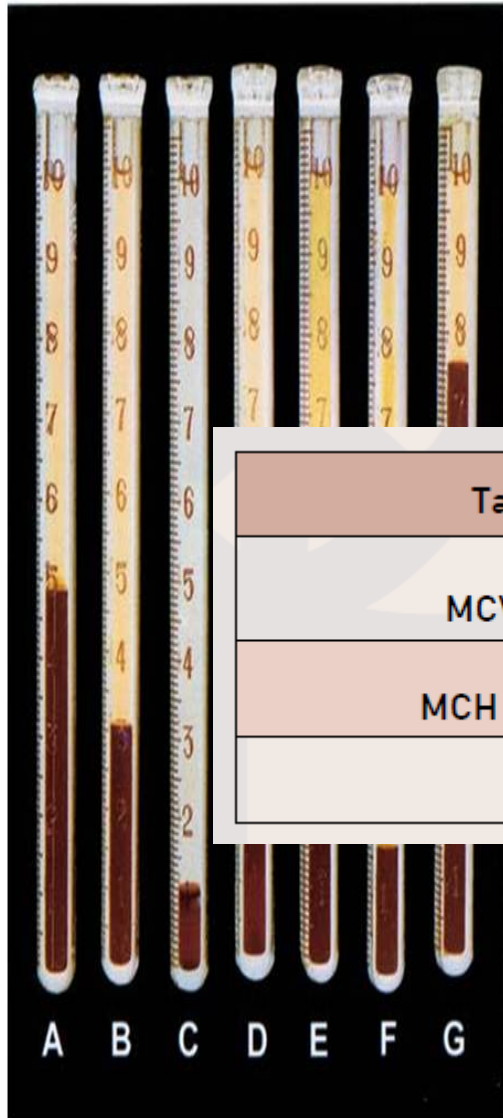


Tam Kan Sayımı

Dr. Mesude Falay
Ankara Numune Eğitim ve Araştırma Hastanesi
Hematoloji Laboratuvarı



Plazma

Tablo 1). Wintrobe indekslerinin hesaplanması

$$\text{MCV (fL)} = \text{HCT (\%)} \times 10 / \text{Eritrosit sayısı (Milyon/}\mu\text{L)}$$

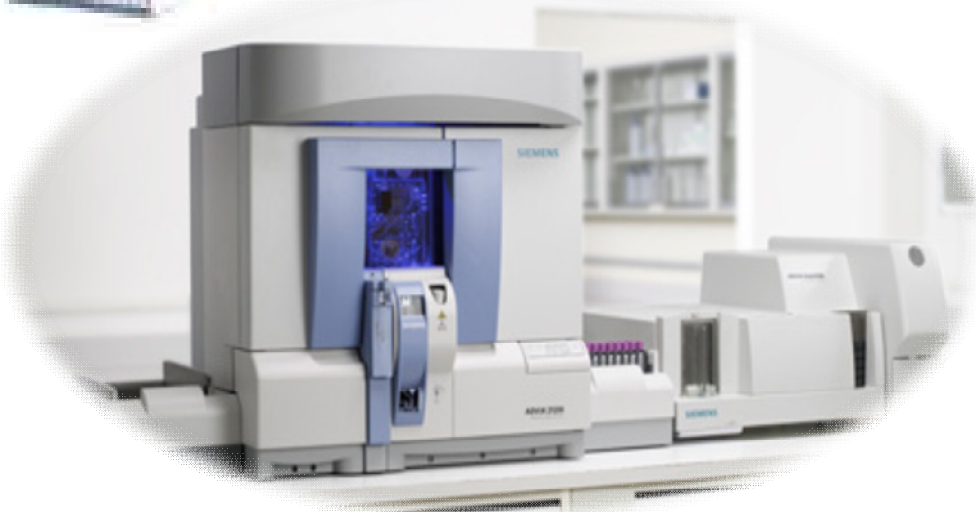
$$\text{MCH (pg)} = \text{HGB (g/dL)} \times 10 / \text{Eritrosit sayısı (Milyon/}\mu\text{L)}$$

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



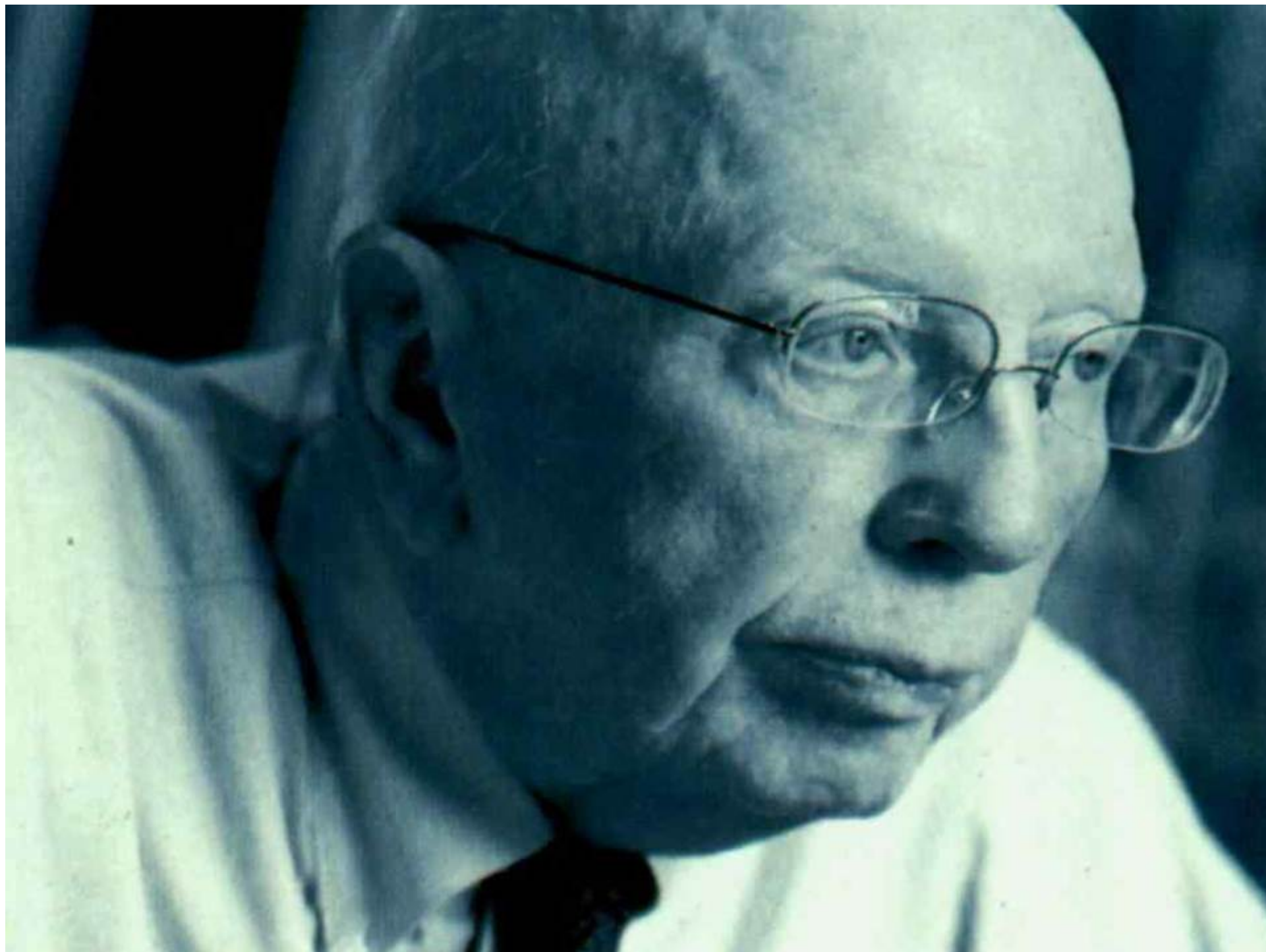
Eritrosit
sütunu
(PCV)





Tam Kan Sayımı cihazlarında kullanılan yöntemler

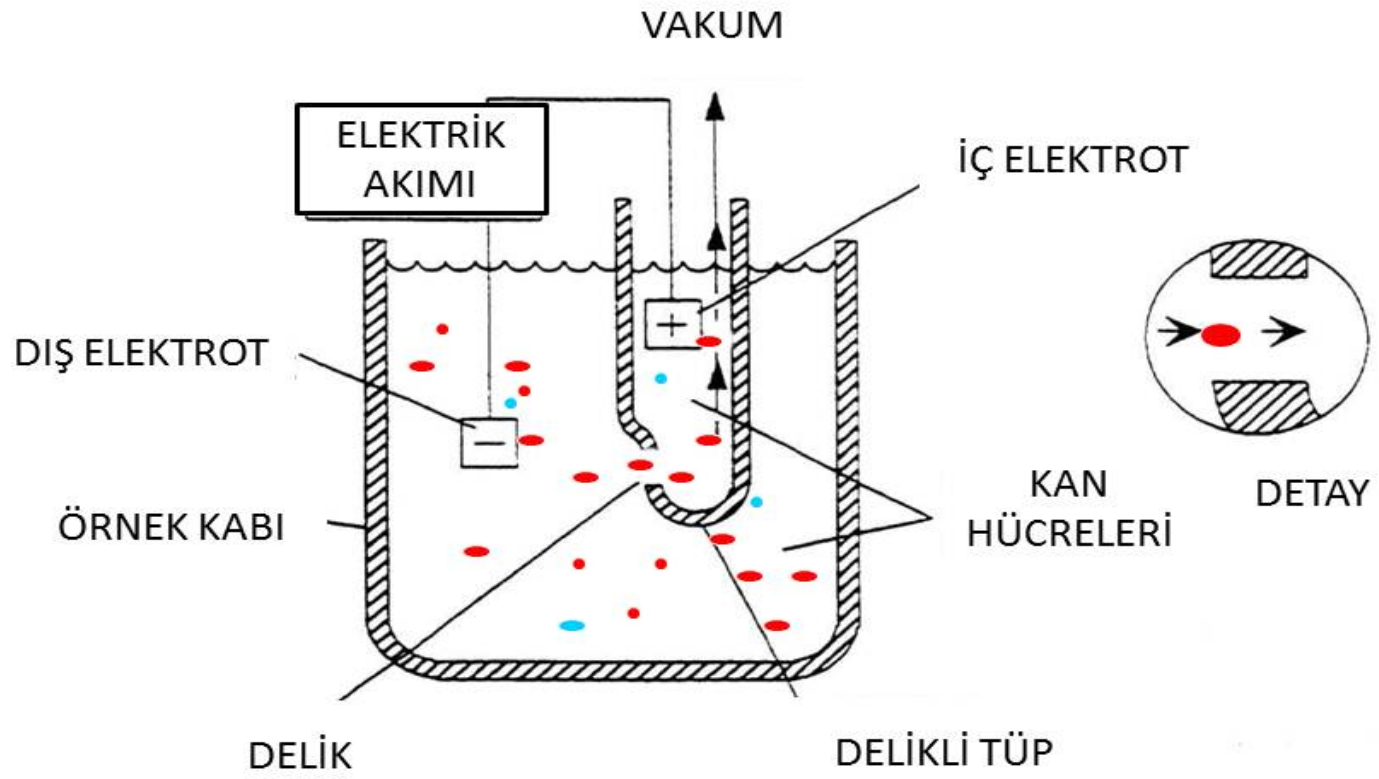
- 1. İmpedans yöntemi



Tam Kan Sayımı cihazlarında kullanılan yöntemler

- 1. İmpedans yöntemi

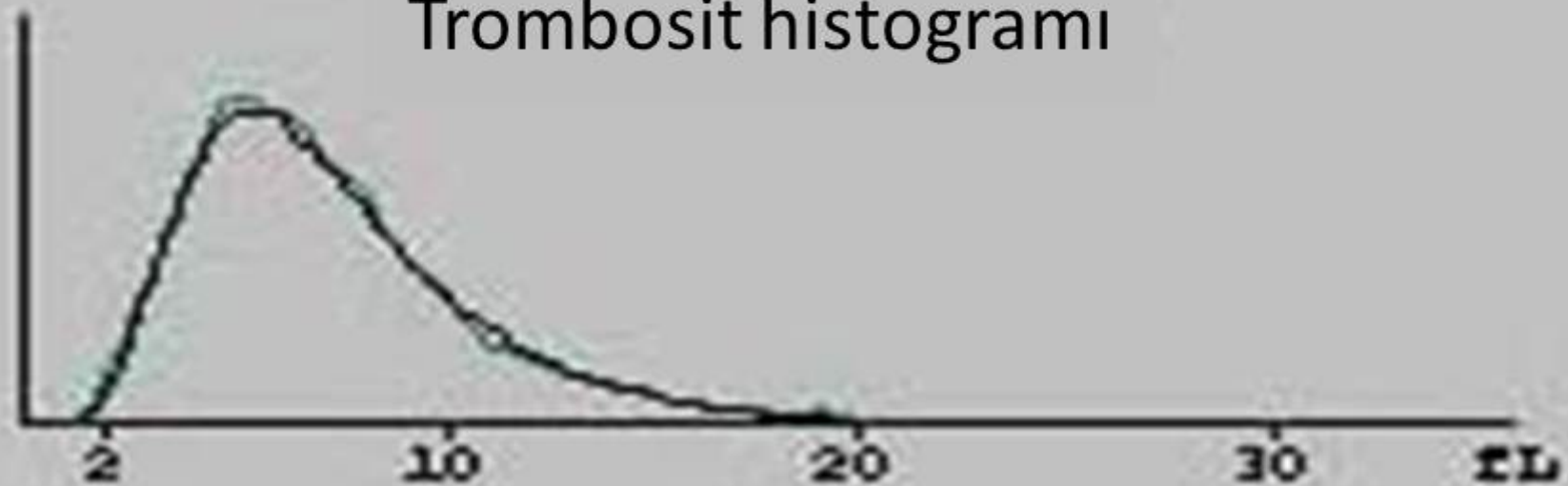
Kan hücreleri ve kanın seyreltildiği çözeltinin iletkenlik özellikleri kullanılmaktadır.



Eritrosit histogramı

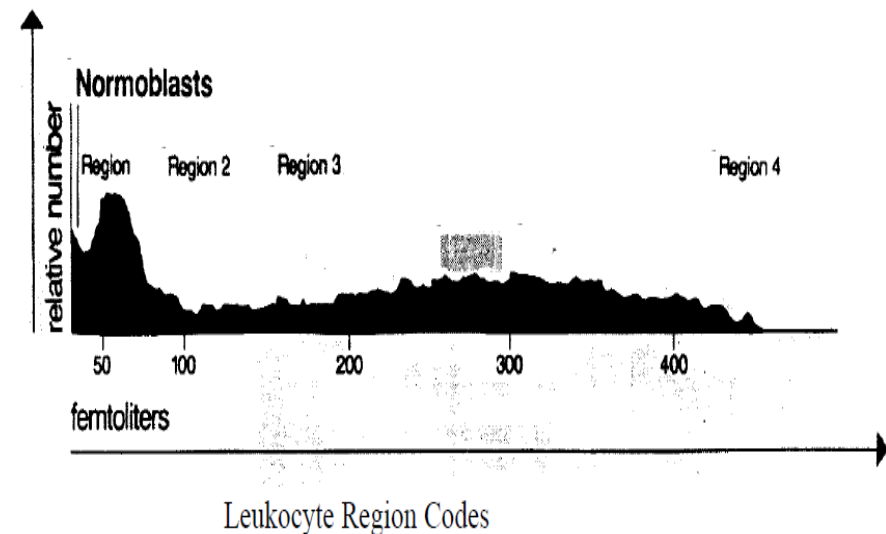
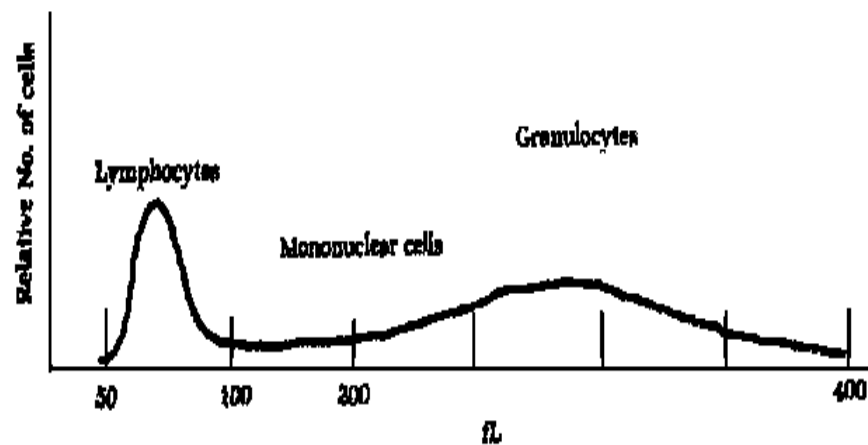


Trombosit histogramı



- İmpedansla lökosit sayımı , eritrositlerin noniyonik deterjanla parçalandığı ayrı bir bölmede yapılmaktadır.

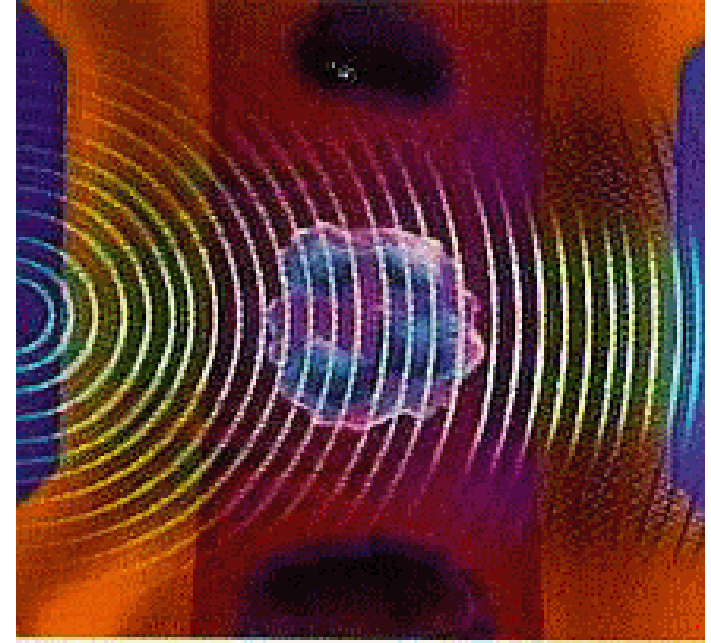
Cell type	Size range	Cells that fall within this size range
Lymphocytes	35-90 fL	lymphs and atypical lymphs
Mononuclear	90-160 fL	Monos, promyelocytes, myelocytes, plasma cells and blasts
Granulocyte	160-450 fL	segs, bands, metas, eos and basos



Tam Kan Sayımı cihazlarında kullanılan yöntemler

- 1. İmpedans yöntemi
- 2. Radyo Dalgaları (VCS)

İmpedans ile lökosit sayımı yapılırken aynı anda da radyo dalgaları ile lökositlerin çekirdekleri, yoğunlukları ve sitoplazma granülleri hakkında bilgi elde edilmektedir.



Tam Kan Sayımı cihazlarında kullanılan yöntemler

- 1. İmpedans yöntemi
- 2. Radyo Dalgaları
- 3. Optik Laser Scatter (Optik Yöntem)

Eritrosit, lökosit , trombositler sayılır

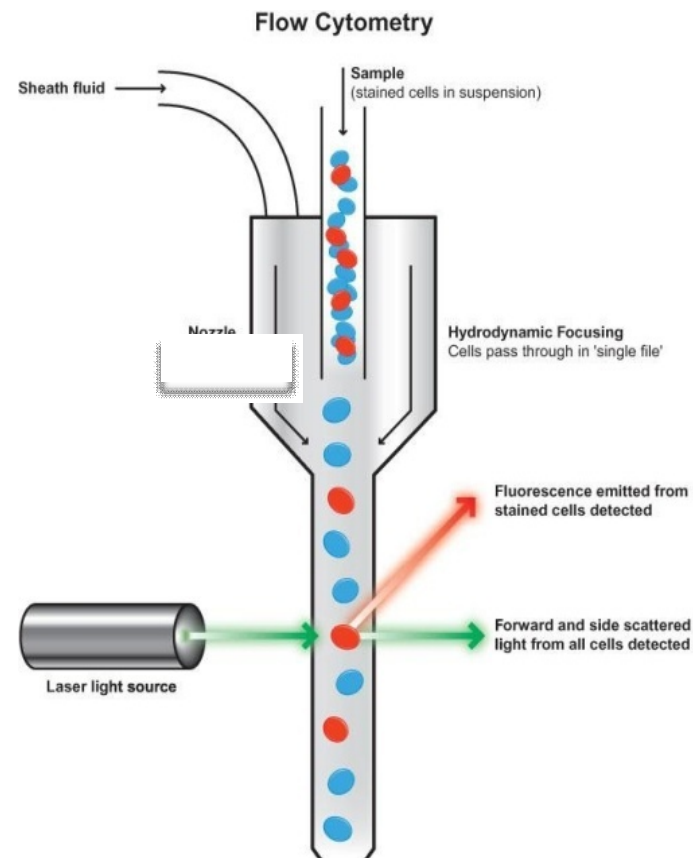
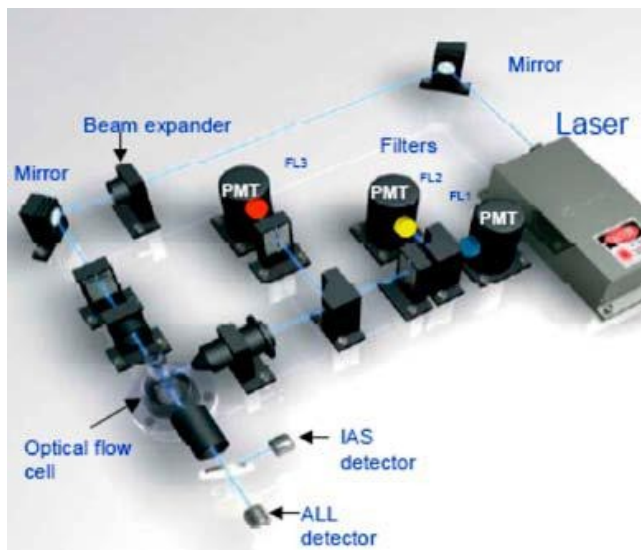
İmpedansla yada tek başına kullanılabilir

Kan hücreleri flow-cell de lazer kaynağının önünden geçerken lazer ışığının saçılmasına neden olurlar.

