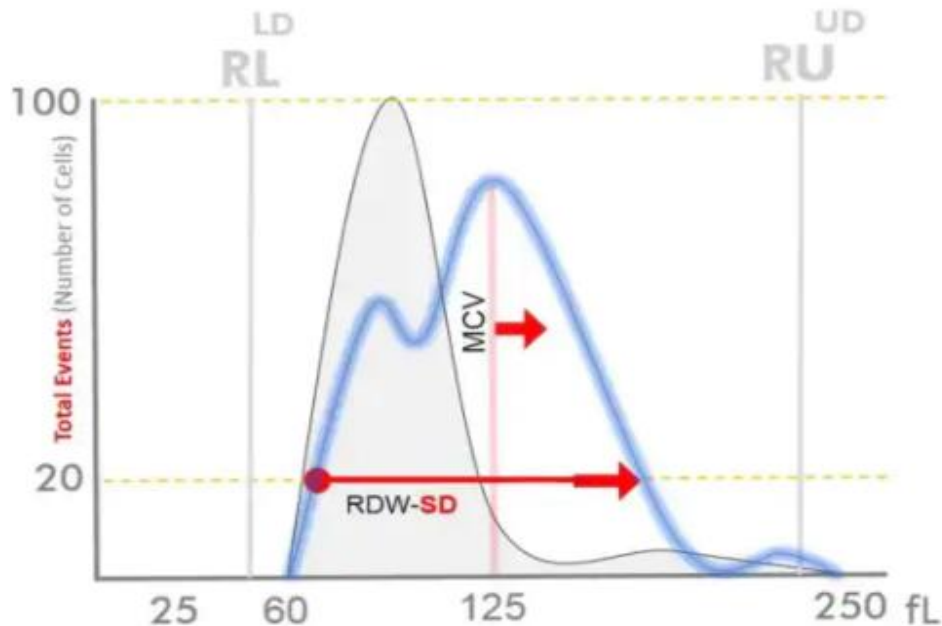


RBCs Histogram

Volume Histogram

Case 6

Recovery from Megaloblastic Anemia



RBCs Histogram

Volume Histogram

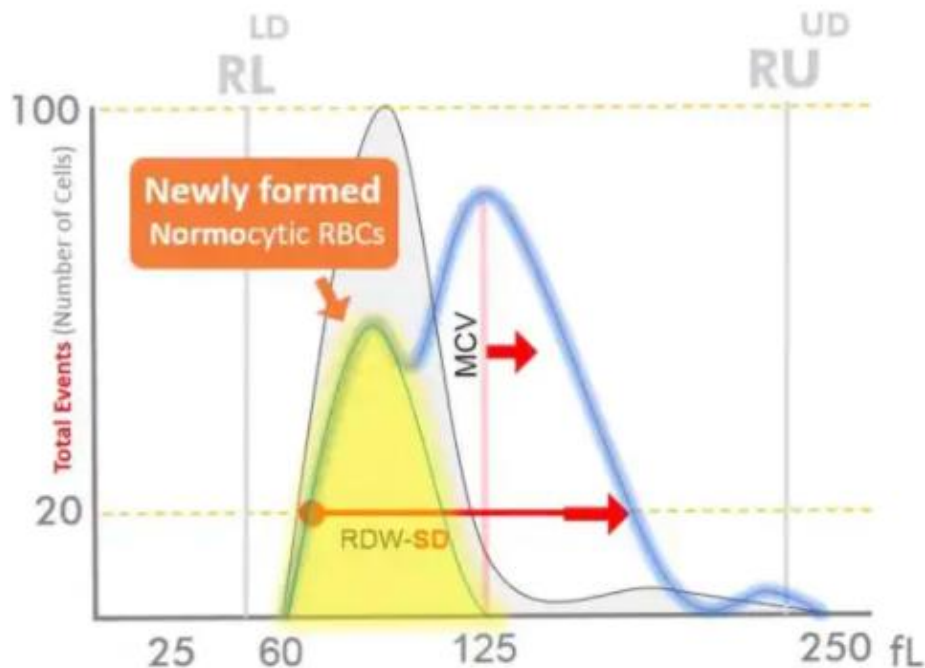
- RBCs count is $\uparrow\uparrow$
- MCV is not normal yet
- More $\uparrow\uparrow\uparrow$ RDW Higher than Untreated cases
- 2 Populations could be easily distinguished :

RBCs Histogram

Volume Histogram

Case 6

Recovery from Megaloblastic Anemia



RBCs Histogram

Volume Histogram

- RBCs count is $\uparrow\uparrow$
- MCV is not normal yet
- More $\uparrow\uparrow\uparrow$ RDW Higher than Untreated cases
- 2 Populations could be easily distinguished :

The **pre-existing** macrocytic RBCs

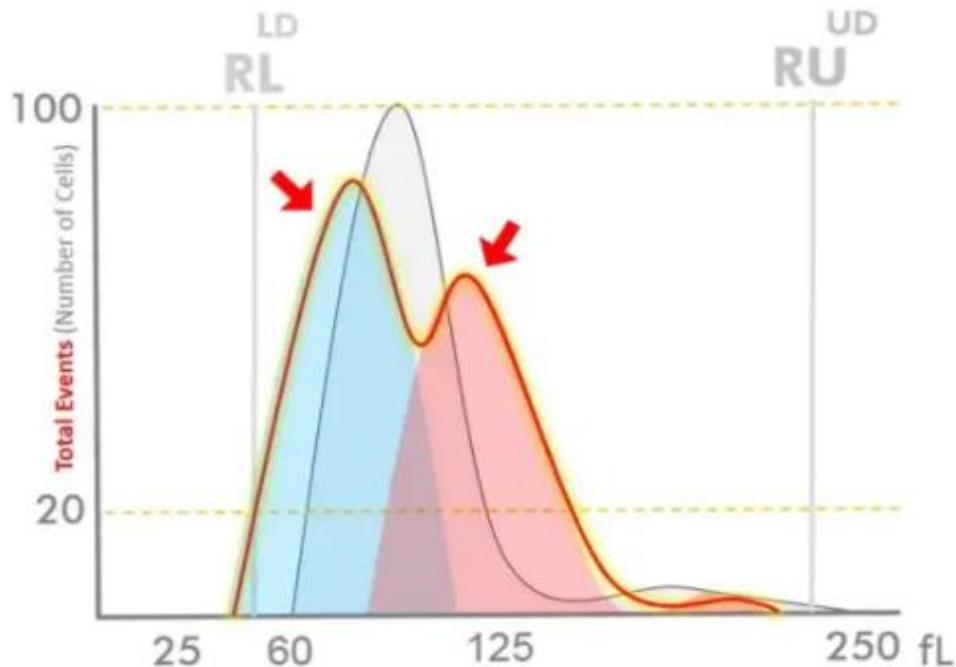
The **newly formed** normocytic RBCs

RBCs Histogram

Volume Histogram

Case 7

Bimodal or **MP** Curve
Multiple Peaks



RBCs Histogram

Volume Histogram

- **Dimorphic RBCs population**

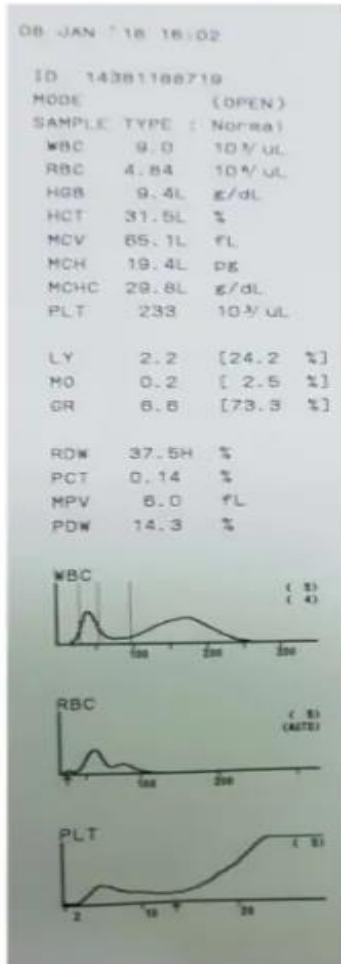
- **Possible causes**

1. Patients on Hematinic treatment
2. Following blood transfusion
3. Dual deficiency anemia

(Iron and Vit.B₁₂/Folic)

RBCs Histogram

Volume Histogram



After blood transfusion-Dimorphic

↑↑↑RDW → RDW base very wide (MP)



Abu Jad Caesar

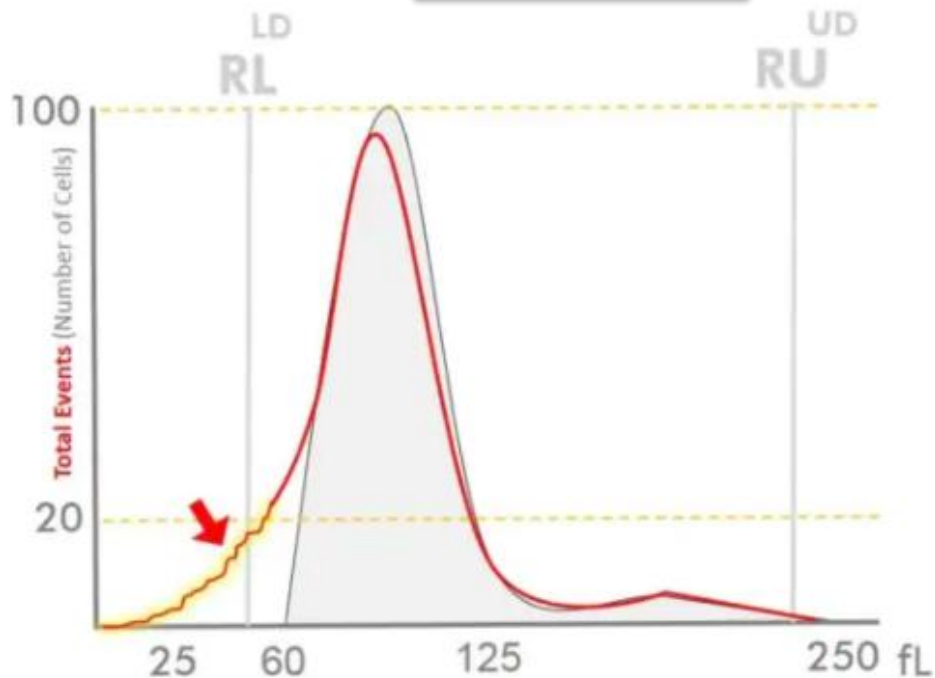
RBCs Histogram

Volume Histogram

Case 8

RBCs Fragmentation

Schistocytes



RBCs Histogram

Volume Histogram

- MCV is normal
- RDW is $\uparrow\uparrow$
- A plateau to the **left** of the curve peak

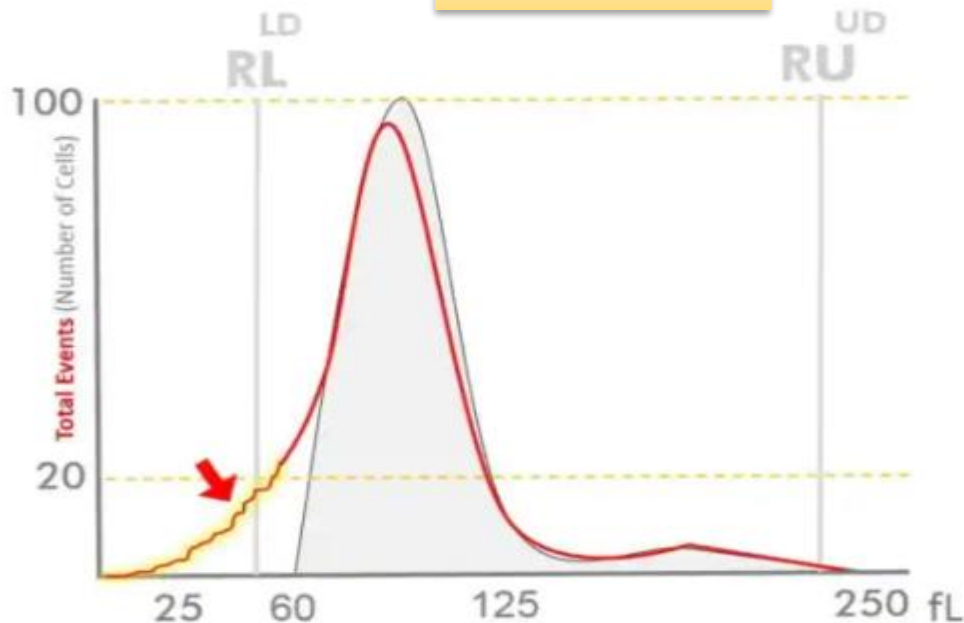
RBCs Histogram

Volume Histogram

Case 8

RBCs Fragmentation

Schistocytes



RBCs Histogram

Volume Histogram

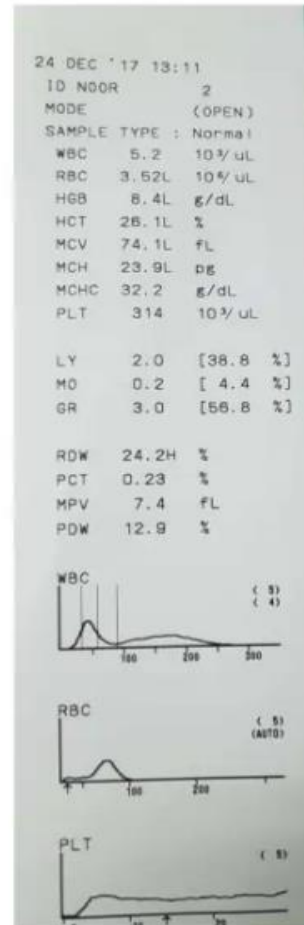
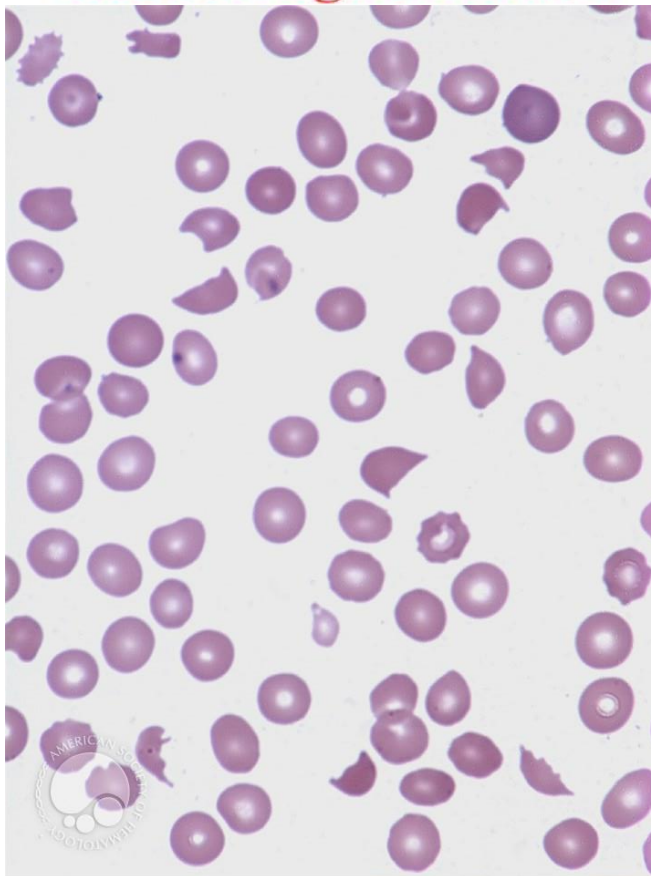
Possible Causes :

- DIC
- TTP
- HUS
- Burns
- Cardiac prosthetic valves
- Metastatic tumors
- Sickle cell anemia
- Megaloblastic anemia
- Cytotoxic chemotherapy
- HELLP

RBCs Histogram

Volume Histogram

RBCs fragmentation



RBCs Histogram

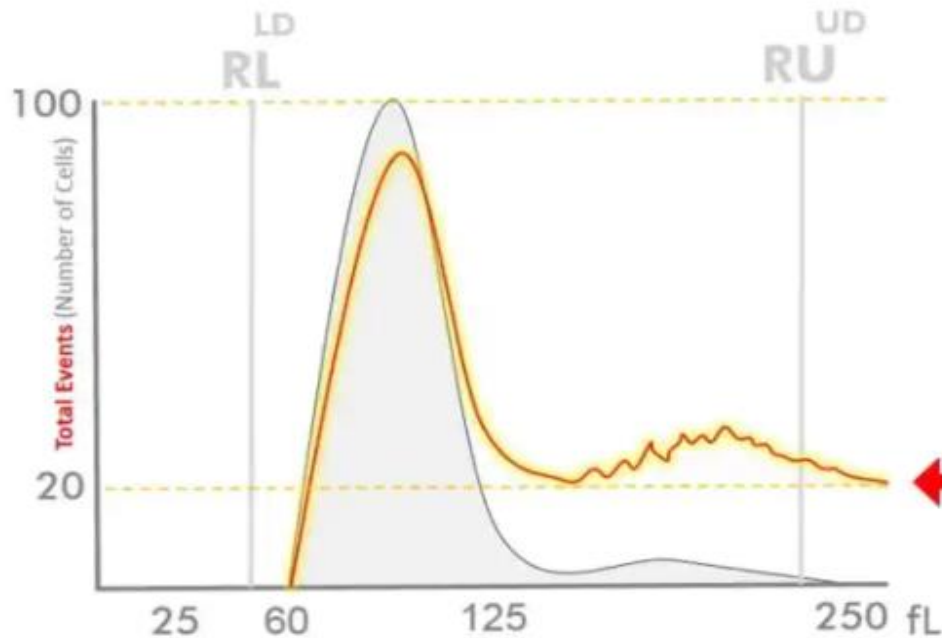
Volume Histogram

Case 9

RBCs Agglutination

RBCs Histogram

Volume Histogram



Normal RBC & PLT Histogram

Parameters

WBC	5.14	[10 ³ /uL]
RBC	4.57	[10 ⁶ /uL]
HGB	13.7 -	[g/dL]
HCT	42.2	[%]
MCV	92.3	[fL]
MCH	30.0	[pg]
MCHC	32.5 -	[g/dL]
RDW-SD	43.4	[fL]
RDW-CV	13.2	[%]
PLT	213	[10 ³ /uL]
PDW	10.7	[fL]
MPV	9.2	[fL]
P-LCR	19.4	[%]
PCT	0.20	[%]

NEUT	2.50	[10 ³ /uL]	48.7 -	[%]
LYMPH	2.15	[10 ³ /uL]	41.8 +	[%]
MONO	0.38	[10 ³ /uL]	7.4	[%]
EO	0.10	[10 ³ /uL]	1.9	[%]
BASO	0.01	[10 ³ /uL]	0.2	[%]

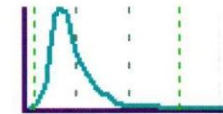
Normal Ranges

(4.40 - 11.30)
(4.50 - 5.90)
(14.0 - 17.4)
(41.5 - 50.4)
(80.0 - 96.0)
(27.5 - 32.5)
(33.4 - 35.0)
(37.0 - 54.0)
(11.0 - 14.5)
(150 - 450)
(9.0 - 14.0)
(9.0 - 12.0)
(13.0 - 43.0)
(0.17 - 0.35)
(54.0 - 62.0)
(25.0 - 35.0)
(2.0 - 8.0)
(0.0 - 1.0)
(0.5 - 3.0)

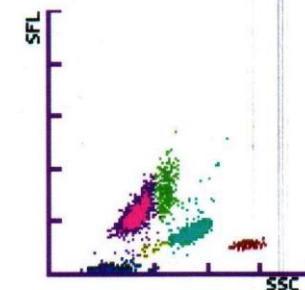
RBC



PLT



DIFF



WBC IP Message(s)

RBC IP Message(s)

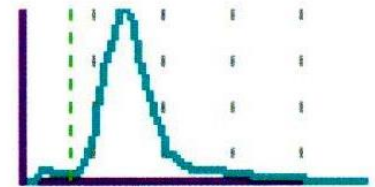
PLT IP Message(s)

IDA

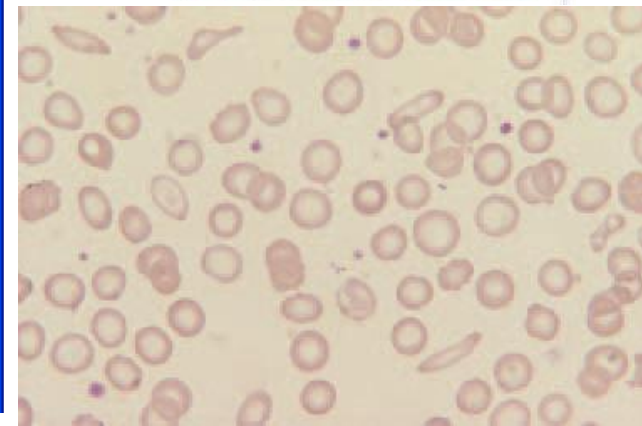
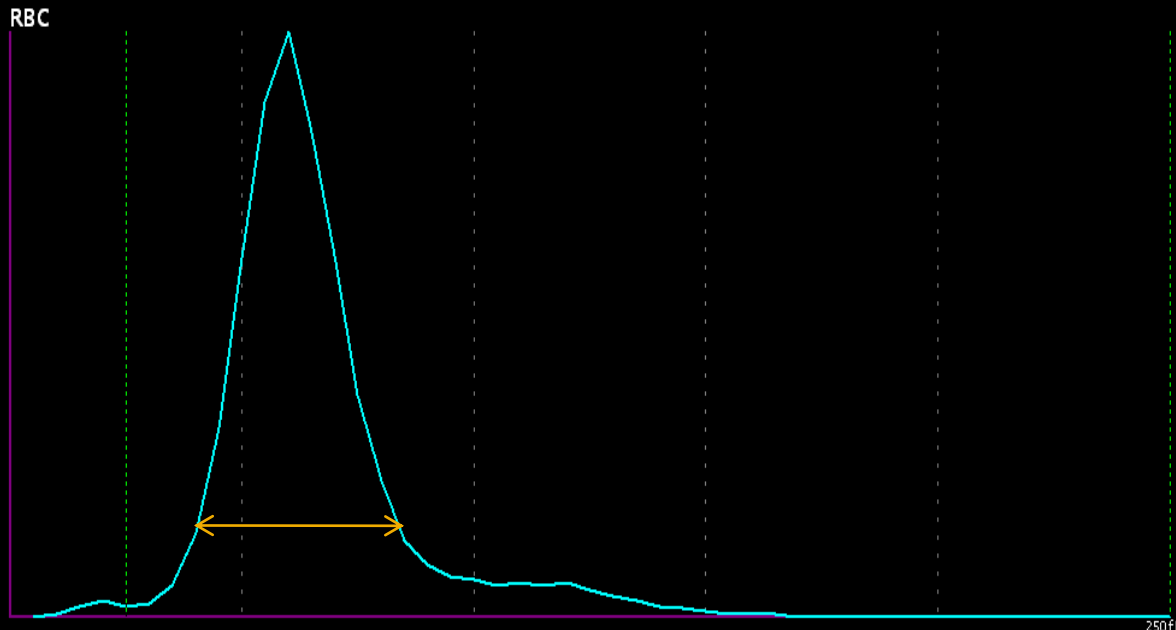
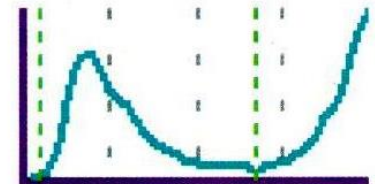
Parameters

WBC	4.74	[10 ³ /uL]
RBC	4.08 -	[10 ⁶ /uL]
HGB	9.5 -	[g/dL]
HCT	29.9 -	[%]
MCV	73.3 -	[fL]
MCH	23.3 -	[pg]
MCHC	31.8	[g/dL]
RDW-SD	51.0	[fL]
RDW-CV	21.4 +	[%]

RBC



PLT

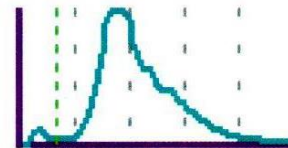


Megaloblastic anemia

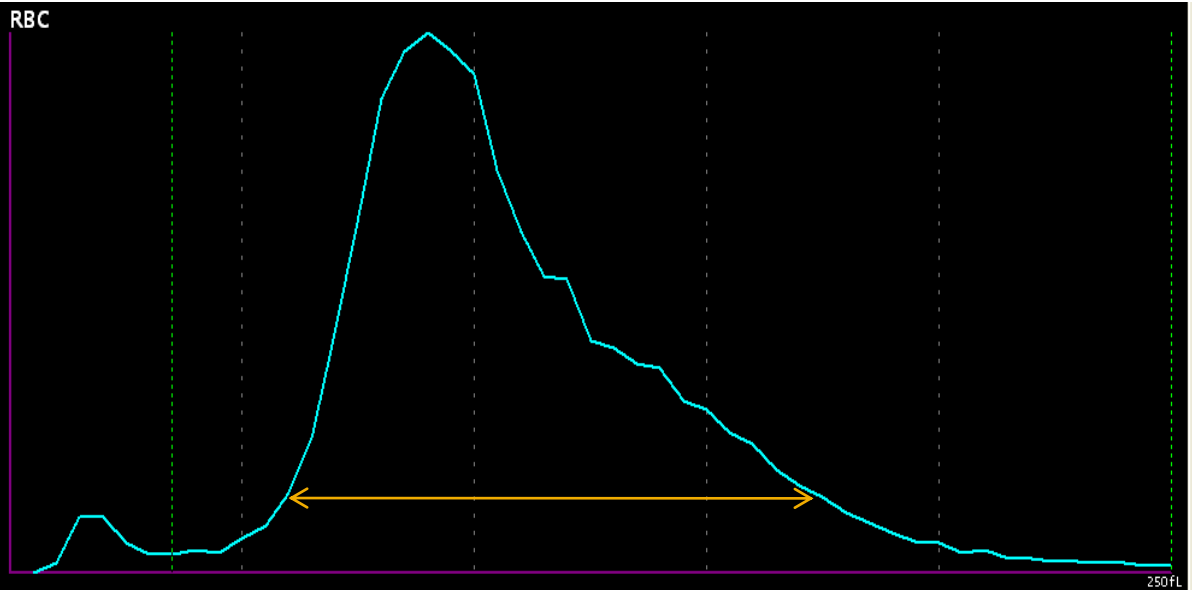
Parameters

WBC	3.70	-	[10 ³ /uL]
RBC	2.86	-	[10 ⁶ /uL]
HGB	9.4	-	[g/dL]
HCT	29.5	-	[%]
MCV	103.1	+	[fL]
MCH	32.9		[pg]
MCHC	31.9		[g/dL]
RDW-SD	92.7	+	[fL]
RDW-CV	27.7	+	[%]

RBC



PLT

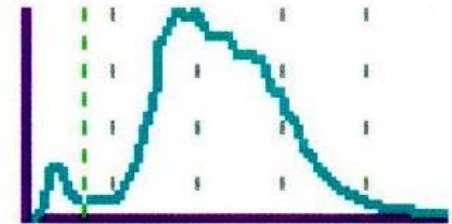


Megaloblastic Anemia

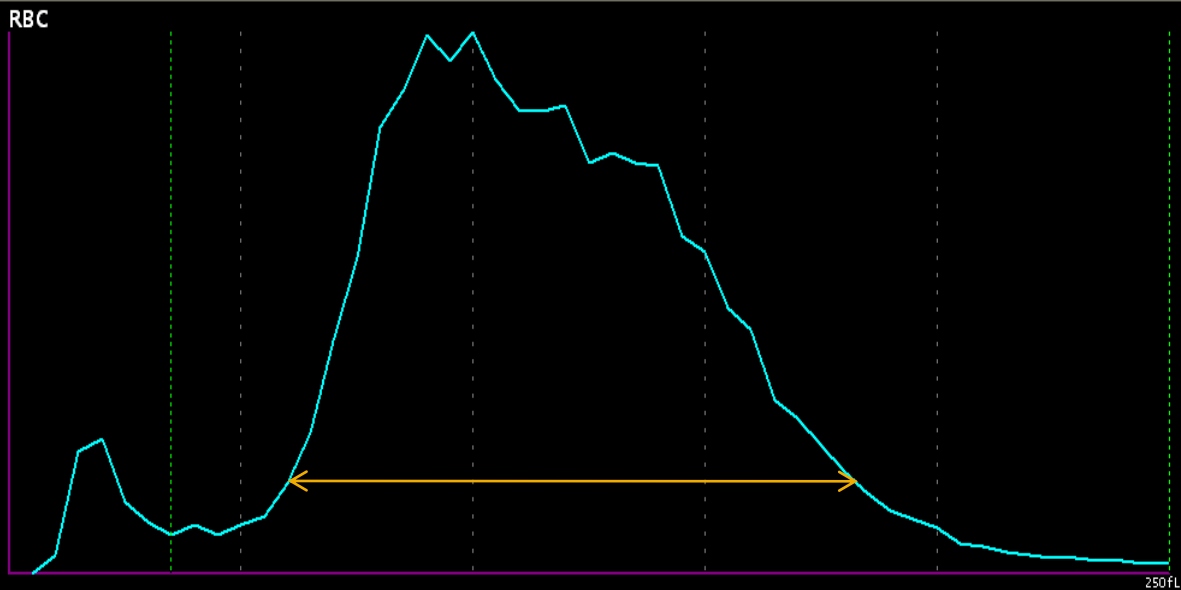
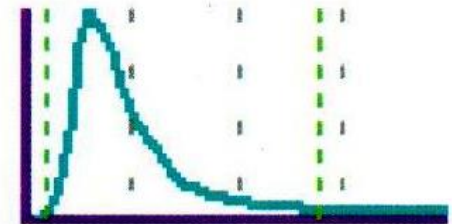
Parameters

WBC	4.29	-	[$10^3/\mu\text{L}$]
RBC	1.85	-	[$10^6/\mu\text{L}$]
HGB	6.6	-	[g/dL]
HCT	20.8	-	[%]
MCV	112.4	+	[fL]
MCH	35.7	+	[pg]
MCHC	31.7	-	[g/dL]
RDW-SD	106.9	+	[fL]
RDW-CV	29.2	+	[%]

RBC



PLT



Hemolytic Anemia

Parameters

WBC 3.21 - [10³/uL]
 RBC 3.50 - [10⁶/uL]
 HGB 10.2 - [g/dL]
 HCT 33.1 - [%]
 MCV 94.6 [fL]
 MCH 29.1 [pg]
 MCHC 30.8 - [g/dL]
 RDW-SD 70.4 + [fL]
 RDW-CV 21.2 + [%]

PLT 259 [10³/uL]
 PDW 15.3 + [fL]
 MPV 11.2 [fL]
 P-LCR 34.5 [%]
 PCT 0.29 [%]

NEUT 1.73 * [10³/uL] 53.9 * [%]
 LYMPH 0.78 - [10³/uL] 24.3 - [%]
 MONO 0.59 + [10³/uL] 18.4 + [%]
 EO 0.09 * [10³/uL] 2.8 * [%]
 BASO 0.02 * [10³/uL] 0.6 * [%]

WBC IP Message(s)

Lymphopenia

Immature Gran?

RBC IP Message(s)

Anisocytosis mod
 Hypochromia

Schisto (mod)

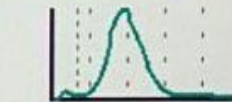
Normal Ranges RBC

(4.50 - 11.00)
 (4.00 - 5.50)
 (12.5 - 17.5)
 (38.0 - 53.0)
 (80.0 - 97.0)
 (26.0 - 34.0)
 (31.0 - 36.0)
 (37.0 - 54.0)
 (11.0 - 14.5)

(150 - 450)
 (9.0 - 14.0)
 (9.0 - 12.0)
 (13.0 - 43.0)
 (0.17 - 0.35)

PLT IP Message(s)

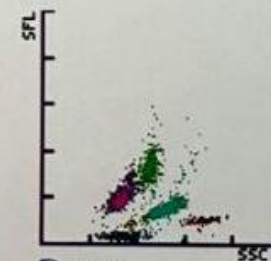
Keratocyte (mild)



PLT



DIFF



P 64

L 23

M 13

NRBC 1% of WBC (100)

sphero (mild)

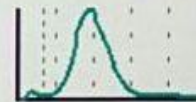
Hemolytic Anemia

Parameters

WBC	3.21 -	[10 ³ /uL]	(4.50 - 11.00)
RBC	3.50 -	[10 ⁶ /uL]	(4.00 - 5.50)
HGB	10.2 -	[g/dL]	(12.5 - 17.5)
HCT	33.1 -	[%]	(38.0 - 53.0)
MCV	94.6	[fL]	(80.0 - 97.0)
MCH	29.1	[pg]	(26.0 - 34.0)
MCHC	30.8 -	[g/dL]	(31.0 - 36.0)
RDW-SD	70.4 +	[fL]	(37.0 - 54.0)
RDW-CV	21.2 +	[%]	(11.0 - 14.5)
PLT	259	[10 ³ /uL]	(150 - 450)
PDW	15.3 +	[fL]	(9.0 - 14.0)
MPV	11.2	[fL]	(9.0 - 12.0)
P-LCR	34.5	[%]	(13.0 - 43.0)
PCT	0.29	[%]	(0.17 - 0.35)
NEUT	1.73 *	[10 ³ /uL]	53.9 * [%] (54.0 - 62.0)
LYMPH	0.78 -	[10 ³ /uL]	24.3 - [%] (25.0 - 35.0)
MONO	0.59 +	[10 ³ /uL]	18.4 + [%] (3.0 - 8.0)
EO	0.09 *	[10 ³ /uL]	2.8 * [%] (0.0 - 1.0)
BASO	0.02 *	[10 ³ /uL]	0.6 * [%] (0.5 - 3.0)

Normal Ranges

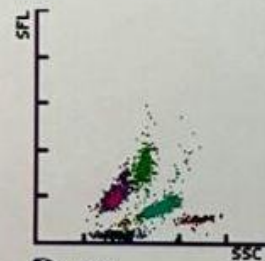
RBC



PLT



DIFF

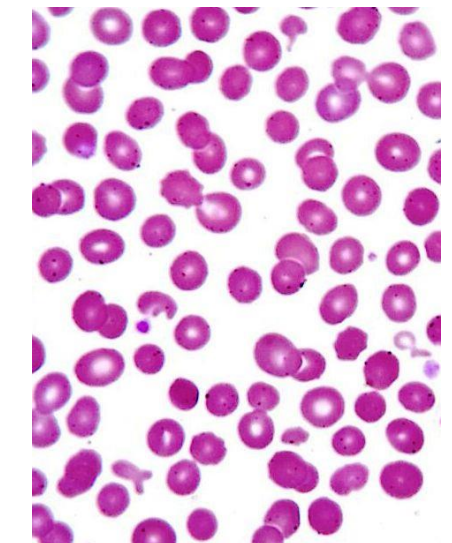
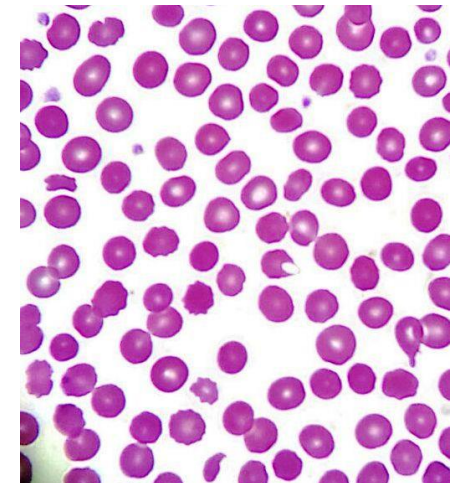


P 64

L 23

M 13

NRBC 1% of WBC (100)



WBC IP Message(s)

RBC IP Message(s)

PLT IP Message(s)

Lymphopenia

Anisocytosis

Hypochromia

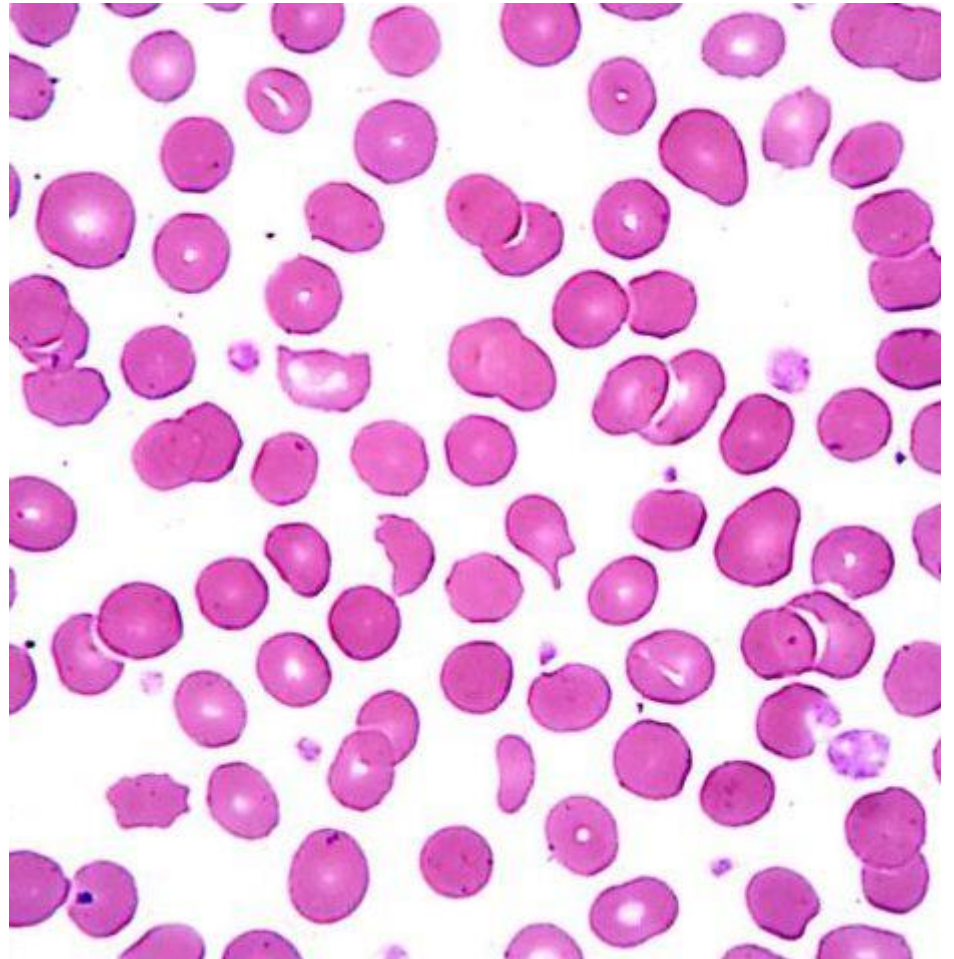
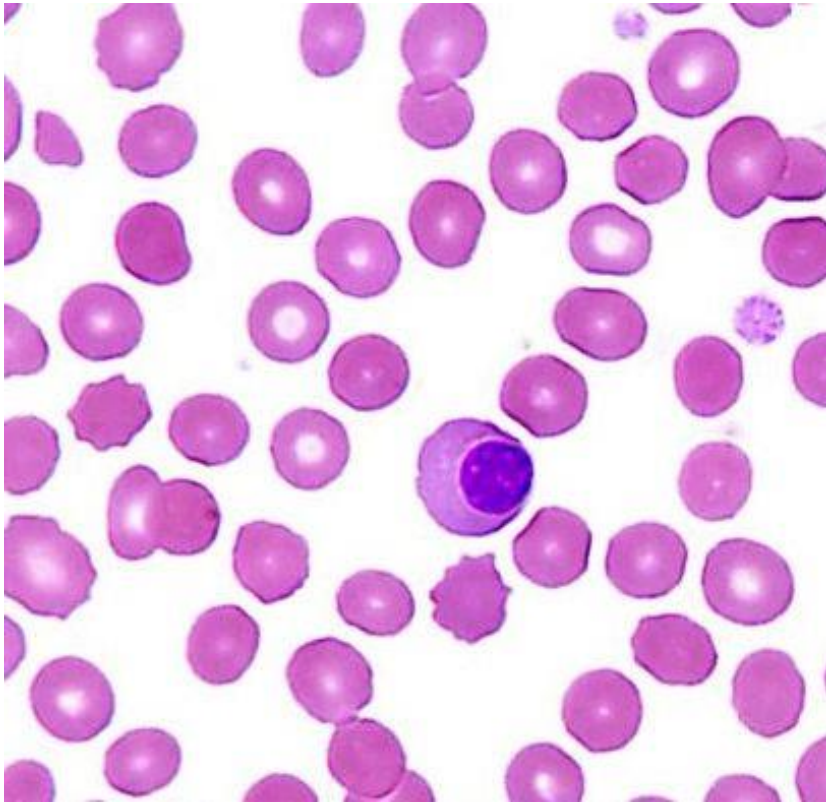
Immature Gran?

Schisto (mod)

Keratocyte (mild)

sphero (mild)

Hemolytic Anemia



Severe Macrocytic Anemia

RDW-SD or CV?

Parameters	Unit	Reference Value
WBC	7.31 [10 ³ /uL]	(4.00 - 11.00)
RBC	3.46- [10 ⁶ /uL]	(4.20 - 5.40)
HGB	14.1 [g/dL]	(12.5 - 16.0)
HCT	41.5 [%]	(37.0 - 47.0)
MCV	119.9+ [fL]	(78.0 - 100.0)
MCH	40.8+ [pg]	(27.0 - 31.0)
MCHC	34.0 [g/dL]	(32.0 - 37.0)
PLT	443 [10 ³ /uL]	(140 - 450)
RDW-SD	56.6+ [fL]	(37.0 - 54.0)
RDW-CV	13.0 [%]	(11.5 - 14.0)
PDW	9.9 [fL]	(9.0 - 17.0)
MPV	8.7- [fL]	(9.0 - 13.0)
P-LCR	15.5- [%]	(17.0 - 47.0)
PCT	0.38+ [%]	(0.17 - 0.35)

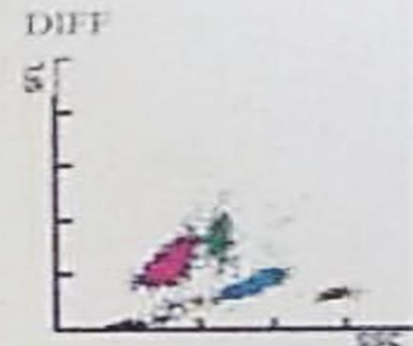
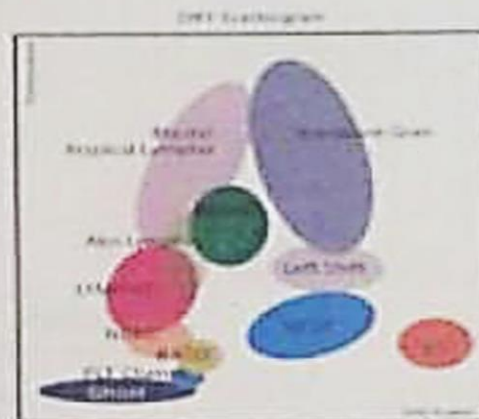
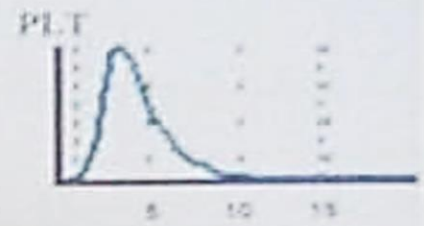
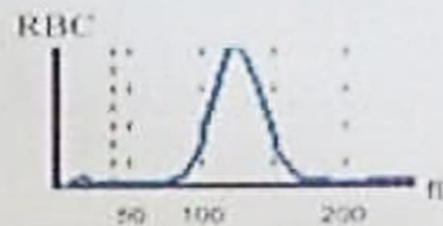
References values has been adjusted by sex and age !

NEUT	3.16 [10 ³ /uL]	43.2 [%]
LYMPH	3.36 [10 ³ /uL]	46.0 [%]
MONO	0.65 [10 ³ /uL]	8.9 [%]
EO	0.11 [10 ³ /uL]	1.5 [%]
BASO	0.03 [10 ³ /uL]	0.4 [%]

WBC IP Message(s)

RBC IP Message(s)
Macrocytosis

PLT IP Message(s)



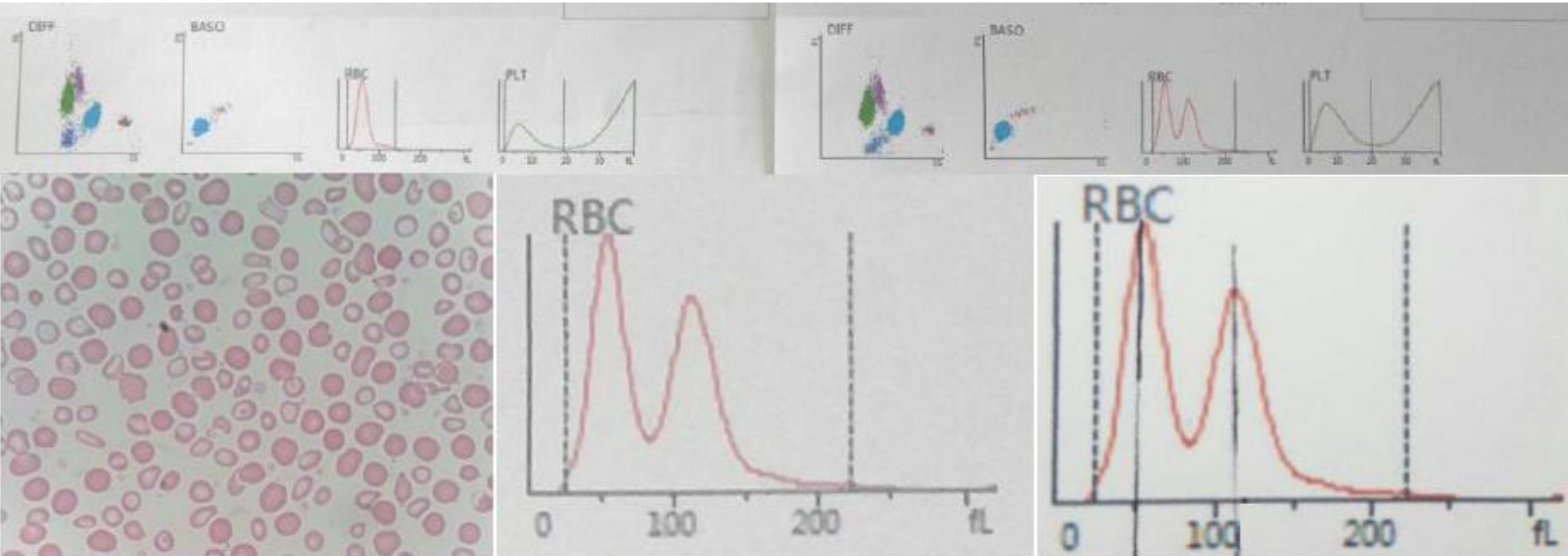
ماکروسیتیک شدید منجر به کاهش کاذب RDW-CV شده است. البته RBC نسبتاً پایین باعث افزایش MCH شده ولی روی MCHC تاثیر نداشته است

فقدان دم راست در هیستوگرام RBC احتمال Cold Agg را رد می کند.

Treatment of severe IDA & Megaloblastic crisis

MP Flag

Multiple Peaks

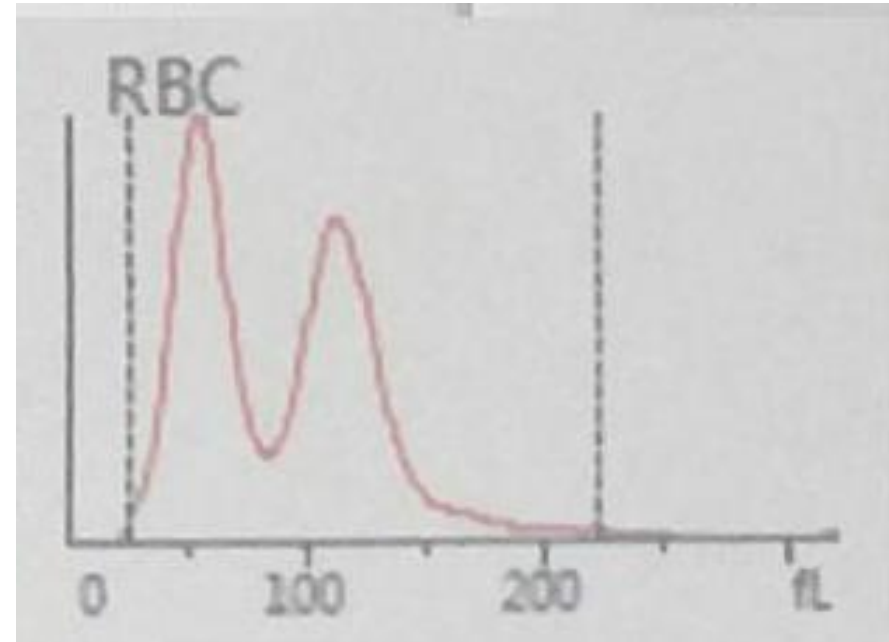
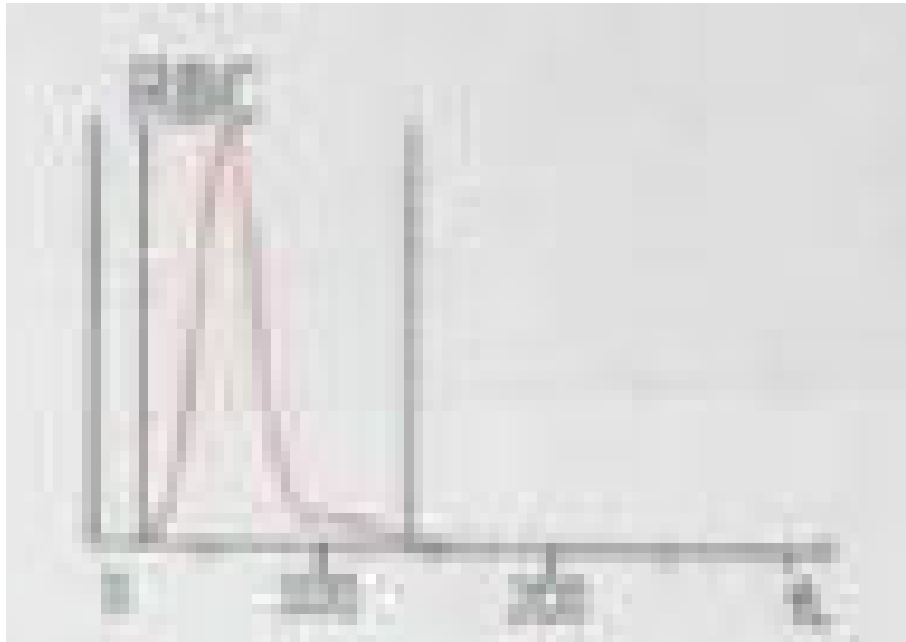


شکل ۶۲-۱۰: بیمار خانم ۳۶ ساله هست که ۳ سال پیش بعد از سزارین و خونریزی شدید با فریتین ۲.۰ و هموگلوبین ۷.۵g/dl مراجعه کرده ولی طی این مدت هرگز به آهن خوراکی پاسخ نپداده تا اینکه مرداد ماه سال ۹۶ پزشک ۴ واحد آهن تزریقی به بیمار تجویز میکند که نتیجه آن فوق العاده موثر بوده و جمعیت جدیدی از اریتروسیت‌ها شکل گرفته و فریتین بیمار اکنون ۲۹۹ و TIBC ۳۳۶ و آهن سرم ۸۳ می‌باشد. ولی یک سوال مهم هنوز باقی مانده که چرا با فقدان پلی کرومازیا و رتیکولوسیتوز (رتیک ۱/۴٪)، جمعیت جدید ماکروسیتوز بوده و پیک قدیمی روی ۵۴fL و جمعیت جدید روی ۱۱۲fL و کاملاً ماکروسیتیک می‌باشد که میانگین دو جمعیت ۷۹fL و مقدار RDW حدود ۴۸٪ شده است. در حل این مسئله وضعیت ماکروسیتوز، اووالوسیتوز و نوتروفیل هیپر سگمانته چک شد که هر سه در مقادیر +۱ مشهود بودند، لذا می‌توان نتیجه گرفت که همزمانی فقر فولات یا کوبالامین نیز شاید سوار بر فقر آهن شده و این رخداد باعث ماکروسیتوز جدید و کاهش رتیک (کریز مگالوبلاستیک) شده است. برای بیمار بررسی بیشتر، سطح فولات و کوبالامین (B9 و B12) درخواست گردید و در عین حال از عدم دریافت فراورده خونی طی مدت مذکور نیز اطمینان حاصل گردید. سوال دوم شمارش RBC بیمار هست که حتی در روزگار شدید آنمی که Hb زیر ۸ بود، کماکان RBC بالای ۵ میلیون بوده و هنوز هم هست و لذا یک تابلوی مینور هم مورد شک هست که متعاقب از بین رفتن پیک دوم و پایداری شرایط می‌بایست بررسی شود. سوال سوم پلاکت بالای ۹۰۰ هزار هست که البته در اریتروپوئز شدید و تحریک BM و طحال می‌تواند به صورت موقتی رخ بدهد.

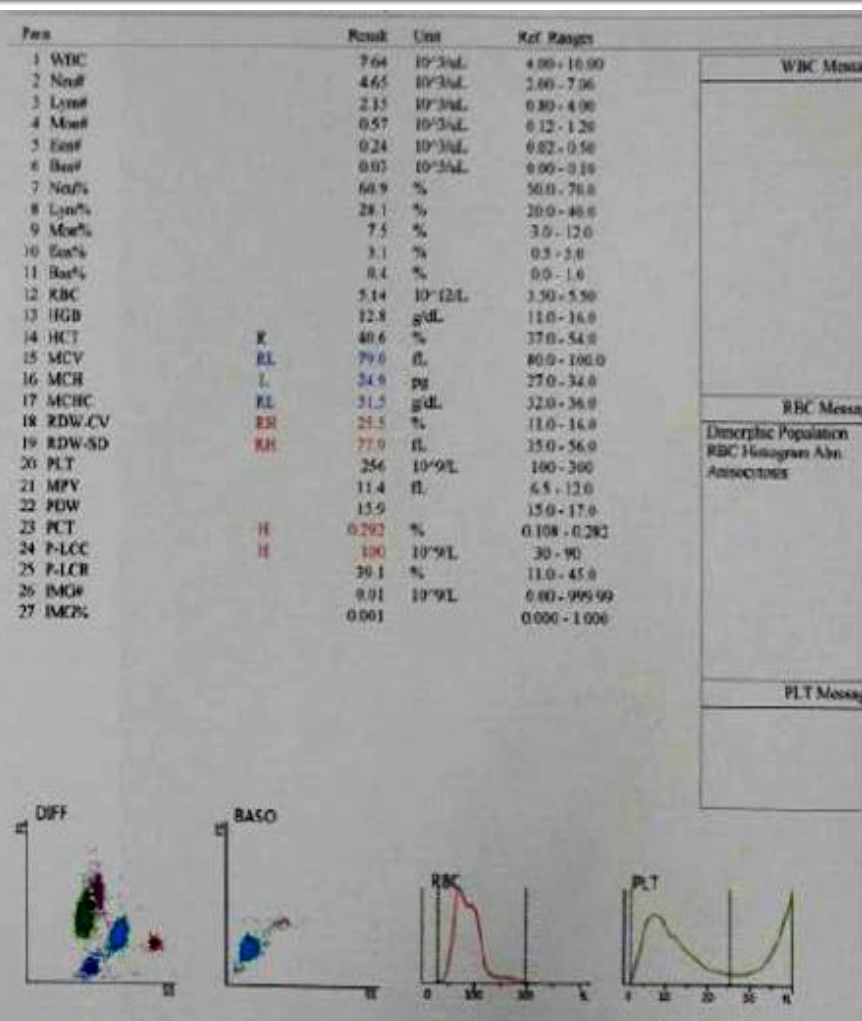
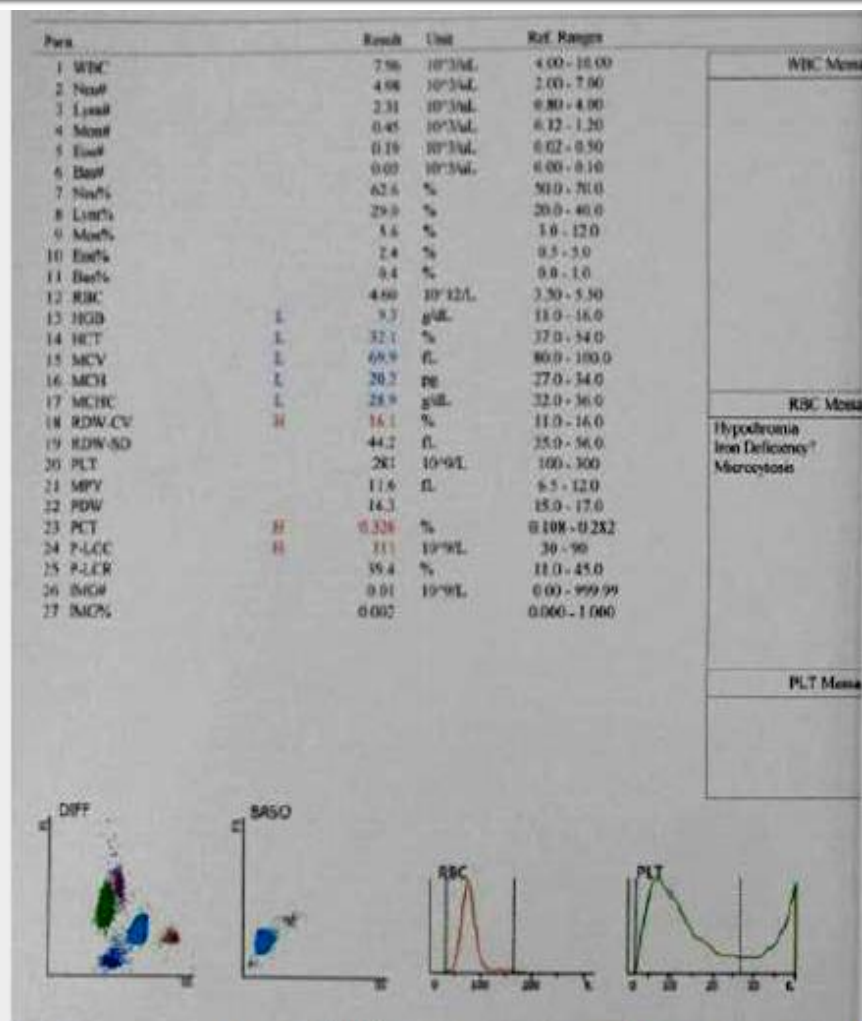
Treatment of severe IDA & Megaloblastic crisis

MP Flag

Multiple Peaks



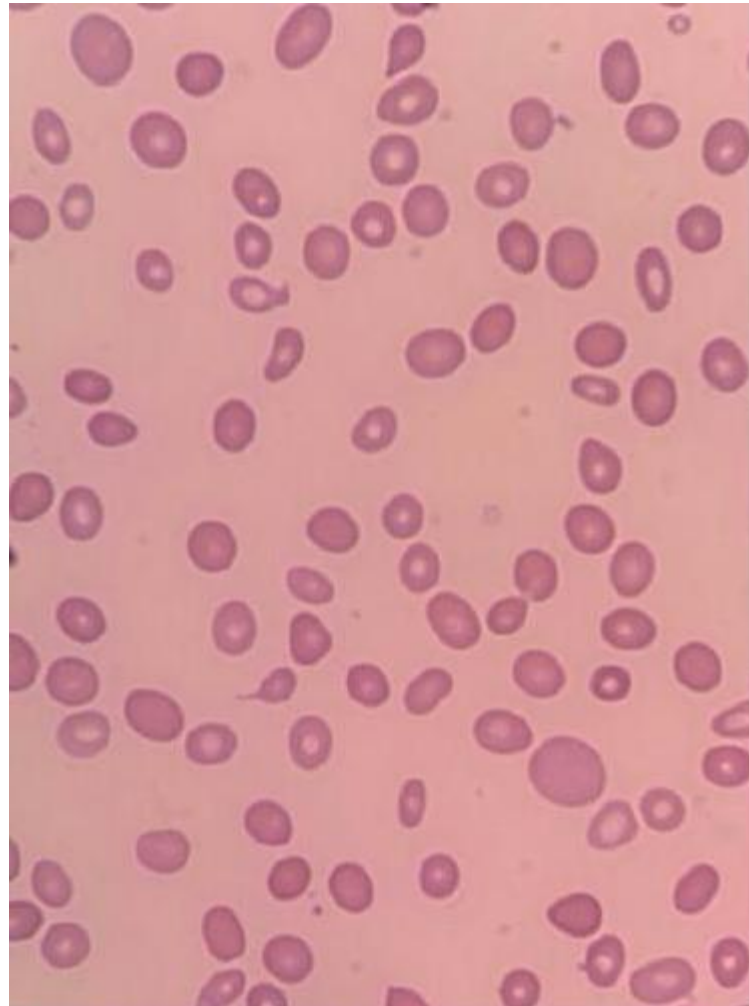
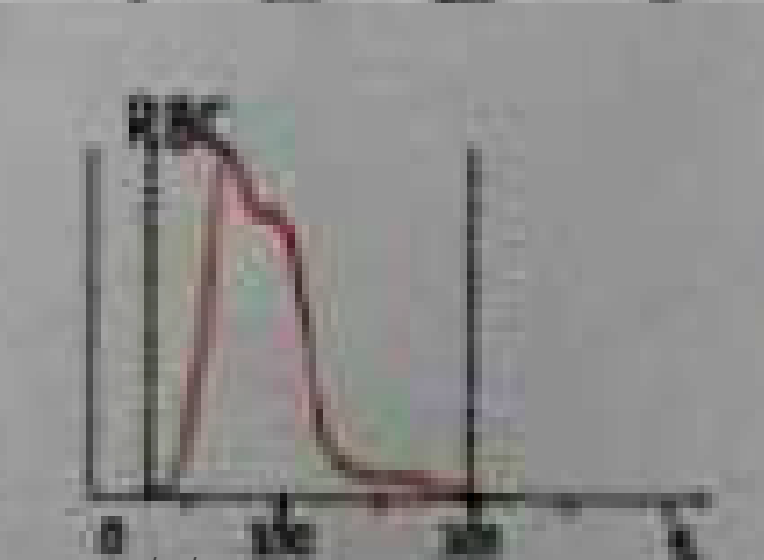
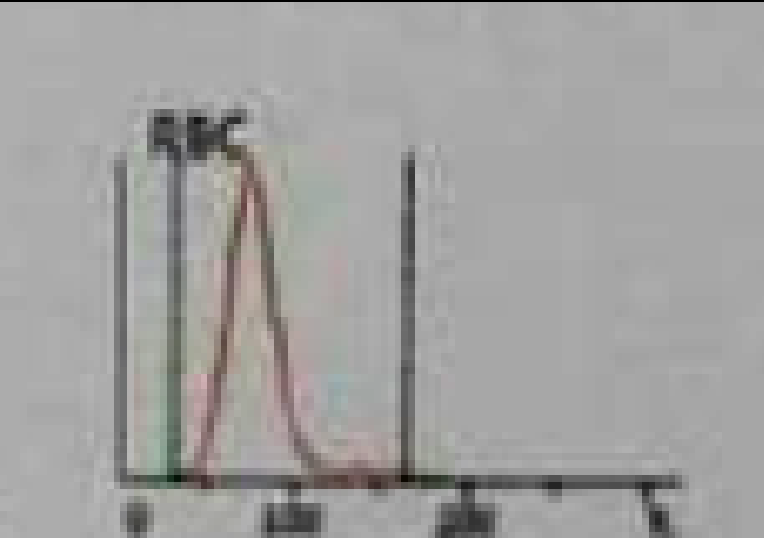
RBC Histogram after treatment of severe IDA



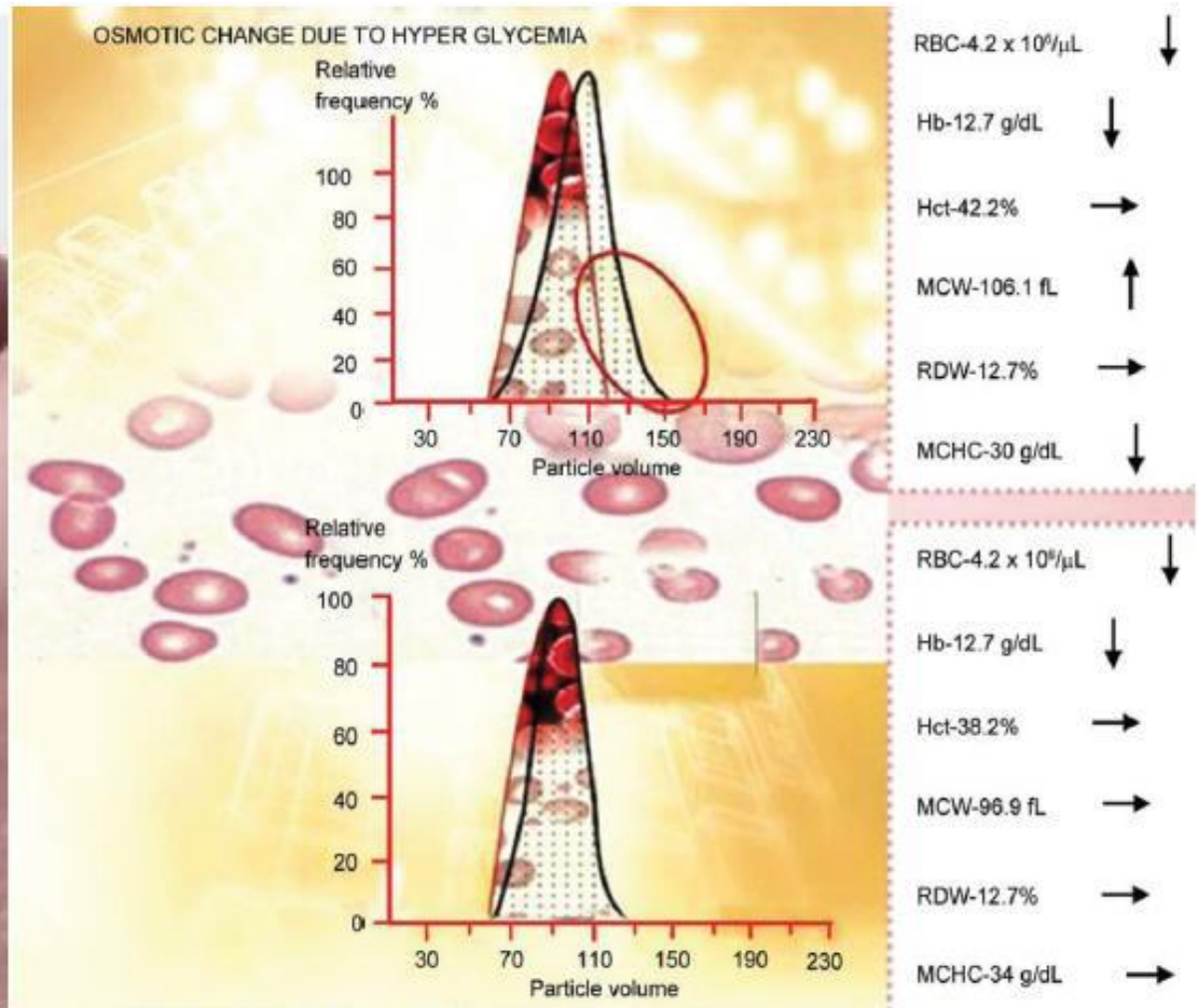
شکل ۶۳-۱۰: بیمار احتمالاً تالاسمیک مینور توام با فقر آهن شدید هست که طی درمان یک ماهه با آهن. فریتین از ۳ به ۱۶ افزایش یافته ولی کماکان TIBC بالای ۴۲۰ هست و هیستوگرام دوقله ای شده است. مقدار RBC, MCV, MCH, MCHC و RDW همگی افزایش محسوس داشته و هیستوگرام RBC دوقله ای شده است.

RBC Histogram after treatment of severe IDA

Dimorphic Picture



Hyperglycemia & Acute swelling phenomenon

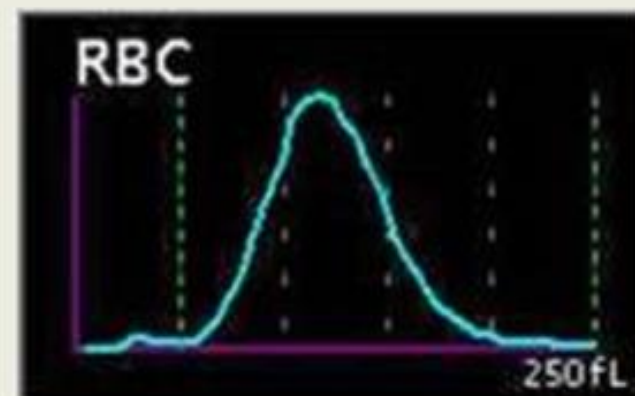


شکل ۱۳۹-۱۰: سندرم تورم حاد ناشی از هیپرگلیسمی که بعد از انکوباسیون در ایزوتون به سایز اصلی خود بازگشته است.