Lökositler floresan boyalarla boyanarak formül de yapılabilir.

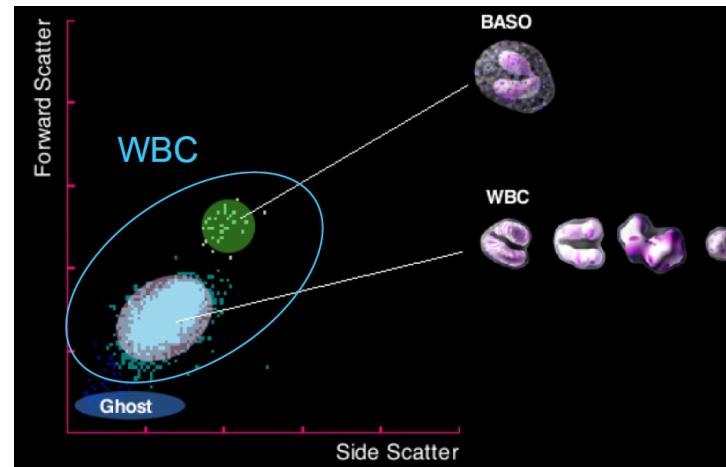
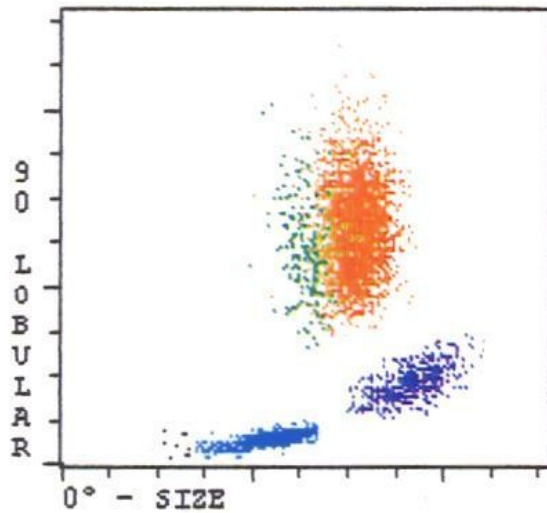
Optical light scatter and Fluorescence

- WBC ,WBC diff, PLT , RBC .
- Laser light (farklı dalga boylarında) :flow cell' den gecerken hücreler büyüklük ve granülaritesine göre seçilir
- Floresan boyalarda kullanılabilir(e.g CD61, CD4, CD8, CD34)



Tam kan sayımı:

- Optical light scatter and Fluorescence
 - forward scatter (FSC) : büyüklük
 - side scatter (SCC): granülarite



Tam Kan Sayım Sistemleri ve kullandıkları yöntemler (5 diff)

Cihazlar

Instrument and manufacturer

Technology used for differential count

Beckman-Coulter Instrumentation

VCS Technology (Volume, Conductivity, and Scatter)

(Coulter STKS, GEN-S, LH 700 series)

1. Impedance with low-frequency electromagnetic current
2. Impedance with high-frequency electromagnetic current
3. Laser light scattering

Sysmex Instrumentation
(Roche Diagnostics Corporation)

1. Impedance with low-frequency direct current
2. Impedance with radiofrequency current
3. Hydrodynamic Focusing

(SE series, XE2100)

Cell Dyne Technology
(Abbott Diagnostics Instrumentation)
Cell-Dyn 1800
Cell-Dyn 3500, 3700

The Coulter Principle

Multiple-Angle Polarized Scatter Separation (MAPSS)

1. Four light-scattering parameters: forward light scatter, orthogonal light scatter, narrow-angle light scatter, and depolarized orthogonal light scatter
2. Hydrodynamic Focusing
3. Use of Flow Cells

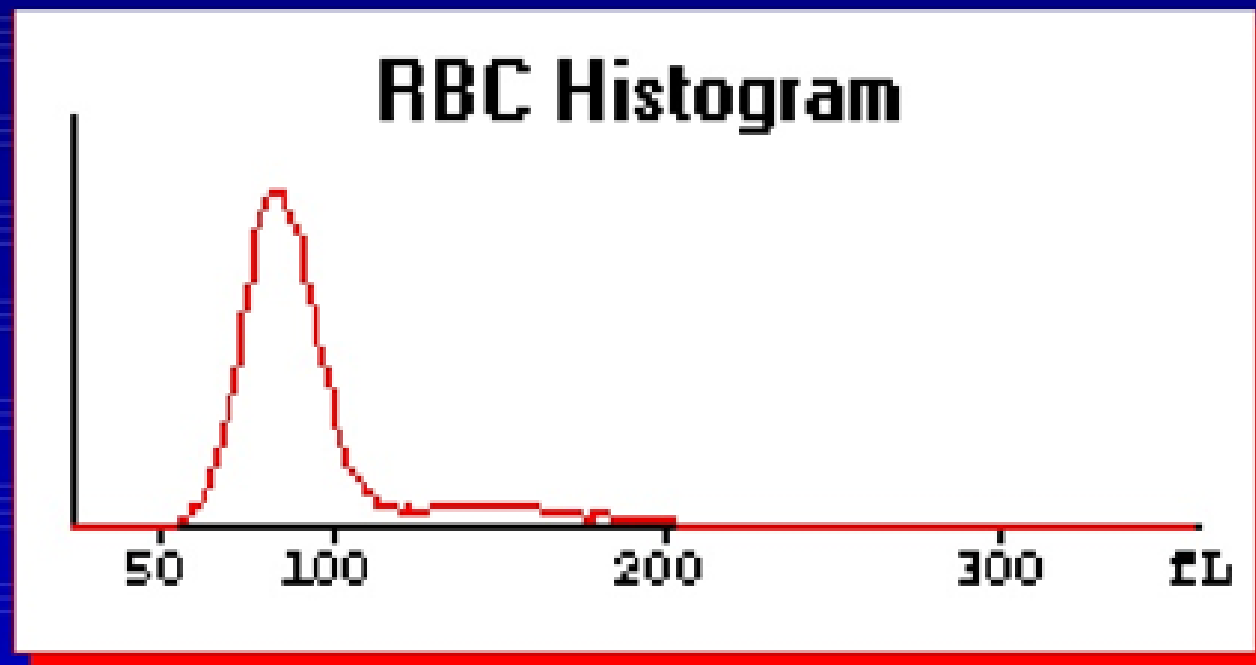
Cell-Dyn Ruby, Sapphire.

1. Multiple-Angle Polarized Scatter Separation (MAPSS)
2. Hydrodynamic Focusing
3. Use of Flow Cells
4. Fluorescence

Eritrosit sayımı ve eritrosit indeksleri

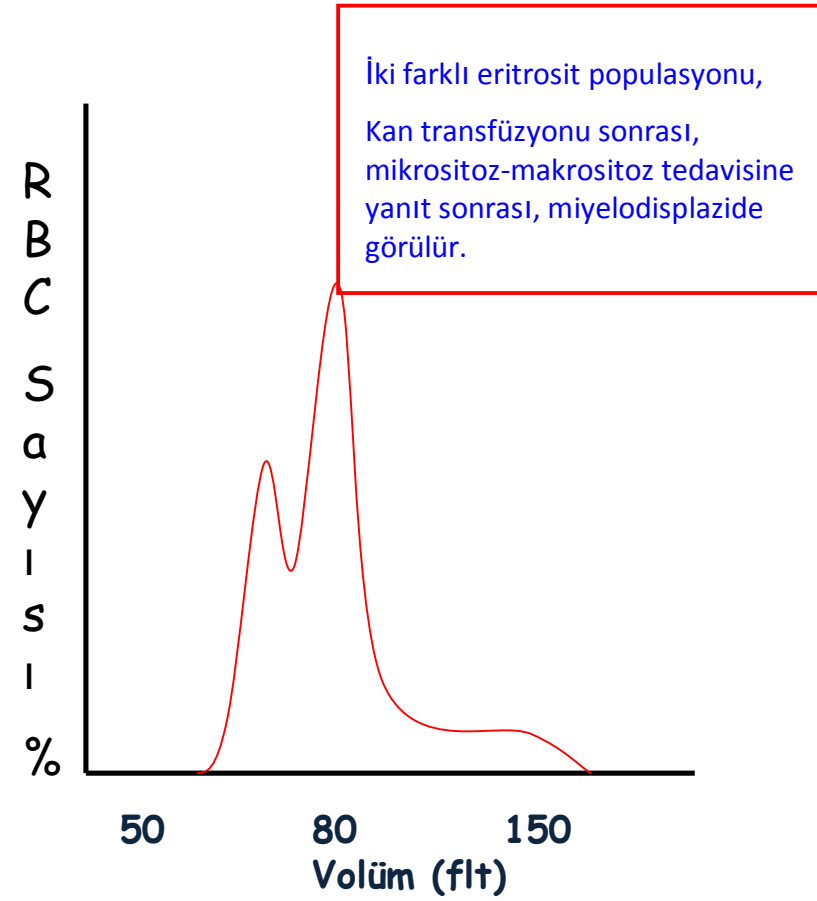
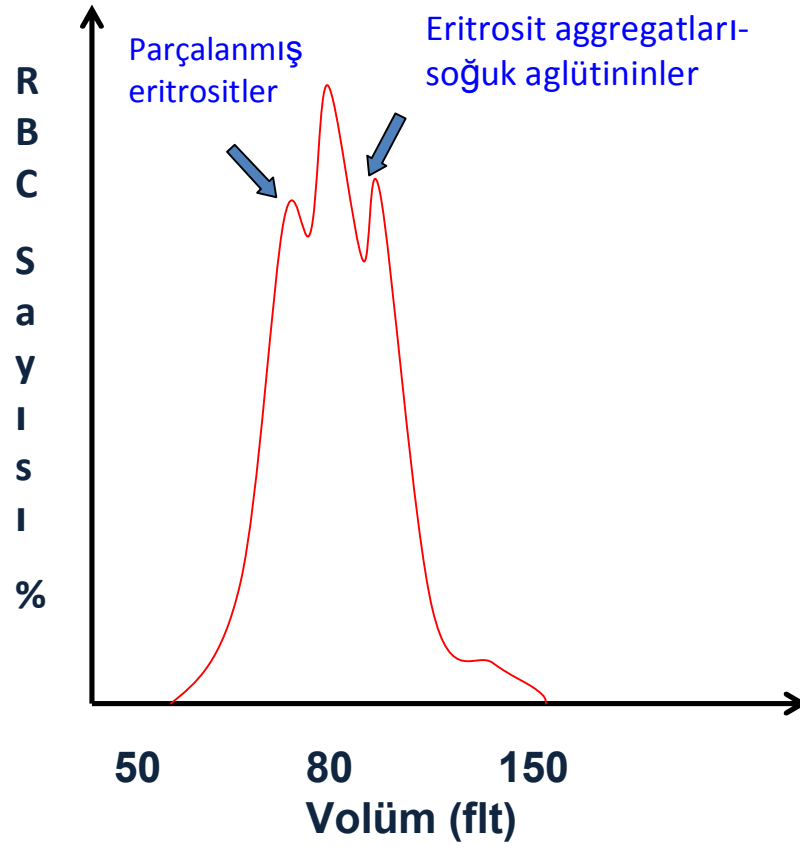
- Eritrosit histogramlarından yararlı bilgiler edinilir
- Şistositlerin ve mikrositlerin varlığında trombositler ve eritrosit histogramı arasında köprü oluşur

RBC HISTOGRAM



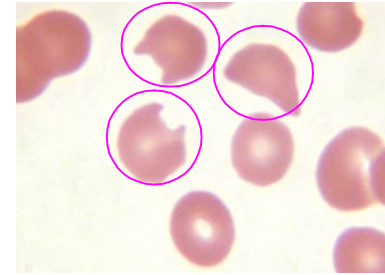
NORMAL

Eritrosit (RBC) Histogramları



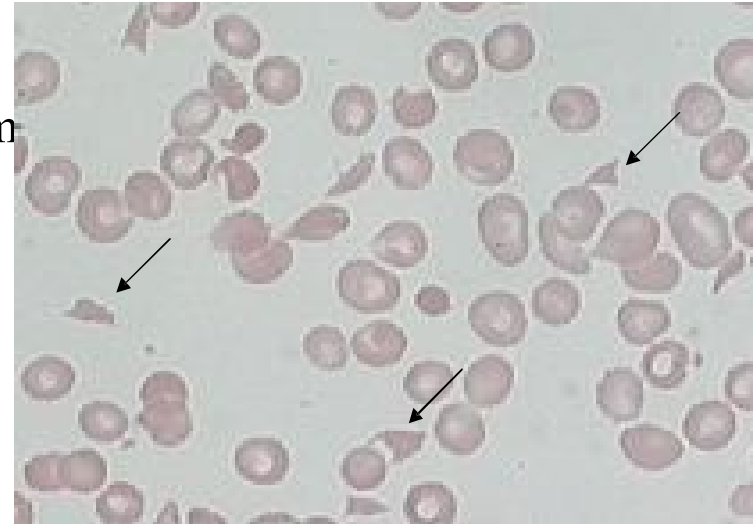
Şiztositler (RBC fragments)

- Şiztosit düzensiz şekilli eritrosit kırıntıları olarak tanımlanır.



- **Hasta grupları:**

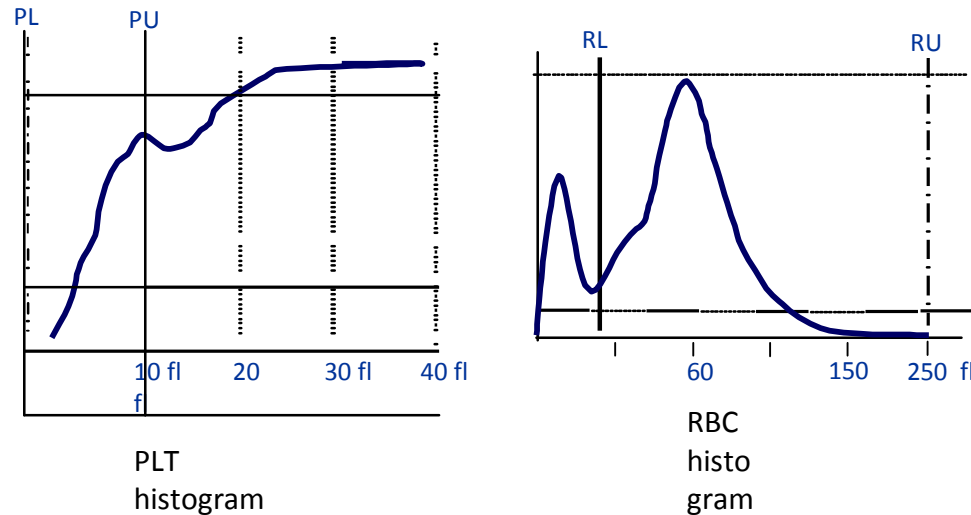
- Mikroanjiopatik hemolitik anem
- Ciddi yanıklar
- Üremi hastaları
- Prostetik kapak



- % 0,1 – 0,6

Şiztositler

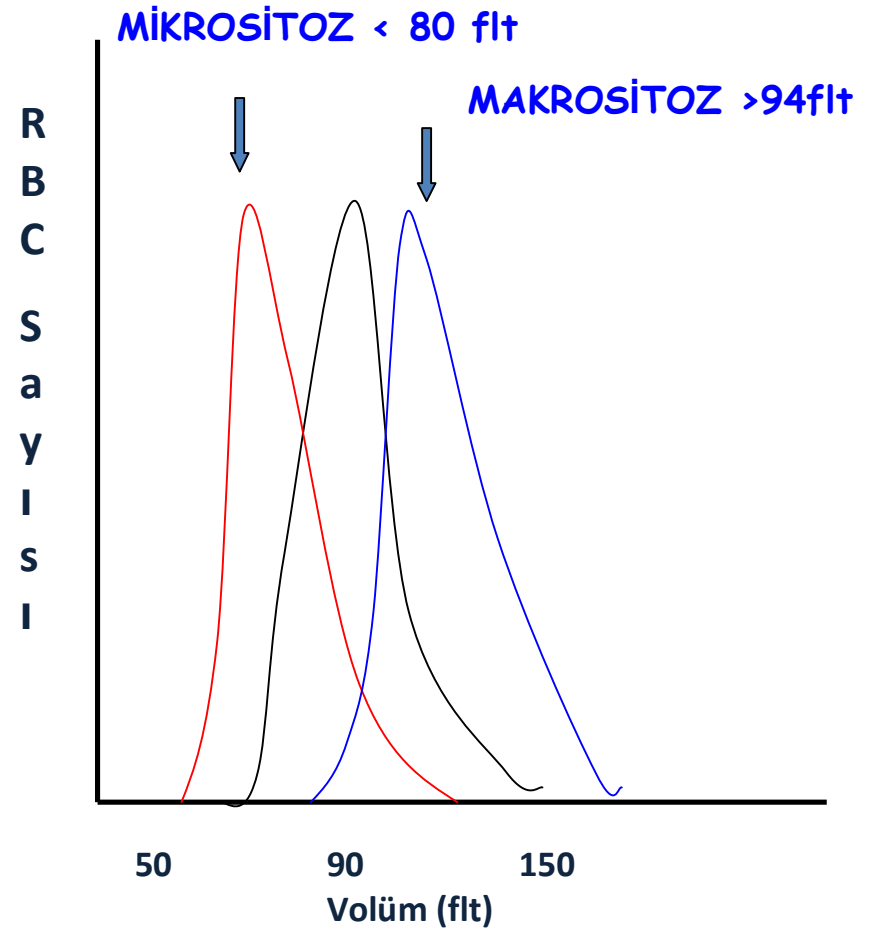
- Şiztositler $\square < 30$ fL, Düşük RNA içeriği
 - PLTler ile aynı büyüklükte olabilir
 - PLT sonuçları hatalı yüksek olabilir.
 - Anormal RBC histogram ve anormal PLT histogram



Ortalama eritrosit hacmi

Mean Corpuscular Volume (MCV)

- Direkt olarak ölçülen parametre,
- $MCV = Hct(\%) \times 10 \div RBC$ (milyon)
- Anemilerin morfolojik sınıflamasında önemli,
- Mikrositoz <80 fl
- Makrositoz >94 fl
- 80-94 Normositler
- Yüksek lenfosit sayısı,
- Hiperglisemi MCV ↑



Hemoglobin

- Siyanhemoglobin yöntemi ile fotometrik olarak ölçülür. (540-550nm)
- Bazı cihazlarda sodyum-lauril-sülfat yöntemi kullanılmaktadır,
- g/dl
- Abbott: Imidazole derivatives
- Beckman-Coulter: ethoxylated amine compounds and/or quaternary ammonium salts
- Siemens: N,N-dimethyl-laurylamine N-oxide
- Sysmex: Pyridinium salts and SLS

$$\text{HCT} = \text{MCV} * \text{Eritrosit sayısı} / 10$$



MCH (Mean Corpuscular Hemoglobin)

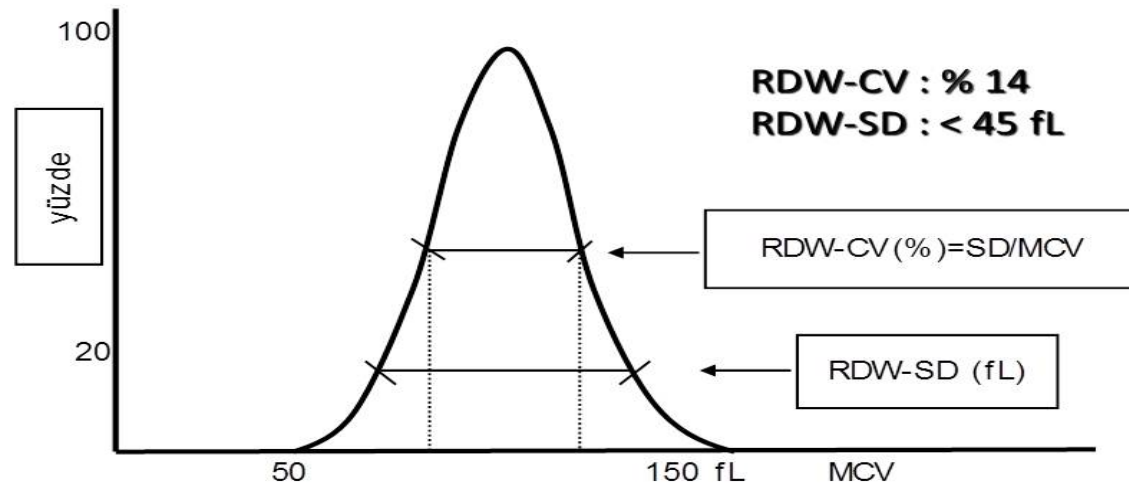
- Eritrosit içindeki ortalama HGB miktarını gösterir
- 30-34 pg
- Mikrositik anemilerde MCH düşük, Makrositik anemilerde MCH yüksek

MCHC (Mean Corpuscular HGB Concentration)

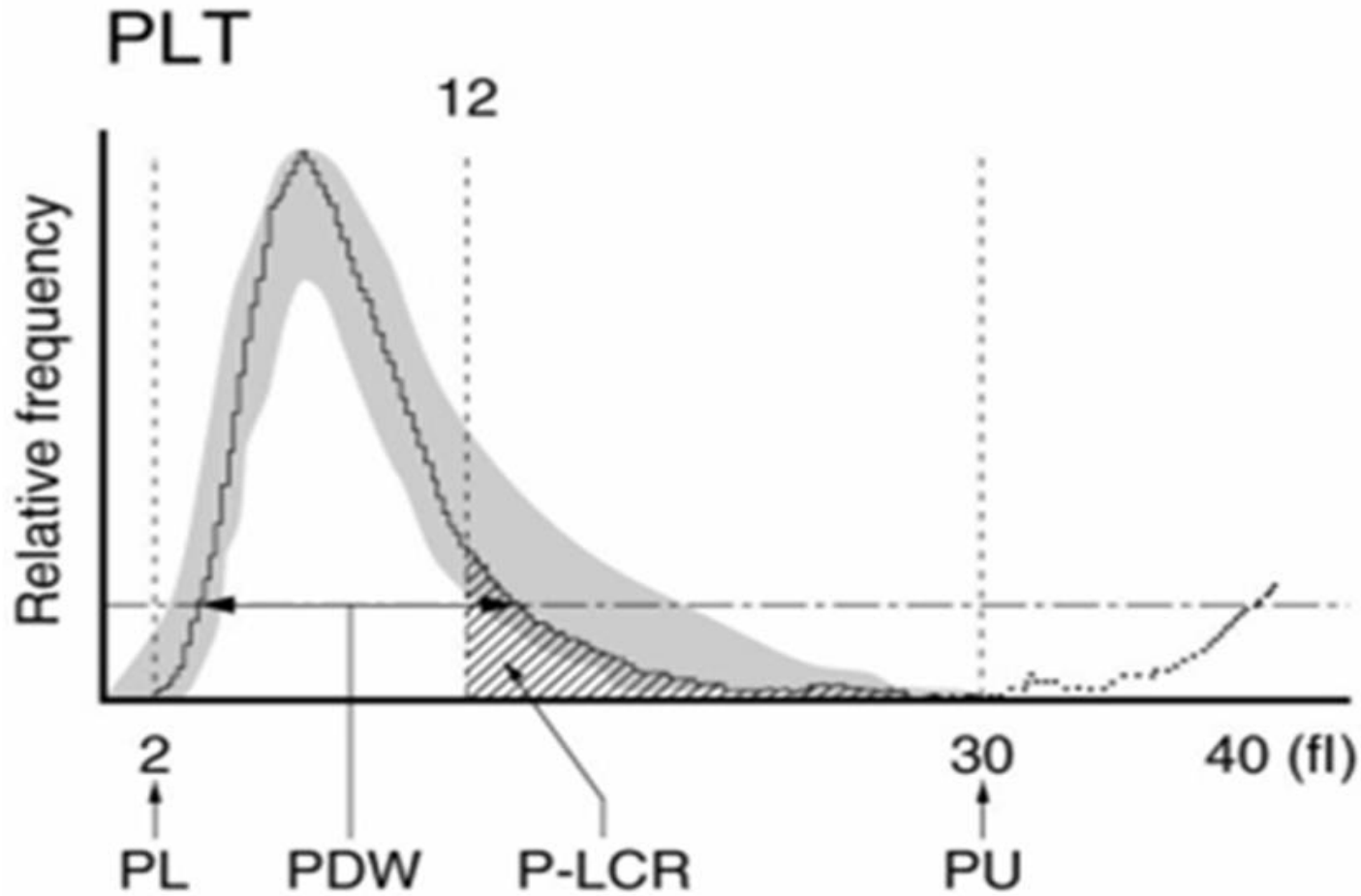
- Eritrositlerin HGB içeriğinin yüzde olarak ifadesidir
- %30-36 g/dl
- HGB, MCV ve RBC sayısından hesaplanır
- Kontrol parametresi olarak kullanılır!!!!

RDW (Red Cell Distribution Width)

- Eritrosit histogramlarından elde edilen istatikselsel bir deęerdir
Periferik kan yayması yapılması için ölçüt



Otomatik Kan Sayımı Cihazlarıyla Trombosit sayımı impedans/optik/immunolojik



- **Plt Histogram**

- Normal platelet histogram (2-20 fL).

- **(0-2)**

- Hava kabarcığı

- toz

- Electronic and Electrical noise

- **20 fL' nin üzeri**

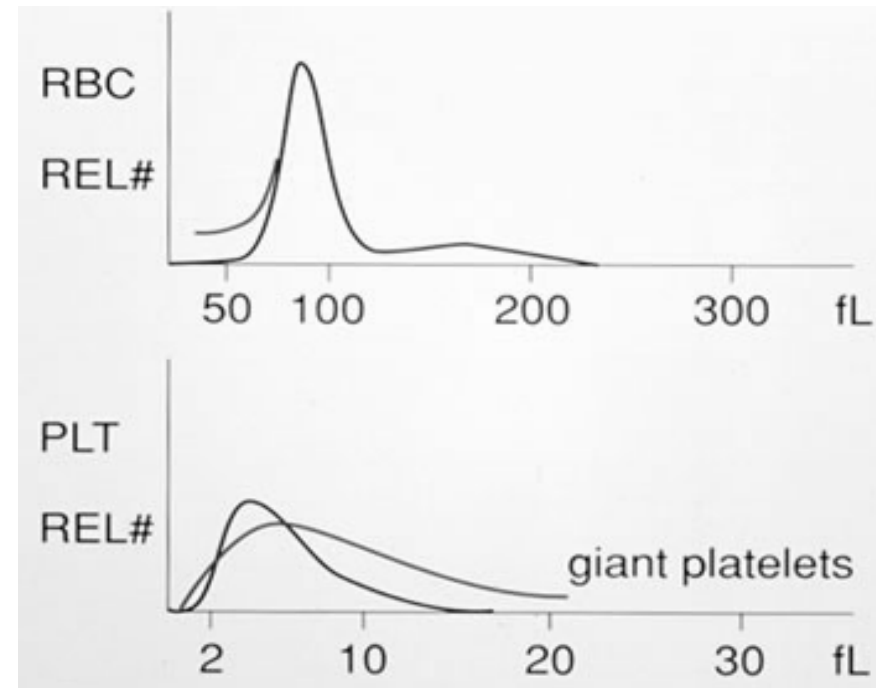
- Mikrosit

- şistosit

- WBC's fragments

- Dev plt

- p1ht1

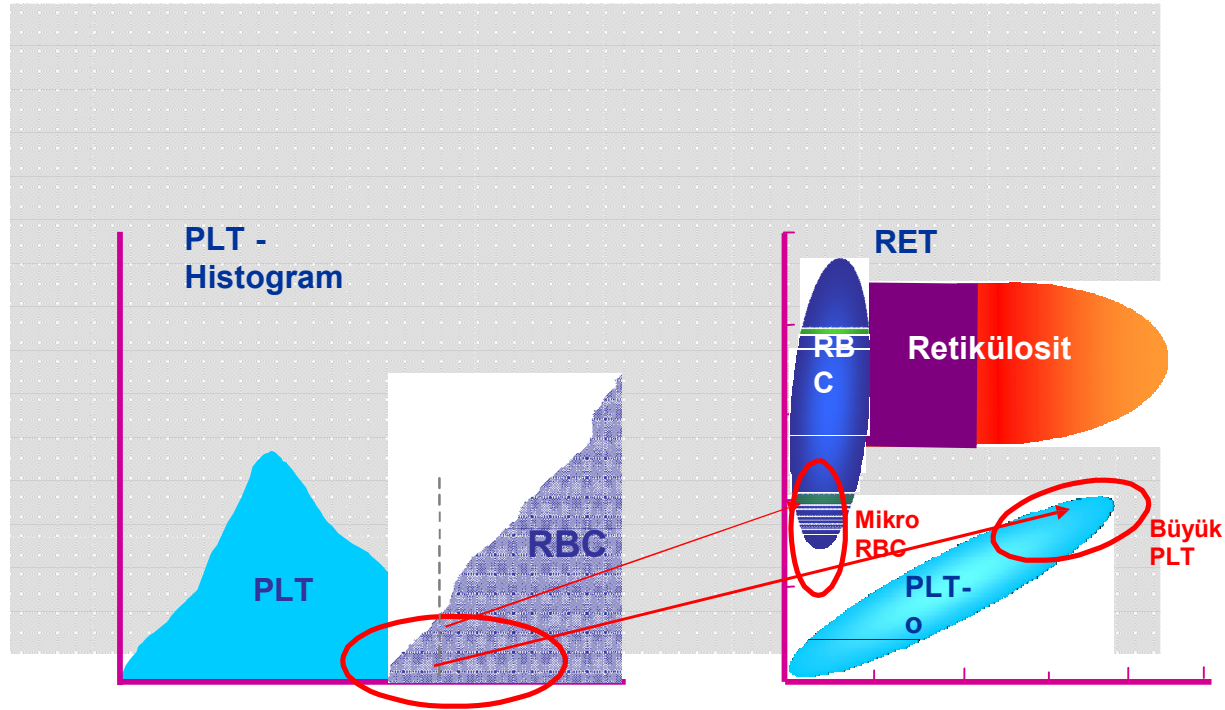


Trombosit sayımı

- **Optik yöntemle** floresan boyalar kullanılarak yapılmaktadır. Sysmex (XE-2100) kan sayımı cihazları RNA/DNA'yı floresan polimethin boyası ile retikülosit kanalında saymaktadır. Özellikle trombosit sayısının düşük ($< 50 \text{ bin}/\mu\text{L}$) ve parçalanmış eritrosit sayısının yüksek olduğu durumlarda optik yöntemle sayımın değeri artar.
- **İmmünolojik sayım**, trombositlerin yüzeyinde bulunan antijenlerin (CD41, CD61) floresan işaretli monoklonal antikörlerle boyanarak *flow cytometry* yöntemi ile sayılmasıdır. Özellikle, düşük trombosit sayılarında immünolojik yöntem en güvenilir olanıdır. İmpedansla trombosit sayısı $30 \text{ bin}/\mu\text{L}$ altında trombosit transfüzyonu gerekirken, immünolojik yöntemle bu sayı $10 \text{ bin}/\mu\text{L}$ 'dir. Bazı kan sayım cihazları immünolojik yöntemle trombosit sayımı yapabilmektedir.
- Eritrosit indekslerine benzer şekilde ortalama trombosit hacmi (*Mean Platelet Volume: MPV*), trombosit dağılımı genişliği (*Platelet Distribution Width, PDW*) ve büyük trombosit oranı (*Platelet Large Cell Ratio, P-LCR*) gibi çeşitli trombosit indeksleri de hesaplanmaktadır .
- **EDTA'lı kanda trombositlerin büyüklükleri kolayca değiştiğinden trombosit indekslerinin pek anlamı yoktur; standardize edilmediğinden de rutin olarak kullanılmamaktadır!!!!!!**

Optik Trombosit Ölçümü (PLT-O)

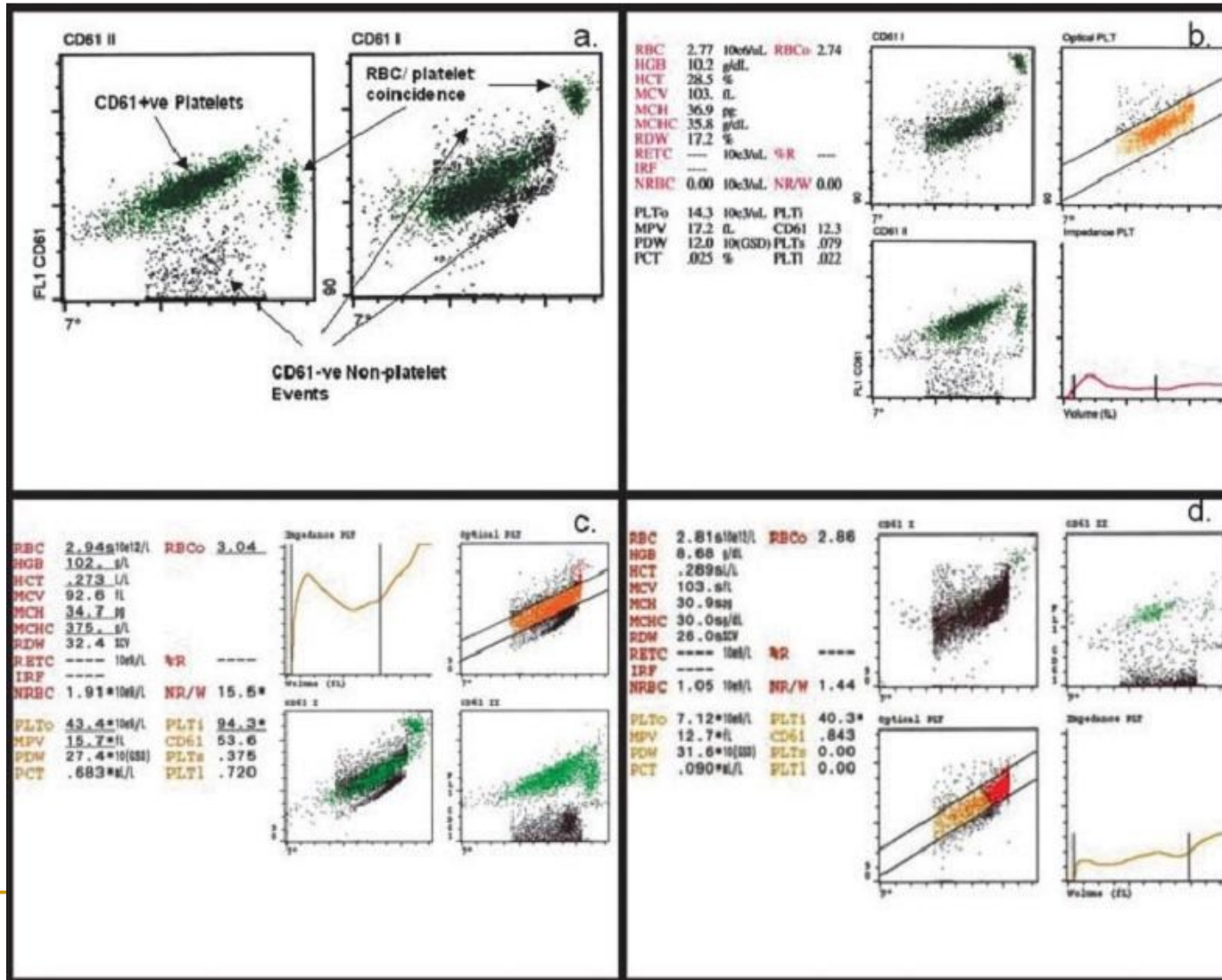
Bazı mikro eritrositlerin ve büyük trombositlerin hücre büyüklükleri aynı olabilir!

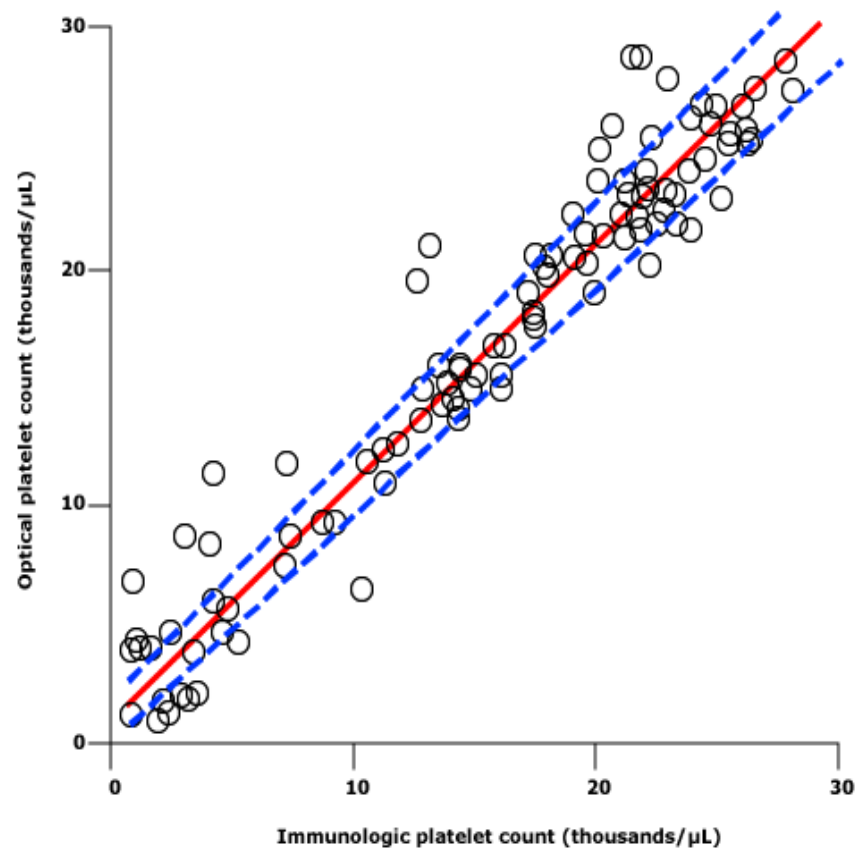


Empedans kanalında eşit
büyüklükteki hücreler
ayrıştırılmaz!
_____ (mikro eritrosit/büyük
trombosit)

RNA bilgisi aynı
büyüklükteki eritrosit ve
trombositleri RET
_____ kanalında
birbirinden **ayırır**

CD61 İmmün Trombosit Sayımı





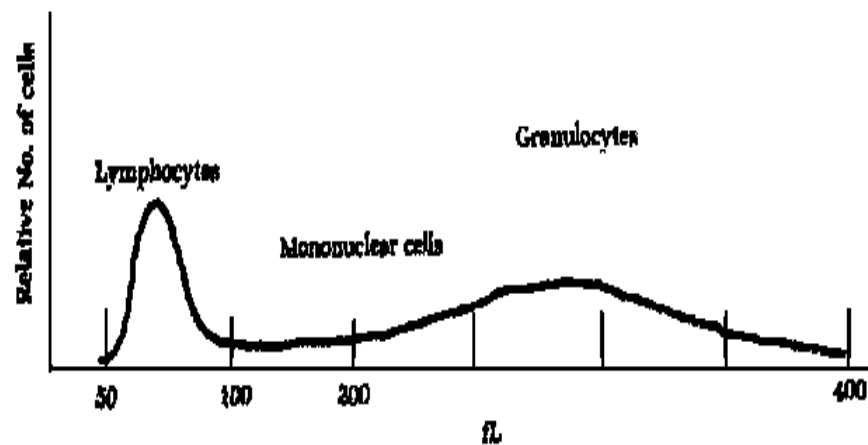
This graph depicts simultaneously-obtained automated platelet counts using an optical method (y-axis) and an immunologic method (anti-CD61, y-axis) for counts in the range from zero to 30,000 platelets/ μ L. The least squares best-fit line for the data points is shown as the red line, while the 95 percent confidence limits are shown by the two dashed blue lines. Note that the majority of the data points well outside of the 95 percent confidence limits are above the best-fit line, suggesting that the optical method is more prone to overestimate platelet counts than the immunologic method in this range.

Redrawn from: Kunz D1, Kunz WS, Scott CS, Gressner AM. Automated CD61 immunoplatelet analysis of thrombocytopenic samples. Br J Haematol 2001; 112:584.