High Serum Glucose

Acute swelling phenomenon

Items				WBC Differential			
Item	Data		Unit	Item	Data		Unit
WBC	7.29	*	$10^3/\mu\text{L}$	NEUT#	5.04	*	$10^3/\mu\text{L}$
RBC	2.57		$10^6/\mu\text{L}$	LYMPH#	1.23	*	$10^3/\mu\text{L}$
HGB	7.5		g/dL	MONO#	0.96	*	$10^3/\mu\text{L}$
HCT	31.1		%	EO#	0.05	*	$10^3/\mu\text{L}$
MCV	121.0	+	fL	BASO#	0.01	*	$10^3/\mu\text{L}$
MCH	29.2		pg	NEUT%	69.1	*	%
MCHC	24.1	-	g/dL	LYMPH%	16.9	*	%
PLT	96	*	$10^3/\mu\text{L}$	MONO%	13.2	*	%
RDW-SD	100.2		fL	EO%	0.7	*	%
RDW-CV	22.7		%	BASO%	0.1	*	%
MPV	14.0	*	fL	Extended Differential			
RET%	0.77		%	WBC			
RET#	1.98		$10^4/\mu\text{L}$	Item	Data		Unit
IRF	0.130		Ratio	IG#	0.06	*	$10^3/\mu\text{L}$
NRBC#	0.00	*	$10^3/\mu\text{L}$	TC%	0.8	*	%
NRBC%	0.0	*	/100WBC				

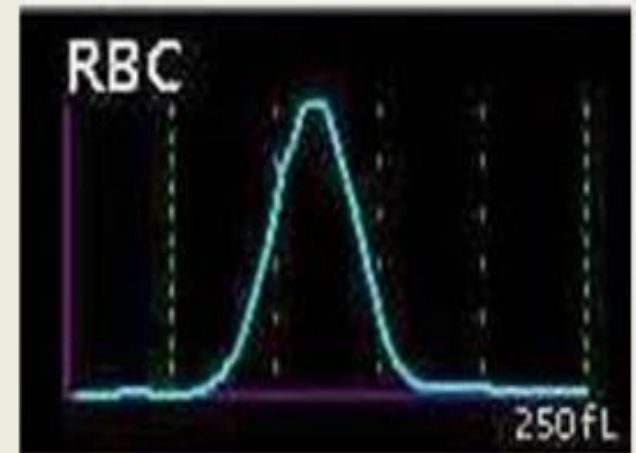


VERY HIGH..... 1494 mg/dL

High Serum Sodium(Na=176)

Acute swelling phenomenon

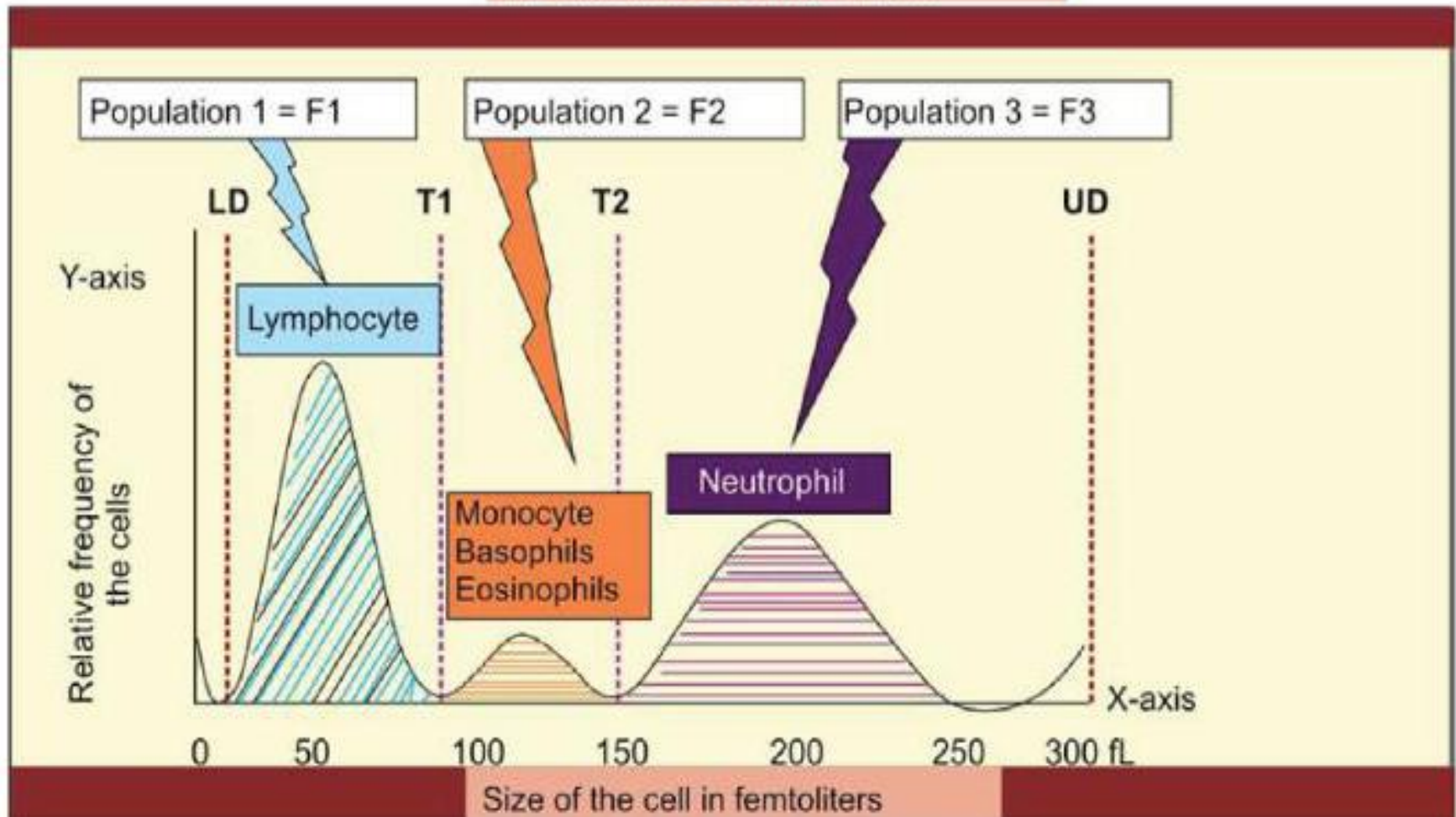
Items			WBC Differential		
Item	Data	Unit	Item	Data	Unit
WBC	2.41	$10^3/\mu\text{L}$	NEUT#	1.44	$10^3/\mu\text{L}$
RBC	2.89	$10^6/\mu\text{L}$	LYMPH#	0.58	$10^3/\mu\text{L}$
HGB	9.4	g/dL	MONO#	0.30	$10^3/\mu\text{L}$
HCT	34.2	%	EO#	0.08	$10^3/\mu\text{L}$
MCV	118.3	fL	BASO#	0.01	$10^3/\mu\text{L}$
MCH	32.5	pg	NEUT%	59.8	%
MCHC	27.5	g/dL	LYMPH%	24.1	%
PLT	124	$10^3/\mu\text{L}$	MONO%	12.4	%
RDW-SD	70.9	fL	EO%	3.3	%
RDW-CV	16.5	%	BASO%	0.4	%
MPV	11.8	fL	Extended Differential		
RET%		%	WBC		
RET#		$10^4/\mu\text{L}$	Item	Data	Unit
IRF		Ratio	IG#	0.00	$10^3/\mu\text{L}$
NRBC#		$10^3/\mu\text{L}$	IG%	0.0	%
NRBC%		/100WBC			



WBCs Histogram

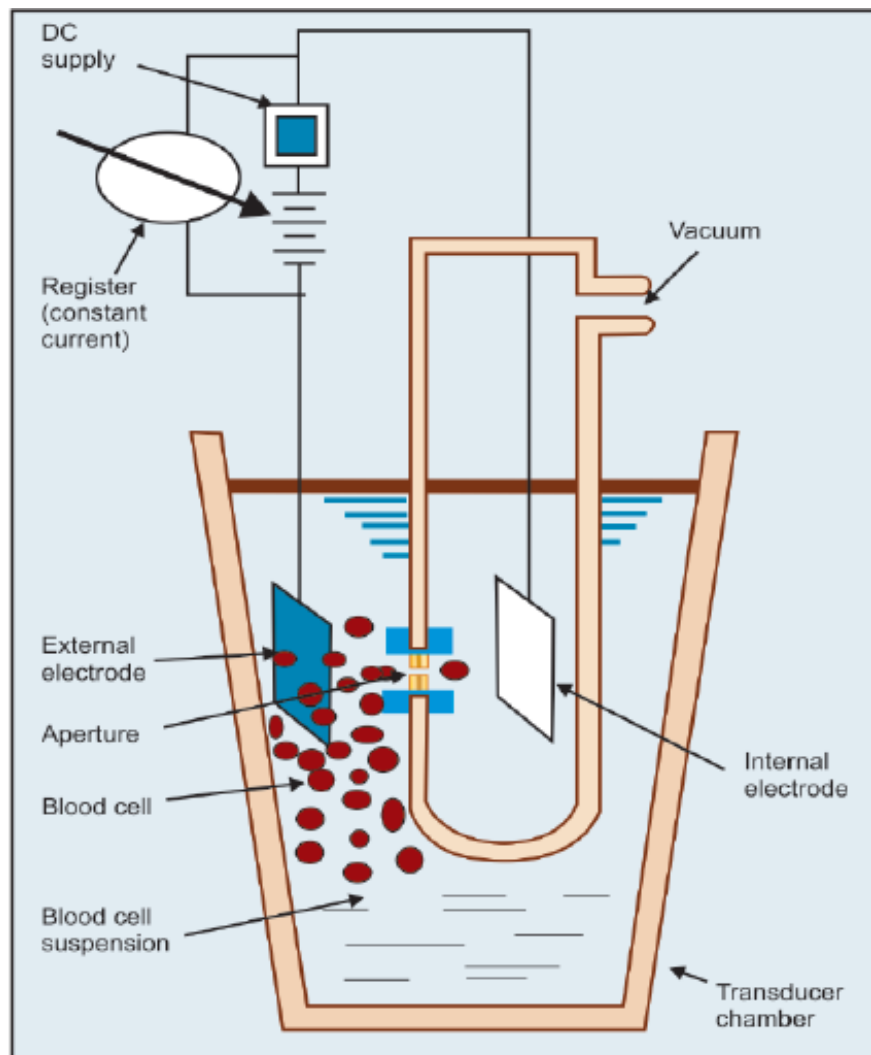
3-Part Differentiation

NORMAL WBC HISTOGRAM

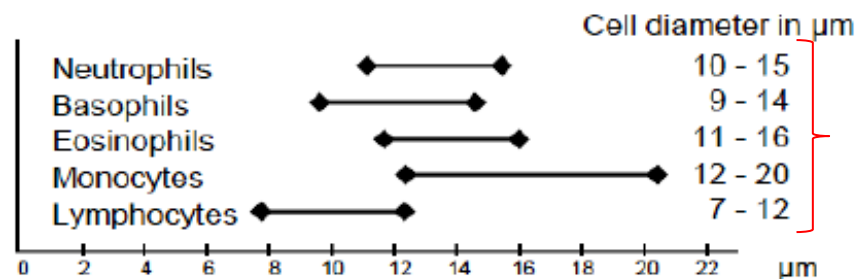


■ WBC detection is between 30 and 300 fL.

WBC Histograms



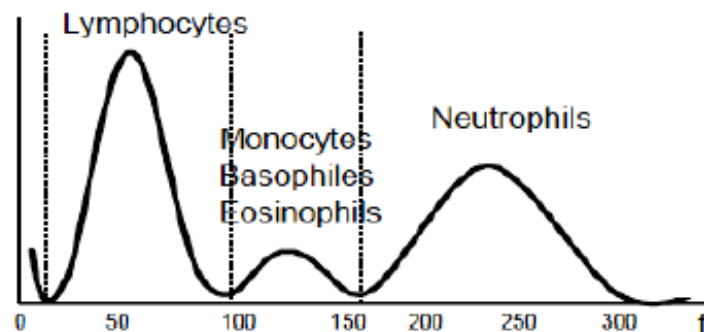
Before adding lysing reagent



After adding lysing reagent

Cell diameter in fl

Cell Type	Cell diameter range (fl)
Lymphocytes	30 - 80
Monocytes	60 - 120
Basophils	70 - 130
Eosinophils	80 - 140
Neutrophils	120 - 250



شکل ۱۲-۱: اختلاف سایز لکوسیت‌ها قبل و بعد از لیز نسبی در سل کانتر

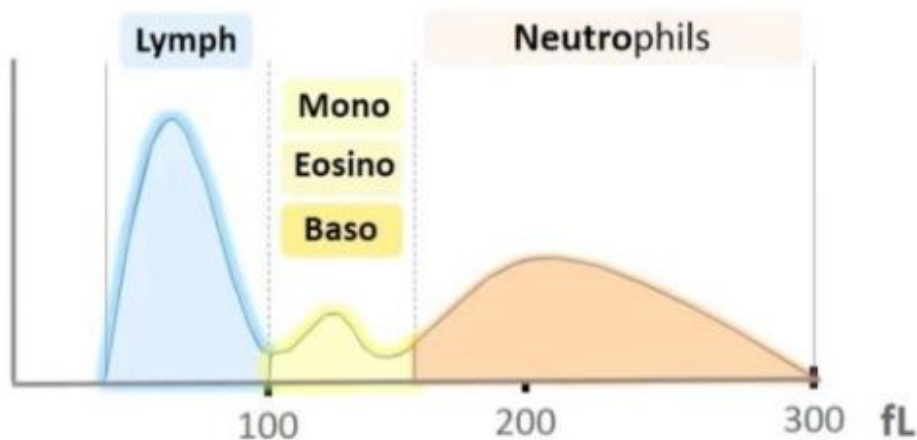
WBCs Histogram

3-Part Differentiation

LYSE Reagent effect on WBCs

After adding **Lyse** Reagent

- Cells will be shown in a histogram according to their **size**.



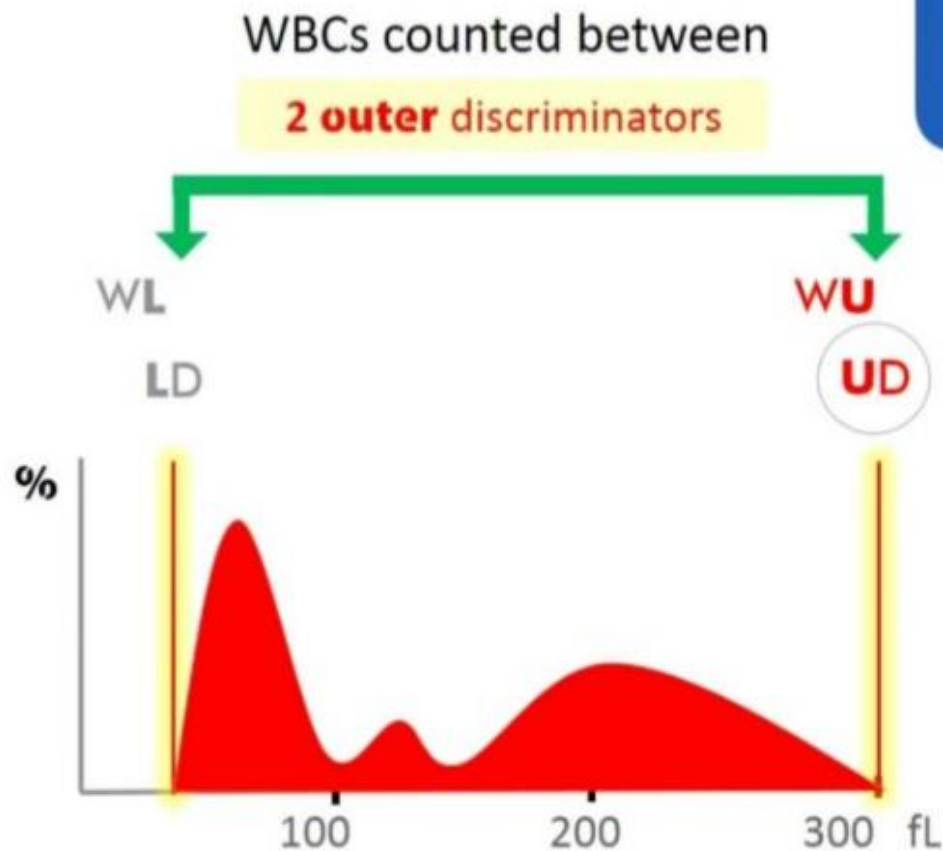
WBCs Histogram

3-Part Differentiation

Lymphocytes	30 – 80	fL
Monocytes	60 – 120	fL
Basophils	70 – 130	fL
Eosinophils	80 – 140	fL
Neutrophils	120 – 250	fL

WBCs Histogram

3-Part Differentiation



WBCs Histogram

3-Part Differentiation

WL WBCs Lower discriminator
= LD Lower Discriminator
[Flexible] fluctuates between 30 – 60 fL

WU WBCs Upper discriminator
= UD Upper Discriminator
[Fixed] at 300 fL

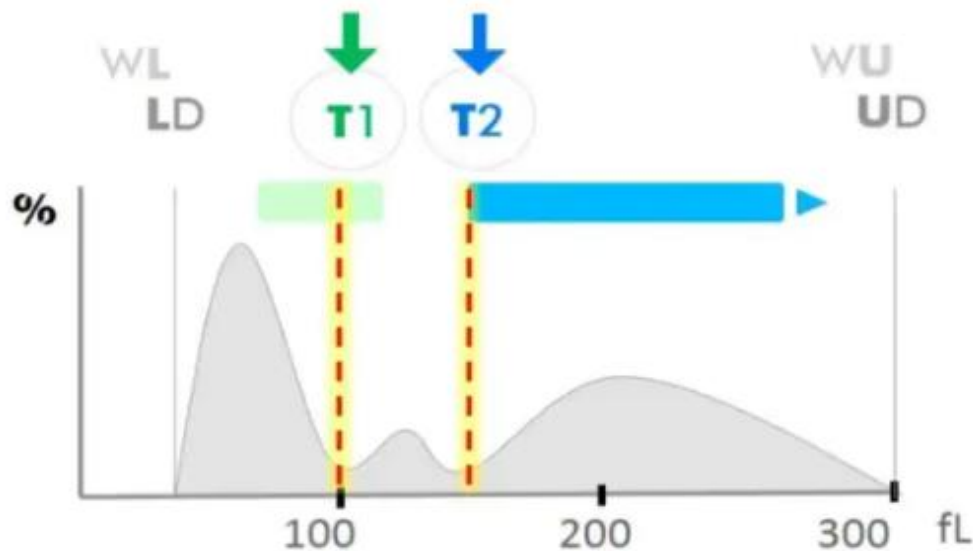
WBCs Histogram

3-Part Differentiation

WBCs histogram consists of **2 Troughs**

[Valleys]

[Detected by **2 inner** discriminators]



WBCs Histogram

3-Part Differentiation

T1 : 78 – 114 fL

T2 : > 150 fL

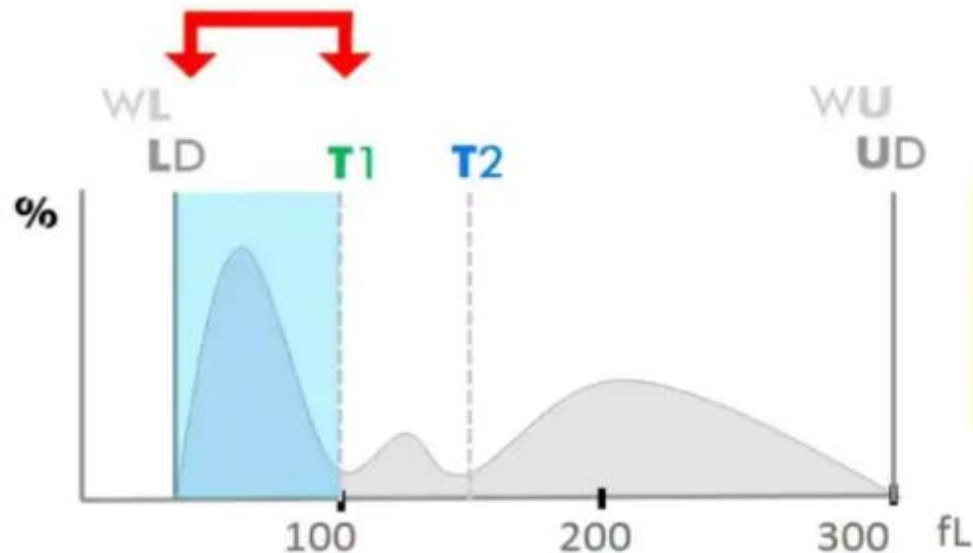
WBCs Histogram

3-Part Differentiation

WBCs histogram consists of **2 Troughs**

[Valleys]

[Detected by **2 inner** discriminators]



WBCs Histogram

3-Part Differentiation

Peak between **LD** and **T1**
represents small cells
i.e. **Lymphocytes**

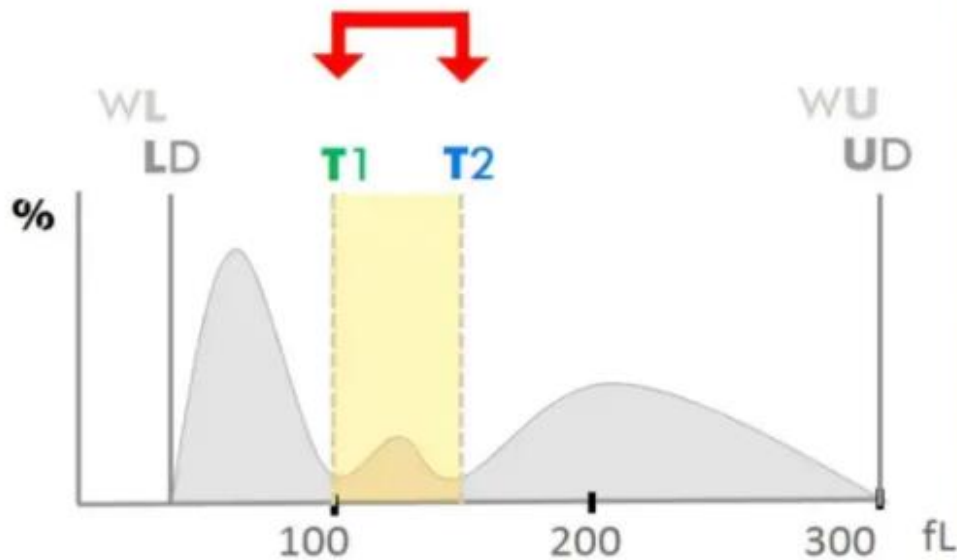
WBCs Histogram

3-Part Differentiation

WBCs histogram consists of **2 Troughs**

[Valleys]

[Detected by **2 inner** discriminators]



WBCs Histogram

3-Part Differentiation

Peak between T1 and T2 includes:

- Eosinophils
- Monocytes
- Basophils
- Blast cells
- Promyelocytes
- Myelocytes
- Metamyelocytes

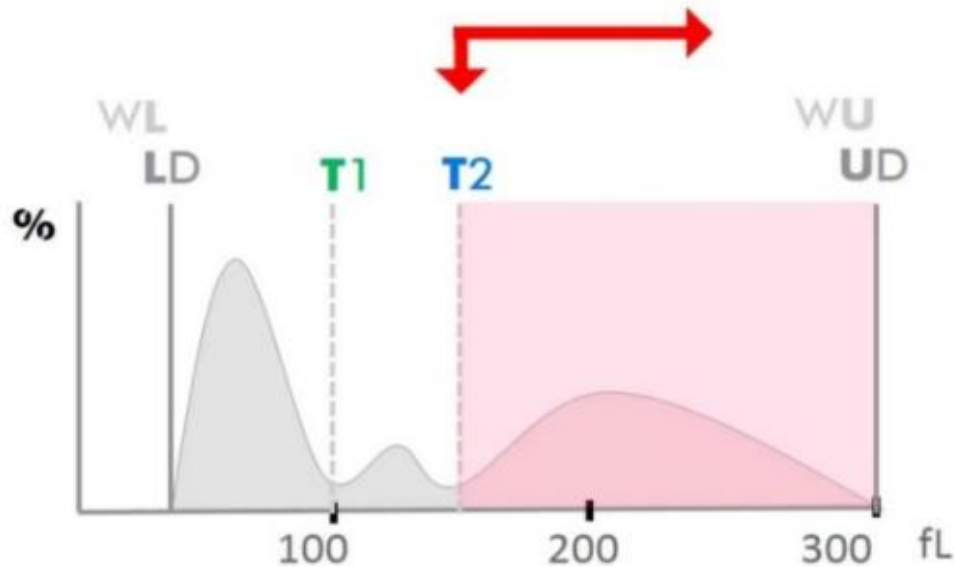
WBCs Histogram

3-Part Differentiation

WBCs histogram consists of **2 Troughs**

[Valleys]

[Detected by **2 inner** discriminators]



WBCs Histogram

3-Part Differentiation

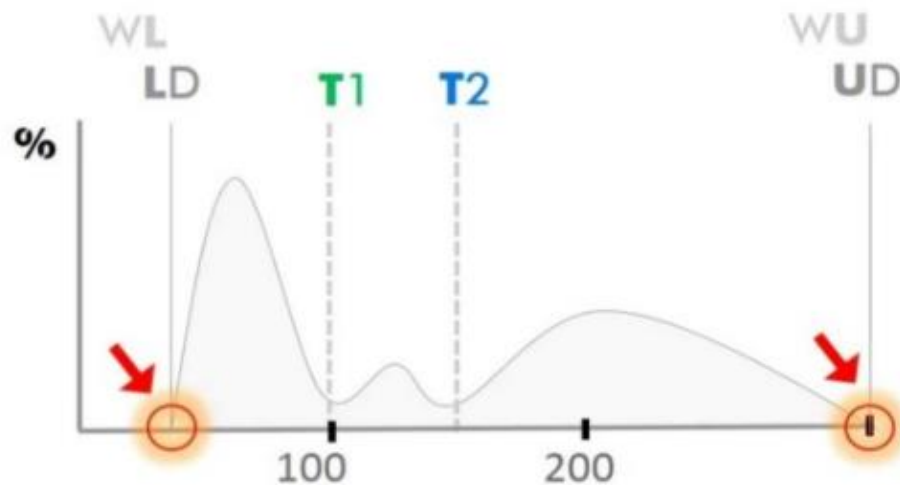
Peak after T2 represents :

Neutrophils **Band cell**

WBCs Histogram

3-Part Differentiation

Important Notes



WBCs Histogram

3-Part Differentiation

The distribution curve should be within the discriminators, **starts** and **ends** at the **base line**.

Less than 20%

3-Part Differentiation

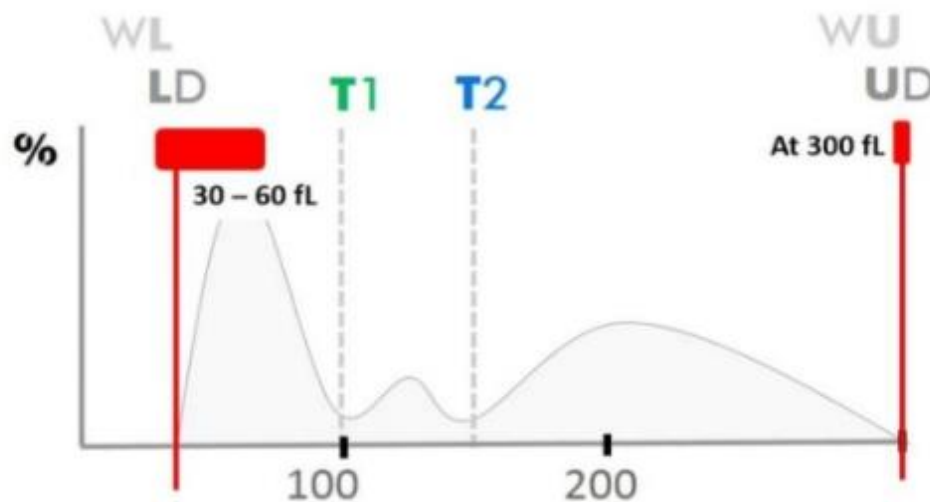
WBCs Histogram

3-Part Differentiation

The LD is **flexible** while UD is **fixed**.

The WBC-channel shows **only WBCs and platelets** (RBCs are lysed)

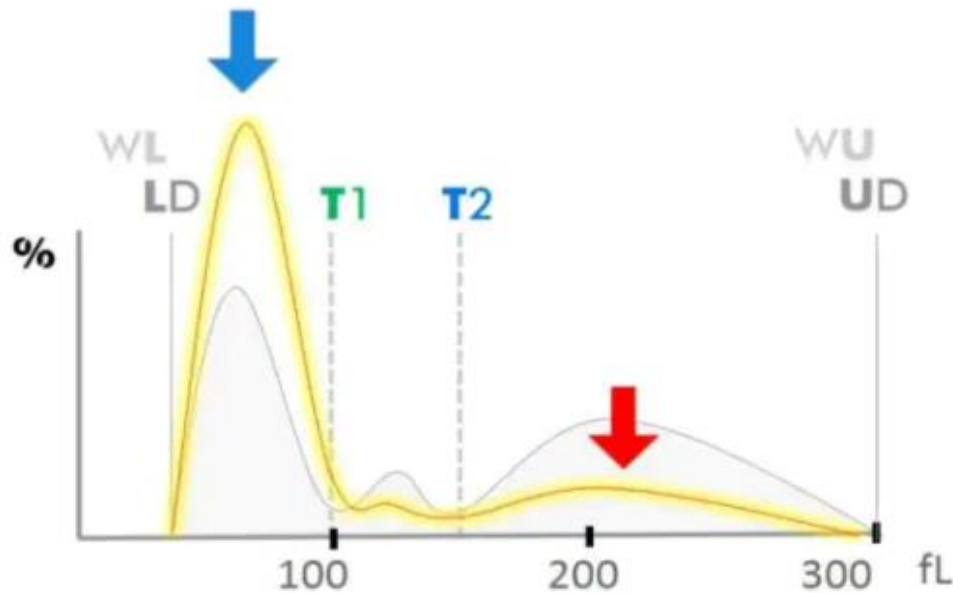
The volume of the platelet is usually between 8 - 12 fl, therefore the LD at the WBC Histogram **seperates** the WBCs from the platelets.
(Platelets were not counted).



WBCs Histogram

3-Part Differentiation

Prominent **peak** with broad distribution between **WL/LD** and **T1**



WBCs Histogram

3-Part Differentiation

In cases of :

- **Lymphocytosis**
- **Neutropenia**

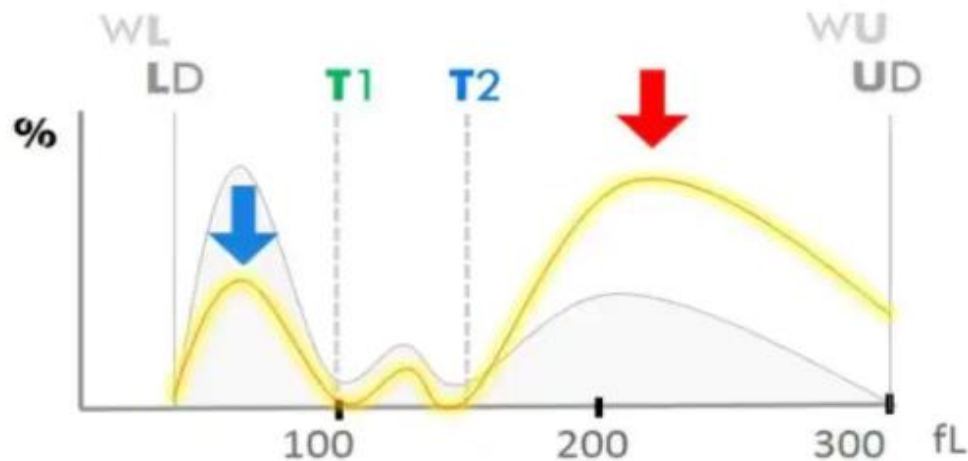
WBCs Histogram

3-Part Differentiation

Prominent **peak** with broad distribution between **T2** and **WU/UD**

WBCs Histogram

3-Part Differentiation



In cases of :

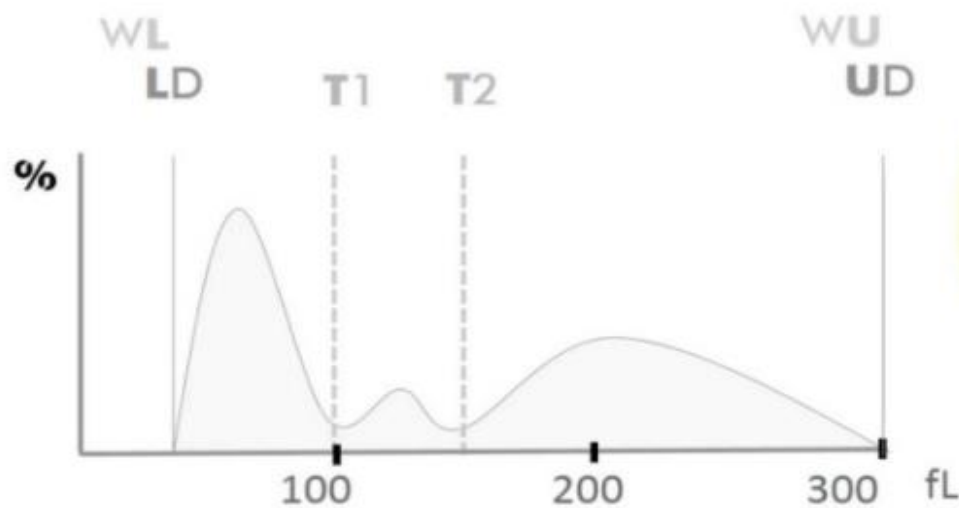
- **Neutrophilia**
- **Lymphopenia**

WBCs Histogram

3-Part Differentiation

WBCs Histogram

3-Part Differentiation



WBCs Histogram

3-Part Differentiation

List of **WBC** error flags in order of priority

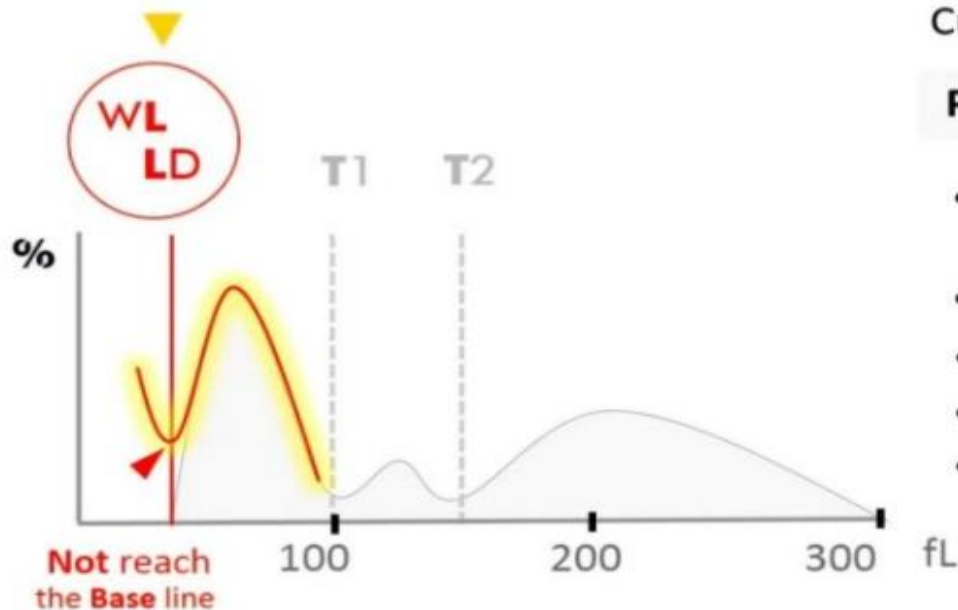
WL	Relative frequency for LOWER discriminator (LD) exceeds the range. May be caused by the inclusion of <u>platelet clumps</u> , <u>large platelets</u> , <u>fibrin</u> , etc.
T1	Lower TROUGH Discriminator, that distinguishes lymphocytes and mixed cells, cannot be determined.
T2	Higher TROUGH Discriminator, that distinguishes mixed cells and neutrophils, cannot be determined.
F1	Small cell histogram error. Relative frequency for T1 exceeds the range.
F2	Middle cell histogram error. Relative frequency for T1 or T2 exceeds the range.
F3	Large cell histogram error. Relative frequency for T2 exceeds the range.
WU	Relative frequency for UPPER discriminator (UD) exceeds the range. May be caused by <u>lyse-resistant red blood cells</u> , or when <u>numerous abnormal blood cells</u> are present.