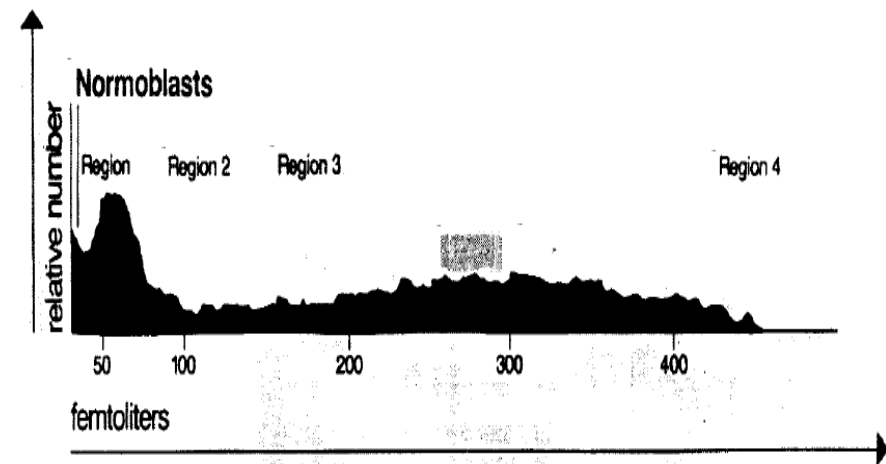
Formül Lökosit

- İmpedansla lökosit sayımı sırasında lökositler büyüklüklerine göre üç gruba ayrılabilir

Cell type	Size range	Cells that fall within this size range
Lymphocytes	35-90 fL	lymphs and atypical lymphs
Mononuclear	90-160 fL	Monos, promyelocytes, myelocytes, plasma cells and blasts
Granulocyte	160-450 fL	segs, bands, metas, eos and basos

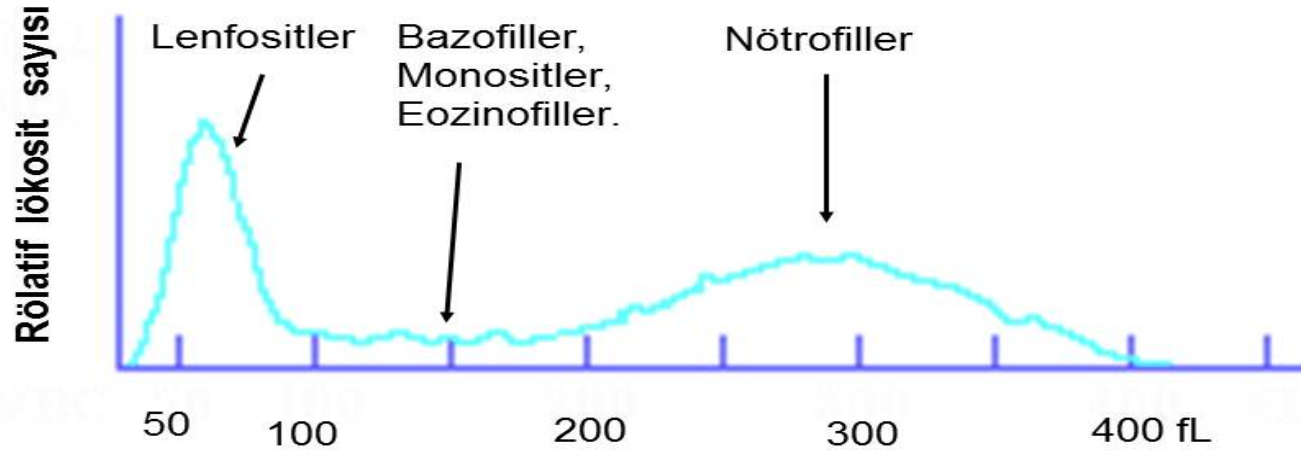


Leukocytes (Coulter)

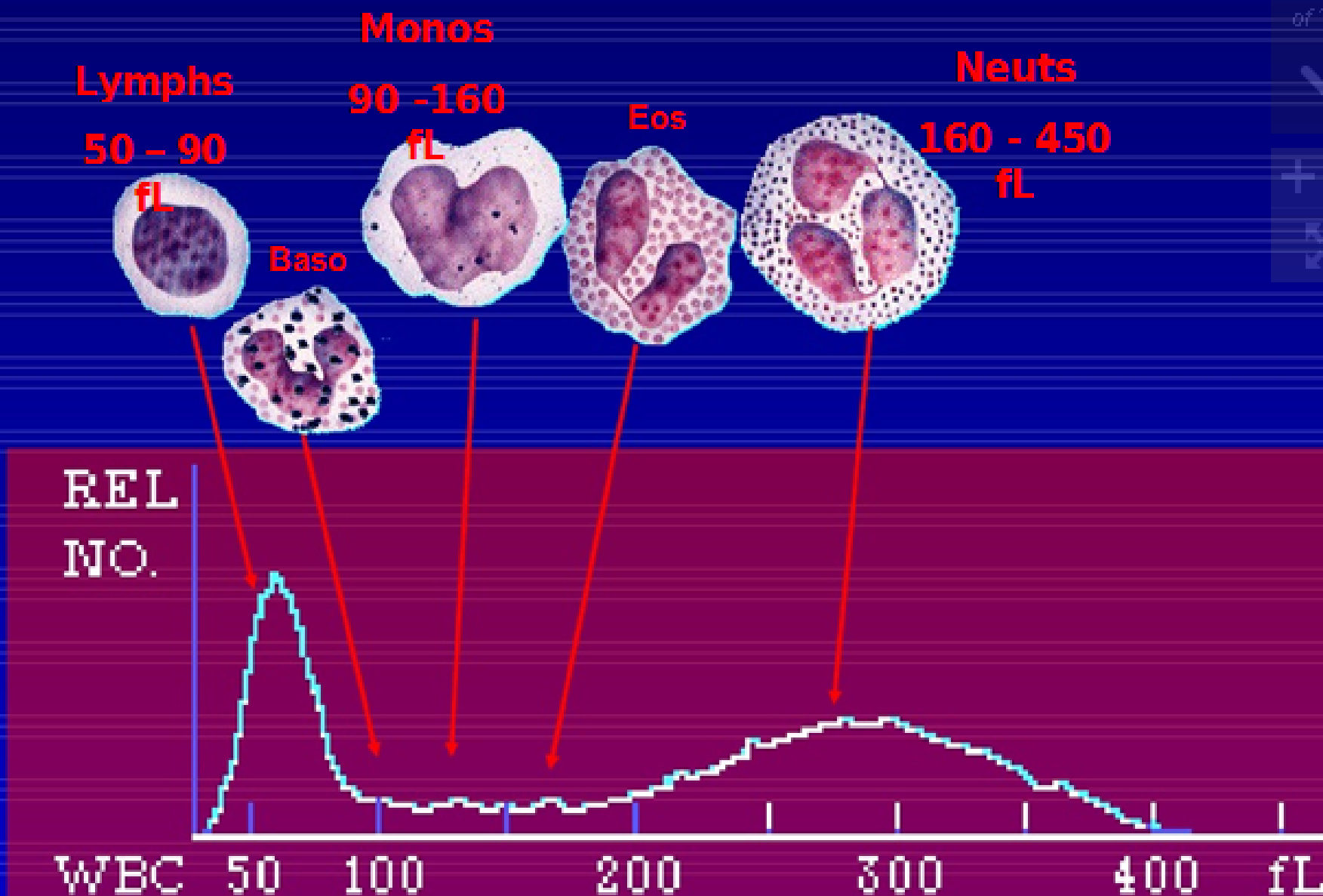


Leukocyte Region Codes

KAN SAYIMI CİHAZLARIYLA LÖKOSİT FORMÜLÜ

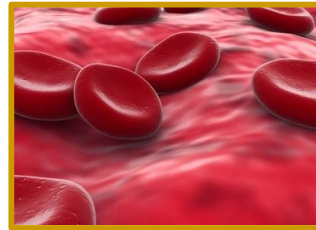


Coulter WBC Histogram

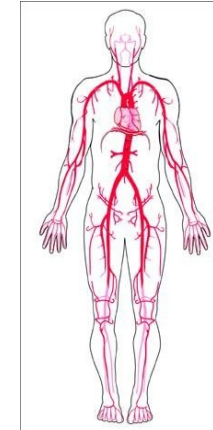


Retikülosit İndeksleri

- Eritrositler uzun ömürlü oldukları için, eritrosit parametreleri zaman bakımından sadece **averaj** bir değer verir.
- Dolayısıyla bu parametreler (MCV, MCH) demir eksikliğini **daha geç gösterir**.
- Retikülositler ise kısa ömürlü olduklarından, kemik iliği aktiviteleri için çok **daha hızlı ve hassas** bir kaynaktır.



120 gün

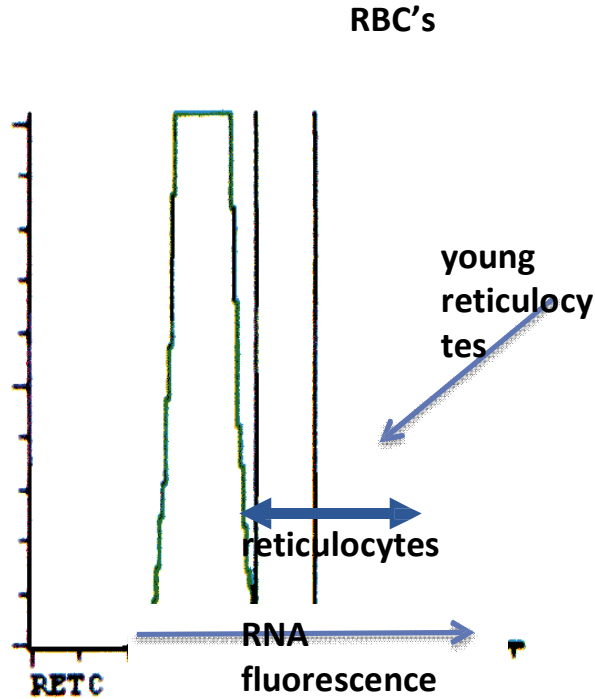


1-2 gün

Retikülosit Sayımı



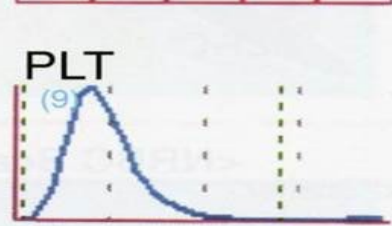
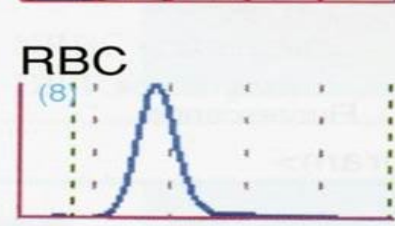
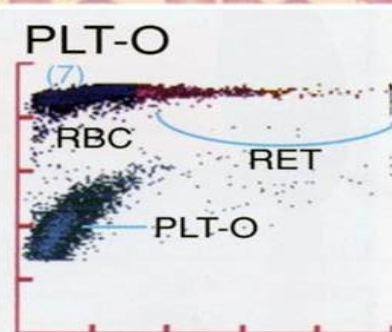
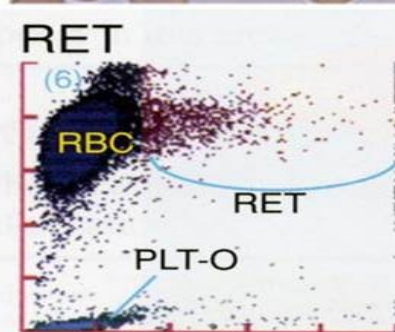
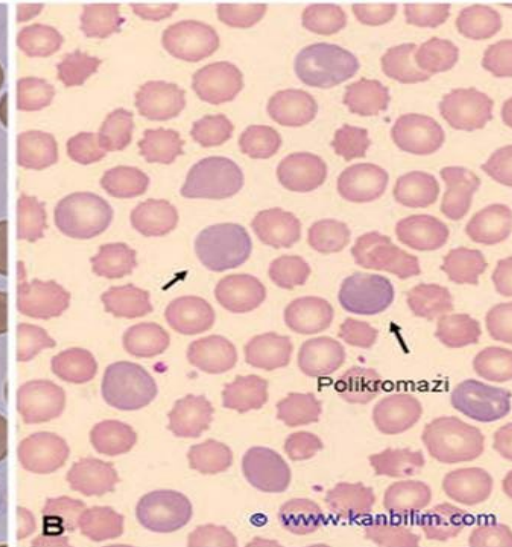
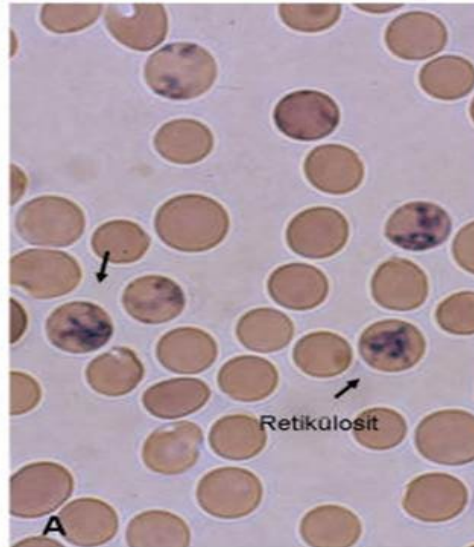
- Olgunlaşmasını tamamlamamış eritrositlerdir.Çekirdeklerini kaybettikten bir süre sonra daha RNA artığı bulunmaktadır.
- RNA **krezil mavisi/yeni metilen mavisi** ile boyanır
- Kİ' nin hematopoetik aktivitesi hakkında bilgi verir



Hemolytic Anemia $\uparrow$
Megaloblastic / Fe eksikliği N/ $\downarrow$
(Prediction of) Recovery after
Hemopoetic Progenitor Cell
Transplantation
Monitoring of Effectiveness of
recombinant EPO

RETİKÜLOSİT SAYIMI

- Retikülosit yüzdesi eritrosit sayısının azaldığı durumlarda yanıltıcı olabileceğinden “*mutlak retikülosit sayısı*” ve “*düzeltilmiş retikülosit sayısı*” hesaplanmalıdır
- **Düzeltilmiş retikülosit yüzdesi = Hastanın retikülosit yüzdesi x Hasta HCT ÷ 45**
- Otomatik retikülosit sayımı 1990 yılında gerçekleşmiş ve kan sayımı cihazları genç retikülositlerin “**Immature Reticulocyte Fraction, (IRF)**” sayımına da olanak sağlamıştır.
- Genç retikülositlerde yaşlılara göre daha çok RNA bulunmaktadır. Artışı kemik iliğinin eritrosit yapımına başladığının ilk göstergesidir.
- IRF, eritropoetin tedavisinde ve kemik iliği naklinde takip parametresi olarak kullanılmaktadır.
- Retikülosit sayımında kullanılan floresan boya aynı zamanda trombositleri de boyadığından bu kanalda optik olarak trombosit sayısını da belirlemek mümkündür



Yeni Parametreler

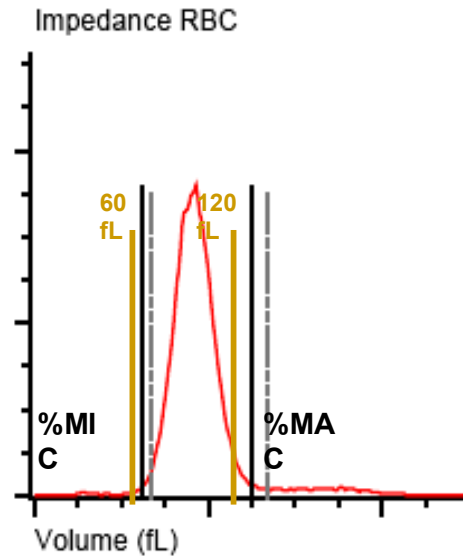
- Microcytic RBC - Macrocytic RBC
- Hypochromic RBC - Hyperchromic RBC
- Hemoglobin Konsantrasyonu
- Dağılım Genişliği (HDW)
- Olgunlaşmamış Retikülosit (IRF)
- Retikülosit Hemoglobin Eşdeğeri (Ret He)
- Ortalama Retikülosit Volumü (MCV_r)
- Retikülosit Hemoglobin İçeriği (CH_r)
- NRBC (eritroblastlar)
- Olgunlaşmamış Granülositler (IG)
- Monoklonal ab ile lökosit sayımı
- Hematopoetic Progenitör Hücre
- Olgunlaşmamış Trombositler (IPF)
- Çekirdekli Trombositler (Reticulated Platelet)
- Optik Trombosit Ölçümü (PLT-O)
- CD61 immün trombosit sayımı

Yeni RBC parametreleri

% Micro ve % Macro

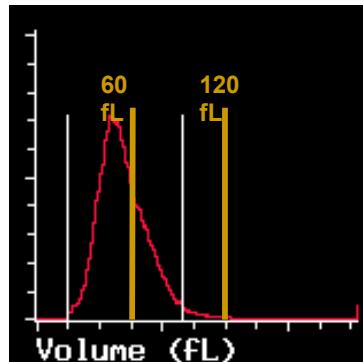
- CBC modunda çalışılır
- < 60 fL : % Microcytic RBCs
- > 120 fL : % Macrocytic RBCs

RBC	5.35	10e6/uL	RBCo	5.38
HGB	16.3	g/dL	%MIC	1.78
HCT	43.9	%	%MAC	.803
MCV	82.0	fL	%HPO	12.3
MCH	30.5	pg	%HPR	.049
MCHC	37.2	g/dL		
RDW	12.1	%		
HDW	10.8	%		
RETC	39.6	10e3/uL	%R	.741
IRF	.229			
NRBC	0.00	10e3/uL	NR/W	0.00
MCVr	85.7	fL		
MCHr	25.0	pg		
CHCr	28.9	g/dL		

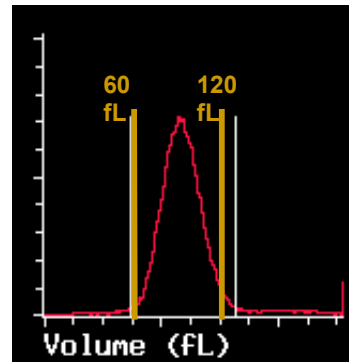
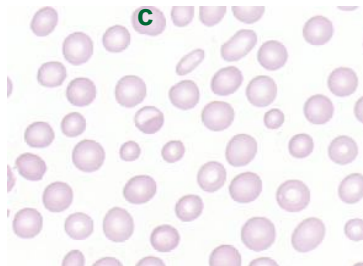


Microcytic, normocytic, macrocytic RBC

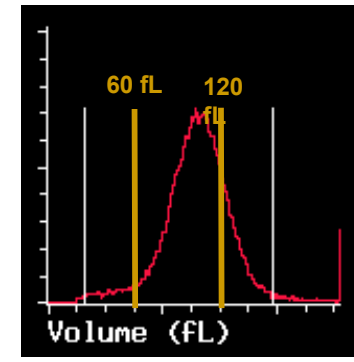
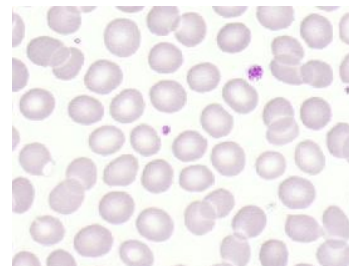
- Histogramlar



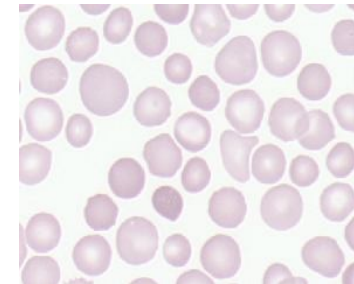
Microcyti



Normocyti



Macrocyti



Yeni RBC parametreleri

CBC + RETC modu

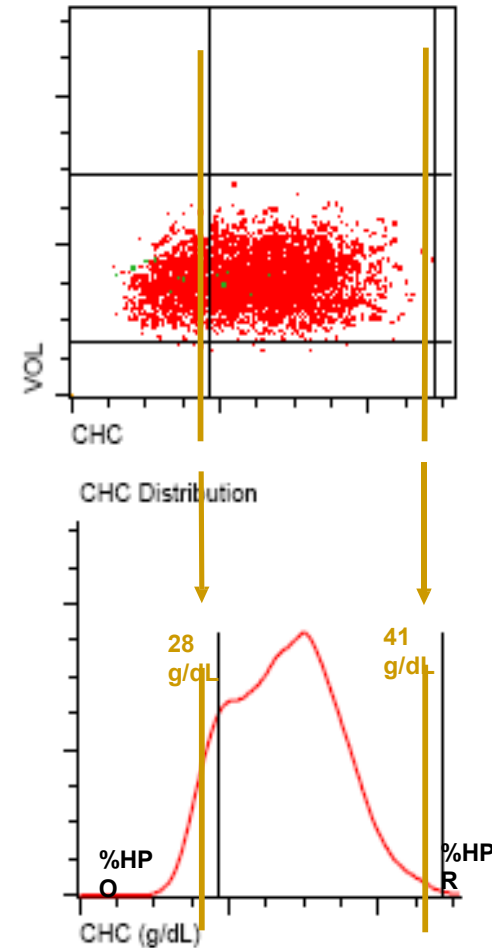
- %HPO hypochromic RBC (%)
- %HPR hyperchromic RBC (%)
- HDW Hgb concentration distribution width
- MCVr mean reticulocyte volume
- MCHr mean reticulocyte Hb content
- M-CHCr mean reticulocyte Hb concentration

RBC	5.35	10e6/uL	RBCo	5.38
HGB	16.3	g/dL	%MIC	1.78
HCT	43.9	%	%MAC	803
MCV	82.0	fL	%HPO	12.3
MCH	30.5	pg	%HPR	.049
MCHC	37.2	g/dL		
RDW	12.1	%		
HDW	10.8	%		
RETC	39.6	10e3/uL	%R	.741
IRF	.229			
NRBC	0.00	10e3/uL	NR/W	0.00
MCVr	85.7	fL		
MCHr	25.0	pg		
CHCr	28.9	g/dL		

Hipokromik ve Hiperkromik RBC

- < 28 g/dL : % Hypochromic RBCs
- > 41 g/dL : % Hyperchromic RBCs

RBC	5.35	10e6/uL	RBCo	5.38
HGB	16.3	g/dL	%MIC	1.78
HCT	43.9	%	%MAC	803
MCV	82.0	fL	%HPO	12.3
MCH	30.5	pg	%HPR	.049
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MCVr	85.7	fL		
MCHr	25.0	pg		
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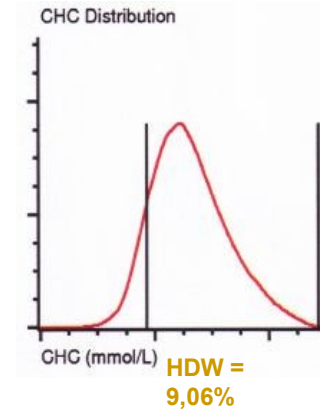
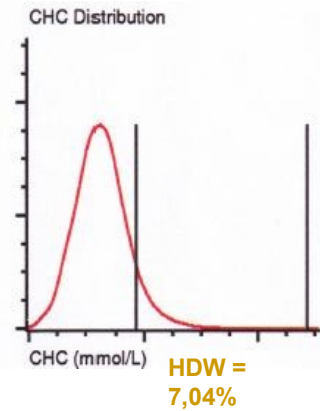
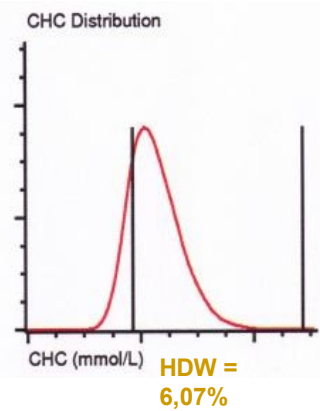
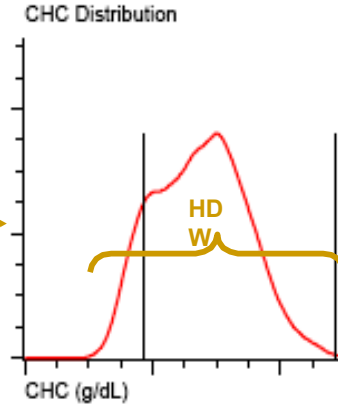


Hemoglobin Konsantrasyonu Dağılım Genişliği

(Hgb concentration Distribution Width - HDW)

Hücresel hemoglobin konsantrasyonu varyasyon katsayısı

RBC	5.35	10e6/uL	RBCo	5.38
HGB	16.3	g/dL	%MIC	1.78
HCT	43.9	%	%MAC	.803
MCV	82.0	fL	%HPO	12.3
MCH	30.5	pg	%HPR	.049
MCHC	37.2	g/dL		
RDW	12.1	%		
HDW	10.8	%		
RETc	39.6	10e3/uL	%R	.741
IRF	.229			
NRBC	0.00	10e3/uL	NR/W	0.00
MCVr	85.7	fL		
MCHr	25.0	pg		
CHCr	28.9	g/dL		



Şistositler

- < 30 fL
- Düşük RNA içeriği
- Boyut ve hemoglobin içeriği ile ayrılır
- Küçük eritrositler ve membran parçacıkları da sayılabilir
- Mikroskopik ve otomatize sayımlar arasında iyi korelasyon ($r=0,75-0,96$), CV %1,42 (%13 konsantrasyonda) -%6 (2,1)
- Sensitivite %91,8 – 100
- Spesifite % 20-52,2
- Pozitif sonuçlar için mikroskopi

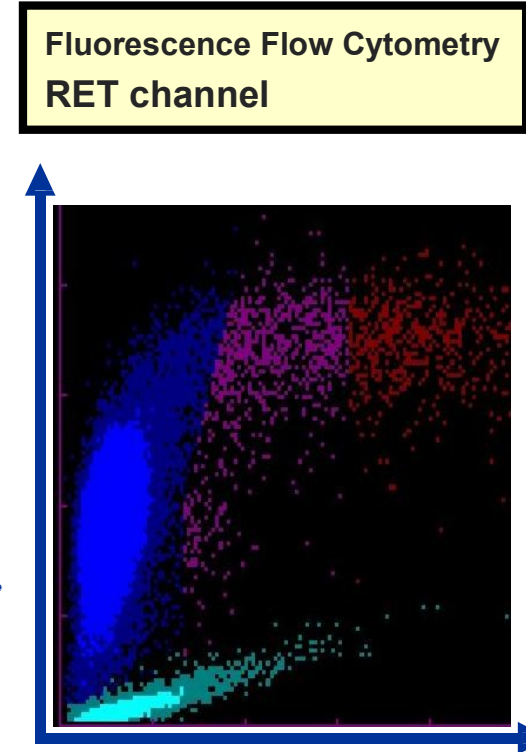
Retikülosit Hemoglobin Eşdeğeri (RET-He)

Sadece retikülosit konsantrasyonuna bakmak yeterli değildir, aynı zamanda üretilen retikülositlerin **büyüklüğü** ve **hemoglobin içeriği** de bu hücrelerin **kalitesi** için önemlidir.

- Demir eksikliğini daha hızlı teşhis etmede
- Retikülositler ileriye saçılan sinyaller ve floresan boya sinyalleri ile diğer hücrelerden ayrıştırılır

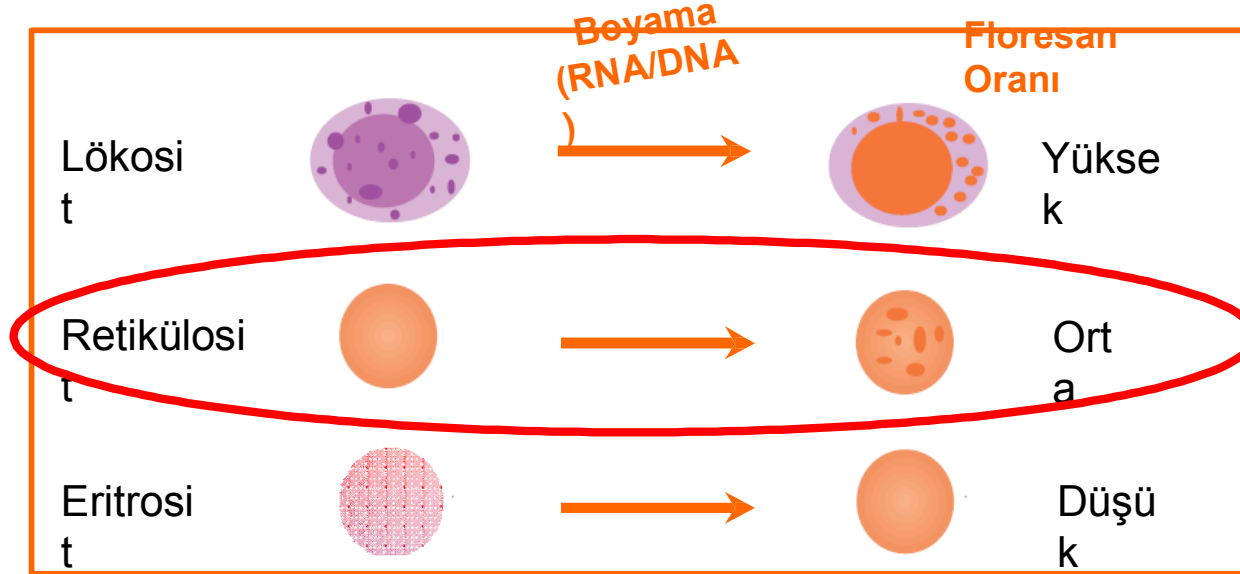
Floresan Yoğunluk

İleriye Saçılan
Işıklar



RET-He

- Floresan boya ile hücrelerdeki nükleik asit içeren partiküller boyanır.
- Böylece her bir hücre grubu RNA/DNA içeriğine göre farklı bir şekilde boyanmış olur (nükleik hücreler daha fazla, eritrositler daha az).
- Retikülositler lökosit ve eritrositlerden ayrıştırılır.



Retikülosit Hemoglobin İçeriği (CHr)

Retikülositlerden Hb sentezini ve demir kullanılabilirliğinin yeterliliğini yansıtır

- ❑ Düşük olması → Demir eksik eritropoez (ferritin ve transferrin ↓)
- ❑ **Pediatride Fe eks için en güvenilir index**
- ❑ i.v. demir tedavisinin izlenmesi (Heterozigot β Talasemi'de daima düşüktür)

Ortalama Retikülosit Volumü (MCV_r)

Demir depoları düşük hastalarda demir tedavisini takiben hızla artar, eritropoezisin iyileşmesi ile aynı hızda azalır

Vit B12 ve folik asit ile tedavi sonrası makrositozda MCV_r ↓

$MCV_r \times \text{retikülosit sayısı} = Hct_r$

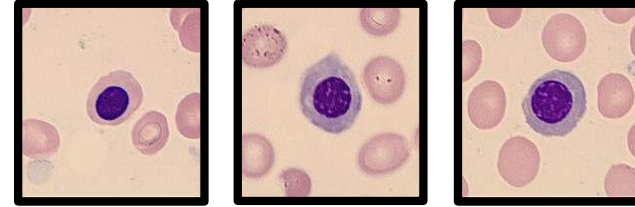
$MCV_r / MCV \rightarrow$ k.i. Transplantasyonu sonrası eritropoetik cevabın en erken işaret

Farklı cihazlardan elde edilen verilerin karşılaştırılması problemlidir

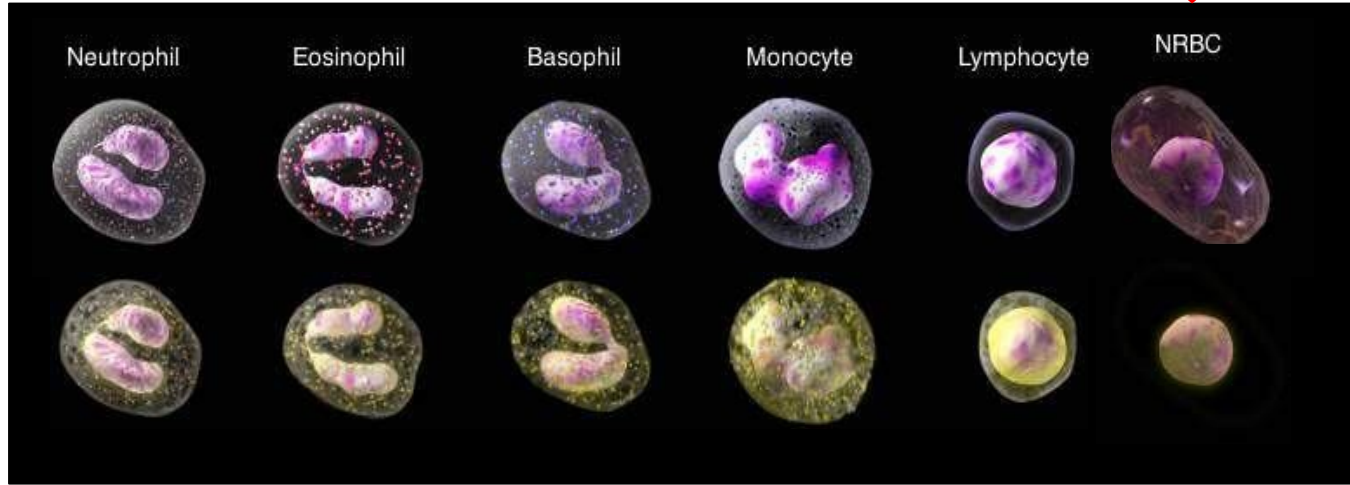
NRBC (eritroblastlar)

- Periferik kanda NRBC :

- Yeni doğmuş bebeklerde
- Yetişkinlerde
 - Hematopoietik stres (kanama, ciddi hipoksi, sepsisemi...)
 - Talasemi
 - Myeloproliferatif hastalıklar
 - k.i. metastazı
- Hastalık ciddiyeti ile doğru orantılı, kök hücre transplantasyonunda kötü prognostik faktör → sayı önemli



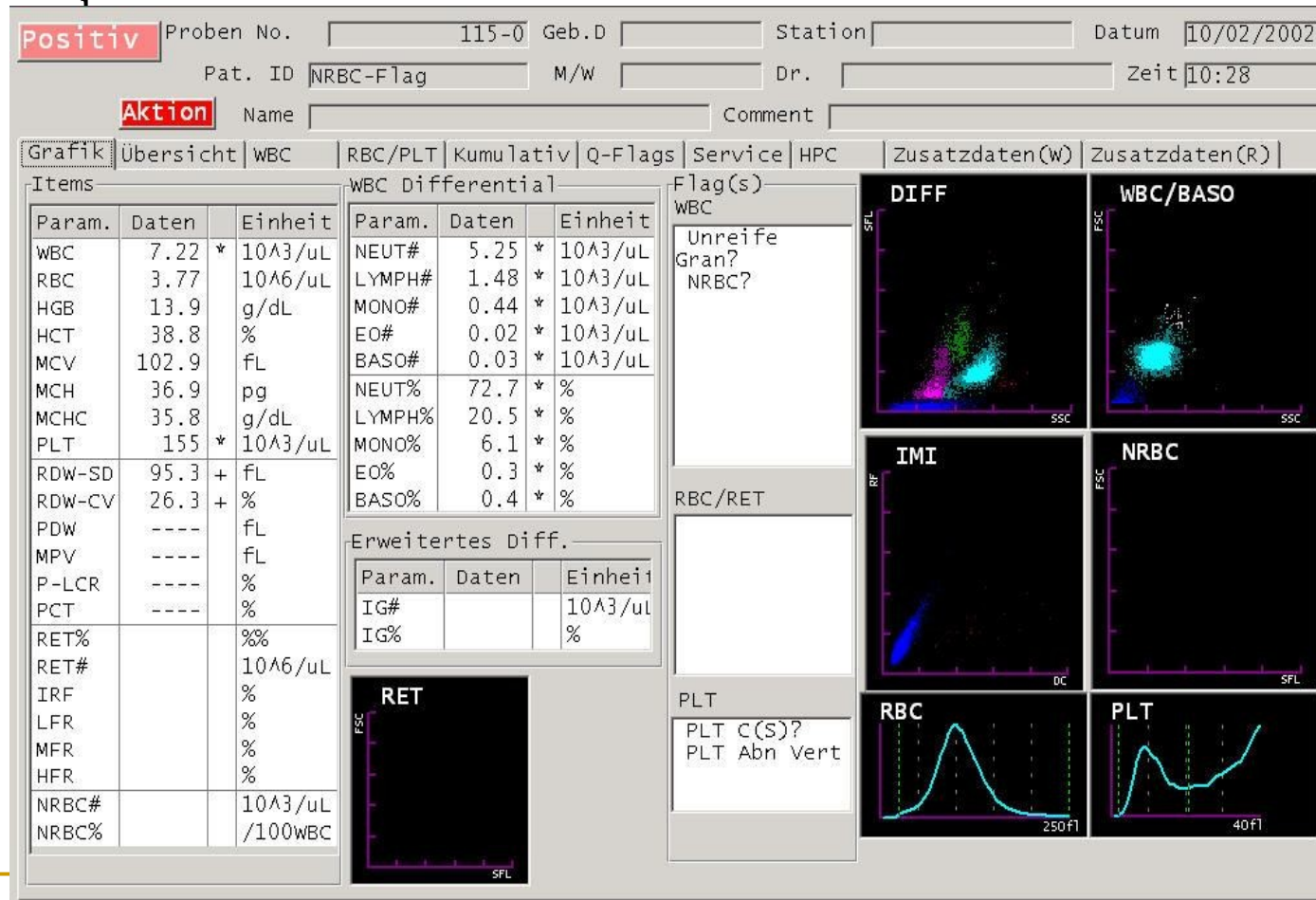
NRBC Eritroblast Ölçümü



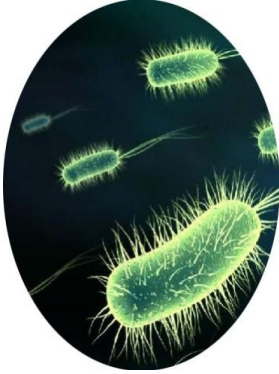
- Eritroblastların **sitoplazmaları çözülür**
- **Çekirdekler tam** olarak kalır ve **boyanır**
- İçerdikleri nükleik aside göre boyandıkları için, eritroblastların floresan yoğunlukları lökositlere göre **daha azdır**
- Bu yöntemle eritroblastlar, çekirdek içermelerine rağmen lökositlerden **net olarak ayrıştırılır**

NRBC – çekirdekli RBCler

- “NRBC?” Uyarı mesajı DIFF kanalından



Olgunlaşmamış Granülositler (IG)



- Ciddi enflamasyon ve enfeksiyon vakalarının teşhisinde!

➔ Periferik kanda artışı

- Sistemik enflamasyon
- Hematolojik malignite
- İlaç tedavisi (kemoterapi, glikokortikoidler gibi)

Spesifite %83 – 97

Sensitivite %35 - 40

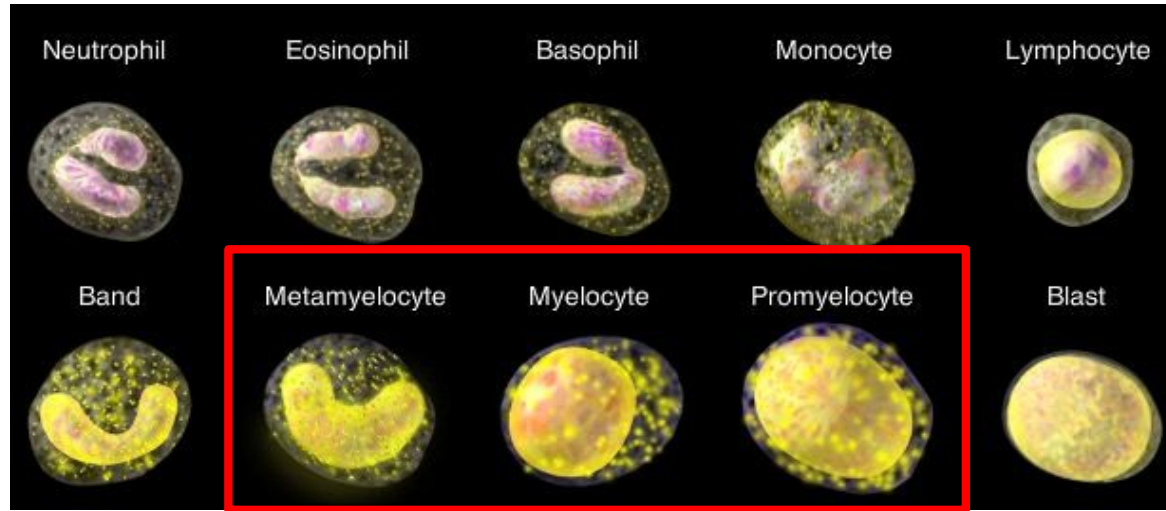
Olgunlaşmamış Granülositler (IG)

Nasıl Ölçülür?

Fluorescence Flow Cytometry teknolojisi ile:

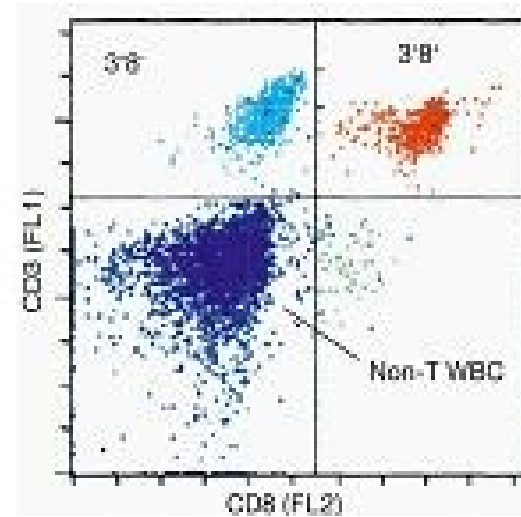
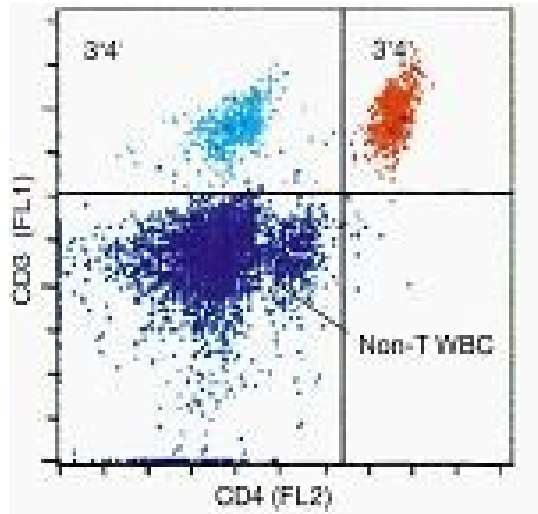
- Yana saçılan ışınlar lökositlerin dahili hücre yapısını belirler
- Floresan boya sinyalleri ise hücrelerin içine girerek nükleik asitleri (RNA/DNA) boyar

Bu sayede IG hücreleri net bir şekilde ayrıştırılır ve tanımlanır!