AG: The particle count equal to or less than the LD exceeds a prescribed range. Probable cause is platelet agglutination, which does not alter WBC count but may result in decreased platelet count. Therefore, this flag is added to the PLT parameter.

WBCs Histogram

3-Part Differentiation

1 LD Flag (WL Flag)



Action

- » All results marked with 'WL' should be controlled.
- » WBC count might be incorrect.

WBCs Histogram

3-Part Differentiation

Curve **does not start** at base line

Possible Causes :

- **PLT Clumps** : Clotted sample
EDTA-Incompatibility
[Re-collect on citrate]
- **Giant Platelets**
- **Nucleated RBCs**
- **Lyse-resistant RBCs**
- **Cryoglobulins** → artifacts

pseudoleukocytosis & pseudothrombocytosis

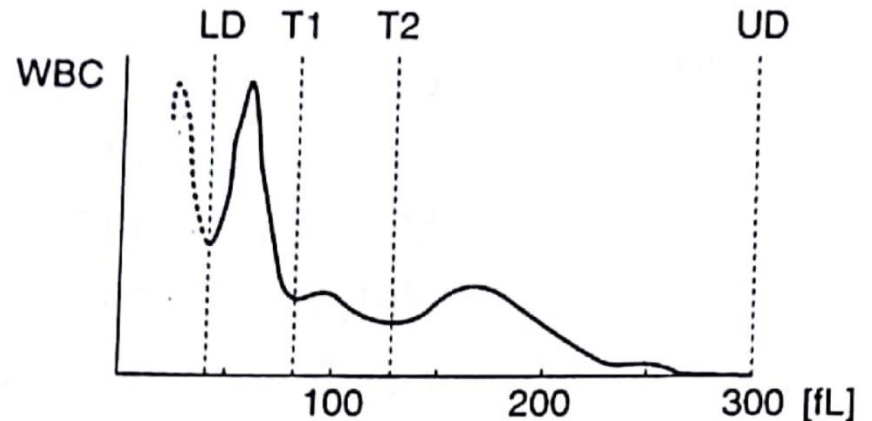
علل لیز نشدن RBC : بالا بودن HbF ، در neonates ،
بیماریهای کبدی و اورمی و nRBC

فلگ WL زمانی درج می شود که هیستوگرام ممیز LD را
در ارتفاع بالاتر از ۴۰٪ قطع کند و این خطا در کنار هر سه
پارت دیف درج می شود.

WBCs Histogram

3-Part Differentiation

• WBC Histogram Error-WL (1A)



Possible causes :

- Nucleated RBC
- Unlysed RBC
- Platelet clumps as in EDTA incompatibility and coagulated sample
- Cold agglutinins
- Impedance noise

WBC	WL 8.5 [$\times 10^3/\mu\text{L}$]
LYM%	WL 39.3 [%]
MXD%	WL 17.6 [%]
NEUT%	WL 43.1 [%]
LYM#	WL 3.4 [$\times 10^3/\mu\text{L}$]
MXD#	WL 1.5 [$\times 10^3/\mu\text{L}$]
NEUT#	WL 3.6 [$\times 10^3/\mu\text{L}$]

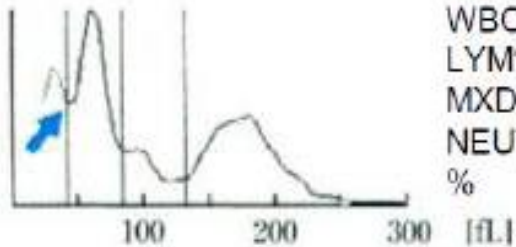
WBCs Histogram

3-Part Differentiation

1

LD Flag (WL Flag)

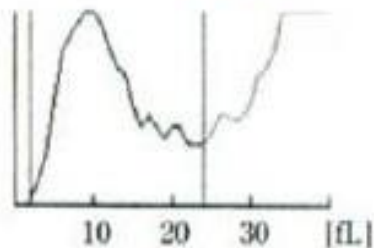
WBC-Histogram



Results

WBC	WL*	6.4 x10 ⁹ /L
LYM%	WL*	41.4%
MXD%	WL*	14.0%
NEUT	WL*	44.6%
	%	

PLT-Histogram

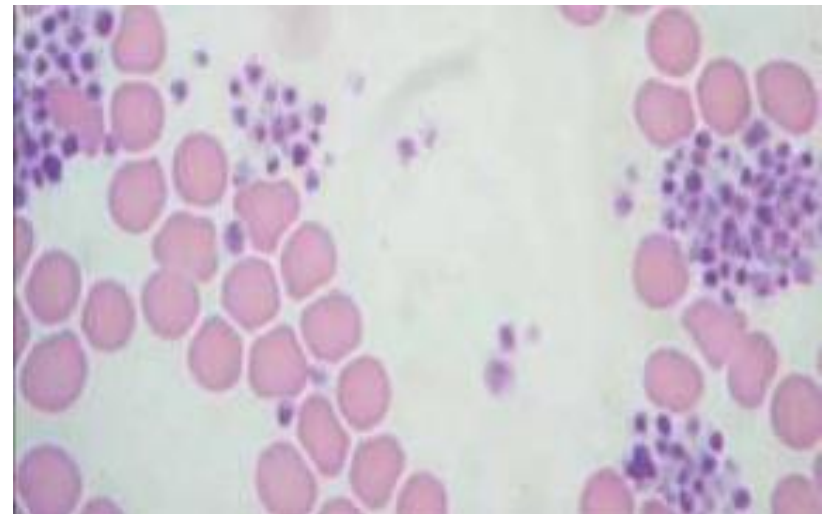


Results

PLT	PU	55 x10 ⁹ /L
PDW	DW	---. -fl
MPV	DW	---. -fl
P-LCR	DW	-. ---%

WBCs Histogram

3-Part Differentiation



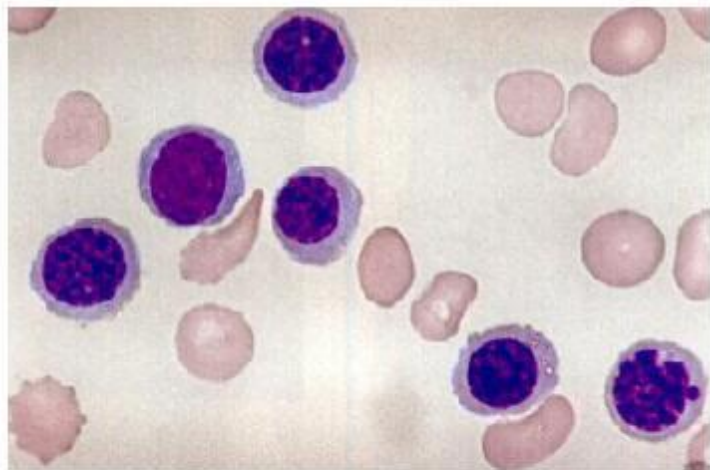
WBCs Histogram

3-Part Differentiation

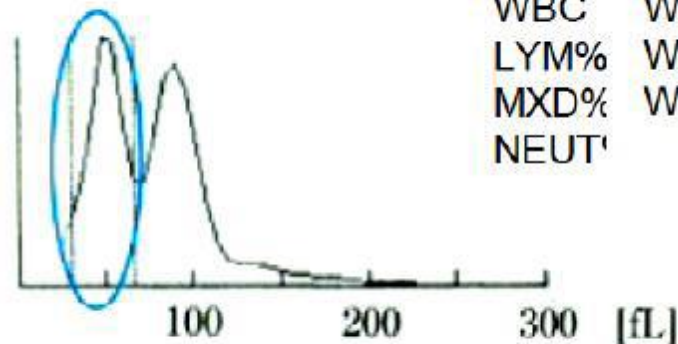
1

LD Flag (WL Flag)

WL & Erythroblastosis



WBC-Histogram



Results

WBC	WL*	56.1 x 10 ⁹ /L
LYM%	WL*	42.7%
MXD%	WL	-.---
NEUT'		-.---

شکل ۲۹-۱۰: اریترو بلاستوز شدید (1352/100WBC) که با تلاقی نور موبلاست‌ها و لنفوسیت‌ها، فراکسیون لکوسیت‌ها بهم خورده و خطای WL و T2 ایجاد شده است. در این شرایط امکان دیف ناحیه MXD و NEUT مقدور نبوده و نقطه چین می‌خورد، مرز بین RBC‌های شبه لیز شده و لنفوسیت اختلال داشته و درصد لنفوسیت نیز افزایش محسوس و کاذب دارد.

WL & T2 flags

WBCs Histogram

3-Part Differentiation

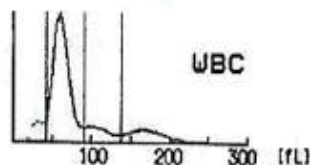
1

LD Flag (WL Flag)

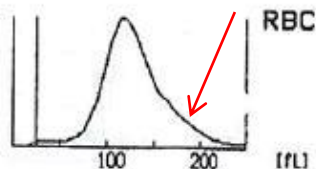
WL & Erythroblastosis

No. 25
Date 83/03/01 15:30
Mode WB

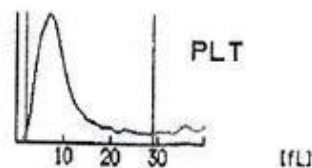
WBC + $28.5 \times 10^9/\mu\text{L}$
RBC - $3.70 \times 10^6/\mu\text{L}$
HGB 14.3g/dL
HCT 45.1%
MCV + 121.9fL
MCH + 38.6pg
MCHC 31.7g/dL
PLT AG $186 \times 10^3/\mu\text{L}$



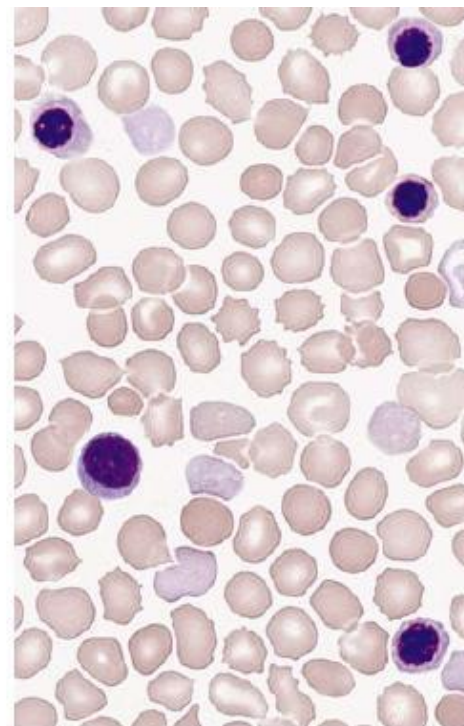
LYM% + 70.6%
MXD% 12.8%
NEUT% - 16.6%
LYM# $20.1 \times 10^9/\mu\text{L}$
MXD# $3.6 \times 10^9/\mu\text{L}$
NEUT# $4.8 \times 10^9/\mu\text{L}$



RDW + 19.2%



PDW 12.0fL
MPV 10.2fL
P-LCR 26.1%



شکل ۱۲۴-۱۰: گزارش CBC دستگاه Sysmex-K21 در اریتروبلاستوز جنینی در نوزاد نارس (۲۸ هفته و ۱۱۰۰ گرم وزن) که باعث افزایش WBC، MCV، MCH و لنفوسیت و کاهش تعداد RBC می شود. نسبت N-RBC نوزاد به WBC حدود ۱۲۵:۱۰۰ (و درصد دستی لنفوسیت ۴۷٪ است که با اصلاح آن از طریق فرمول $\text{True WBC} = (\text{WBC} \times 100) / (100 + \text{NRBC})$ ، شمارش واقعی

لکوسیت به ۱۲۶۰۰ می رسد.

وجود nRBC باعث می شود WBC و لنفوسیت و MCV بصورت کاذب افزایش پیدا کند

WBCs Histogram

3-Part Differentiation

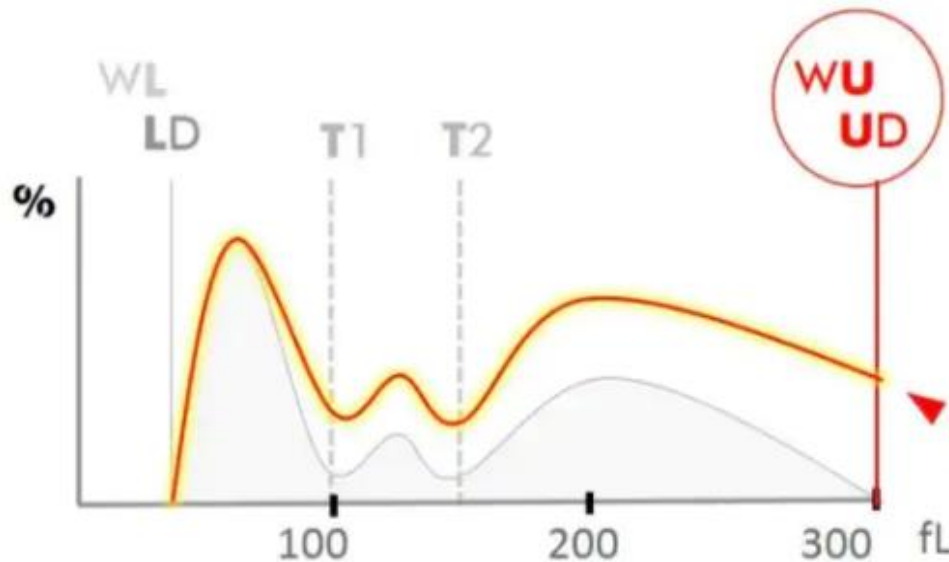
2

UD Flag (WU Flag)

WBCs Histogram

3-Part Differentiation

Curve does **not end** at base line



Possible Causes :

- **Sever neutrophilia**
(Linearity exceeds the limit)

Pre-dilution [e.g. 1:5]
of the sample might help
to obtain **correct** results.

Not reach the Base line

- WBC aggregation
- Satellite phenomenon
- Hypersegmentation

WBCs Histogram

3-Part Differentiation

2

UD Flag (WU Flag)

PLT satellitism vs Neutrophil aggregation

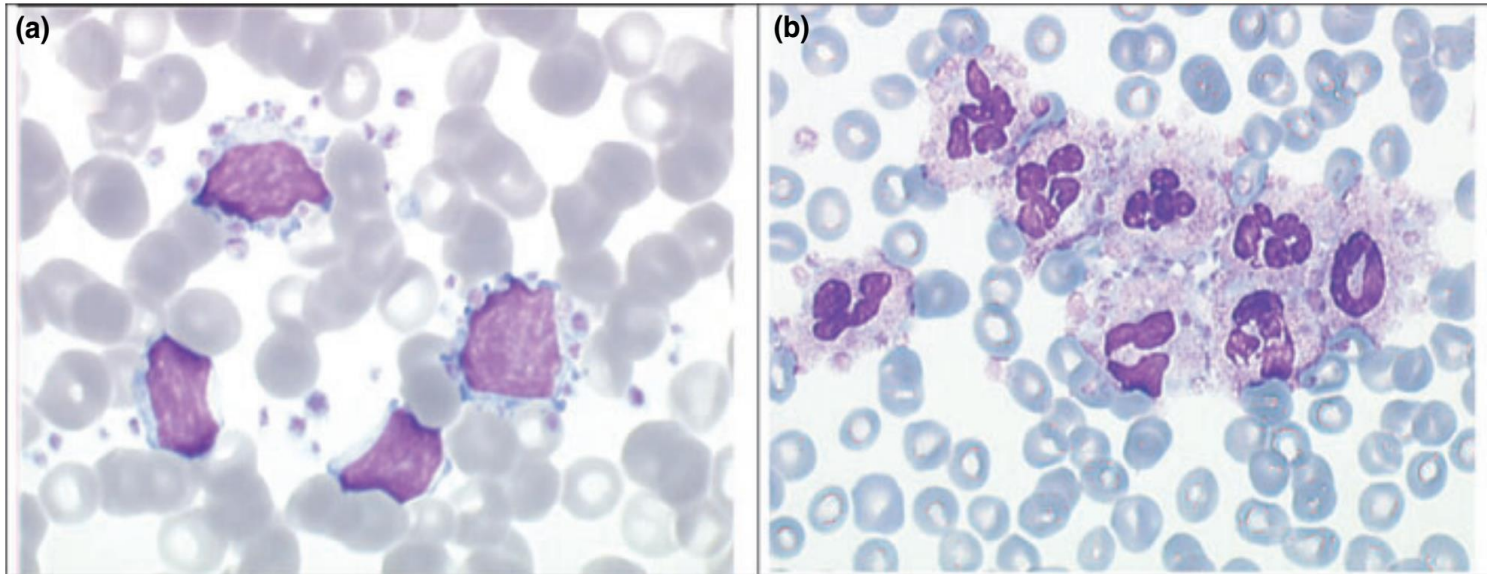


Figure 3. (a) Platelets surrounding lymphocytes in a patient known for chronic lymphocytic leukaemia. (b) Neutrophil-Platelet aggregates; that latter situation is related to PLT satellitism around polymorphs: PLT are 'bridges' between PLT-neutrophil rosettes, generating peculiar clumps, differing from neutrophil aggregates, as no PLT is observed within the latter (peripheral blood smears; MGG staining).

WBCs Histogram

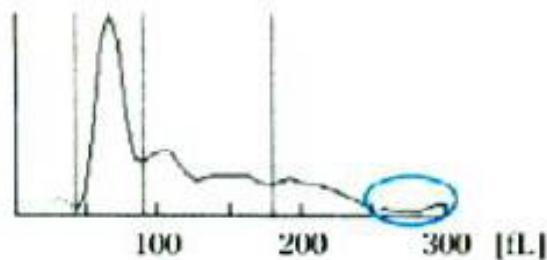
3-Part Differentiation

2

UD Flag (WU Flag)

Neutrophil aggregation

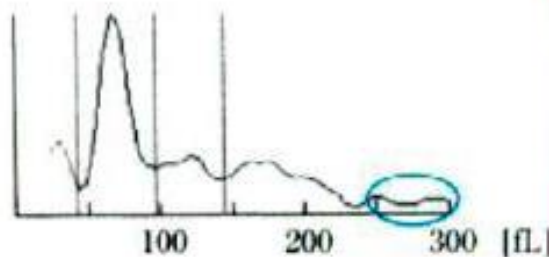
WBC-Histogram



Results

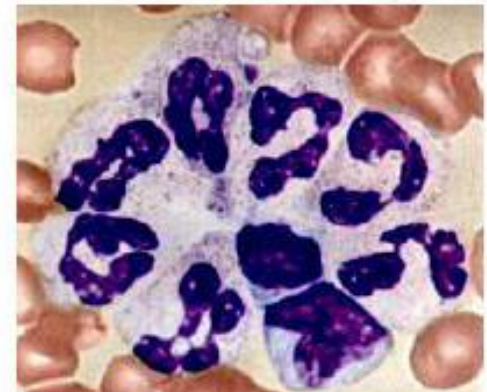
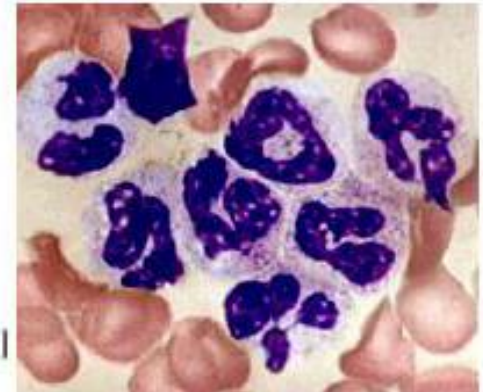
WBC - $2.3 \times 10^9/L$
LYM% 39.7%
MXD% + 32.2%
NEUT%- 28.1%

WBC-Histogram



Results

WBC - $2.1 \times 10^9/L$
LYM% 41.9%
MXD% 17.5%
NEUT%- 40.6%



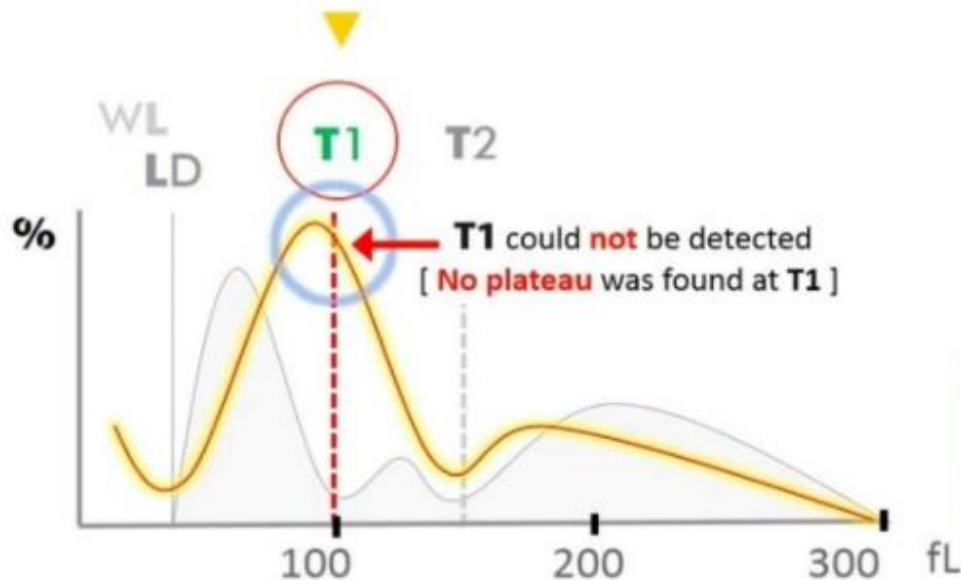
WBCs Histogram

3-Part Differentiation

3

T1 Flag

No differentiation between lymphocytes and mixed cells



T1: 78-114 fL

WBCs Histogram

3-Part Differentiation

Causes :

- Blast cells
- Eosinophilia
- Basophilia
- Plasma cells
- Abnormal/variant lymphs

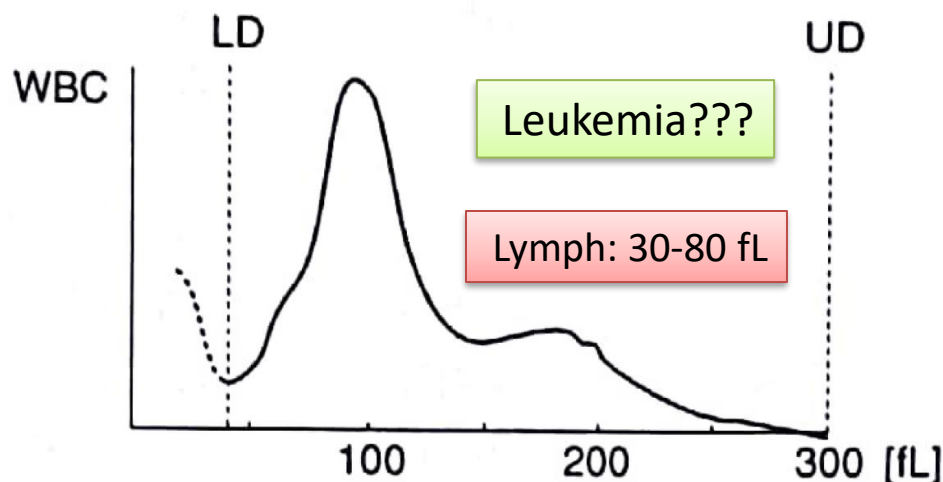
**Blood Smear is
Recommended**

WBCs Histogram

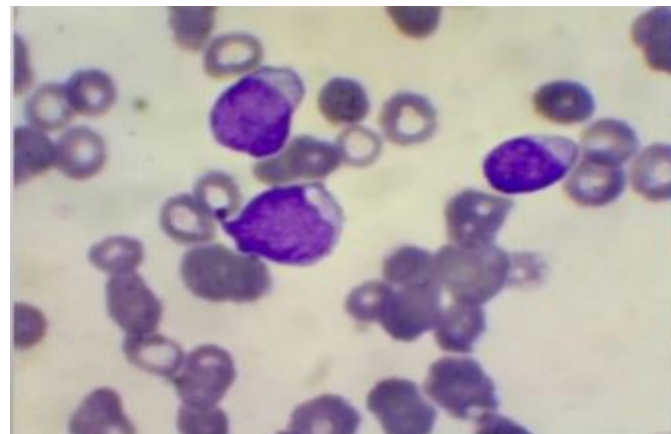
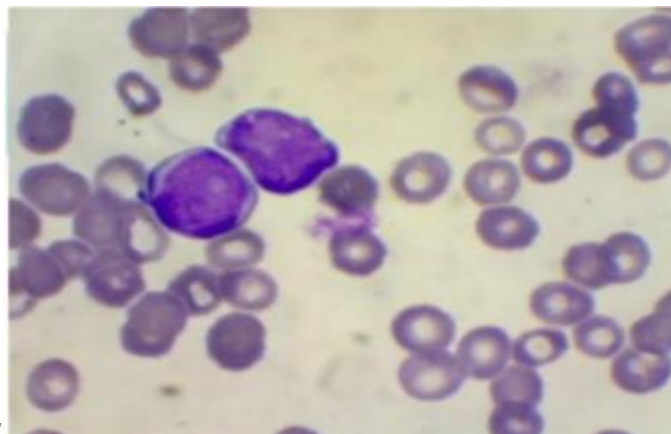
3-Part Differentiation

T1: 78-114 fL

WBC Histogram Error-T1 (2A)



WBC		+15.4 [$\times 10^3/\mu\text{L}$]
LYM%	T1	---.- [%]
MXD%	T1	---.- [%]
NEUT%	T1	---.- [%]
LYM#	T1	---.- [$\times 10^3/\mu\text{L}$]
MXD#	T1	---.- [$\times 10^3/\mu\text{L}$]
NEUT#	T1	---.- [$\times 10^3/\mu\text{L}$]



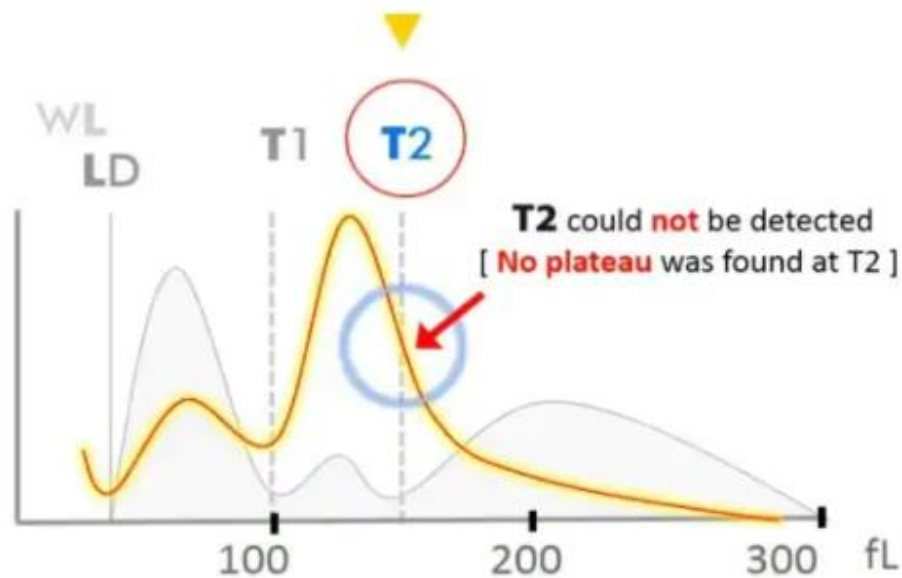
WBCs Histogram

3-Part Differentiation

4

T2 Flag

No differentiation between mixed cells and neutrophils



T2: > 150 fL

WBCs Histogram

3-Part Differentiation

Causes :

- Eosinophilia
- Immature granulocytes
- Abnormal cell populations

Blood Smear is Recommended

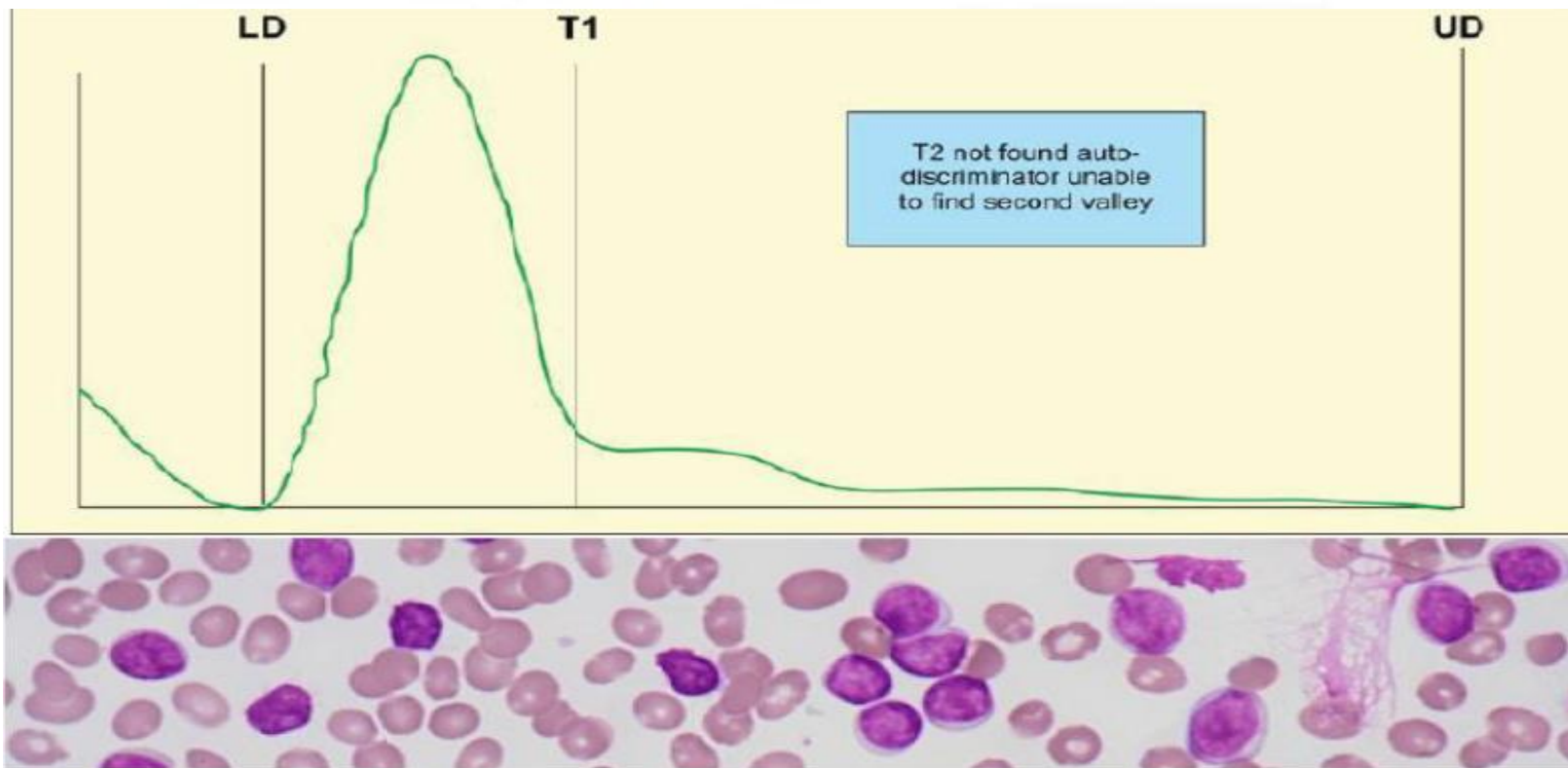
WBCs Histogram

3-Part Differentiation

4

T2 Flag

No differentiation between mixed cells and neutrophils



WBCs Histogram

3-Part Differentiation

بیمار مبتلا به CLL و ۹۷٪ لنفوسیت آبنرمال و فقدان سایر انواع لکوسیت است.

سل کانتر نمی تواند دره T2 را شناسایی کند و نتیجه دیف را نمی دهد و Flag های T2، F2 و F3 می دهد.