Monoklonal Antikor Aplikasyonu

- Optik ve flöresan ölçüm yapabilen analizör
- Geniş bir seride monoklonal antikorlar kullanılır (Lenfoit, myeloid ve trombosit belirteçleri)
- Anormal sayımların ayrıntılandırılması, malign ve reaktif ayrımı

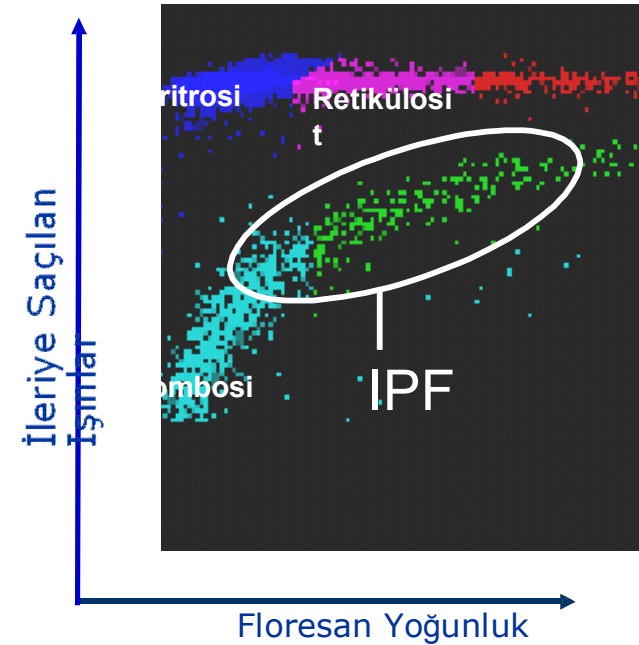


Hematopoetik Progenitör Hücre (HPC)

- CD34 HPC'de bulunan bir yüzey glikoproteini
- CD34+ → Periferik kök hücre nakli için zamanlama
- Eritrosit ve trombositler parçalanır
- Olgunlaşmamış myeloid hücreler dışındaki tüm lökositler de membranların lipid içeriğine göre parçalanır
- Radyo frekans ve doğru akım yoluyla analiz
- Hızlı ve ekonomik ancak CD34+ kök hücreleriyle benzer parçalanma özellikleri gösteren CD34- hücreler de kaydedilebilir
- Özel yazılım gerektirir

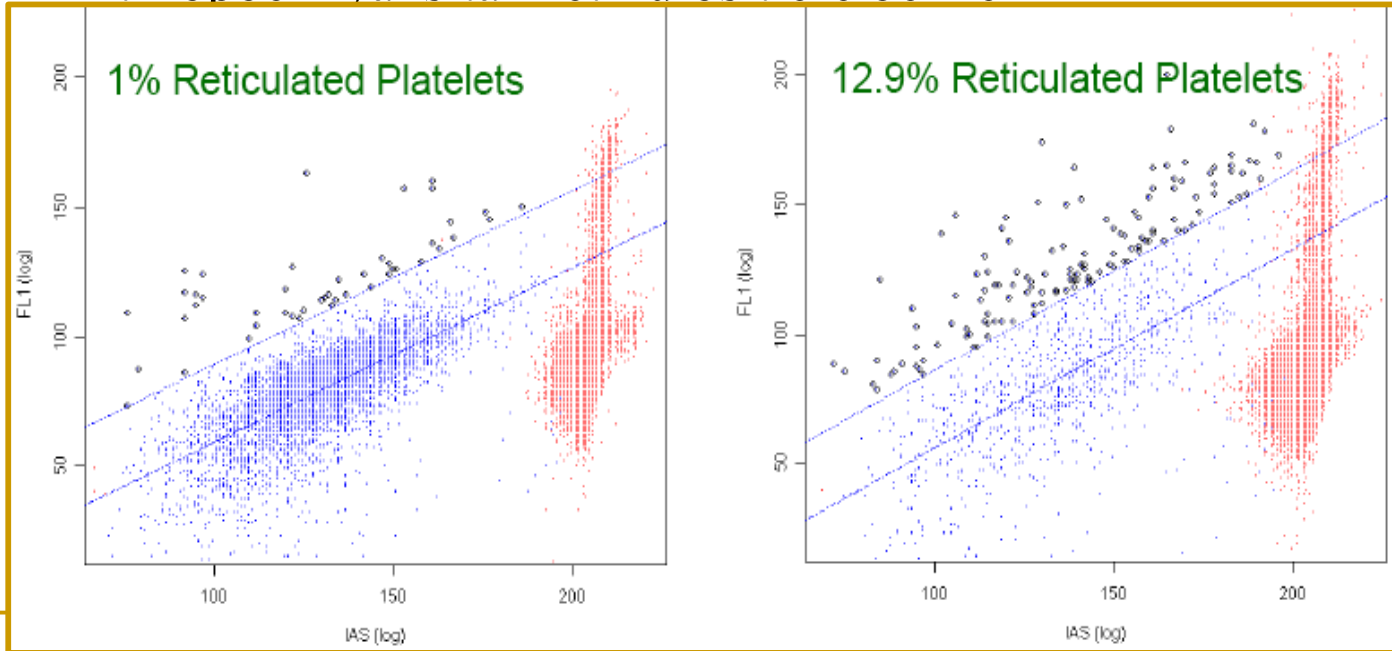
Olgunlaşmamış Trombositler (IPF)

- Genel **trombosit üretimi** için önemli bir göstergedir.
- Olgunlaşma(normal) trombositlere göre daha çok RNA içerdiklerinden floresan yoğunlukları fazladır
- IPF= Olgunlaşmamış tr / toplam tr
- Normal trombositlerden belirgin bir şekilde ayrılır
- ■ % 1,1 - % 6,1



Çekirdekli Trombositler

- RNA içeren trombositler
- 1-2%
- Çekirdekli trombositler megakaryopoietik aktiviteyi yansıtır
- Erithropoezi vansıtan retikülositlere benzer



Digital görüntüleme sistemleri

Uniform Printing of a Precise Blood Volume

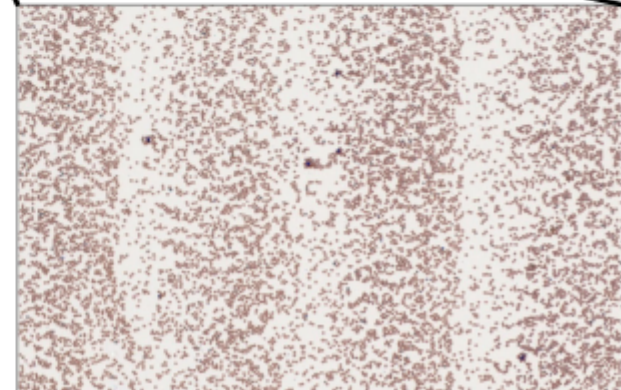
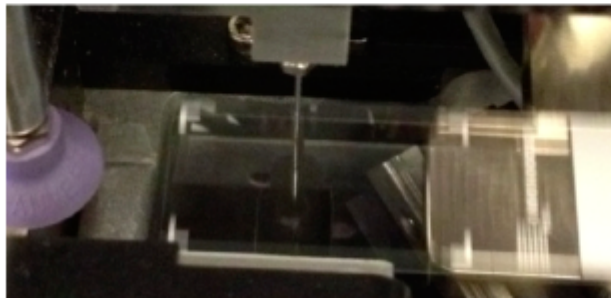
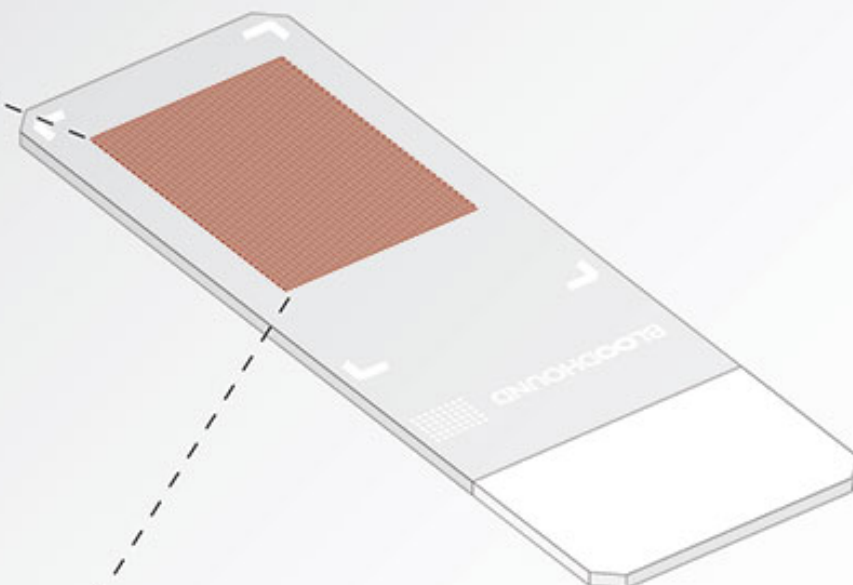


Table 2. Performance characteristic testing comparison for image-based haematology systems

Performance validation testing	Digital image cell locator	Digital image CBC/Diff/Retic
Precision/repeatability	YES	YES
Within run	YES	YES
Between run (day to day)	YES	YES
Between techs	YES	YES
Accuracy	YES	YES (Reference H20-A2)
Comparability	YES	YES (compare to H20-A2 and predicate haematology analyser)
Sensitivity/Specificity	YES	YES
Sample stability	NO	YES
Carryover	NO	YES/NO (if cartridge system)
Linearity	NO	YES
Limit of blank (LoB)/Limit of detection (LoD)/Limit of quantitation (LoQ)	NO	YES – May be modified or adapted to analyser methodology
Reference intervals	NO	YES

CBC, complete blood count.

Bloodhound™ Integrated Hematology System Parameters		
WBC	White Blood Cell Count	10 ³ /μL
RBC	Red Blood Cell Count	10 ⁶ /μL
HGB	Hemoglobin Concentration	g/dL
HCT	Hematocrit	%
MCV	Mean Corpuscular Volume	fL
MCH	Mean Corpuscular Hemoglobin	pg
MCHC	Mean Corpuscular Hemoglobin Concentration	g/dL
PLT	Platelet Count	10 ³ /μL
RDW	Red Cell Distribution Width	%
RDW-SD	Red Cell Distribution Width - Standard Deviation	fL
MPV	Mean Platelet Volume	fL
#NEUT	Neutrophil Absolute Count	10 ³ /μL
%NEUT	Neutrophil Percent	%
#LYMPH	Lymphocyte Absolute Count	10 ³ /μL
%LYMPH	Lymphocyte Percent	%
#MONO	Monocyte Absolute Count	10 ³ /μL
%MONO	Monocyte Percent	%
#EOS	Eosinophil Absolute Count	10 ³ /μL
%EOS	Eosinophil Percent	%
#BASO	Basophil Absolute Count	10 ³ /μL
%BASO	Basophil Percent	%
#UC	Unclassified Cell Count	10 ³ /μL
%UC	Unclassified Cell Percent	%
#NRBC	Nucleated Red Blood Cell Count	10 ³ /μL
%NRBC	Nucleated Red Blood Cell Percent	%
#RETIC	Reticulocyte Count	10 ⁶ /μL
%RETIC	Reticulocyte Percent	%
HGBr	Reticulocyte Hemoglobin	pg



Classify White Blood Cells - Press Spacebar to Continue

Total Cells:556
Cells Found by User:0
Total Cells In View:200
Case ID:1000004141
Review ID:1000003525
Image ID:1000000980
Facility:100001

77x

0 - Unclassified (Count: 187)

⏮ Patient Name: .
Barcode Result: Barcode NA
9.04 um

NEXTSLIDE
IMAGING

1. WBC

2. RBC

3. Platelet

4. Complete

13 of 100 Classified

Show at most 200 Cells.

Code	Class Description	Hot Key	Pct
1	Neutrophil	NumPad1	
2	Lymphocyte	NumPad2	
3	Monocyte	NumPad3	
4	Banded Granulocyte	NumPad4	
5	Reactive Lymphocyte	NumPad5	
6	Eosinophil	NumPad6	
7	Basophil	NumPad9	
8	Metamyelocyte	NumPad7	
9	Myelocyte	NumPad8	
10	Promyelocyte	P	
11	Blast Cell	B	
12	Nucleated Red Cell	N	

Toxic Granulation	None	Present
Toxic Vacuolation	None	Present
Dohle Bodies	None	Present
Auer Rods	None	Present
Clefted Lymphocytes	None	Present
Hairy Projection	None	Present
Smudge Cells	None	Present

Figure 1. Default white blood cell (WBC) review screen in the Nextslide Digital Review Network system. The unclassified WBC images are presented on the left. Morphology codes for WBC can be entered or edited on the right.

Table 3. Percentage of Positive Agreement Between the Nextslide Digital Review Network System and Microscopy for White Blood Cell (WBC), Red Blood Cell (RBC), and Platelet Morphology Review				
Cell Type	Morphology Code	Occurrences, No.	Concordance, %	Overall Concordance, %
WBC	Toxic granulations	28	78.57	76.10
	Döhle bodies	5	80.00	
	Vacuolations	38	84.21	
	Smudge cells	32	65.63	
	Clefted lymphocytes	10	60.00	
	Hairy projections	2	100.00	
	Auer rods	2	100.00	
RBC	Anisocytosis	54	74.07	76.50
	Poikilocytosis	31	83.87	
	Microcytosis	7	71.43	
	Macrocytosis	2	50.00	
	Hypochromasia	3	100.00	
	Polychromasia	35	80.00	
	Echinocytes (burr cells)	19	89.47	
	Dacrocytes (teardrops)	16	81.25	
	Spherocytes	6	66.67	
	Target cells	2	50.00	
	Acanthocytes	1	100.00	
	Ovalocytes	28	71.43	
	Schistocytes	5	80.00	
	Stomatocytes	2	0.00	
	Howell-Jolly bodies	1	100.00	
	Basophilic stipplings	5	40.00	
Platelet	Clumped platelets	3	66.67	87.50
	Platelet satellitosis	5	100.00	
	Large platelets	8	100.00	
	Giant platelets	8	75.00	
Overall				77.12

Table 1

New Parameters: Proposed Clinical Applications and Technical Limitations

Parameter	Availability*	Proposed Clinical Applications	Limitations	References
Hematopoietic progenitor cells	XE 2100	Surrogate for CD34 stem cell quantitation before peripheral harvesting	Reduced availability; measurement depends on time between sampling and analysis; high imprecision	4, 5
Immature granulocytes	XE 2100	Diagnosis of bacterial infections in appropriate clinical setting	Reduced availability	6-8
Nucleated RBCs	Sapphire; Pentra 120 DX; LH 750; ADVIA 2120; XE 2100	Diagnosis of hematologic diseases; prognostic factor in patients from surgery department or undergoing stem cell transplantation; evaluation of the efficacy of transfusion therapy in thalassemic syndromes	Higher performance on fluorescence-based methods	9-14
Immature reticulocyte fraction	Sapphire; Pentra 120 DX; LH 750; ADVIA 2120; XE 2100	Classification of anemias; monitoring the efficacy of therapy in nutritional anemia; early identification of marrow regeneration (after bone marrow transplantation or chemotherapy); verify aplastic anemia; timing for stem cell collection	Not standardized; reference intervals method-dependent; higher sensitivity in fluorescence-based analyzers	15-21
Reticulocyte indices				
Mean reticulocyte hemoglobin content	ADVIA 2120; XE 2100	Diagnosis of iron-deficient erythropoiesis (absolute or functional); monitoring response to iron supplements; monitoring erythropoietin treatment during dialysis	Reduced availability	22-27
Mean reticulocyte volume	Pentra 120 DX; LH 750; ADVIA 2120	Diagnosis of iron-deficient erythropoiesis; early monitoring of response to treatment in nutritional anemia; early signs of erythropoietic recovery following bone marrow transplantation; evaluation of erythropoietin abuse in sports	Not standardized; reference intervals method-dependent	17, 28-32
RBC fragments (schistocytes)	ADVIA 2120; XE 2100	Diagnosis and monitoring of microangiopathies	Reduced availability; not standardized; definition based only on size and hemoglobin content	33-35
Reticulated platelets	XE 2100	Differential diagnosis of thrombocytopenia; prediction of total platelet recovery after chemotherapy or stem cell transplantation; risk index of thrombosis in patient with thrombocytosis; timing for prophylactic platelet transfusion; evaluation of platelet turnover	Reduced availability; not standardized	36-44

* Sapphire, Abbott, Abbott Park, IL; Pentra 120 DX, ABX-Horiba, Montpellier, France; LH 750, Beckman Coulter, Hialeah, FL; ADVIA 2120, Siemens Diagnostics, Tarrytown, NY; XE-2100, Sysmex, Kobe, Japan.

Table 1. Recently introduced haematology parameters and their clinical utility

Instrument and manufacturer	Parameter	Clinical utility
Abbott Sapphire Beckman Coulter LH 750 Horiba Medical Pentra Siemens Advia Sysmex XE series	Nudeated red blood cell count	Automatic correction of WBC and lymphocyte counts where necessary, fewer manual microscopic counts. Diagnosis of haematological diseases and damage to bone marrow environment
Horiba Medical Pentra* Sysmex X series	Immature granulocyte count	Diagnosis of infection and inflammatory states
Abbott Cell-Dyn 6 Sapphire Beckman Coulter LH 750 Horiba Medical Pentra Siemens Advia Sysmex XE series Siemens Advia	Immature reticulocyte fraction	Monitoring of bone marrow regeneration post transplant or chemotherapy. Classification of anaemias and monitoring of treatment
Sysmex XE series	Percentage hypochromic red cells, reticulocyte haemoglobin content	Functional iron deficiency
	Percentage hypochromic red cells, reticulocyte haemoglobin concentration	Assessment of the availability of iron for erythropoiesis
Abbott Sapphire	Percentage hypochromic red cells*, reticulocyte haemoglobin content*, mean reticulocyte volume*	
Beckman Coulter LH 750	Mean reticulocyte volume*	
	Low haemoglobin density*	
	Red cell size factor*	
Horiba Medical Sysmex XE series	Mean reticulocyte volume*	
	Immature platelet fraction	Differential diagnosis of thrombocytopenia, prediction of platelet recovery post transplant or chemotherapy
Siemens Advia* Sysmex XE series*	Fragmented red cell count	Diagnosis and monitoring of microangiopathies
Abbott Cell-Dyn 6 Sapphire	Monoclonal antibody application	Immunophenotyping, replaces traditional flow cytometer for some protocols and low volume of samples
Beckman Coulter LH 750	White cell volume, conductivity and scatter measurements*	Advanced flags for diagnosis of specific diseases which cause changes to white cell populations
Sysmex XE series	High fluorescent lymphocytes*, NEUT-X*	Diagnosis and monitoring of bacterial or viral sepsis Diagnosis of Myelodysplastic Syndrome (in combination of anaemia)

*Nonreportable parameter, for research use only.

Automated Blood Cell Counts

State of the Art

Mauro Buttarello, MD, and Mario Plebani, MD

Key Words: Blood cell analyzers; CBC count; Leukocyte differential count; Reticulocytes; Reticulocyte indices; Immature platelet fraction

determining the fraction of immature platelets by using a simplified method opens the door to new applications. It is also desirable that, as with the high standardization for basic CBC parameters, a continued effort be made for the parameters (ie, RDW, IRF, MCVr, and MPV) for which results provided are still too different when produced by different analyzers. To reach these goals, cooperation between long-standing (ie, International Council for Standardization in Haematology and the National Committee for Clinical Laboratory Standards, now the Clinical and Laboratory Standards Institute) and recent (International Society of Laboratory Hematology) organizations interested in hematologic standardization and the manufacturers is fundamental. It should be remembered that despite the essential role of automation in the modern hematology laboratory, microscopic control of pathologic samples remains indispensable, so much so that in certain cases, it alone is diagnostic.¹³⁴ Moreover, knowledge of the limits of the specific analyzer in use is of paramount importance for the correct interpretation of results. These considerations require that clinical laboratories performing hematologic diagnostics have personnel with specific training and profound knowledge in laboratory hematology.

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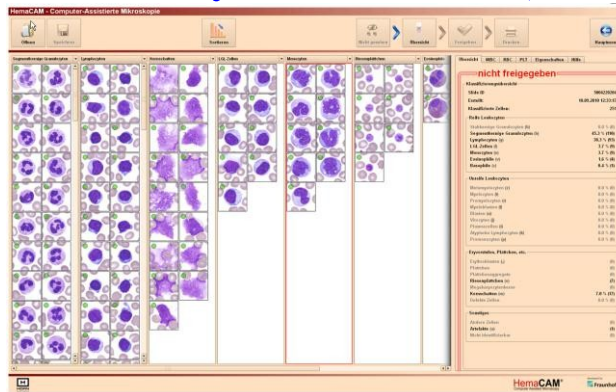
- STANDARDİZE DEĞİL!!!!!!!

Yakın gelecekte tam kan sayım sistemleri???

- ✓ Multiple 'in line' analyser
- ✓ Slide-Maker-Stainer
- ✓ Auto-validation in LIS
- ✓ Automated reflex/morphology etc.
 - ✓ Including digital morphology
- ✓ All Full blood analysis “in line” (ESR, HbA1c,.....)



Total Laboratory
Automation



Digital Morphology



Laboratuvar test sonuçları

(iyi laboratuvar uygulaması, GLP)



Klinik tanı

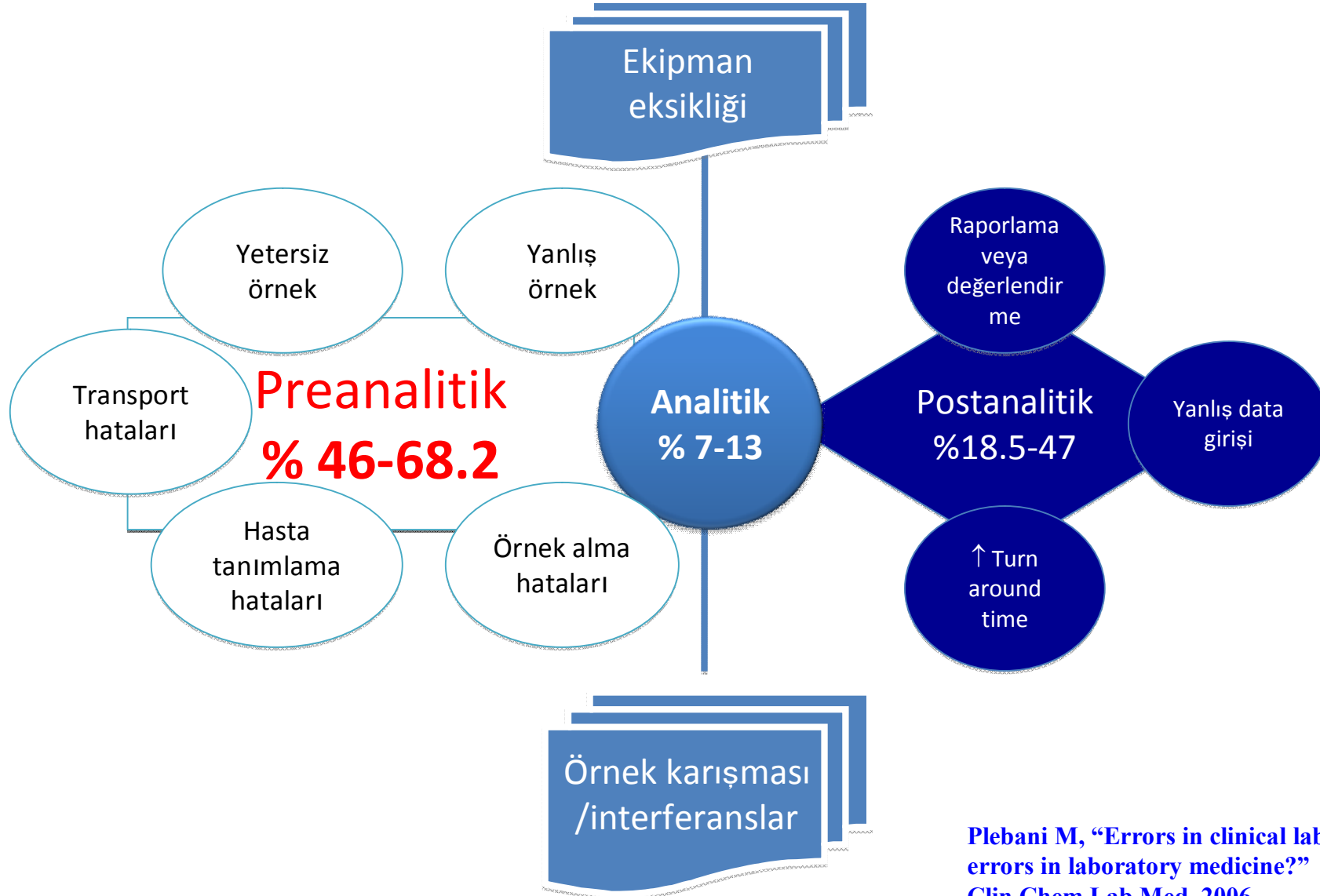


Klinik karar (Hasta)

İyi laboratuvar Pratiği

- Pre-analitik basamak
- Analitik basamak
- Post-analitik basamak

Laboratuvar sürecinin 3 fazındaki hatalar ve oranları



Plebani M, "Errors in clinical laboratories are errors in laboratory medicine?"
Clin Chem Lab Med, 2006.

Product Name: Infohance 2012 - Release Date: January 2012



H26-A2
Vol. 30 No. 14
Replaces H26-A and H38-P
Vol. 16 No. 12 and Vol. 19 No. 7

Validation, Verification, and Quality Assurance of Automated Hematology Analyzers; Approved Standard—Second Edition

This document provides guidance for the validation, verification, calibration, quality assurance (QA), and quality control (QC) of automated multichannel hematology analyzers for manufacturers, end-user clinical laboratories, accrediting organizations, and regulatory bodies. In addition, end-user clinical laboratories will find guidance for establishment of clinically reportable intervals and for QA for preexamination and examination aspects of their systems.

A standard for global application developed through the Clinical and Laboratory Standards Institute consensus process.



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CLSI

International Journal of Laboratory Hematology

The Official Journal of the International Society for Laboratory Hematology



ORIGINAL ARTICLE

INTERNATIONAL JOURNAL OF LABORATORY HEMATOLOGY

ICSH guidelines for the evaluation of blood cell analysers including those used for differential leucocyte and reticulocyte counting

INTERNATIONAL COUNCIL FOR STANDARDIZATION IN HAEMATOLOGY, WRITING GROUP: C. BRIGGS*, N. CULP[†], B. DAVIS[‡], G. D'ONOFRIO[§], G. ZINI[¶], S. J. MACHIN[¶], ON BEHALF OF THE INTERNATIONAL COUNCIL FOR STANDARDIZATION OF HAEMATOLOGY

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Keywords
Hematology analyser,
evaluation/validation, digital
imaging, flow cytometry,
regulatory science

SUMMARY

This revision is intended to update the 1994 ICSH guidelines. It is based on those guidelines but is updated to include new methods, such as digital image analysis for blood cells, a flow cytometric method intended to replace the reference manual 400 cell differential, and numerous new cell indices not identified morphologically are introduced. Haematology analysers are becoming increasingly complex and with technological advancements in instrumentation with more and more quantitative parameters are being reported in the complete blood count. It is imperative therefore that before an instrument is used for testing patient samples, it must undergo an evaluation by an organization or laboratory independent of the manufacturer. The evaluation should demonstrate the performance, advantages and limitations of instruments and methods. These evaluations may be performed by an accredited haematology laboratory where the results are published in a peer-reviewed journal and compared with the validations performed by the manufacturer. A less extensive validation/transference of the equipment or method should be performed by the local laboratory on instruments prior to reporting of results.

INTRODUCTION

Since the previously published ICSH guidelines in 1994, haematology analysers have evolved greatly.

Automated analysers speed up the workflow in the laboratory and improve precision as more cells are counted and cell classifications are based on more measured objective properties (light scatter, fluorescence,

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1

ICSH

(published in the International Journal of Laboratory Hematology, the official ISLH journal)

Hematoloji laboratuvarı – Kalite programı

- Hematoloji laboratuvarında her cihaz için; uygulanan bütün işlemlerin etraflı yazılmış ve laboratuvar yetkilisi tarafından onaylanmış SOP dosyaları hazırlanmalı
- Dosyalarda SOP'ler gözden geçirilme, revize edilmeli

Pre-analitik basamak

Örnek kabulünde

- Laboratuvar kabul kontrolü (SOP' de tanımlanmış)
 - kantite,
 - kalite
 - barkod,
 - istem,
 - Pıhtı, eksik , hemoliz, fazla vb.
 - Uygun sürede (6 saat) içinde lab' a gelmemiş, aşırı ısınmış/donmuş örnek

**Uygun olmayan örnek
reddedilir**

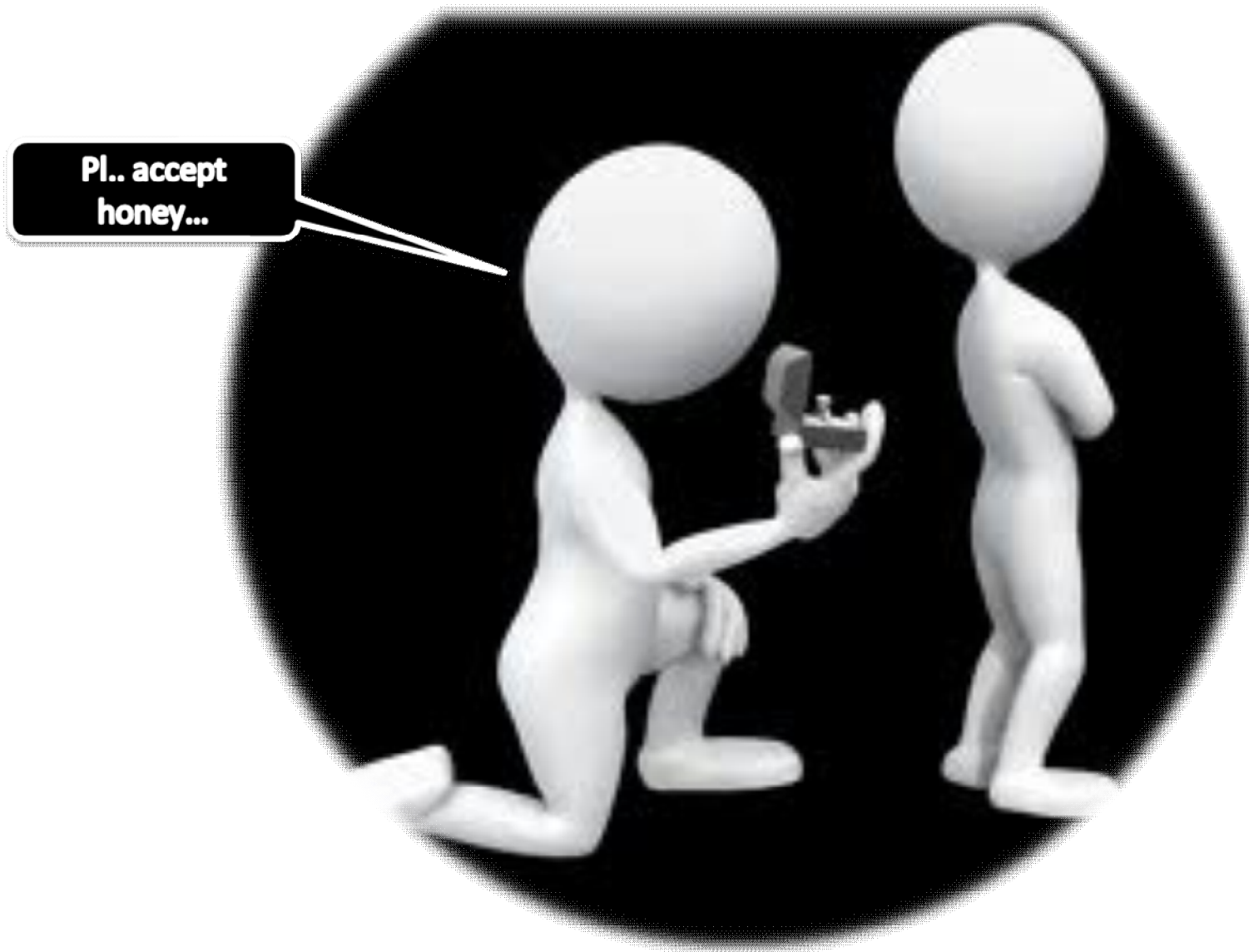
Pıhtılı örnekten
kaçınılmalı

Antikoagülanlı tüpler
(K2EDTA)



Tüp iyice karıştırılmalı
8-10 defa alt üst edilmeli

At sample acceptance counter...



Kan Sayım Cihazlarının Kalibrasyonu

- Cihazların kalibrasyonları üretildikten sonra yapılmıştır
- Kurulum sırasında teknik servis tarafından kontrolleri yapılır ve gerekirse ayarlanır
- Büyük tamir –parça değişiminden sonra veya reaktif değişiminden sonra yeniden ayarlama yapılır
- İç-dış kalite kontrollerinde sorun olmadığı sürece gerek yoktur.
- Firmanın önerdiği aralıklarla ayarlar kontrol edilip yapılır

İç kalite kontrol

- Üç seviye kontrol
- Eski kontroller
- Sonucu bilinen taze kan örneği
- Hareketli ortalama (Moving averages (Bull's algorithm))
- MCHC %30-36

Delta check

GÜNLÜK İÇ KALİTE KONTROL

1.

- **Start up ve background sayım kontrolü** (Monitor for acceptable background.....if required take corrective action)

2.

- **QC ekranından üç seviye kontrol (Low level, normal level and high level)** (Monitor L-J chart, apply Westergard's interpretation, monitor %CV.....if required take corrective action)

3

- **QC ekranında tutulan örnekler** (Monitor %CV.....if required take corrective action)

Kontrol sonuçlarında hata olduğunda...

Uyarı kuralı = her basamağı tekrar kontrol

Redetme kuralı = “out of control”

- Çalışmayı durdur
- Problemi bul ve düzelt
- Kontrolleri ve hasta örneklerini tekrarla
- Problem çözülene kadar sonuç verme

İç kalite kontrol için diğer yapılacaklar



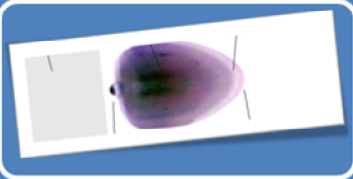
Başka bir analizörle karşılaştırma çalışması



Başka cihazla farklı modda dene



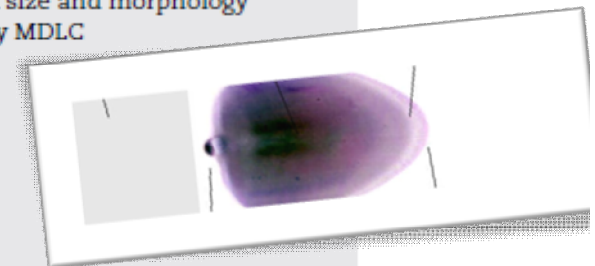
Solüsyonları değiştir, lotları kontrol et



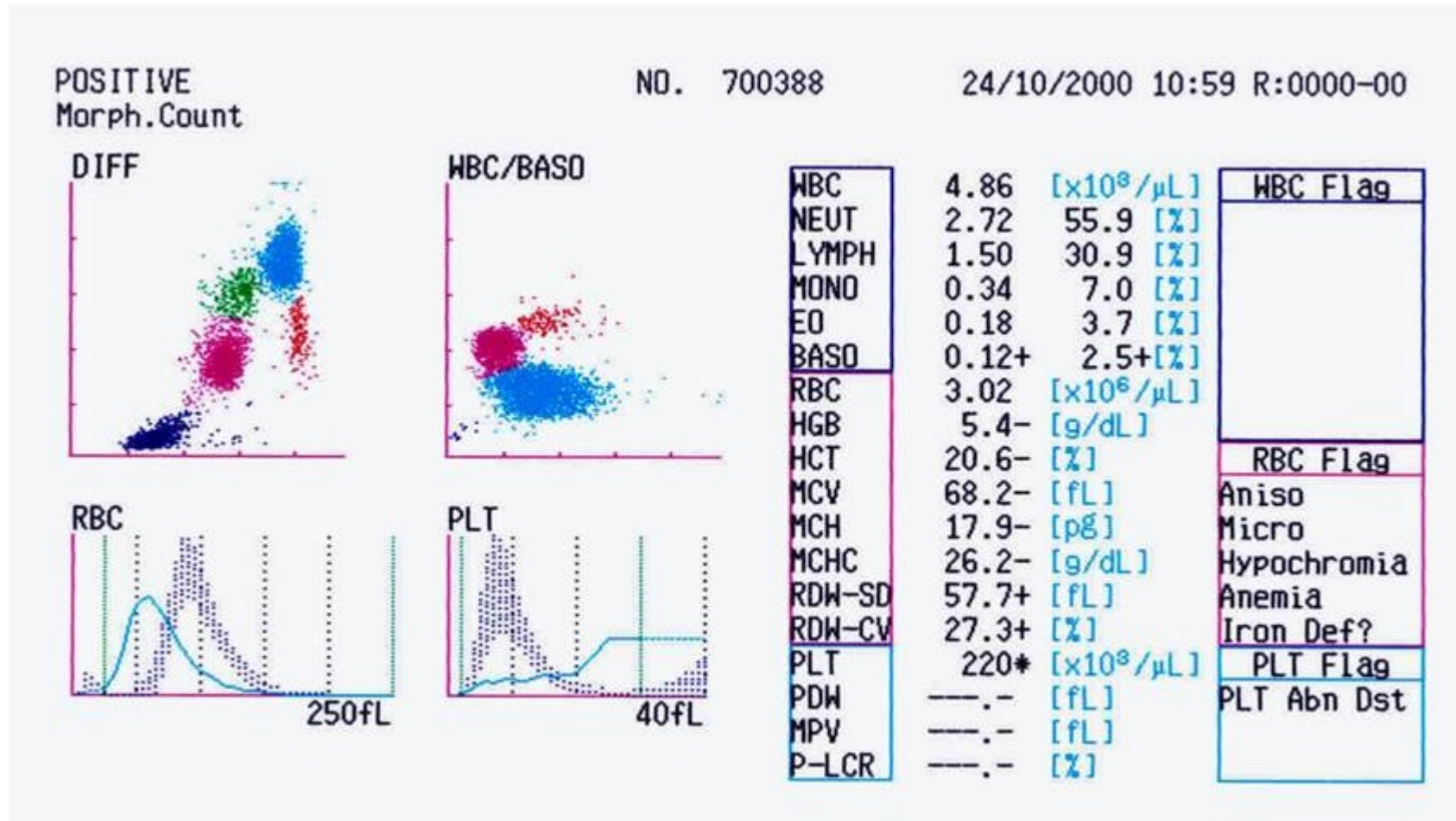
Periferik yayma yaparak kontrol et

Table 1 – Adapted International Society of Laboratory Hematology review criteria for automated complete blood counts.