WBCs Histogram

3-Part Differentiation

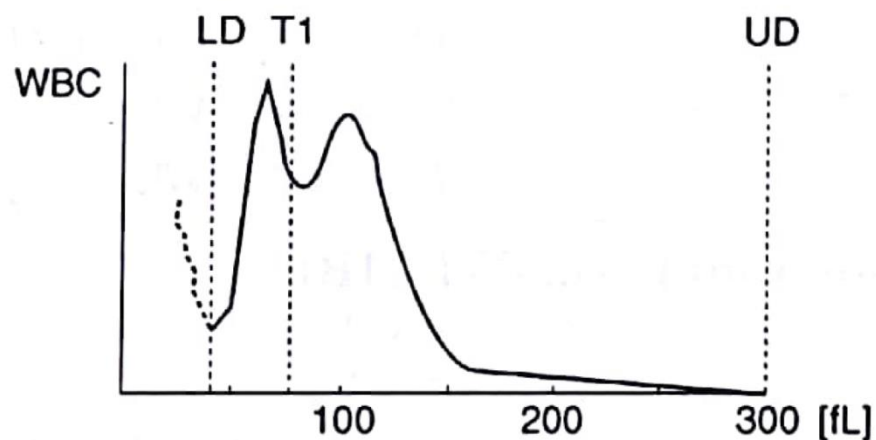
4

T2 Flag

No differentiation between mixed cells and neutrophils

Severe left shift or
Eosinophila

- WBC Histogram Error-T2 (3A)



WBC		5.2 [$\times 10^3/\mu\text{L}$]
LYM%	F1	29.7 [%]
MXD%	T2	---.- [%]
NEUT%	T2	---.- [%]
LYM#	F1	1.5 [$\times 10^3/\mu\text{L}$]
MXD#	T2	---.- [$\times 10^3/\mu\text{L}$]
NEUT#	T2	---.- [$\times 10^3/\mu\text{L}$]

WBCs Histogram

3-Part Differentiation

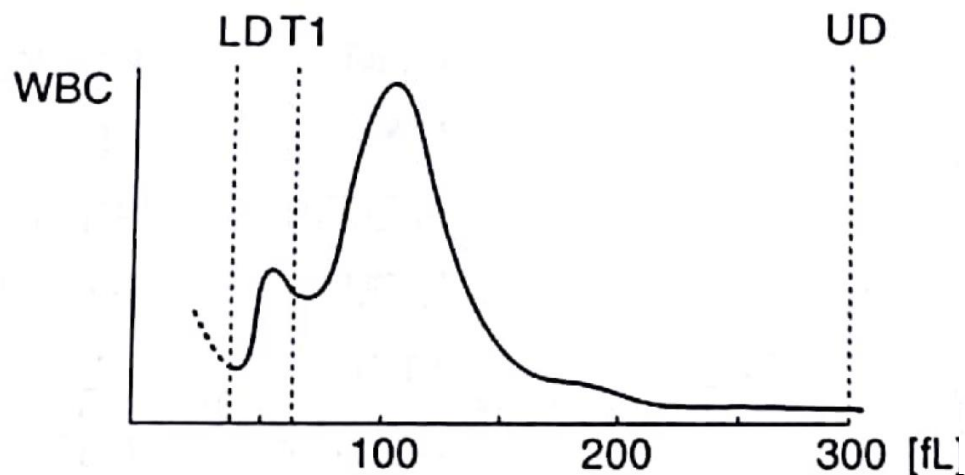
4

T2 Flag

No differentiation between mixed cells and neutrophils

Severe left shift or
Eosinophila or ...

WBC Histogram Error-T2 (3C)



WBC		+15.2 [$\times 10^3/\mu\text{L}$]
LYM%		12.9 [%]
MXD%	T2	---.- [%]
NEUT%	T2	---.- [%]
LYM#		1.6 [$\times 10^3/\mu\text{L}$]
MXD#	T2	---.- [$\times 10^3/\mu\text{L}$]
NEUT#	T2	---.- [$\times 10^3/\mu\text{L}$]

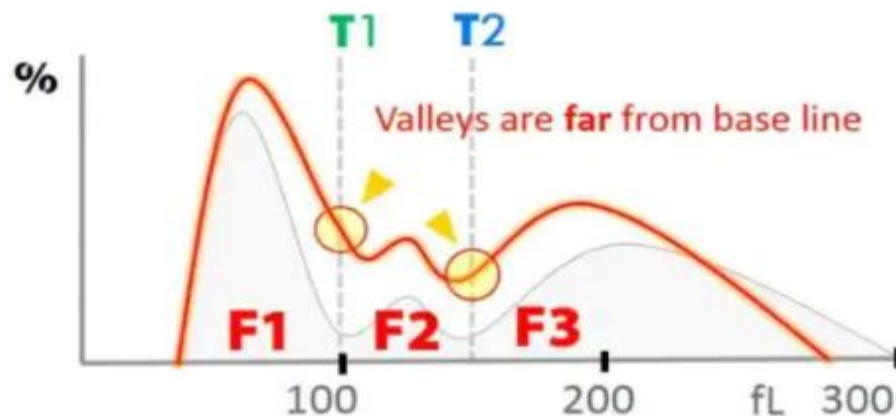
WBCs Histogram

3-Part Differentiation

5

F1, F2, F3 Flags

F = Fraction



WBCs Histogram

3-Part Differentiation

F1 (small cells) Flag

Height of T1 exceeds limit of 40%

F2 (medium cells) Flag

Height of T1 and T2 exceeds limit of 40% and 50% Respectively

F3 (Large cells) Flag

Height of T2 exceeds limit of 50%

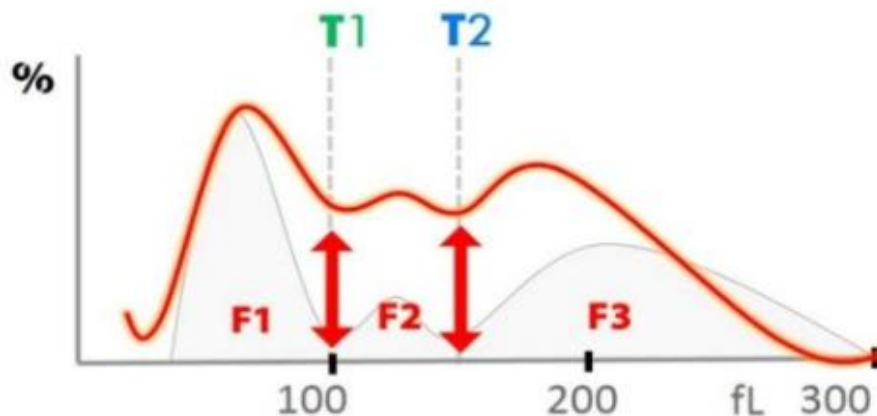
WBCs Histogram

3-Part Differentiation

5 F1, F2, F3 Flags

F = Fraction

T1 or T2 discriminators are set but there is **no clear separation** between the different populations.



WBCs Histogram

3-Part Differentiation

- WBC total count is **correct** (provided that no other flags)
- **T1** and **T2** were detected.
- The troughs are **away** from the basis line.
- It is possible that the fractions are **mixed**; i.e. **F1** and **F2**, or **F2** and **F3** **merge** into each other over large areas.

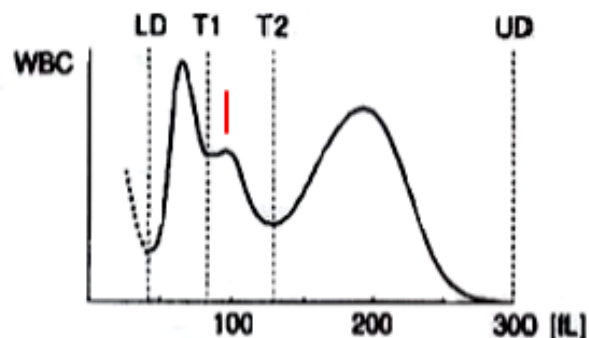
**Manual Differentiation
is necessary**

WBCs Histogram

3-Part Differentiation

5 F1, F2, F3 Flags

WBC Histogram Error - F1

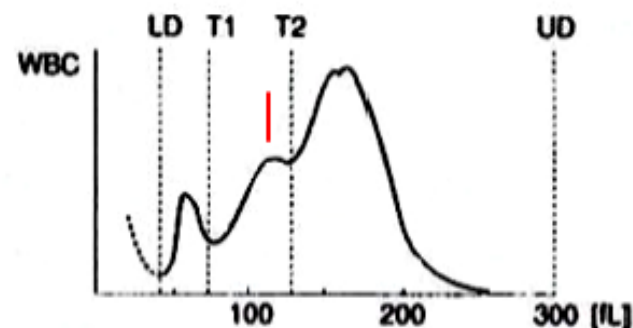


WBC	6.7 [$\times 10^3/\mu\text{L}$]
LYM%	F1 28.3 [%]
MXD%	F2 17.4 [%]
NEUT%	54.3 [%]
LYM#	F1 1.9 [$\times 10^3/\mu\text{L}$]
MXD#	F2 1.2 [$\times 10^3/\mu\text{L}$]
NEUT#	3.6 [$\times 10^3/\mu\text{L}$]

WBCs Histogram

3-Part Differentiation

WBC Histogram Error - F2



WBC	+18.5 [$\times 10^3/\mu\text{L}$]
LYM%	9.5 [%]
MXD%	F2 +23.7 [%]
NEUT%	F3 66.8 [%]
LYM#	1.1 [$\times 10^3/\mu\text{L}$]
MXD#	F2 2.7 [$\times 10^3/\mu\text{L}$]
NEUT#	F3 7.7 [$\times 10^3/\mu\text{L}$]

شکل ۲۷-۱: تصویر فлаг F1 و F2 سل کانتر سیستمس K-21 برای یک افزایش بلاست (F1) و افزایش انوزینوفیل (F2)

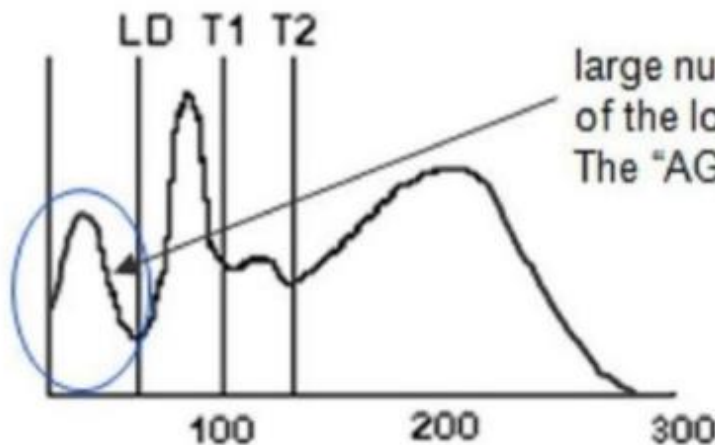
WBCs Histogram

3-Part Differentiation

6

AG flag → Aggregation

Abnormal curve in front of lower discriminator



large numbers of particles are detected in front of the lower WBC discriminator.

The "AG" flag is shown on the PLT result.

Large platelet clumps (> 30fl) are detected in the area before the lower WBC discriminator. Due to their enhanced size they may not affect the PLT histogram curve. If "AG" mark will be generated, the sample should be checked for platelet clumps (e.g. microscopic slide review).

WBCs Histogram

3-Part Differentiation

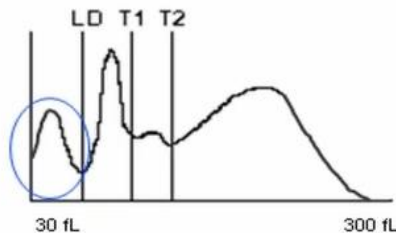
WBCs Histogram

3-Part Differentiation

6

AG flag → Aggregation

Abnormal curve in front of lower discriminator



WBCs Histogram

3-Part Differentiation

■ Probable cause is PLT aggregation which does not alter WBC count but may result in decreased PLT count.

■ Therefore this flag is added to the PLT parameters PLT count.

■ Possible interference

- » PLT aggregation
- » EDTA incompatibility
- » Lyse resistant RBC
- » NRBC
- » Large PLT Giant PLT

■ Action:

- » PLT count should be checked for PLT aggregation.
- » The result is not correct and might be low.
- » Sample should be recollected and measured again.

6

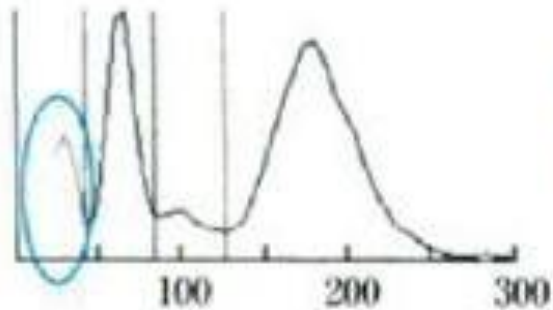
AG flag → Aggregation

WBCs Histogram

3-Part Differentiation

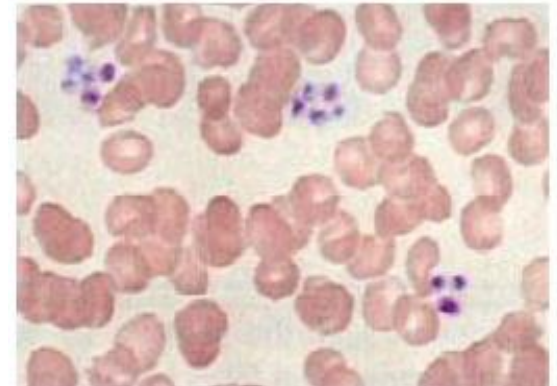
Abnormal curve in front of lower discriminator

WBC-Histogram

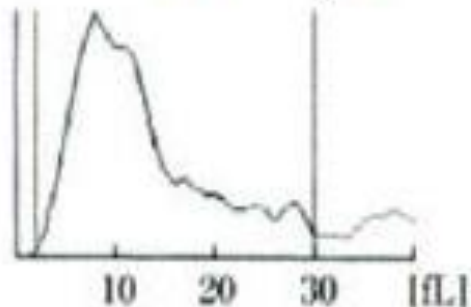


Results

WBC	6.0 x10 ⁹ /L
LYM%	27.5%
MXD%	7.9%
NEUT	64.4%
%	



PLT-Histogram



Results

PLT	86 x10 ⁹ /L
PDW	+ 18.6fl
MPV	12.8fl
P-LCR	+ 43.7%

WBCs Histogram

3-Part Differentiation

WBCs Histogram

3-Part Differentiation

Questions

Which Histogram is **suitable** for the diagnosis ?

WBCs Histogram

3-Part Differentiation

Question 1

Which Histogram is **suitable** for the diagnosis ?

A



B



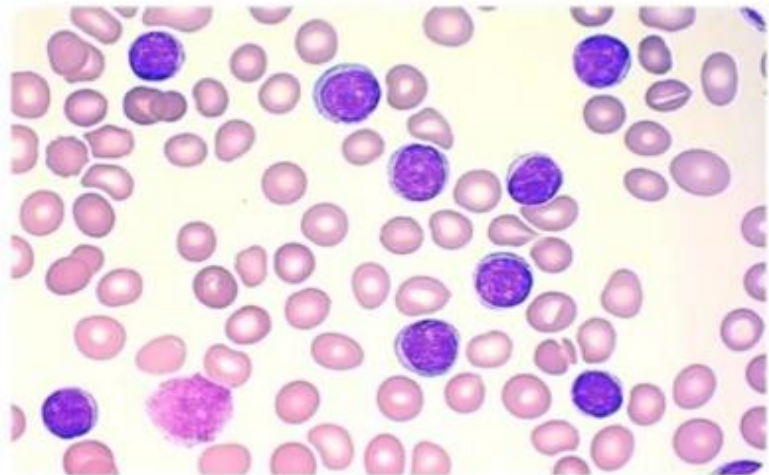
C



WBCs Histogram

3-Part Differentiation

Chronic **Lymphocytic** Leukemia



WBCs Histogram

3-Part Differentiation

Question 1

Which Histogram is **suitable** for the diagnosis ?

Peak

At **small** cell region

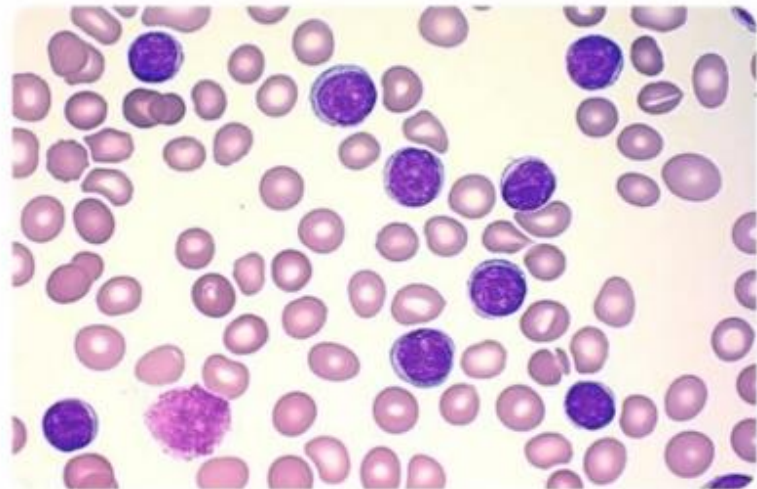
Between WL and T1



WBCs Histogram

3-Part Differentiation

Chronic **Lymphocytic** Leukemia

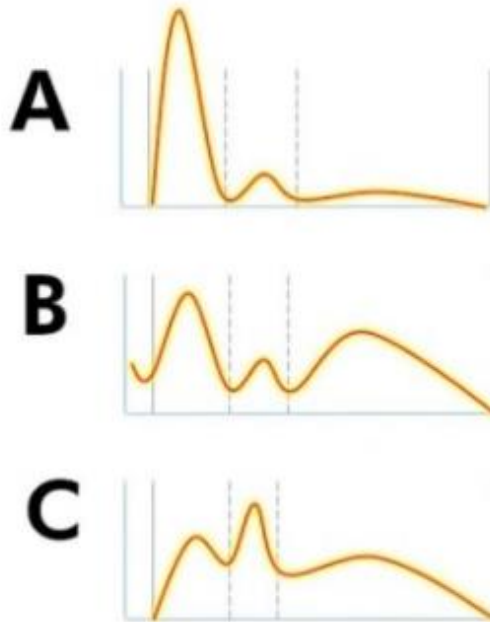


WBCs Histogram

3-Part Differentiation

Question 2

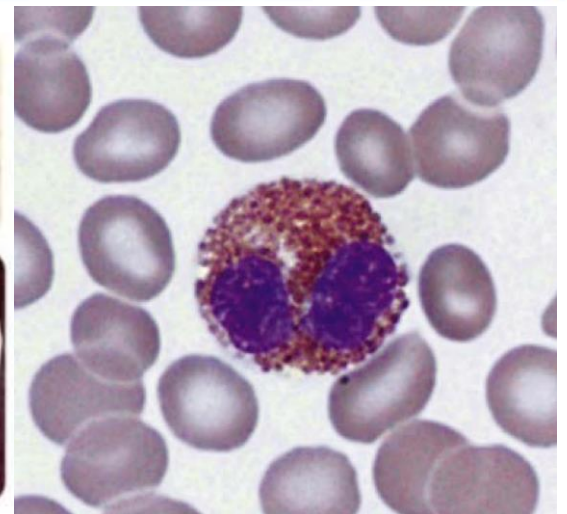
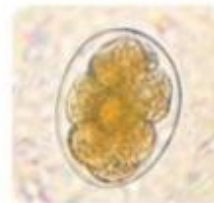
Which Histogram is **suitable** for the diagnosis ?



WBCs Histogram

3-Part Differentiation

Hookworm infection



WBCs Histogram

3-Part Differentiation

Question 2

Which Histogram is **suitable** for the diagnosis ?

Peak

At **medium** cell region

Between T1 and T2

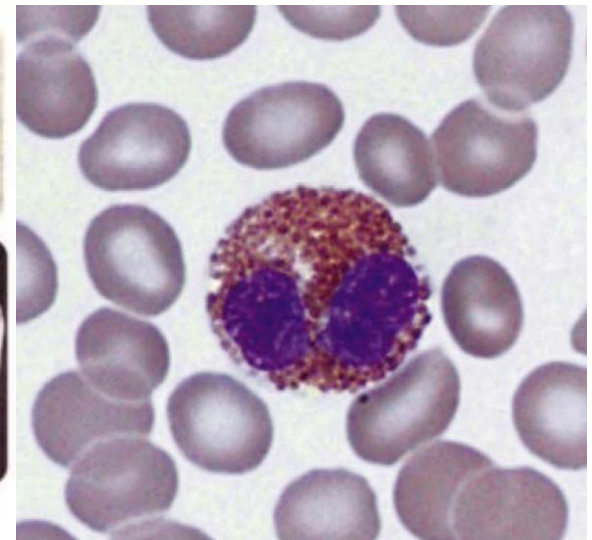
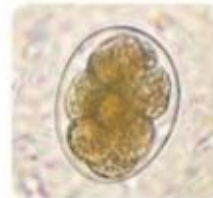
C



WBCs Histogram

3-Part Differentiation

Hookworm infection



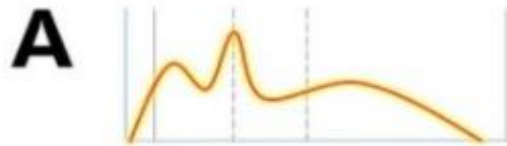
Eosinophils

WBCs Histogram

3-Part Differentiation

Question 3

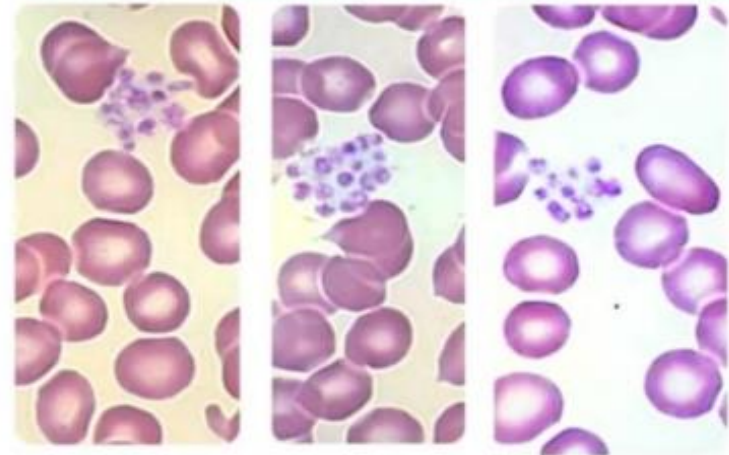
Which Histogram is **suitable** for the diagnosis ?



WBCs Histogram

3-Part Differentiation

EDTA-induced
Pseudothrombocytopenia



WBCs Histogram

3-Part Differentiation

Question 3

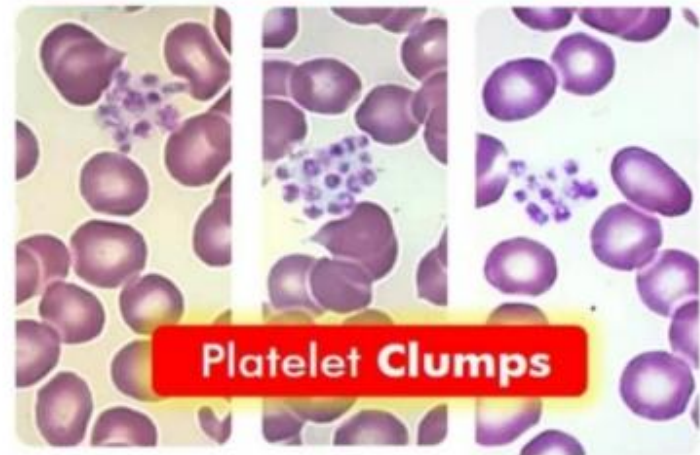
Which Histogram is **suitable** for the diagnosis ?



WBCs Histogram

3-Part Differentiation

EDTA-induced
Pseudothrombocytopenia



WBCs Histogram

3-Part Differentiation

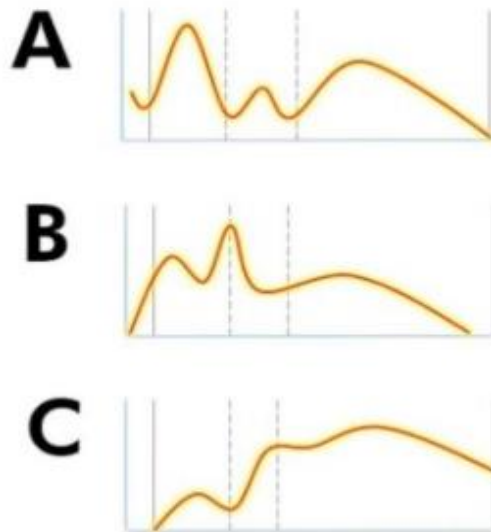
Question 4

Which Histogram is **suitable** for the diagnosis ?

WBCs Histogram

3-Part Differentiation

Chronic Myeloid Leukemia



WBCs Histogram

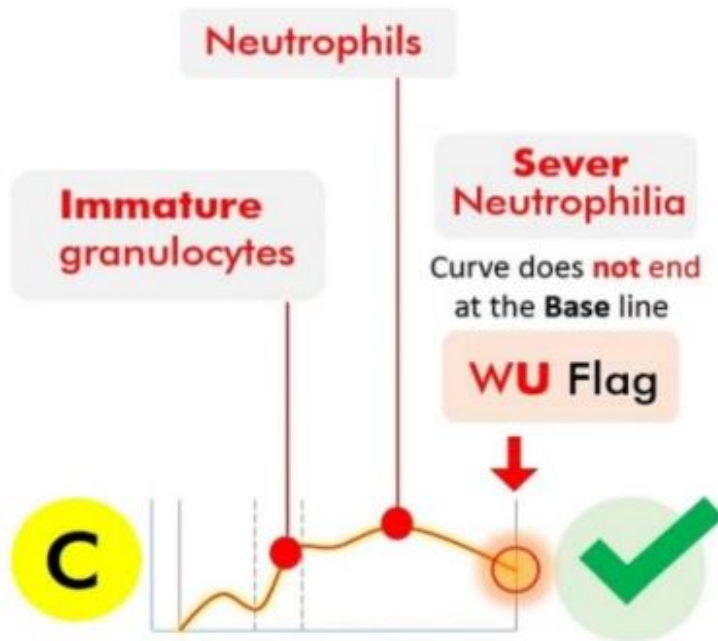
3-Part Differentiation

Question 4

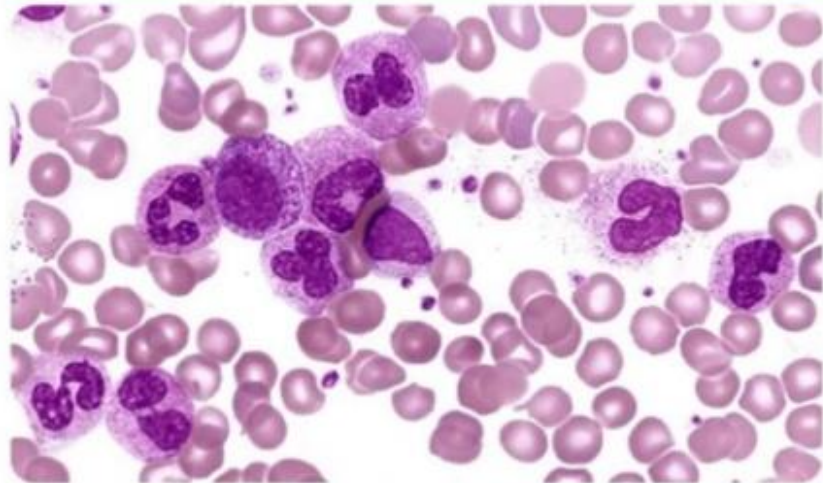
Which Histogram is **suitable** for the diagnosis ?

WBCs Histogram

3-Part Differentiation



Chronic Myeloid Leukemia

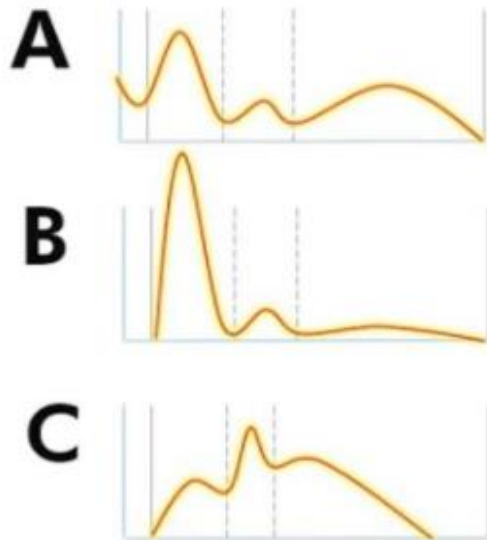


WBCs Histogram

3-Part Differentiation

Question 5

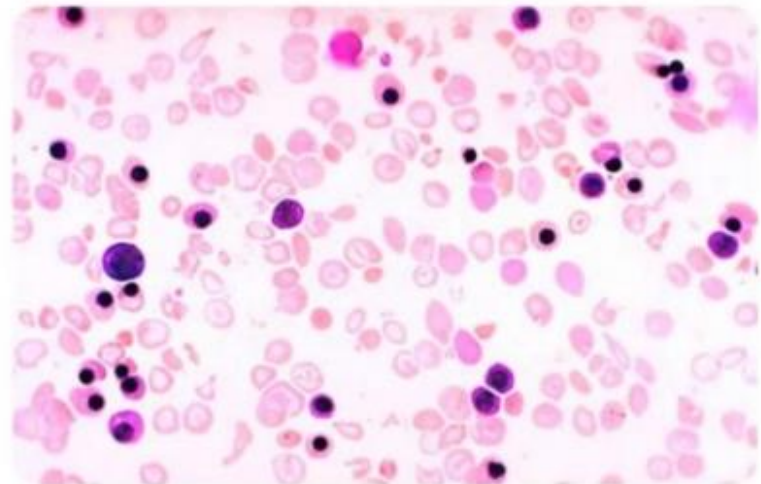
Which Histogram is **suitable** for the diagnosis ?



WBCs Histogram

3-Part Differentiation

β Thalassemia Major

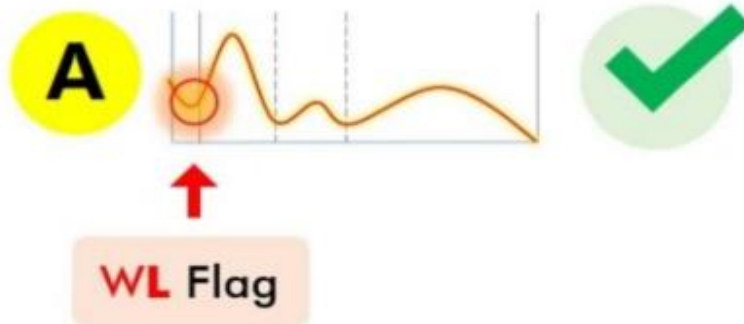


WBCs Histogram

3-Part Differentiation

Question 5

Which Histogram is **suitable** for the diagnosis ?



WBCs Histogram does **not start** at the **Base** line

WBCs Histogram

3-Part Differentiation

β Thalassemia Major



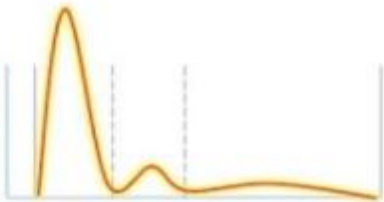
WBCs Histogram

3-Part Differentiation

Question 6

Which Histogram is **suitable** for the diagnosis ?

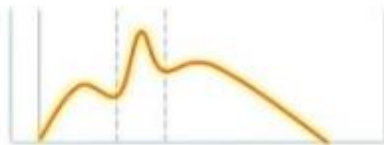
A



B



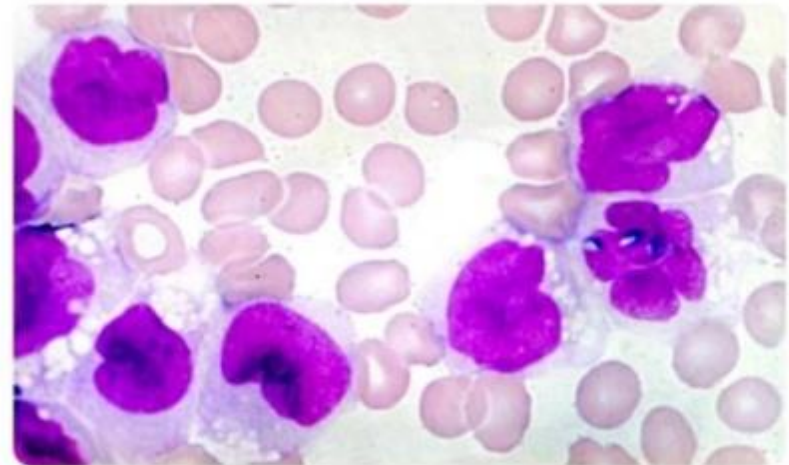
C



WBCs Histogram

3-Part Differentiation

AML M5



WBCs Histogram

3-Part Differentiation

Question 6

Which Histogram is **suitable** for the diagnosis ?

WBCs Histogram

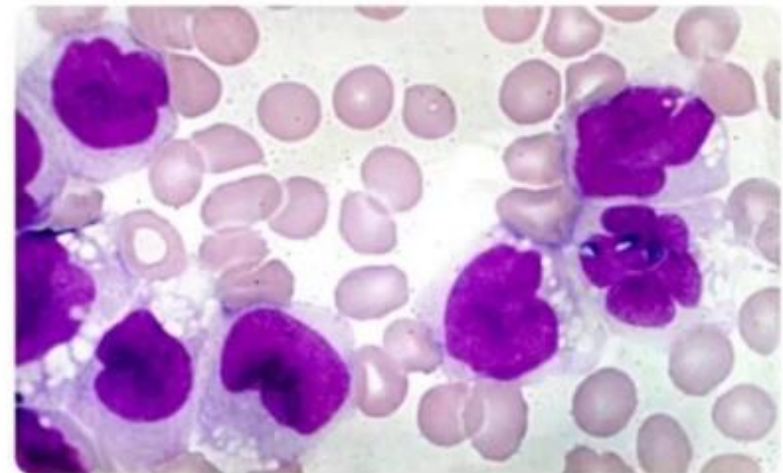
3-Part Differentiation

AML M5

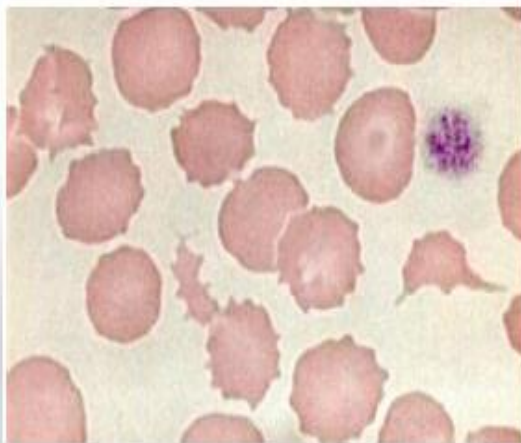
Peak

At **medium** cell region

C

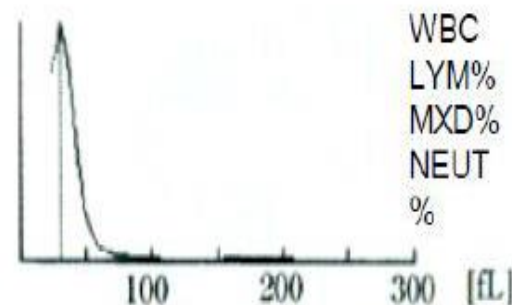


Lysing resistant RBCs & WBC histogram



WBC-Histogram

Results



WBC	WL *	49.4 x10 ⁹ /L
LYM%	WL	-.---
MXD%	WL	-.---
NEUT	WL	-.---
%		

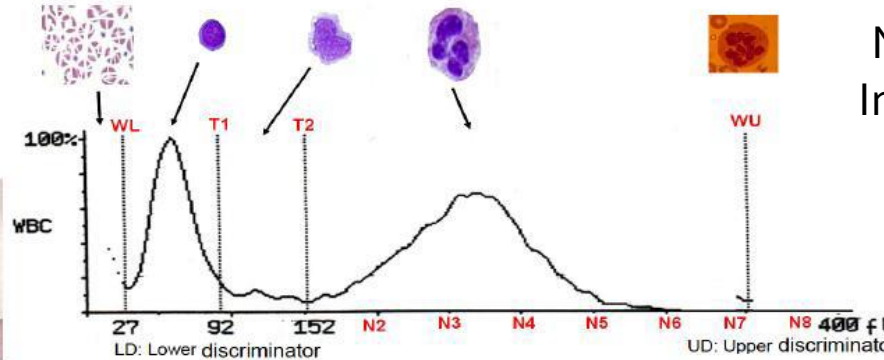
شکل ۱۸-۱۰: گاهی در بیماران کبدی و نوزادان و برخی از هموگلوبینوپاتی‌ها (به خصوص S، C و F) و نمونه خون سرد یا یخچالی، RBCها نسبت به لیز مقاوم بوده و لذا به دلیل عدم لیز، قله بارزی را در مرز WL تشکیل می‌دهند که اغلب خط WL را در ارتفاع بالایی قطع کرده و علاوه بر افزایش کاذب WBC باعث بروز فلاگ WL و * (ستاره: علامت عدم اعتبار نتیجه) نیز می‌شود. در این موارد بهتر است پلاسمای خون برداشته و با محلول Cellpack (ایزوتون دستگاه) جایگزین شود یا اینکه خون با همین محلول رقیق شده و بعد از ۵ دقیقه به دستگاه داده شود. در بیماران کبدی مشاهده آکانتوسیت، جایانت پلاکت، تارگت سل و ماکروسیت کمک کننده می‌باشد.

علل لیز نشدن RBC: بالا بودن HbF (neonates)،
بیماریهای کبدی و اورمی و حضور تعداد زیاد nRBC

WBC Histogram & clinical Application

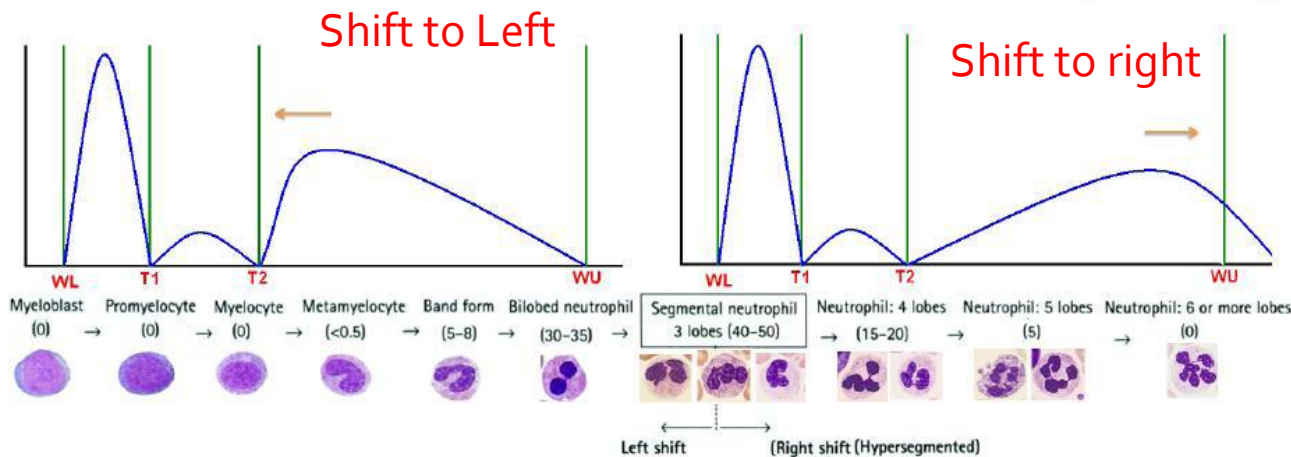
PLT aggregation

nRBC



Normal Band in Adults <5%
In Pediatrics <8%

شکل ۱۹-۱: جایگاه نوتروفیل‌های ۲ تا ۸ سگمنته در ناحیه LCR که خطای WU می‌تواند نشانه حضور نوتروفیل هیپرسگمنته یا آگریگاسیون لکوسیتی باشد که در واکنش TRALI، آلویمونیزاسیون لکوسیتی و عوارض ناشی از ضد انعقاد EDTA دیده می‌شود.

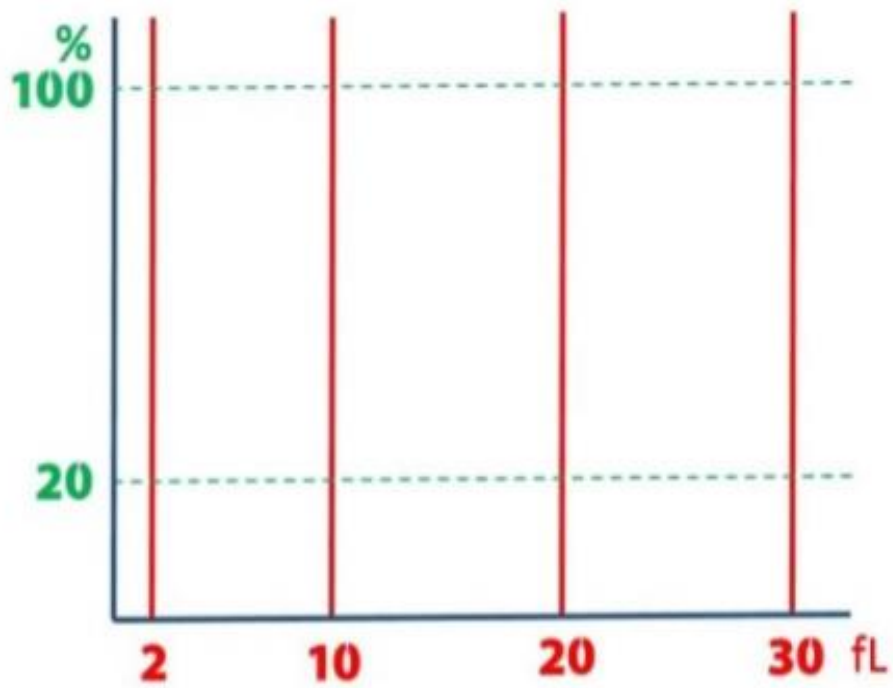


CGP-MGP

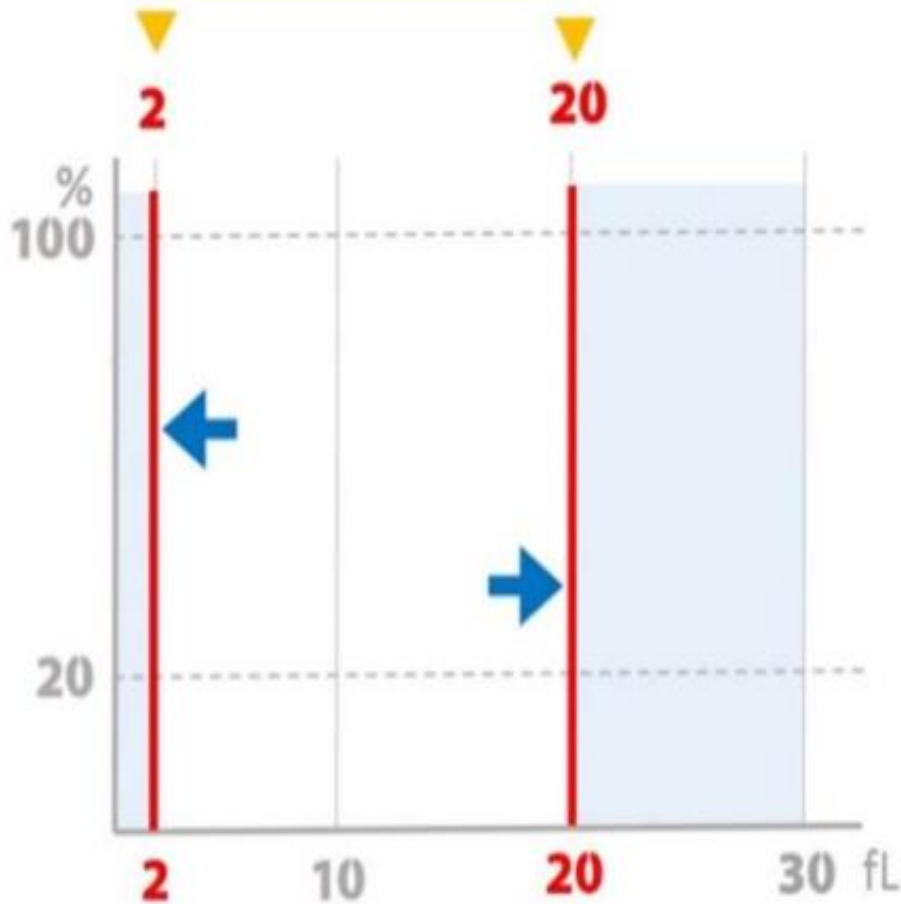
Number of lobes						Number of lobes					
	1	2	3	4	5		1	2	3	4	5
% of neutrophils	35%	25%	21%	17%	2%	%	1%	10%	33%	34%	22%

شکل ۲۰-۱: درصد رده‌های مختلف نوتروفیلی در خون محیطی که بیشترین آنها را نوتروفیل‌های ۳ لوبه تشکیل می‌دهند. برای تخمین شمارش لکوسیت از روی PBS می‌بایست میانگین لکوسیت‌های ۱۰ میدان 400X را در عدد ۳۰۰۰ ضرب نمود.

Platelet Histogram



Platelets counted
between 2 and 20 fL



Platelet Histogram

Platelets counted
between 2 and 20 fL.

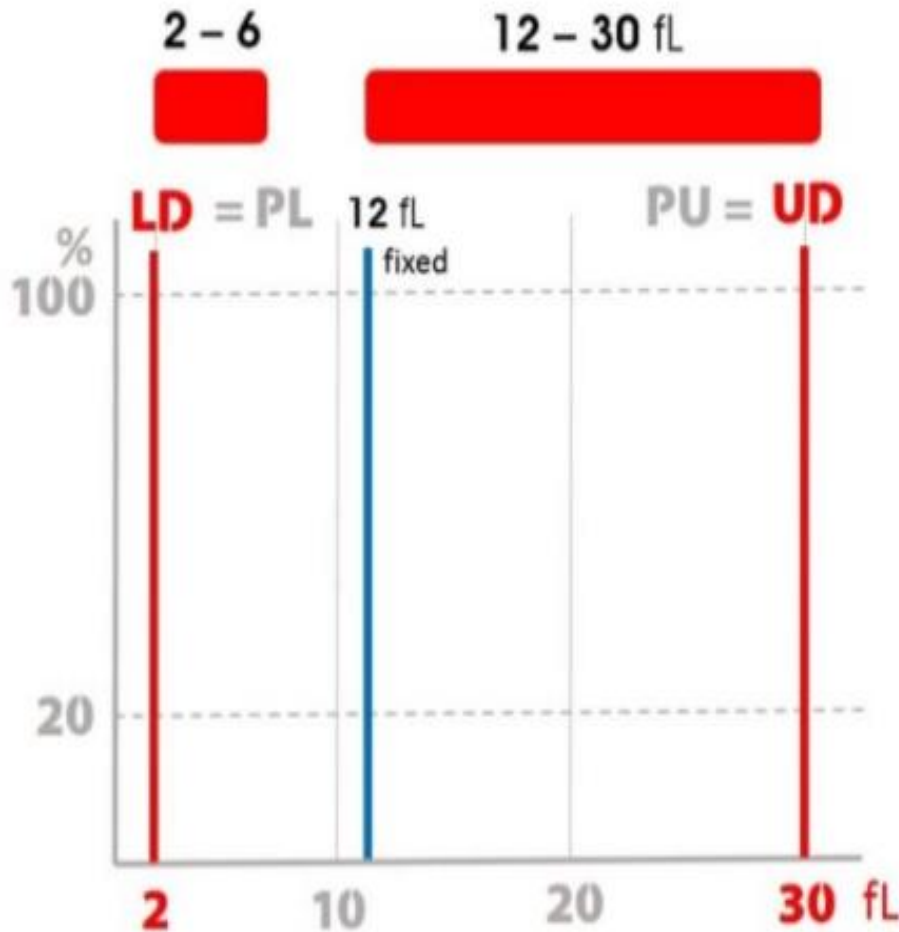
2 fL interference

- Dust
- EDTA particles
- Air bubbles
- Electric Noise

20 fL interference

- RBCs fragments
- WBCs fragments
- Large PLTs
- Giant PLTs

Platelet Histogram



2 flexible Discriminators are used

LD (Lower Discriminator)

Sometimes called **PL** (Platelet LD)

Flexible Discriminator between 2 – 6 fL

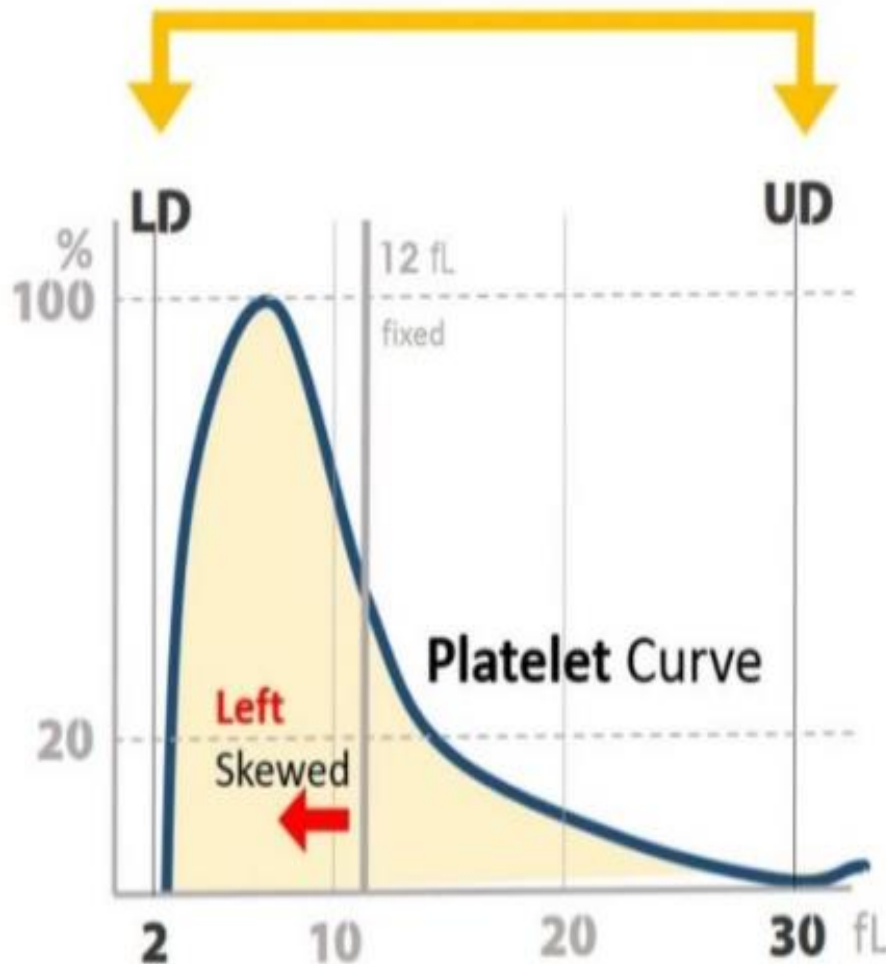
UD (Upper Discriminator)

Sometimes called **PU** (Platelet UD)

Flexible Discriminator between 12 – 30 fL

A **3rd Fixed** Discriminator at 12 fL may be used.

Platelet Histogram

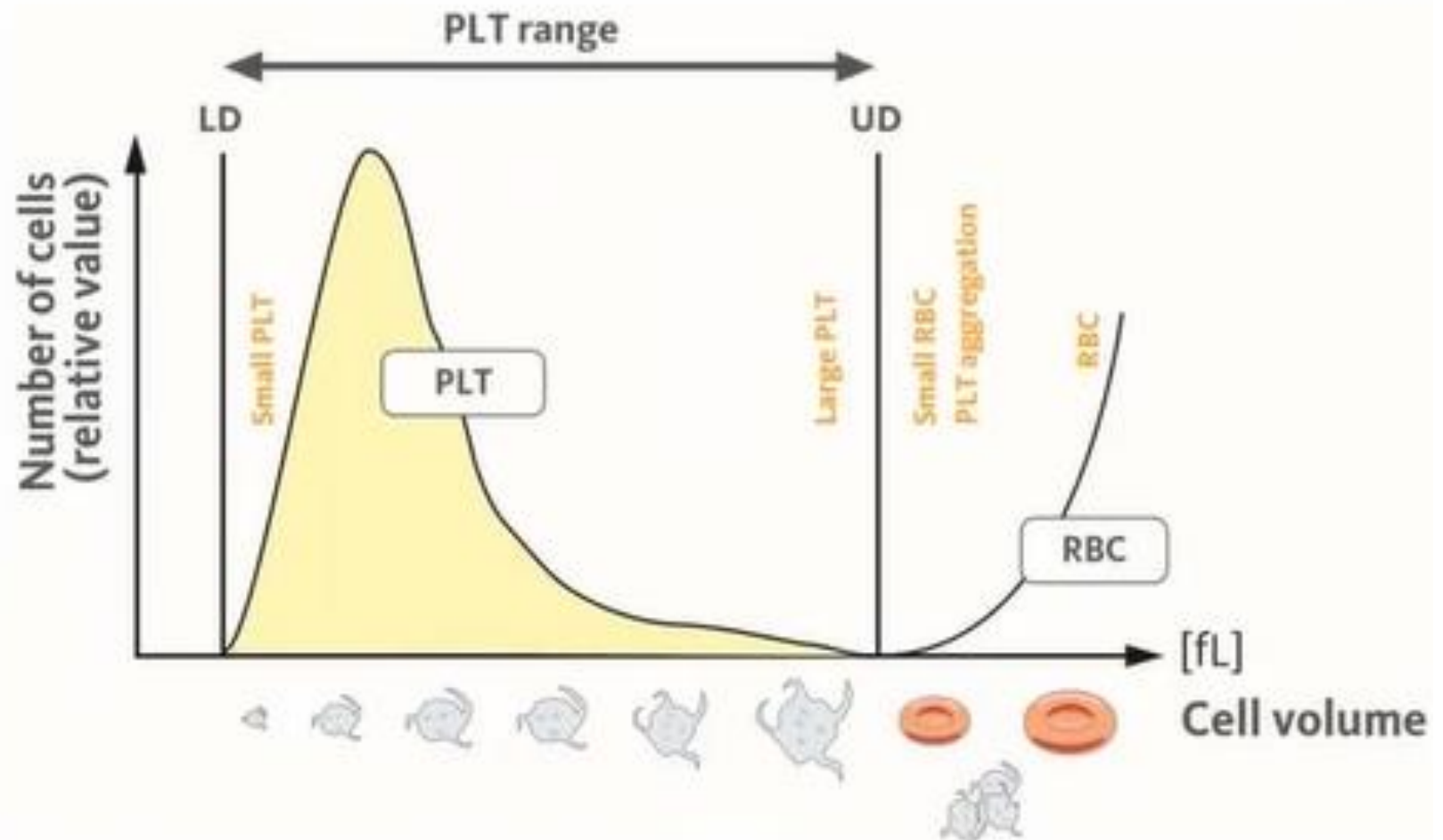


- The platelet histogram curve should lay **within** the **LD** and **UD**.

Normally, The platelet curve is **left-skewed**.

The platelet histogram curve **starts** and **ends** on the **base line**.

Platelet Histogram

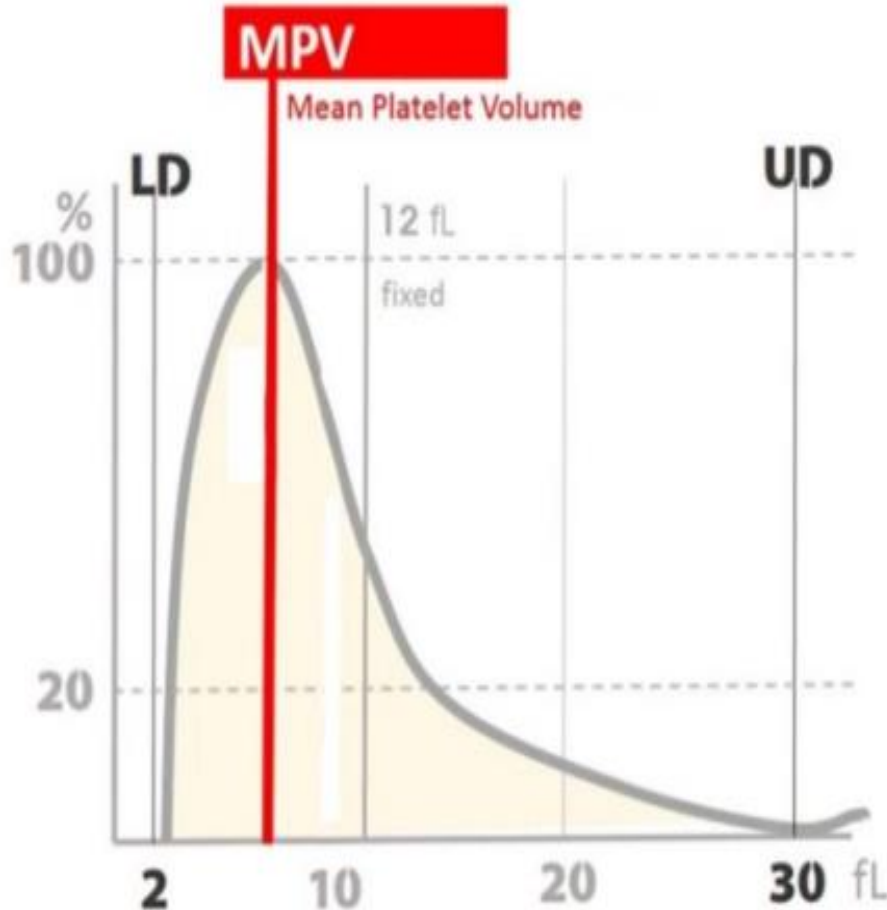


Parameters of Platelets Histogram

- MPV
- PDW
- P-LCR



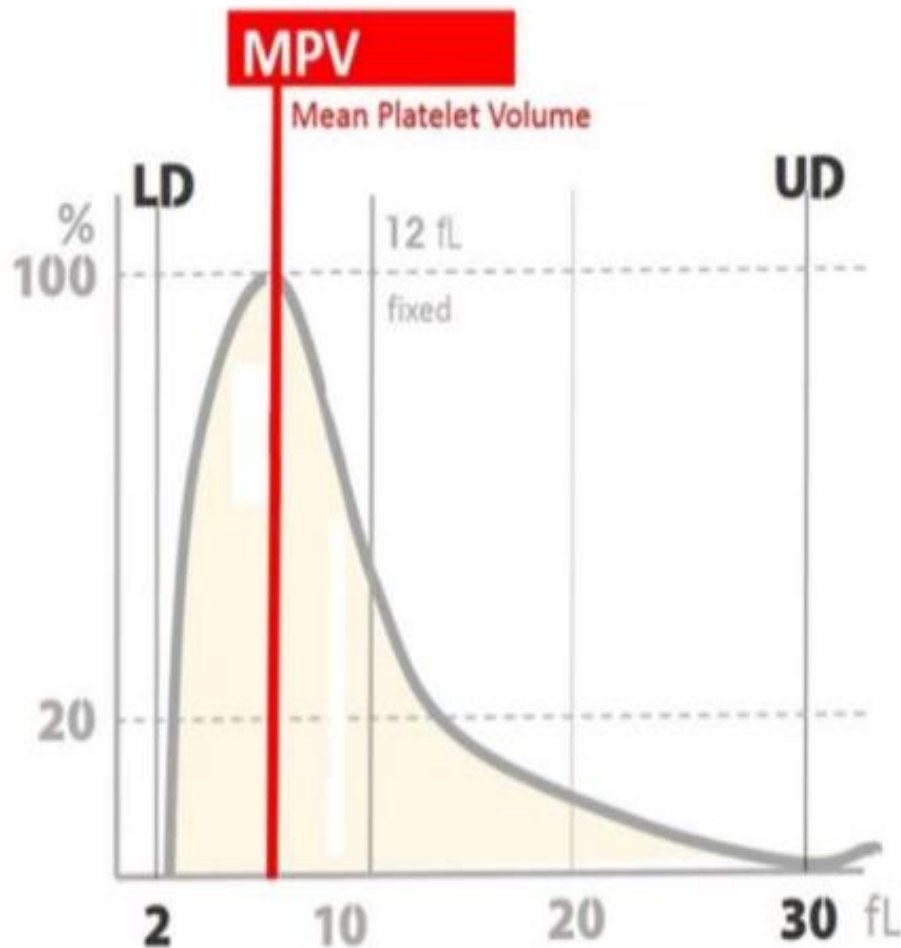
Platelet Histogram



- The MPV is a measure of the **average volume** of the counted platelets. (analogous to the erythrocytic MCV)

$$\text{MPV (fL)} = \frac{\text{Platelet crit} \text{ PCT (\%)}}{\text{PLT (x } 10^3/\mu\text{L) Platelet count}}$$

- Reference Range : 8 – 12 fL**



Platelet Histogram

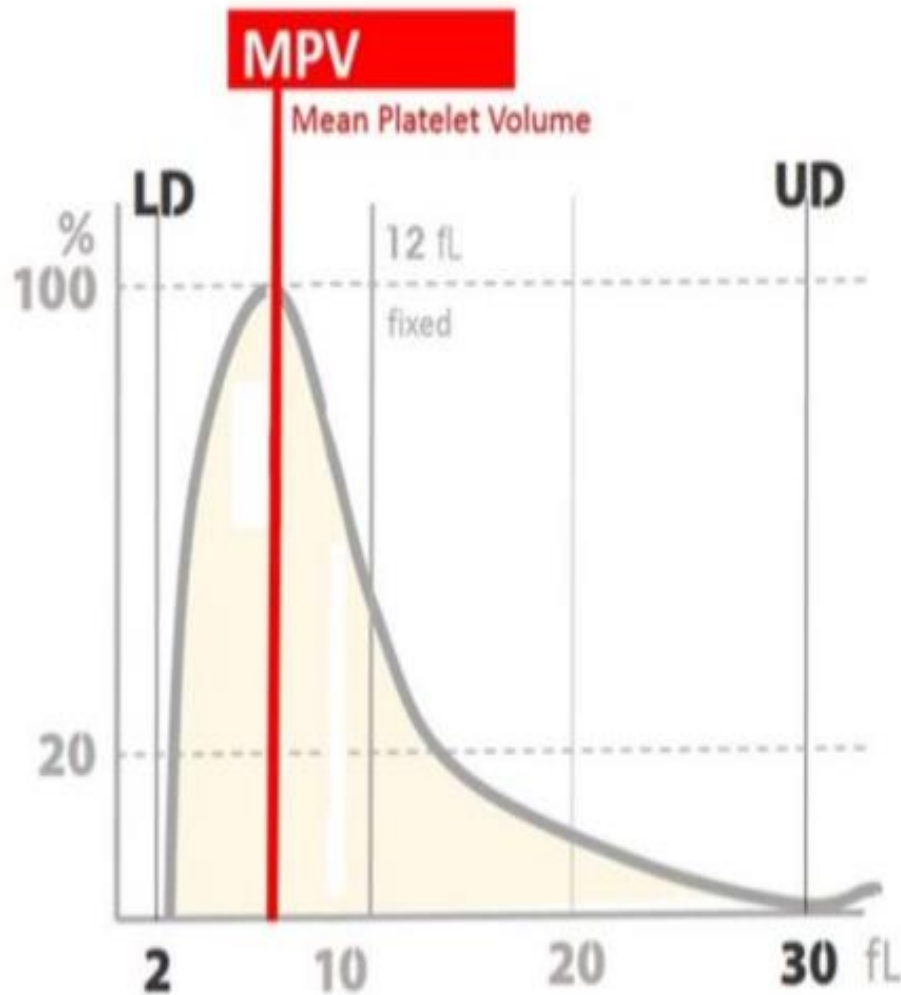
- The range of platelet size (MPV) **varies** according to the platelet count.
- In normal patients, MPV has an **inverse relationship** with platelet count.
[↑ MPV is seen in thrombocytopenia]
- MPV is used to **discriminate** between :

Reactive
Thrombocytosis

↑ PLT Count
Normal MPV

Malignant
Thrombocytosis

↑ PLT Count
↑ MPV



Platelet Histogram

Causes of

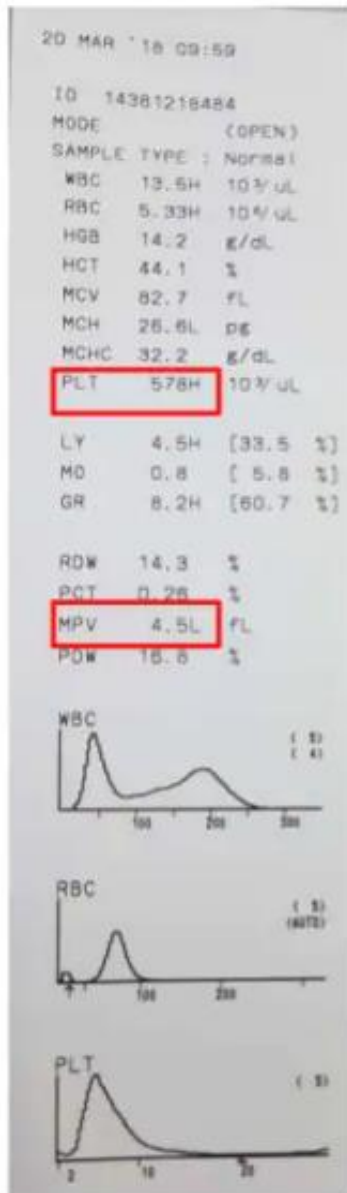
↑ MPV

- Splenectomy
- CML
- Myelofibrosis
- ITP
- Sickle Cell Anemia

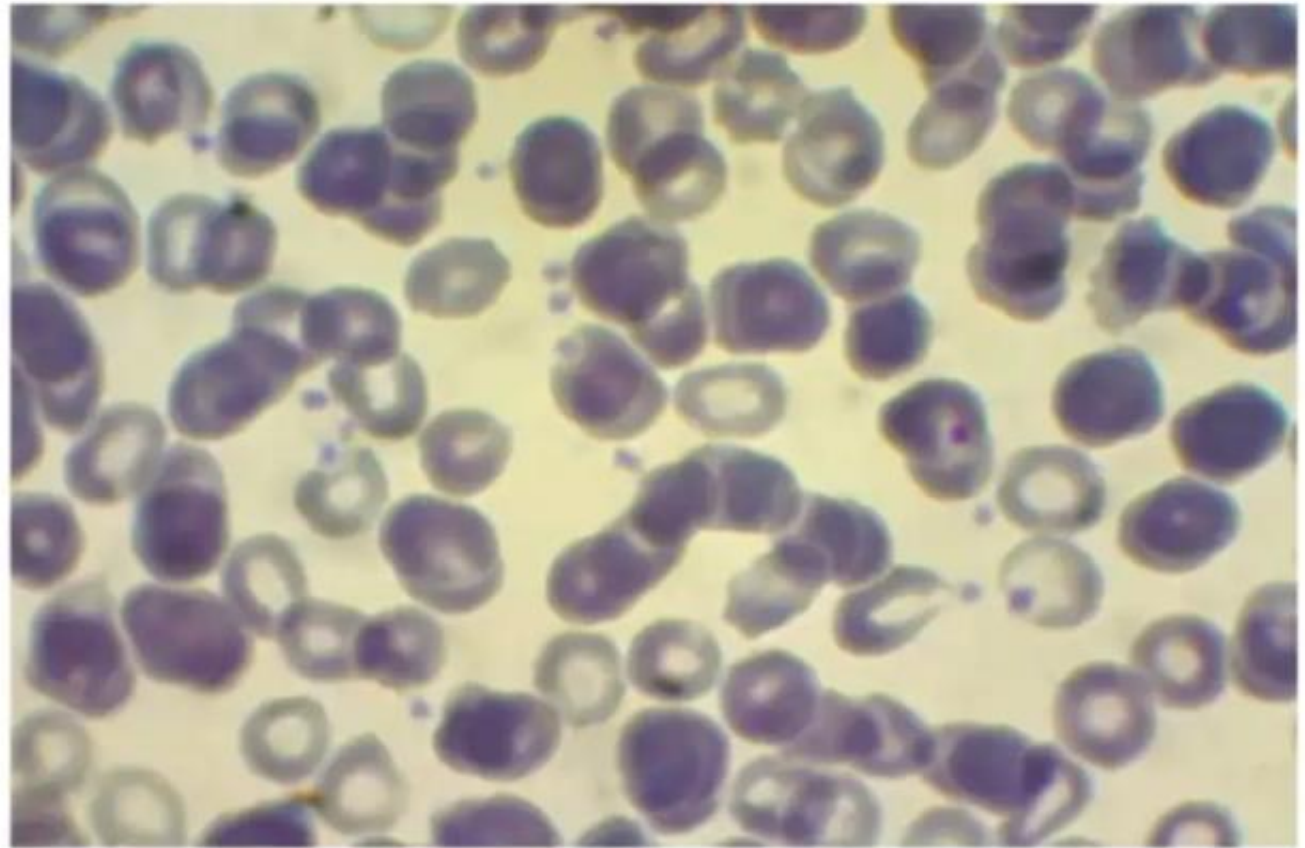
Causes of

↓ MPV

- Hypersplenism
- Aplastic Anemia
- Megaloblastic a.
- Chemotherapy



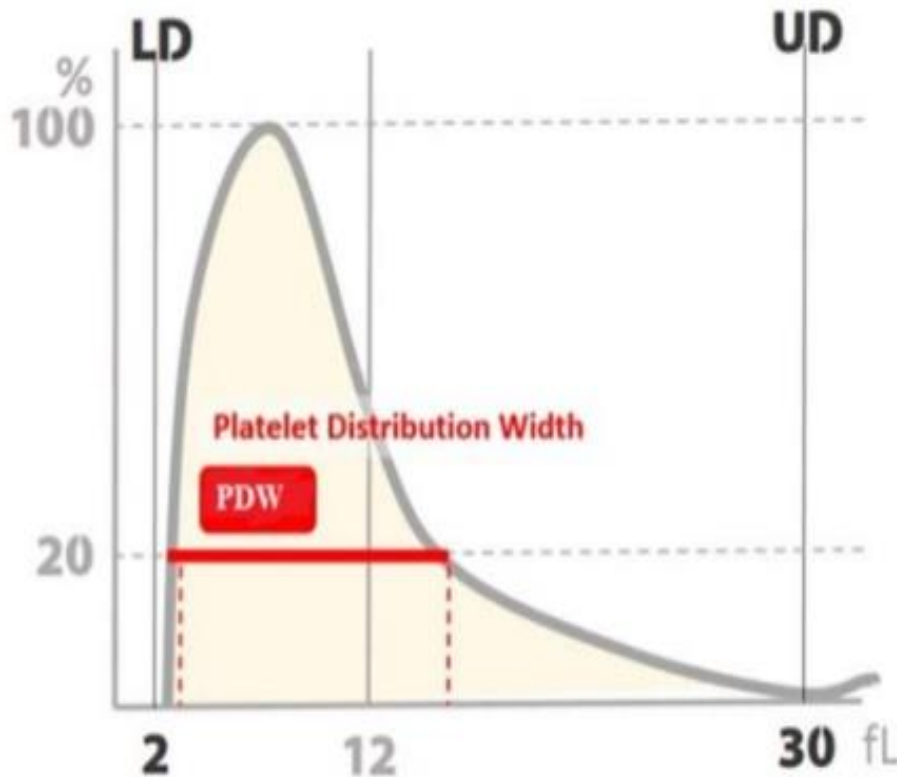
Thrombocytosis → Low MPV



Abu Jad Caesar

PDW

Platelet Distribution Width



Platelet Histogram

- It is a measure of variation of platelet size.
- It is a **coefficient of variation** percentage which is calculated by $SD/MPV \times 100$.
- **Reference range : 9 – 14% (15%)**
- PDW also could be expressed by drawing an arbitrary line at a height of 20%.

Causes of ↑ PDW

- Aplastic Anemia
- Megaloblastic a.
- CML
- Chemotherapy

Causes of False ↑ :

- PLT Clumps
- Micro RBCs
- Fragments
- (Schistocytes)

P-LCR

Platelet Large Cell Ratio



Platelet Histogram

- It's a ratio of **large** platelets.
- Reference range : 15 - 35 %

Causes of ↑ P-LCR :

- (Schistocytes)

Parameters of Platelets Histogram

$\uparrow$ PDW + $\uparrow$ P-LCR + $\uparrow$ MPV

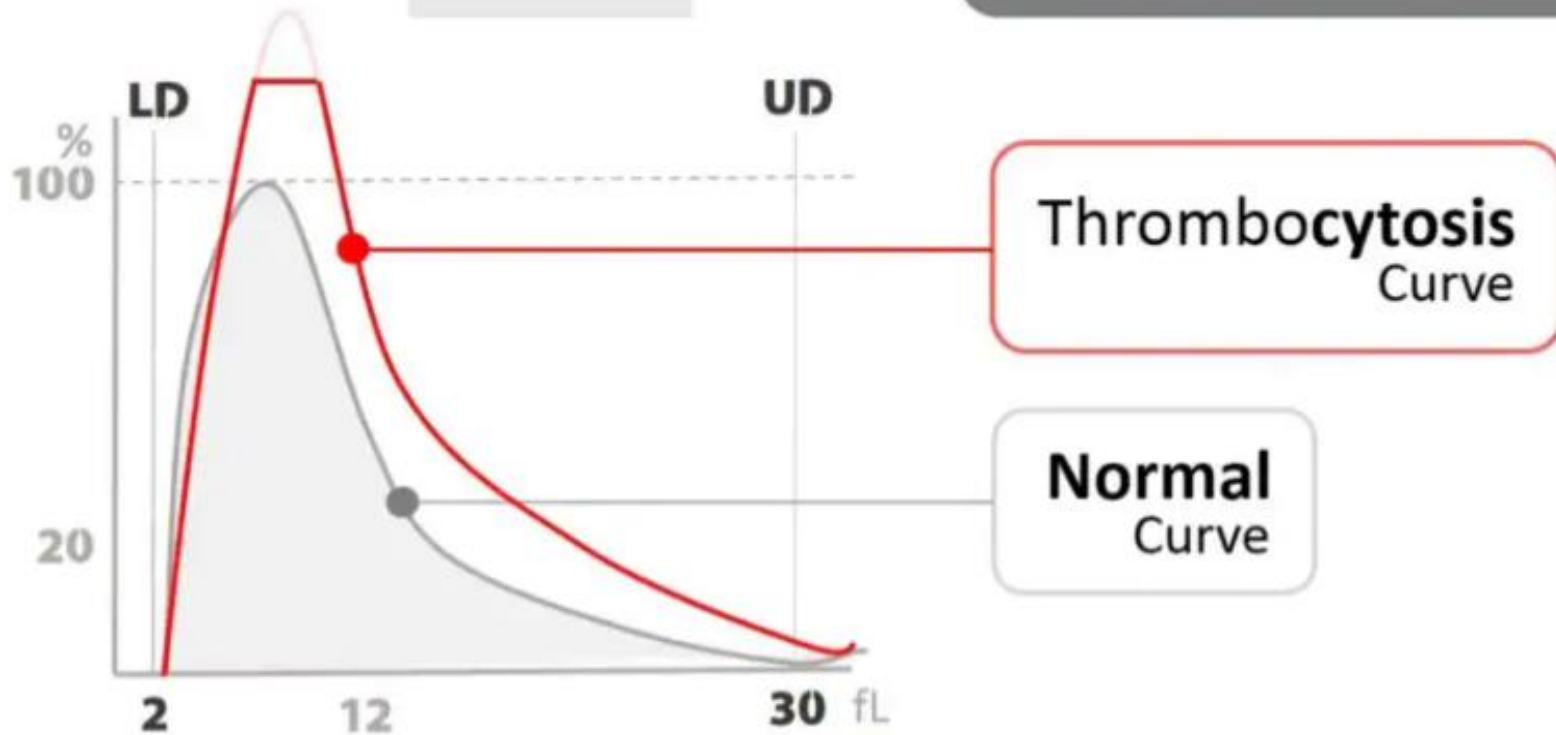


Autoimmune thrombocytopenic purpura (ITP)

Thrombocytosis

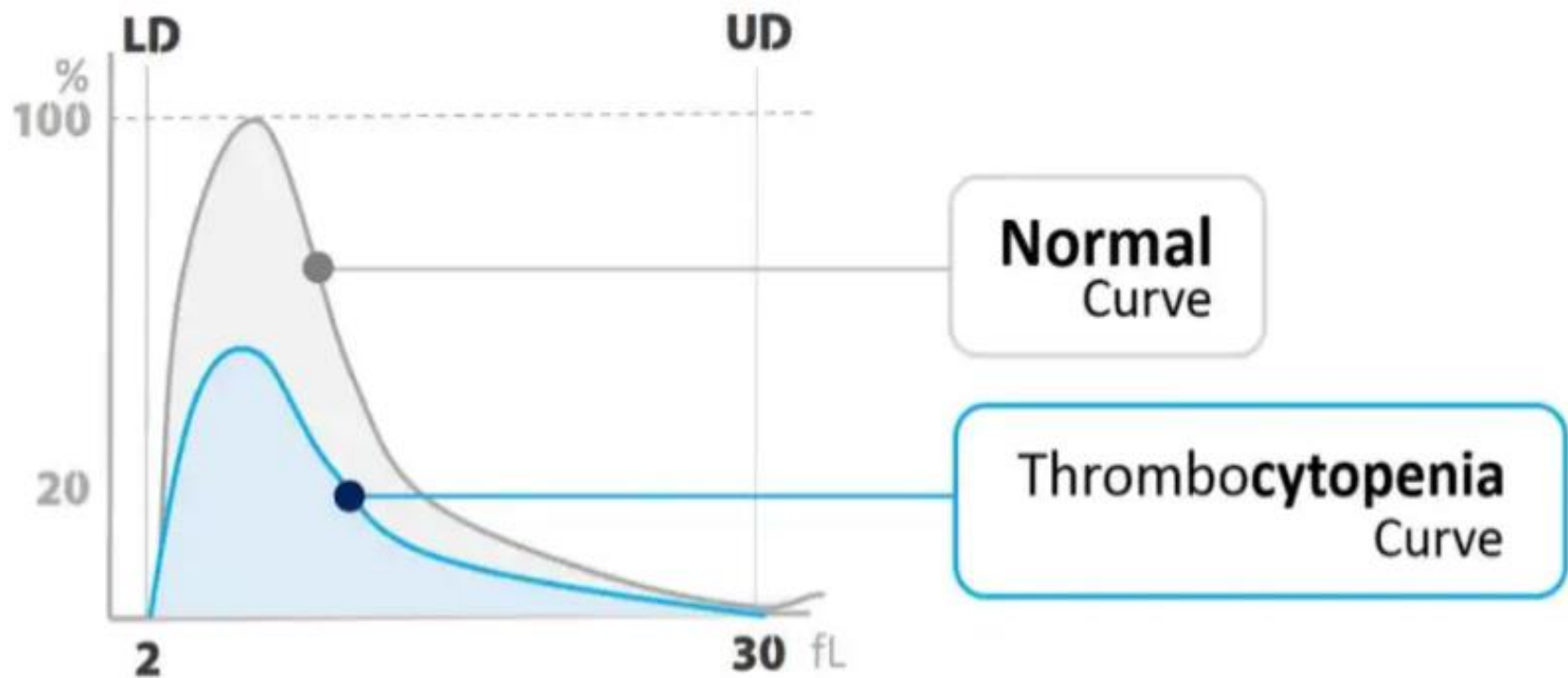


Platelet Histogram

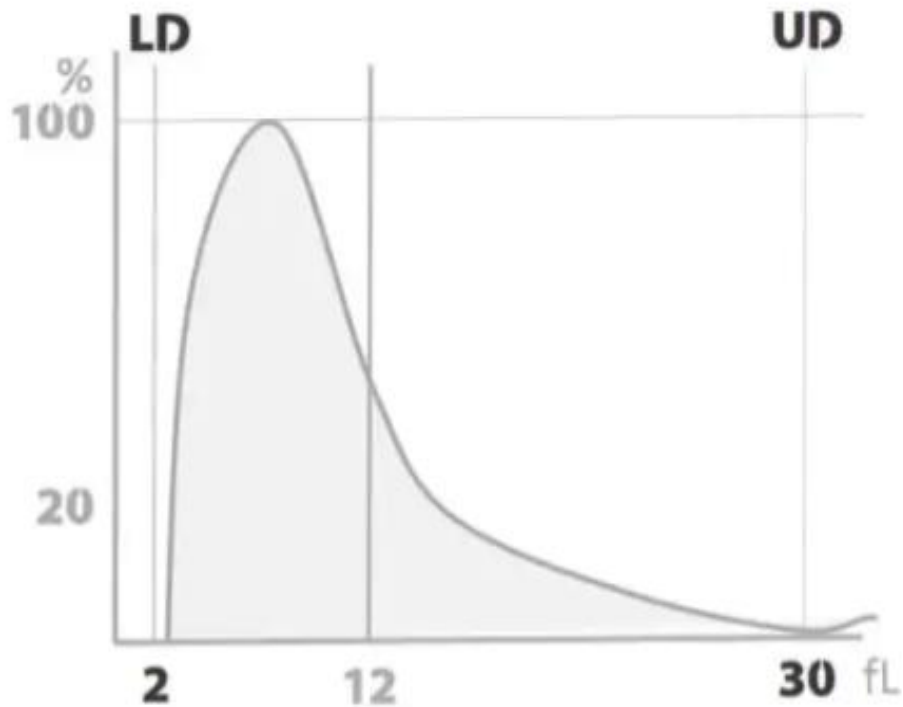


Thrombocytopenia ▼

Platelet Histogram



Platelet Histogram



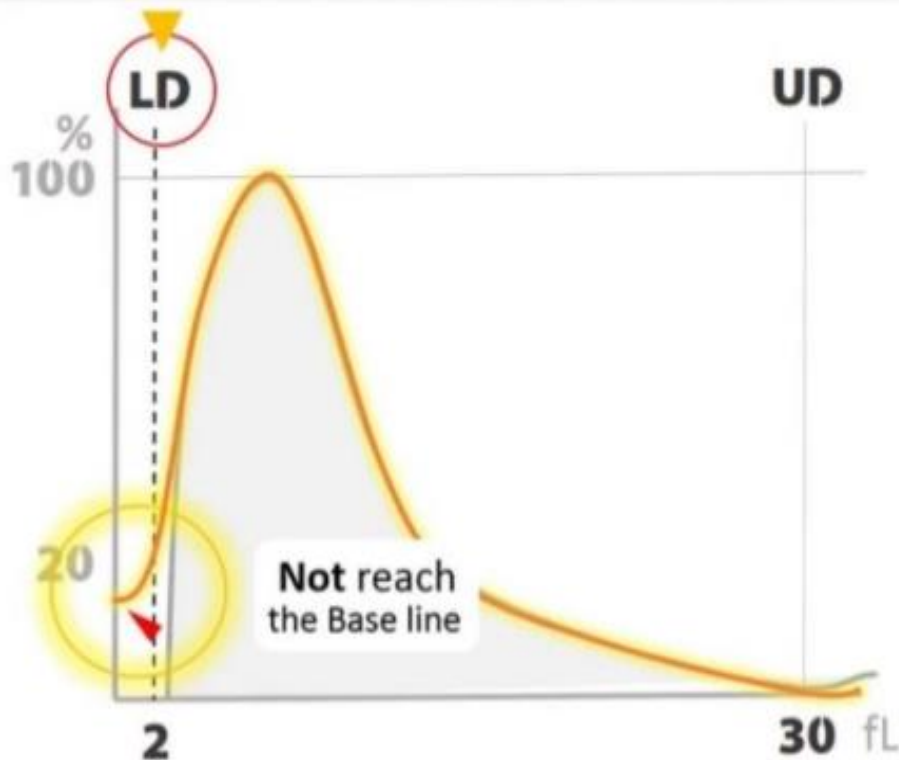
Platelet Histogram

List of **PLT** error flags in order of priority

PL	Relative frequency for LOWER discriminator (LD) exceeds the range. May be caused by <u>electrical noise</u> .
PU	Relative frequency for the UPPER discriminator (UD) exceeds the range. May be caused by electrical noise, <u>platelet clumps</u> , etc.
MP	Two or more peaks in the histogram.
DW	Particle distribution width error when the 20% frequency does not cross the histogram <u>two times</u> . The peak is taken as 100%.
AG	The particle count equal to or less than the LD exceeds a prescribed range. Probable cause is platelet agglutination, which does not alter the WBC count, but may result in decrease platelet count.

1

LD Flag (PL Flag)



Platelet Histogram

This flag appears when lower discriminator (LD) exceeds **preset height** by **10%**.

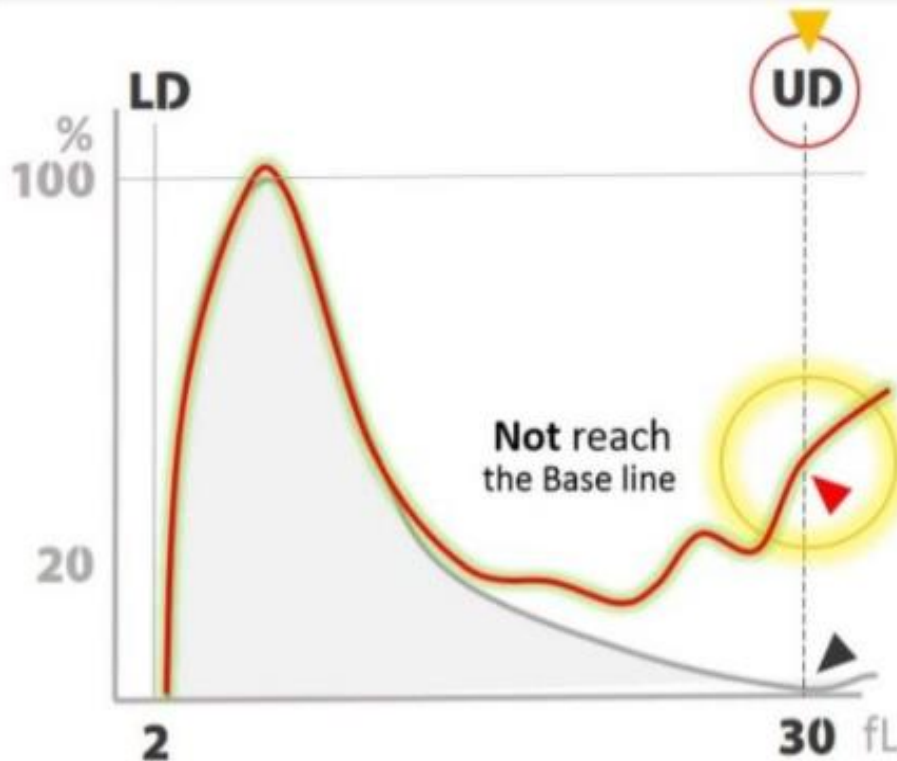
Possible Causes :

- High blank value
- Cell fragments
- Contaminated reagent
- High numbers of bacteria

PLTs count, P-LCR and MPV show PL flag

2

UD Flag (PU Flag)



Platelet Histogram

- Abnormal **height** at upper discriminator.
- This flag appears when UD exceeds the preset height by **> 40%**.

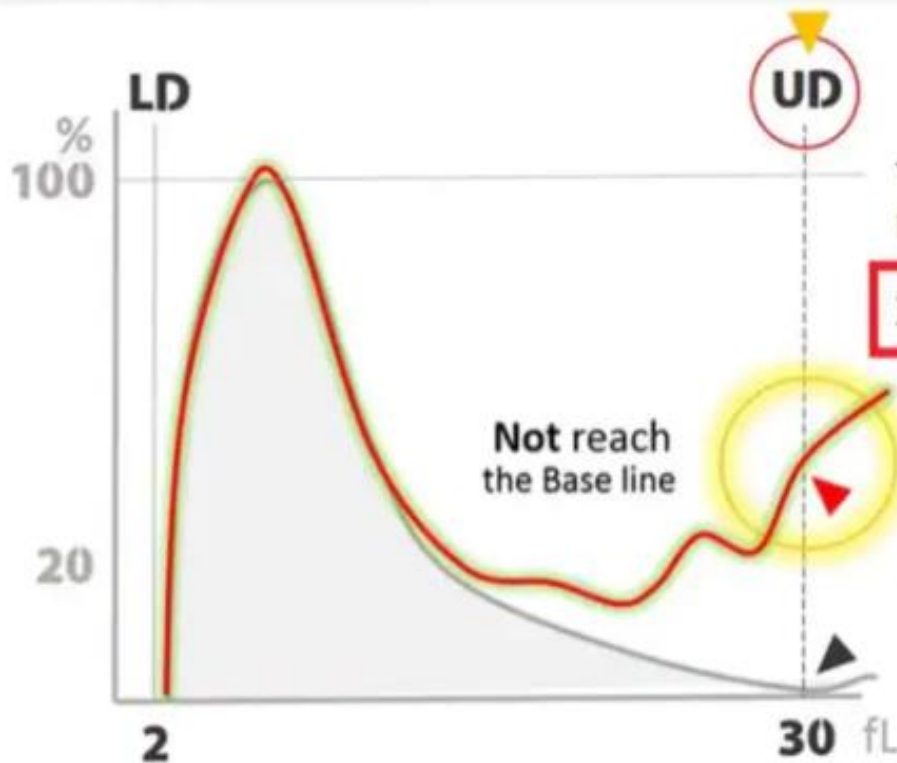
Possible Causes :

- **PLT Clumps** | Clotted sample
EDTA-Incompatibility
[Re-collect on citrate]
(X1.1)
- **Giant Platelets**
- **Micro RBCs**
- **(Schistocytes)**

2

UD Flag (PU Flag)

Platelet Histogram



When collect blood in citrate, multiply by 1.1 or use this formula ?

$PLT \text{ count} + (PLT \text{ count} \times \text{Dilution with citrate})$

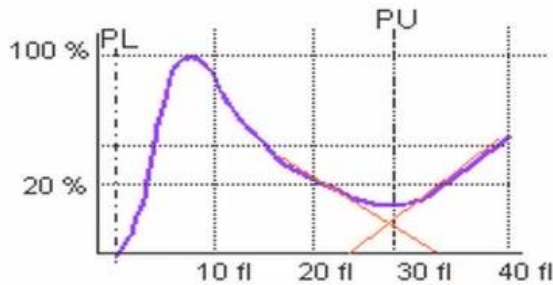
Dilution with citrate= 10%

10% of citrate present with 90% blood

2

UD Flag (PU Flag)

Platelet Histogram



Possible cause:

- EDTA incompatibility
- Clotted sample
- Microcytes/schistocytes
- Giant Platelets
- PLT aggregates

■ Example A curve

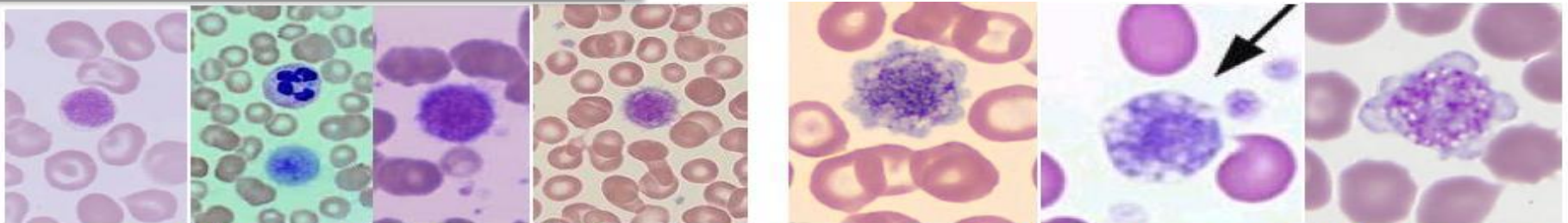
- » Overlapping of both populations (PLT / RBC) is equal.

■ Note:

- » Extreme low MCV and the presence of many RBC fragments might interfere with the PLT result.

■ Actions:

- » The platelet result might be false and should be checked and controlled with alternative methods.



شکل ۳۸-۱۰: تفاوت جایانت پلاکت رتیکولار (جوان) با یک جایانت پلاکت معمولی که هر دو باعث افزایش اندکس P-LCR می شوند ولی فقط پلاکت رتیکولار مقدار IPF را افزایش می دهد.

2

UD Flag (PU Flag)

Platelet Histogram

SAMPLE TYPE : Normal

WBC 7.8 $10^3/\mu\text{L}$

RBC 3.85L $10^6/\mu\text{L}$

HGB 4.7L g/dL

HCT 17.8L %

MCV 46.6L fL

MCH 12.9L pg

MCHC 26.4L g/dL

PLT 267 $10^3/\mu\text{L}$

LY 2.4 [31.5 %]

MO 0.2 [3.0 %]

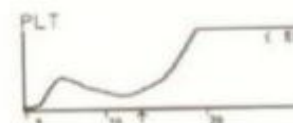
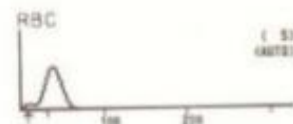
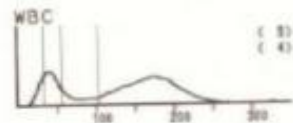
GR 5.0 [65.5 %]

RDW 20.1H %

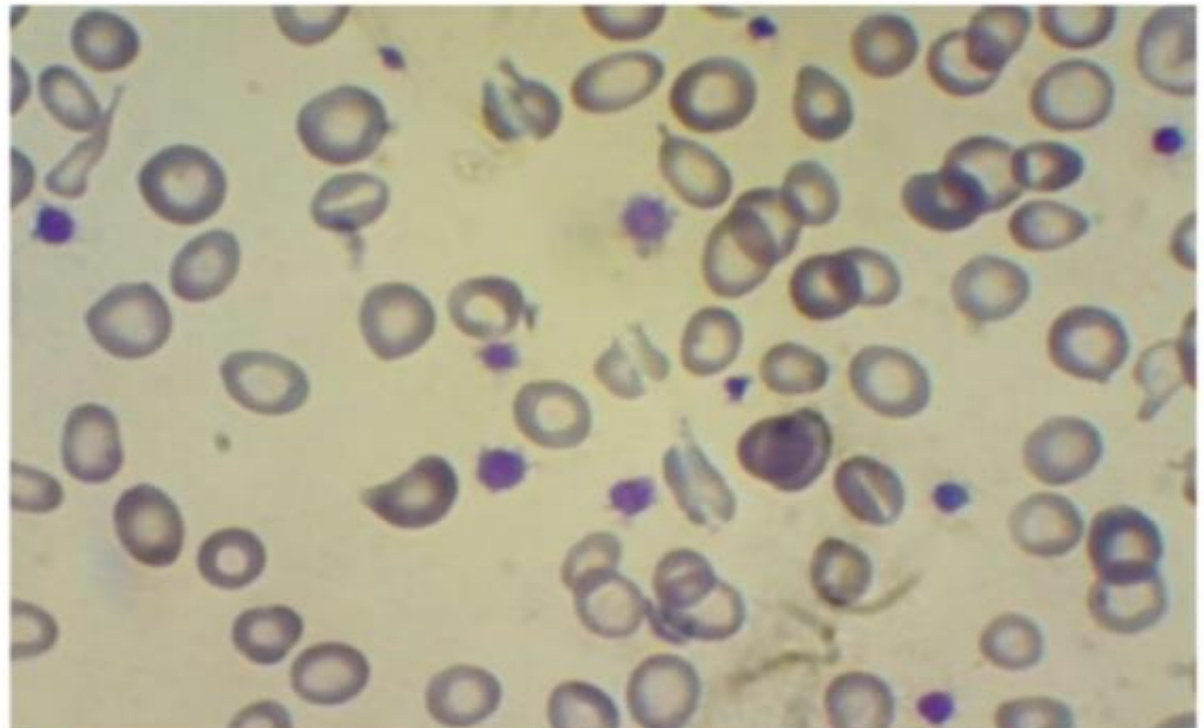
PCT 0.17 %

MPV 5.0 fL

PDW 14.7 %

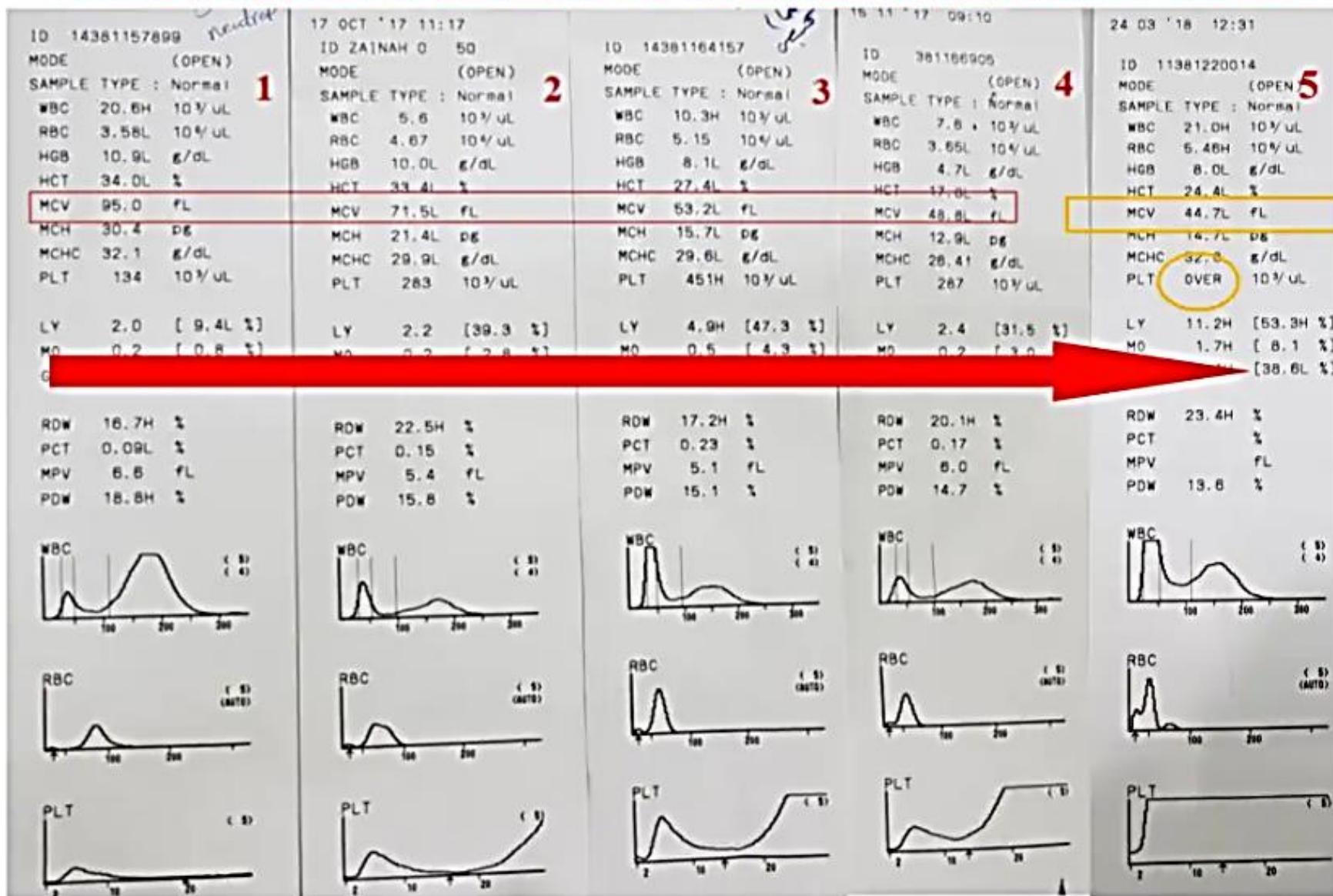


Giant PLTs and Micro RBCs → PLT/RBC overlap



Abu Jad Caesar

PLT/RBC overlap: Extreme microRBC's → no clear separation in volume between PLT & RBCs

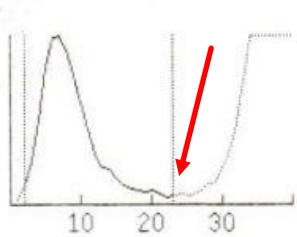


Abu Jad Caesar

2

UD Flag (PU Flag)

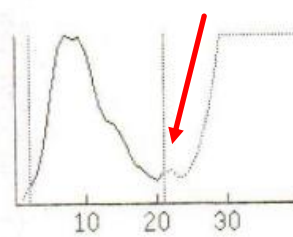
Platelet Histogram



PLT

[fL]

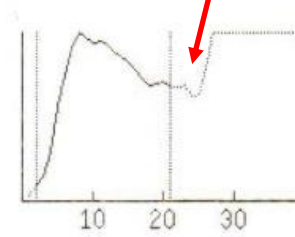
PDW PL* 11.8fL
MPV PL* 8.6fL
P_LCR PL* 16.6%



PLT

[fL]

PDW 15.2fL
MPV 10.0fL
P_LCR 28.3%



PLT

[fL]

PDW DW ---.-fL
MPV PU ---.-fL
P_LCR PU ---.-%

WBC 7.0×10³/μL
RBC 6.01×10⁶/μL
HGB 13.3g/dL
HCT 42.1%
MCV - 70.0fL
MCH - 22.1Pg
MCHC 31.6g/dL
PLT PL* 194×10³/μL

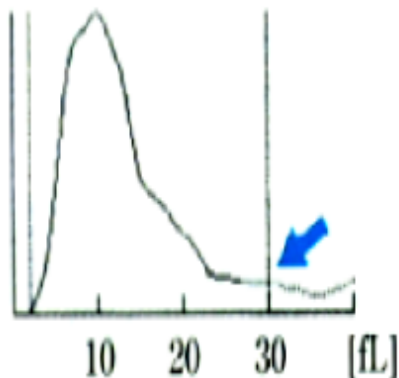
WBC 9.3×10³/μL
RBC ! 7.67×10⁶/μL
HGB 15.5g/dL
HCT 49.5%
MCV - 64.5fL
MCH - 20.2Pg
MCHC 31.3g/dL
PLT 229×10³/μL

WBC 7.7×10³/μL
RBC 5.30×10⁶/μL
HGB - 9.5g/dL
HCT - 32.4%
MCV - 61.1fL
MCH - 17.9Pg
MCHC - 29.3g/dL
PLT PU* 216×10³/μL

PLT is incorrect Low/high??

- In case of platelet aggregation, the PLT count is **incorrect low**.
 - Check EDTA incompatibility –e.g. re-collect the sample and use citrate as anticoagulance to avoid clumping caused by EDTA  **X 1.1** or
 - Add Amikacin to CBC vial let stay for 15 min. then shake for 1-2 min and apply to cell counter
- In case of extreme microerythrocytes or fragmented RBC the PLT count might be **incorrect high**.
 - PLT results should be confirmed with alternative methods

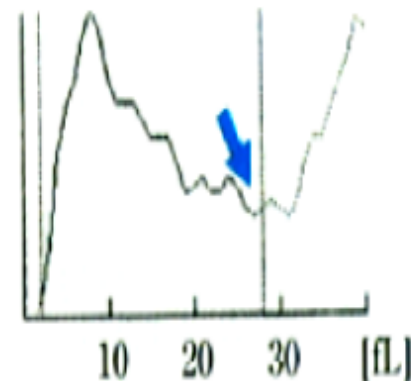
PLT-Histogram



Results

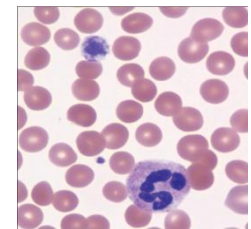
PLT		237 x10 ⁹ /L
PDW	+	18.0fl
MPV		12.4fl
P-LCR	+	44.1%

PLT-Histogram



Results

PLT	PU	71 x10 ⁹ /L
PDW	DW	---.fl
MPV	DW	---.fl
P-LCR	DW	-.---%

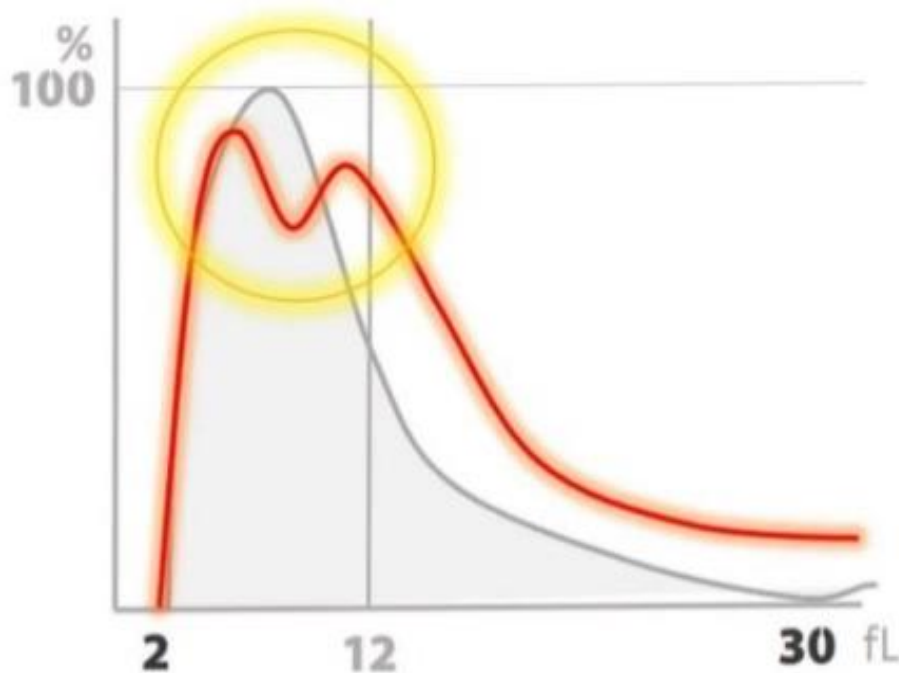


شکل ۴۰: هرچند هر دو گراف به دلیل میل منحنی به سمت راست با حضور جایانت پلاکت همراه هست، ولی در گراف سمت چپ، دم سمت راست هیستوگرام در ارتفاع پایینی ممیز UD/PU را قطع کرده و لذا شمارش آن صحیح می باشد ولی در گراف راست، محل قطع شدن ممیز PU در ارتفاع بالاتری بوده و احتمالاً تعدادی از پلاکت های خیلی بزرگ و رتیکولار وارد محدوده شمارش RBC شدند و حتی احتمال دارد قسمتی از RBC های خیلی کوچک یا شیسیتوسیت نیز وارد محدوده شمارش پلاکت شده باشند، لذا به دلیل فلاگ PU و DW، نتایج آن به صورت نقطه چین بوده و قطعاً شمارش دستگاه می بایست با PBS تایید و چک شود. در گراف سمت راست، پلاکت ها بزرگ تر و رتیکولارتر از گراف سمت چپ هستند. لازم به ذکر است که پلاکت رتیکولار و رتیکولوسیت هر دو مانند جایانت پلاکت و ماکروسیت بزرگ هستند ولی برخلاف آنها به دلیل داشتن RNA با تیاژول یا آکریدین نارنجی فلورسنت هم می شوند و لذا IPF و IRF بالایی هم دارند.

3

MP Flag **M**ultiple **P**eaks

Platelet Histogram



Possible Causes :

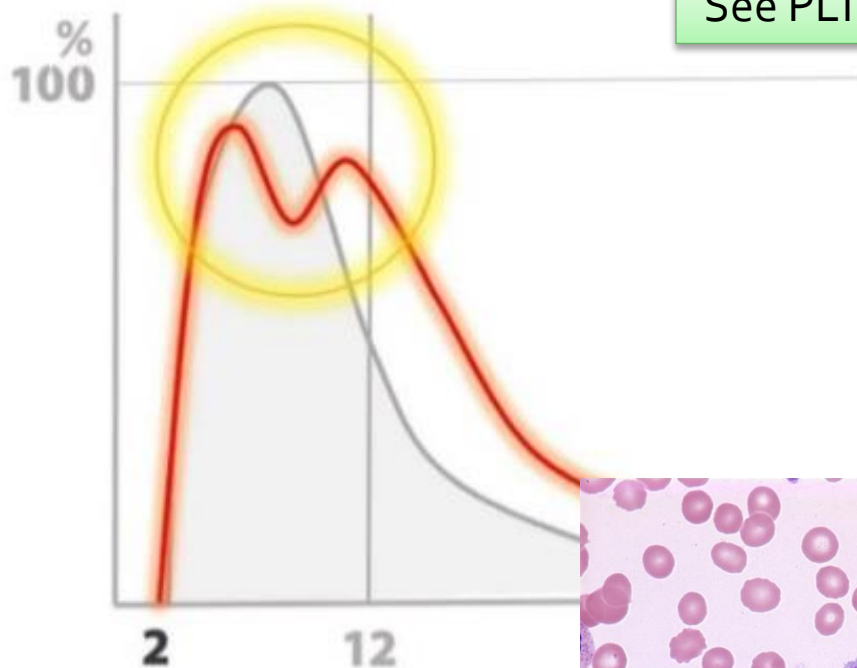
- Platelet transfusion
- Recovery from chemotherapy
- Platelet aggregation

3

MP Flag **M**ultiple **P**eaks

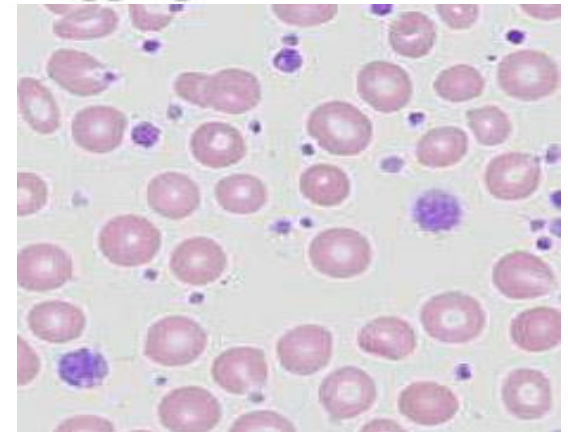
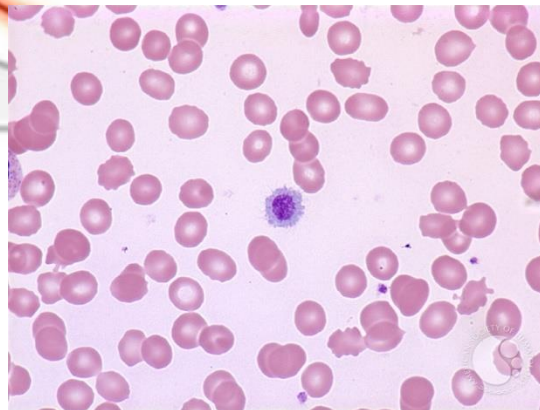
Platelet Histogram

See PLT anisocytosis after chemotherapy



Possible Causes :

- Platelet transfusion
- Recovery from chemotherapy
- Platelet aggregation



Beta-Thal- minor

Platelet Histogram

Parameters

WBC	9.94	[10 ³ /uL]	
RBC	6.14 +	[10 ⁶ /uL]	
HGB	11.5 -	[g/dL]	
HCT	35.2 -	[%]	
MCV	57.3 -	[fL]	
MCH	18.7 -	[pg]	
MCHC	32.7	[g/dL]	
RDW-SD	36.4 -	[fL]	
RDW-CV	20.0 +	[%]	
PLT	230 *	[10 ³ /uL]	
PDW	----	[fL]	
MPV	----	[fL]	
P-LCR	----	[%]	
PCT	----	[%]	
NEUT	8.23 +	[10 ³ /uL]	82.8 + [%]
LYMPH	0.95 -	[10 ³ /uL]	9.6 - [%]
MONO	0.51 +	[10 ³ /uL]	5.1 [%]
EO	0.23 +	[10 ³ /uL]	2.3 [%]
BASO	0.02	[10 ³ /uL]	0.2 [%]

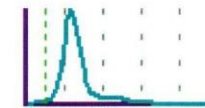
WBC IP Message(s)

RBC IP Message(s)

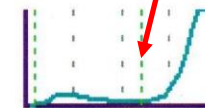
Normal Ranges

(4.50 - 11.50)
(4.10 - 5.10)
(12.0 - 16.0)
(36.0 - 46.0)
(78.0 - 90.0)
(25.0 - 35.0)
(31.0 - 36.0)
(37.0 - 54.0)
(11.0 - 14.5)
(150 - 450)
(9.0 - 14.0)
(9.0 - 12.0)
(13.0 - 43.0)
(0.17 - 0.35)
(37.0 - 72.0)
(20.0 - 50.0)
(0.0 - 14.0)
(0.0 - 1.0)
(0.0 - 6.0)

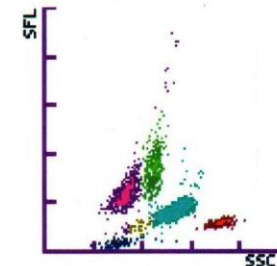
RBC



PLT



DIFF



PLT IP Message(s)

PLT Abn Distribution

Beta-Thal- minor

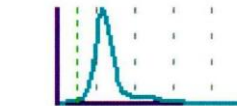
Platelet Histogram

Parameters

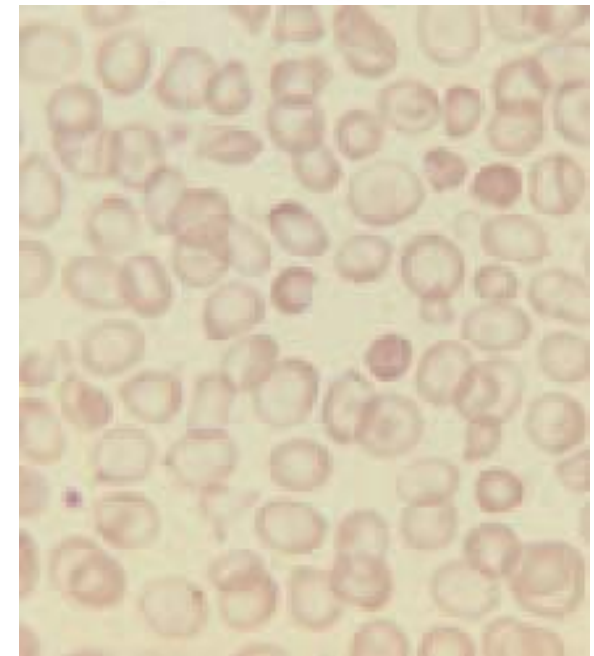
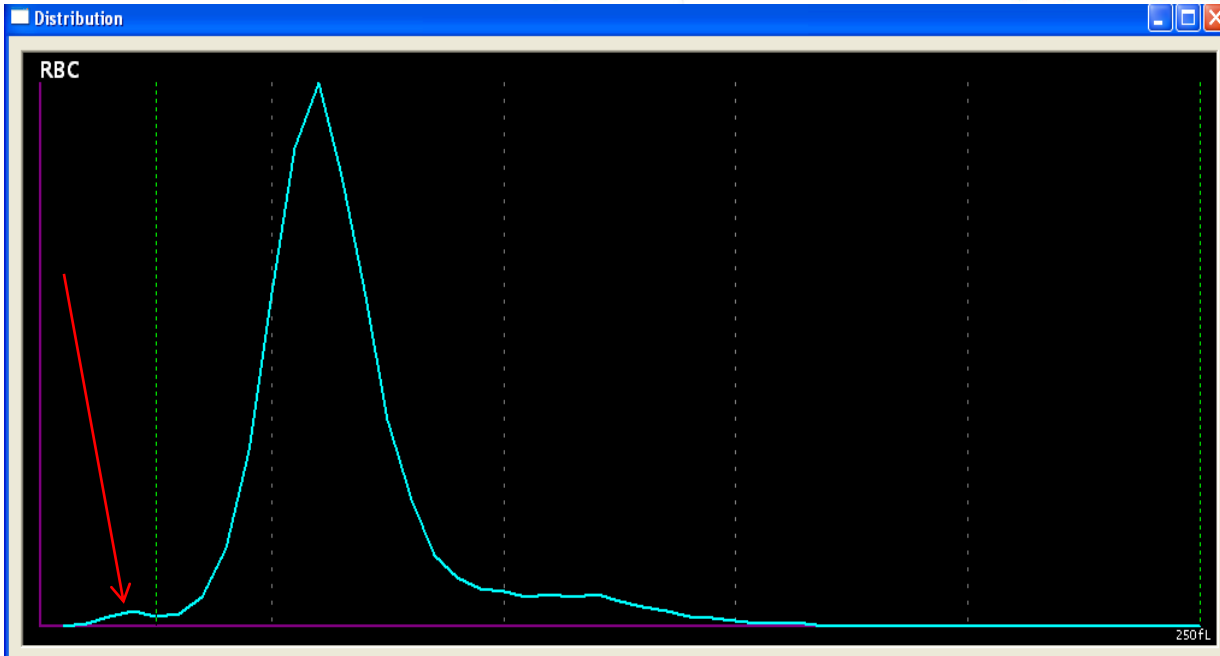
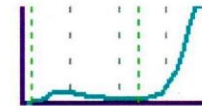
WBC	9.94	[10 ³ /uL]
RBC	6.14 +	[10 ⁶ /uL]
HGB	11.5 -	[g/dL]
HCT	35.2 -	[%]
MCV	57.3 -	[fL]
MCH	18.7 -	[pg]
MCHC	32.7	[g/dL]
RDW-SD	36.4 -	[fL]
RDW-CV	20.0 +	[%]

Normal Ranges RBC

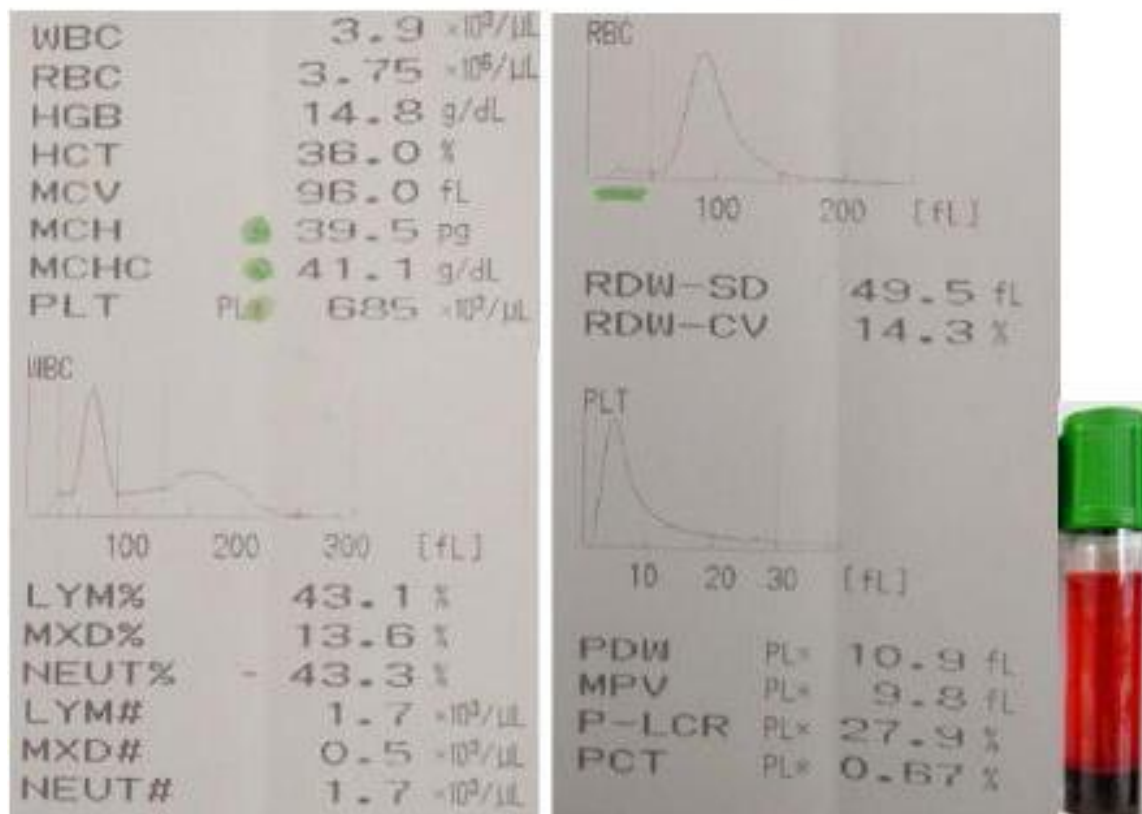
(4.50 - 11.50)
(4.10 - 5.10)
(12.0 - 16.0)
(36.0 - 46.0)
(78.0 - 90.0)
(25.0 - 35.0)
(31.0 - 36.0)
(37.0 - 54.0)
(11.0 - 14.5)



PLT

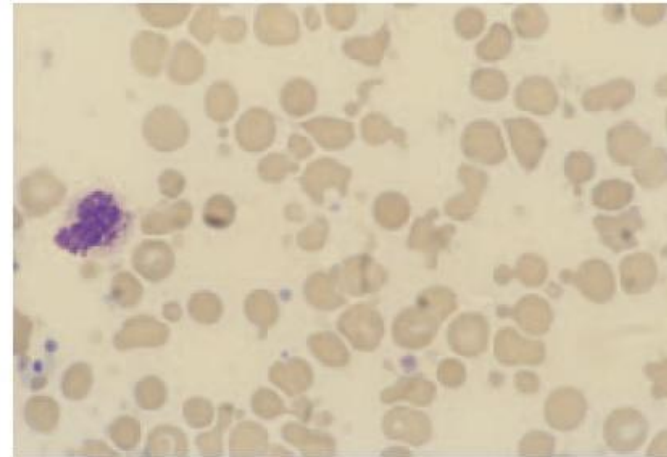
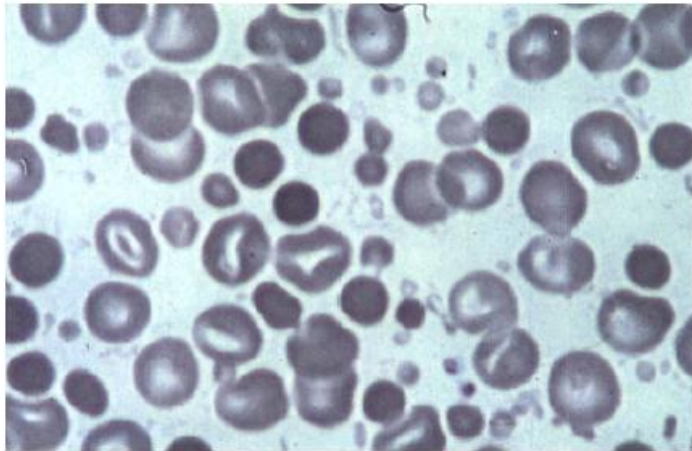


تأثیر همولیز شدید بر نتایج سل کانتر



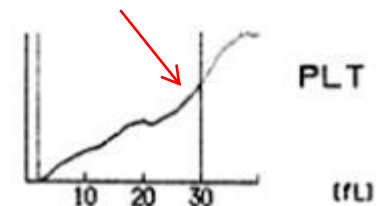
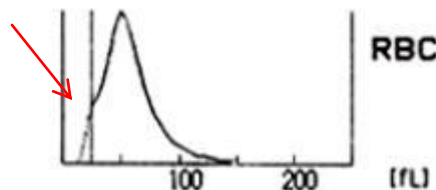
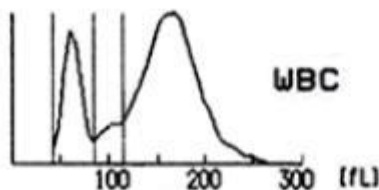
نمونه خون همولیز شده و نتایج آزمایش اشتباه و کاذب است. در این نمونه مقدار Hgb و MCV تغییر نمی کند اما بدلیل لیز شدن شمارش RBC کاهش می یابد و مقدار MCH و MCHC بطور غیر منتظره ای افزایش می یابد. توجه نمایید که MCHC بالاتر از ۳۶ امکان پذیر نیست و هموگلوبین شروع به کریستالیزه شدن می کند. در نمونه ای که لیز شده ذرات و بقایای RBC با پلاکت ها شمرده می شوند که لازم است اسمیر بررسی شود.

تأثیر میکروسیتوز و شیتوسیتوز شدید در منحنی RBC و PLT



شکل ۱۳۵-۱۰: میکروسیتوز شدید ناشی از سوختگی و حرارت دیدن نمونه که با شمارش بجای پلاکت باعث افزایش PLT، PDW، MPV و RDW و MCV می‌شوند.

WBC		$5.8 \times 10^3 / \mu L$	LVM%	21.0%			
RBC	RL*	$5.65 \times 10^6 / \mu L$	MXD%	7.2%			
HGB	-	8.4 g/dL	NEUT%	71.8%			
HCT	RL*	32.5%	LVM#	$1.2 \times 10^3 / \mu L$			
MCV	RL*	57.5 fL	MXD#	$0.4 \times 10^3 / \mu L$			
MCH	RL*	14.9 pg	NEUT#	$4.2 \times 10^3 / \mu L$			
MCHC	RL*	25.8 g/dL					
PLT	PU!	<u>$1884 \times 10^3 / \mu L$</u>	RDW	RL*	32.3%	PDW	DW ---, -fL
						MPV	PU ---, -fL
						P-LCR	PU ---, -%



شکل ۹۰-۱۰: افزایش شدید ولی کاذب پلاکت در اثر حضور شیتوسیت‌ها در خون محیطی که در دستگاه‌های روش ایمیدانز اغلب به عنوان پلاکت شمرده می‌شوند. ۱۱/۲

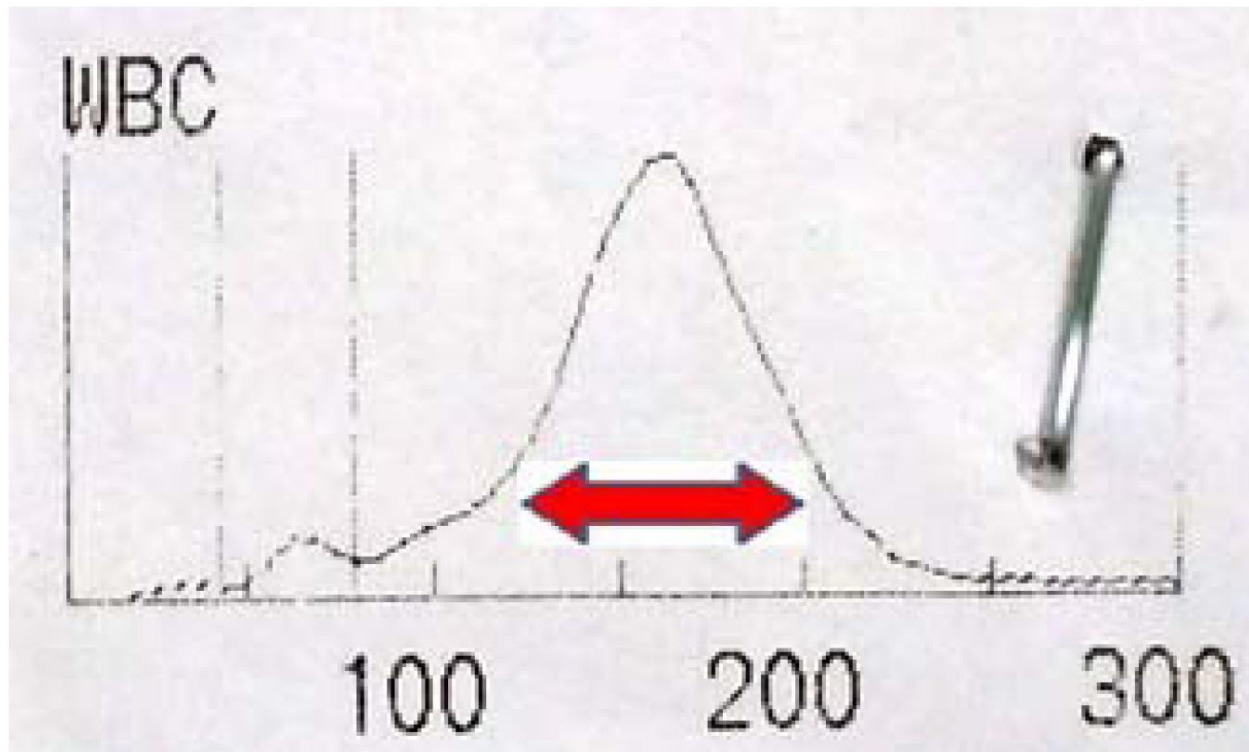
Examples of PLT, MPV

- PLT....decreased
- MPV...increased
 - **ITP, TTP, hepatitis C, thyrotoxicosis, Lupus, H.pylori infections, HIV**
- PLT....increased
- MPV...increased
 - **MPN(ET,CML,MF,...)**
- PLT....decreased
- MPV...normal
 - **BM failure(malignancies,metastasis, AA,...)**
- PLT....increased
- MPV...decreased
 - **Reactive states, inflammation, rheumatologic diseases infections.....Kawasaki,...**
 - **Wiskott–Aldrich syndrome: MPV....very low & PLT count decreased**

Clinical Utility of Blood Cell Histogram

Interpretation Case-1

- Case 1: A 56-year-old male presented with tiredness and early satiety. He had a massive splenomegaly. His Hb= 9.7 g/dl, WBC = of 64,000 per mm

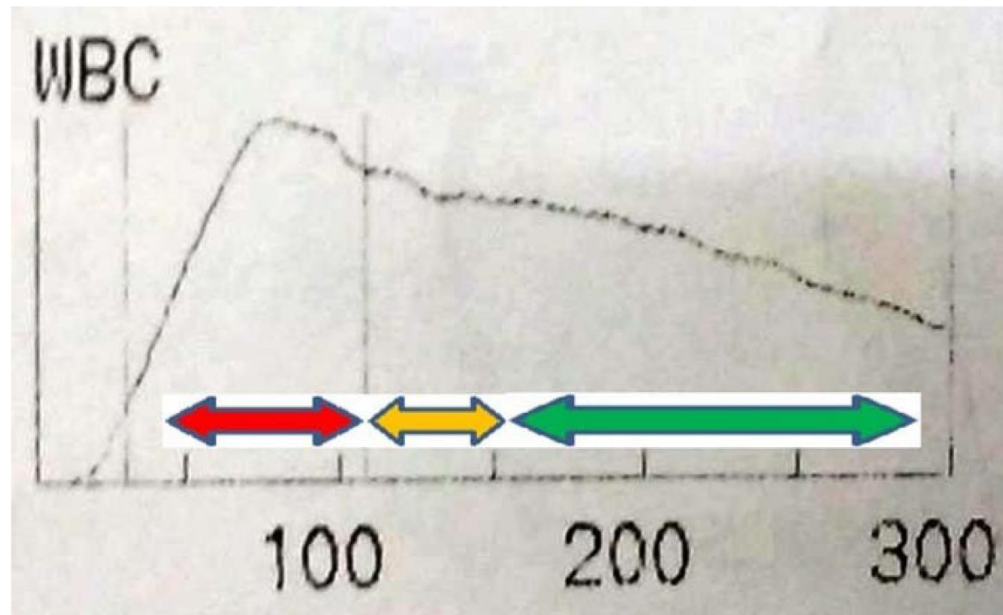


Clinical Utility of Blood Cell Histogram Interpretation Case-1

- Patient in the case 1 has **leukocytosis** with WBC distribution curve showing a peak in the **region of neutrophils**
- The two differential diagnoses for this WBC distribution were **CML in chronic phase** and **leukemoid reaction**
- As the patient was having **massive splenomegaly**, diagnosis was in favour of CML
- The **myelocytes** outnumber the more mature **metamyelocytes** in CML and are seen as the myelocyte bulge in WBC histogram (**red** arrow)

Clinical Utility of Blood Cell Histogram Interpretation Case-2

- Case 2: A 44-year-old male presented with tiredness and massive splenomegaly. His Hb =8.0 g/dl, WBC =2,57,000 per mm , PLT= 96,000 per mm



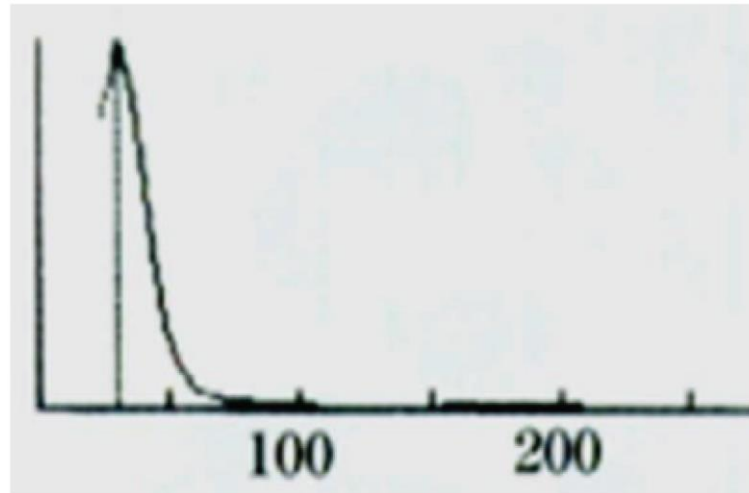
Blast cells -red arrow, basophils -yellow arrow, and cells at various stages of myeloid maturation are shown in green arrow.

Clinical Utility of Blood Cell Histogram Interpretation Case-2

- The patient in case 2 was in **blast crisis of CML**
- In the WBC distribution curve, the maximum number of cells was seen in the region of **blasts** (Red arrow)
- Blast cells occupy the region between 70-120 fL
- Cells at **various stages of myeloid maturation** were also increased (Green arrow)
- There was also marked **basophilia** (Yellow arrow)
- Diagnosis of blastic phase of CML from peripheral blood requires $\geq 20\%$ blasts, which was obvious in this histogram

Clinical Utility of Blood Cell Histogram Interpretation Case-3

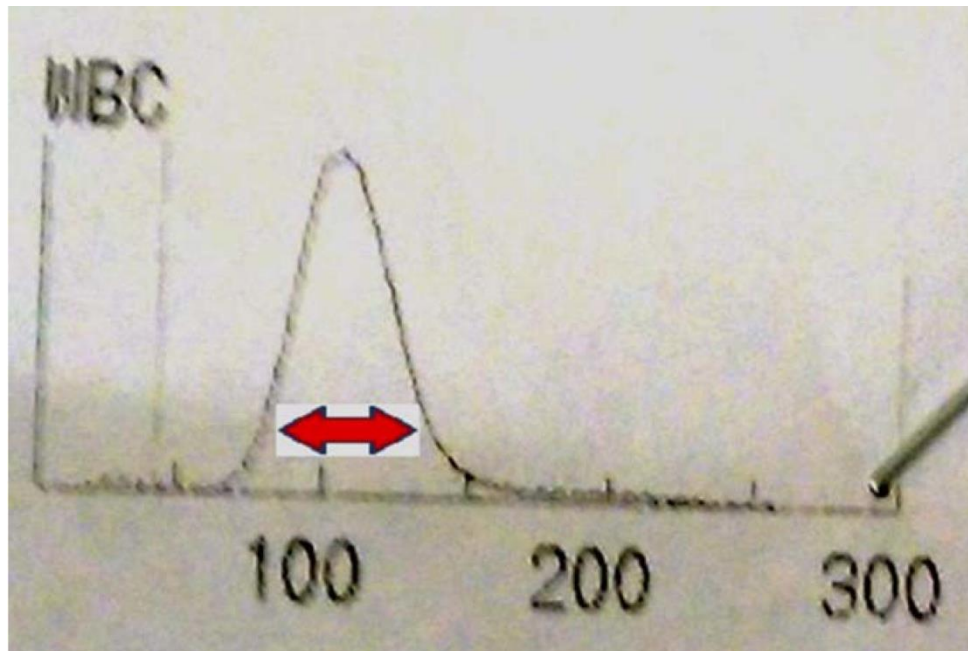
- Case 3: CBC of a 30-year-old female was done as a part of preoperative evaluation for an elective surgery. The WBC = 3.49 million/micL



- The **WBC count** in case 3 was **3.49 million/mm³**, which is in the range of RBC count. The histogram here shows a sliding curve. **Insufficient lysis of RBCs** due to the lack of stromatolyser solution resulted in the entry of RBCs into the WBC counting block, causing a spuriously high count. The **differential diagnoses** for spurious elevations of the WBC count are **platelet clumps**, **nucleated RBCs**, **incomplete lysis of RBCs**, **cryoglobulins**, and **cryofibrinogen**

Clinical Utility of Blood Cell Histogram Interpretation Case-4

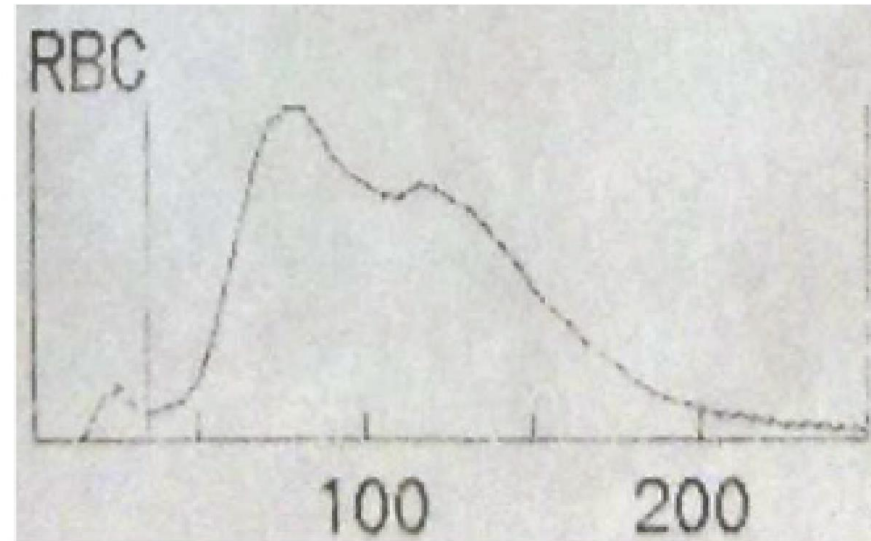
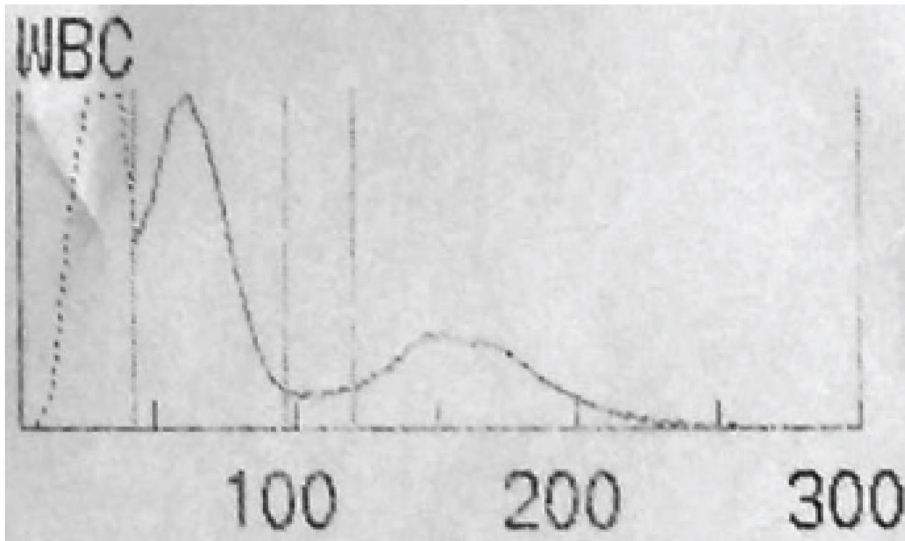
- A 63-year-old male came to Outpatient Department (OPD) with **tiredness** and intermittent **fever**. His Hb =6.6 g/dl, WBC =41,500, and PLT=20,000



- The patient in case 4 has very high WBC count, all seen in the area of **blasts** (Red arrow). This is suggestive of **acute leukemia**

Clinical Utility of Blood Cell Histogram Interpretation Case-5

- A 17-year-old female presented with **tiredness**. Hb =7.8 g/dl and WBC =24,200



- In the WBC histogram, the dotted curve before the lymphocyte peak indicates **nRBC**. nRBCs will be counted as WBC in the cell counter. Looking into the RBC distribution curve, there are two peaks. The second peak in the RBC distribution (with higher MCV) curve indicates **reticulocytes**. Presence of significant number of **nRBCs** and **reticulocytes** are highly suggestive of **hemolytic anemia**

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