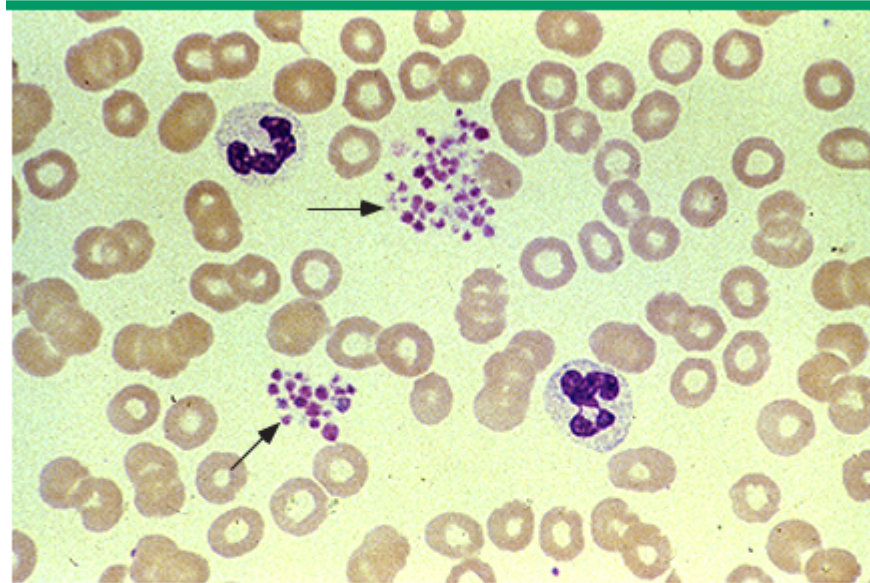
Criterion or rule	Parameter	Adapted ISLH criteria	Action proposed
1	WBC	$<4.0 \times 10^3/\mu\text{L}$ or $>30.0 \times 10^3/\mu\text{L}$	BSR – confirm leukopenia or leukocytosis by blood smear estimate
2	Neutrophils [#]	$<1.0 \times 10^3/\mu\text{L}$ or $>20.0 \times 10^3/\mu\text{L}$	BSR and if necessary MDLC
3	Lymphocytes [#]	$>5.0 \times 10^3/\mu\text{L}$ (adults) and $>7.0 \times 10^3/\mu\text{L}$ (children aged ≤ 12 years)	
4	Monocytes [#]	$>1.5 \times 10^3/\mu\text{L}$ (adults) and $>3.0 \times 10^3/\mu\text{L}$ (children aged ≤ 12 years)	
5	Eosinophils [#]	$>2.0 \times 10^3/\mu\text{L}$	
6	Basophils [#]	$>0.5 \times 10^3/\mu\text{L}$	
7	Hemoglobin	$<7.0 \text{ g/dL}$ or $>18.5 \text{ g/dL}$	BSR – check RBC morphology
8	MCV	$<75 \text{ fL}$ or $>105 \text{ fL}$	BSR – check microcytosis and macrocytosis
9	MCHC	$<30 \text{ g/dL}$	BSR – check hypochromia, verify time passed after sample collection
10	MCHC	$>36.5 \text{ g/dL}$	BSR – check lipemia, hemolysis, hyperleukocytosis, RBC agglutination, and blood: anticoagulant relation and presence of spherocytes
11	RDW-CV	$>22.0\%$	BSR – check anisocytosis
12	Reticulocytes	$>100 \times 10^9/\mu\text{L}$	BSR – check polychromatophilia
13	Platelets	$<100 \times 10^3/\mu\text{L}$ or $>1000 \times 10^3/\mu\text{L}$	BSR – estimate platelet count on blood smear to confirm thrombocytopenia or thrombocytosis
14	MPV	$<5.0 \text{ fL}$ or $\geq 12.5 \text{ fL}$	BSR – check platelet size and morphology
15	Suspect flags	Immature Granulocytes?; Immature Granulocytes present (Promyelocytes, myelocytes, and metamyelocytes) Left shift? Atypical lymphocytes? Abnormal Lymphocytes/blasts?; Blasts? (Myeloblasts, lymphoblasts, or atypical lymphocytes) Nucleated RBC? Fragments? (Schistocytes) Dimorphic RBC population (Erythrocyte population heterogeneous in size) RBC lyse resistance Platelet clumps (generated by scattergram)? and Platelet clumps (generated by the impedance)	BSR and if positive, count erythroblasts/100 leukocytes. If $>10\%$, correct total leukocyte count BSR – check presence of schistocytes Check RBC histogram, observe RDW value and verify anisocytosis on blood smear Rerun sample. If positive, BSR and check for nucleated RBC and abnormal RBC morphology Search for clots in the sample and platelet aggregates on blood smear



Küçük eritrositler (<50 fL) eritrosit histogramında yer almazlar, trombosit histogramına girebilirler. Bu durumda eritrosit sayısı düşüktür, daha önemlisi trombosit sayısı olduğundan daha yüksek bulunur .PY yapılmalı



seudothrombocytopenia due to platelet clumping in EDTA

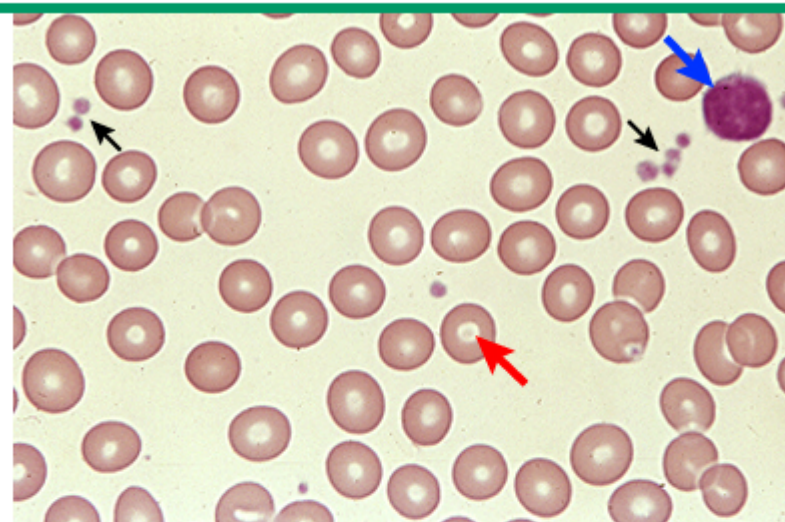


This peripheral blood smear shows platelet clumping (arrows) in an EDTA-anticoagulated blood sample. This patient had an EDTA-dependent platelet agglutinin which caused in vitro platelet clumping, resulting in an artifactually low platelet count (ie, pseudothrombocytopenia). No platelet clumping was seen, and the platelet count was normal, in a blood sample from this patient anticoagulated with sodium citrate.

produced with permission from Beutler, E, Lichtman, MA, Coller, BS, et al, Hematology, 5th ed, McGraw-Hill, New York, 1995.

Graphic 68949 Version 2.0

Normal peripheral blood smear



High power view of a normal peripheral blood smear. Several platelets (black arrows) and a normal lymphocyte (blue arrow) can also be seen. The red cells are of relatively uniform size and shape. The diameter of the normal red cell should approximate that of the nucleus of the small lymphocyte; central pallor (red arrow) should equal one-third of its diameter.

Courtesy of Carola von Kapff, SH (ASCP).

Graphic 59683 Version 2.0

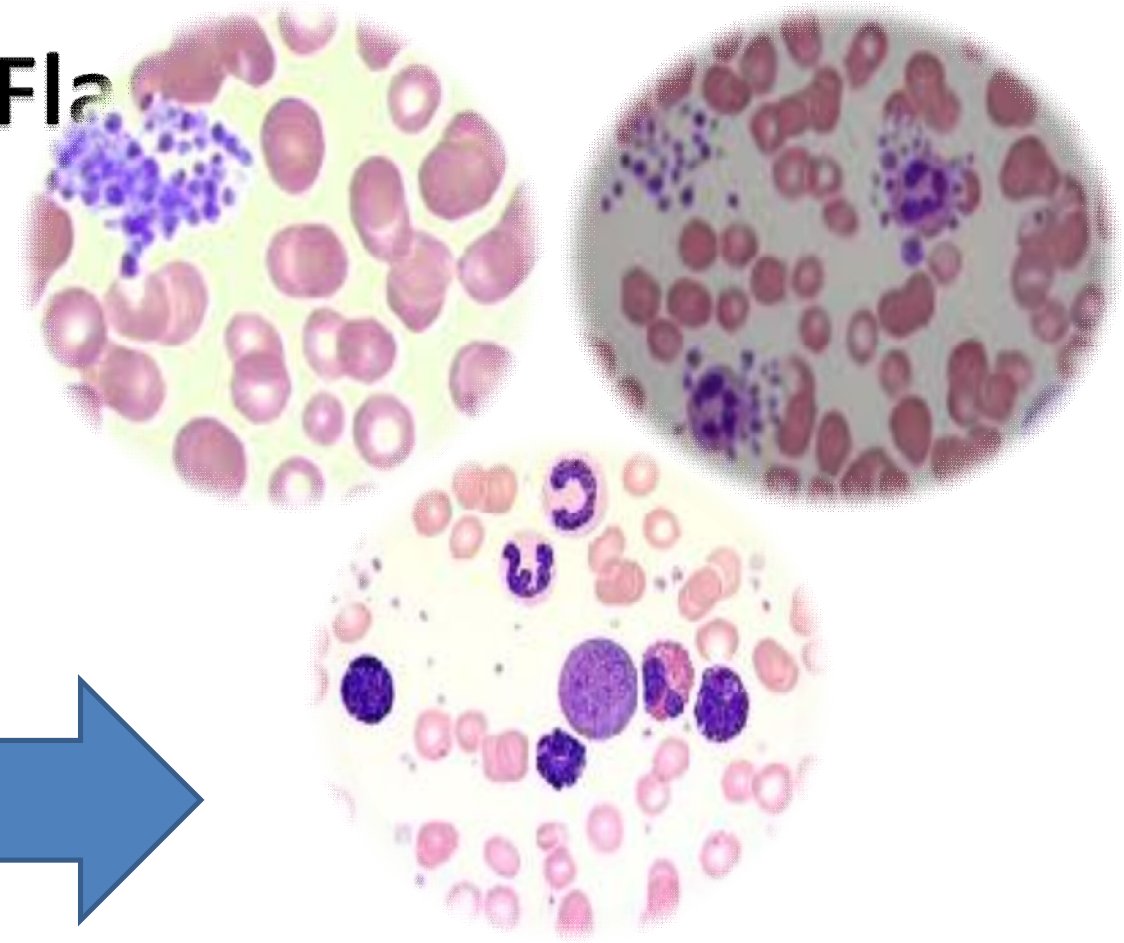
Fla

- ? Platelet clumps
- Thrombocytopenia
- ? Basophilia

PBS

Analyzer

PBS : küme trombosit /
Bazofili

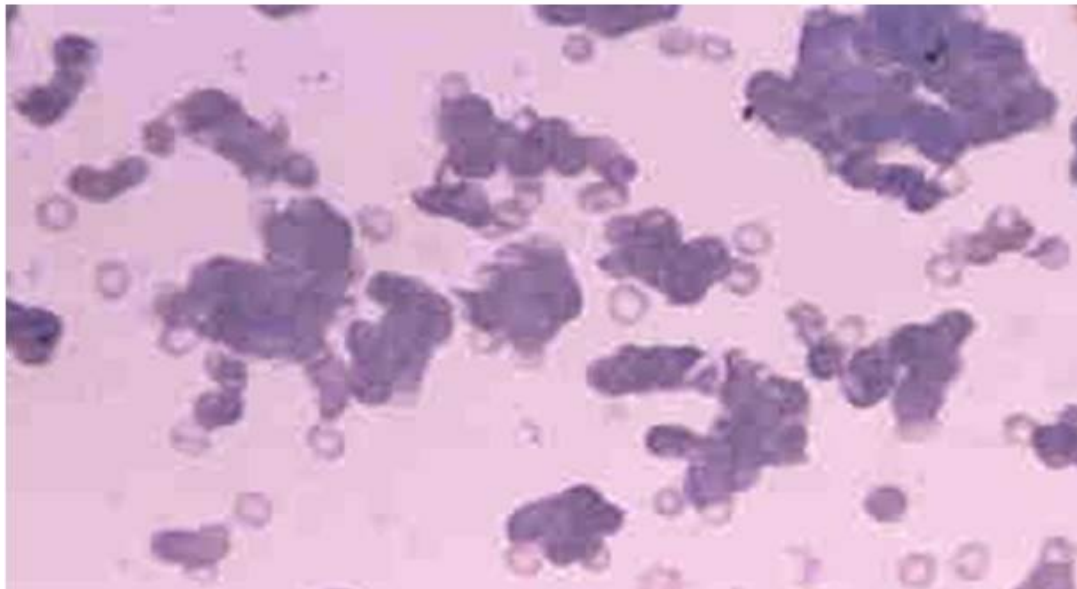


Soğuk aglutinin

Abnormal RBC Pop

RBC	2.23	*V
HGB	9.9	L
HCT	21.8	VL
MCV	97.9	H
MCH	44.4	VH
MCHC	45.4	VH
RDW	20.5	RH

SUSPECT FLAGS:
NRBCs
Dimorphic RBC Pop



- **NRBC:**

WBC yalancı yükseklik/ lenfositoz

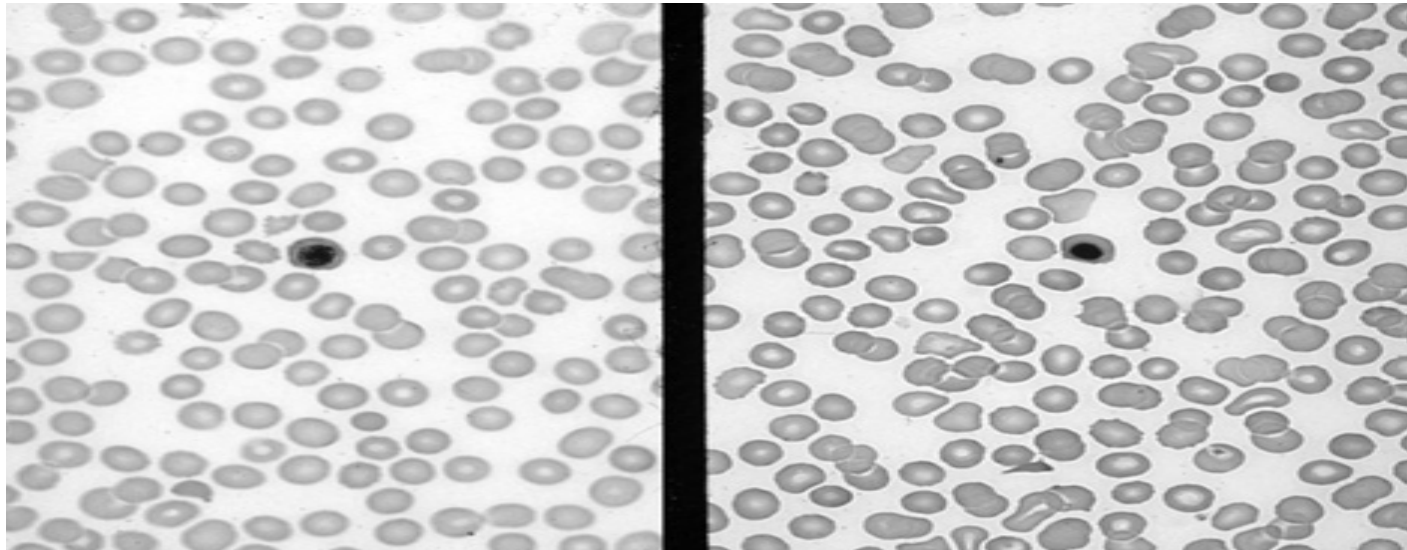


Table 1. Abnormal samples and potential interfering substances that should be included in the evaluation of the haematology analyser

WBC	RBC	Platelets	Interfering substances
Extreme leucocytosis	Sickle cells	Giant platelets	Haemolysis
Extreme leucopenia	Target cells	Platelet clumps	Cryoglobulins
Neutrophilia			
Lymphocytosis			
Monocytosis			
Eosinophilia			
Basophilia			
Blast cells	Fragmented cells	Immature Platelets	Paraproteins
Atypical lymphocytes	Microcytic cells	CD61 labelled platelets	High bilirubin
Smear/smudge cells	Macrocytic cells		Lipaemia
Immature granulocytes	Spherocytes		
Left shift/band neutrophils	Extreme polycythaemia		
CD3/CD4/CD8 Lymphocytes	Extreme anaemia		
	Nucleated red blood cells		
	Reticulocytosis		
	IRF		
	Low Retic Hb Conc/Content		
	Howell–Jolly bodies		
	Heinz bodies		
	Pappenheimer bodies		
	Malarial parasites		

WBC, white blood cell count; RBC, red blood cell count; IRF, immature reticulocyte fraction; Hb, haemoglobin; Retic, reticulocytes; Conc, concentration.

Kan Sayım İnterferans

● WBC

1. RBC anomalileri, hemolize dirençli
2. Nucleated RBCs
3. Fragmented WBCs
4. 35 fl den büyük parçalanmamış partiküller
5. Büyük trombosit kümeleri
6. Örneğin fibrin, hücre parçası vb debri içermesi
7. pediatrik/onkoloji örnekleri

► RBC

1. WBC' nin yüksekliği (99.9' un üstü)
2. Yüksek oranda dev trombosit
3. RBC' nin Aglutinasyon
4. RBC' nin 36 fL' den küçük olması
5. Örneğin fibrin, hücre parçası vb debri içermesi
6. pediatrik/onkoloji örnekleri

Kan Sayım İnterferans

- **Hgb**

1. yüksek WBC
2. lipemi
3. Heparin
4. RBC anomalileri, hemolize dirençli
5. yüksek trigliserid
6. yüksek bilirubin

- ▶ **MCV**

1. yüksek WBC
2. Yüksek oranda dev trombosit
3. RBC' nin Aglutinasyon
4. RBC' nin 36 fL' den küçük olması

Kan Sayım İnterferans

► Plt

1. Normalden küçük RBC
2. şistosit
3. Küme trombosit
4. debri

● RDW

1. yüksek WBC
2. Yüksek oranda dev trombosit
3. RBC' nin 36 fL' den düşük olması
4. 2 farklı RBC hücre grubu
5. RBC aglutinasyonu

Kan Sayım İnterferans

- **MPV**

Bilinenler :platelet sayısı, şekli, EDTA

- **Hct**

RBC ve MCV

- **MCH**

Hgb ve RBC

- **MCHC**

Hgb, RBC ve MCV

Abnormal sonuç???

- **Plt < 40,000**

1. Örneği kontrol et (pıhtı, az/çok örnek.)
2. Küme trombosit, RBC parçası, mikro RBC için PY yap

- **WBC +++++**

1. Isotonik ile sulandırma 1:2 ya da ötesi (nihai sonuç için, sulandırılan sonucu sulandırma faktörü ile çarpın); nihai WBC yi RBC den çıkarın, hct hesapla, MCV yi doğru olandan hesaplayın ($MCV = Hct/RBC \times 10$)
2. “Yüksek WBC nedeniyle Hgb, MCH,MCHC rapor edilemiyor,

Abnormal sonuç???

- **Plt +++++**

1. Mikrosit ve parçalanmış eritrosit açısından PY
2. Eğer mevcutsa, plt tahmini ?, anlaşıyorlarsa, manual plt sayımı yap
3. Isotonik ile 1:2 numune sulandırın, sulandırılan sonucu sulandırma faktörü ile çarpın

- **RBC > 7.0**

1. Isotonik ile 1:2 sulandırın, sulandırma sonucunu sulandırma faktörü ile çarpın, Hgb i gözden geçirin ve MCH, MCHC tekrar hesaplayın

DIŞ Kalite Kontrol



RIQAS

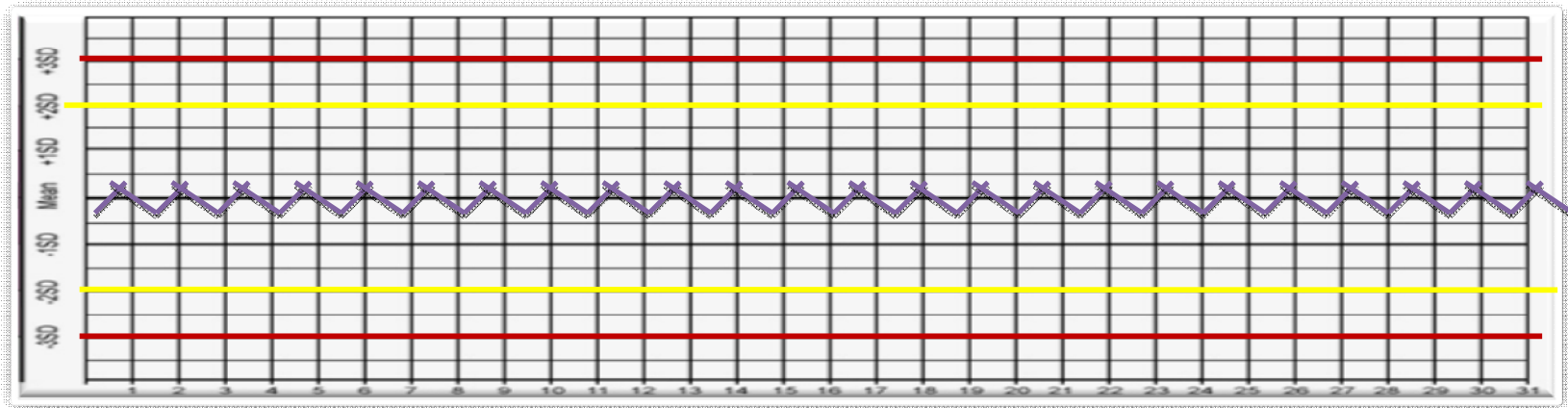


>17'nin
DKD programı var

I prefer the 'xyz' analyzer because its 2SD QC charts are amazingly flat



2SD



Full Blood Count (FB surveys)

Analytes

	Units
White blood count (WBC)	$10^9/\text{L}$
Haemoglobin (Hb)	g/dL
Red blood count (RBC)	$10^{12}/\text{L}$
Haematocrit (Hct)	L/L
Mean cell volume (MCV)	fL
Mean cell haemoglobin (MCH)	pg
Mean cell haemoglobin concentration (MCHC)	g/dL
Platelet count (PLT)	$10^9/\text{L}$

Specimens

Two whole blood specimens are issued per distribution, with 12 distributions per year on a monthly basis (24 specimens per year). The specimens are prepared from pooled human blood donations in CPD-A1 anticoagulant (collected from individually consented donors by the UK National Blood Service), partially fixed and treated with antibiotics. Red cell and haemoglobin, white cell and platelet concentration may be varied. Blood is dispensed aseptically, using an automated mixing-dispensing system to ensure identical aliquots. The shelf life of the unopened specimens is at least two weeks post distribution at 4°C.

time that the survey is open. The shelf life of the unopened specimens is at least two weeks post distribution at 4°C.

Automated Differential Leucocyte Count (DL surveys)

- **Analytes**

Three or five population differential leucocyte count as determined by automated analysers. Performance monitoring is undertaken on Neutrophil and Lymphocyte counts only.

- **Specimens**

Two specimens are distributed, six times per year (12 specimens per year). Seven types of commercially prepared survey material are available and the choice of material type (matrix) is instrument dependent. Analysis of results is by material type and in some cases by individual instrument type. Table 2 lists the matrices that are currently distributed.

Table 2. Survey material types (matrices) for ADLC

Matrix	Instruments
A 3 population diff	Serono (Baker), Sysmex and Cell Dyn
B 3 population diff	ABX, Beckman Coulter
C 5 population diff	Cell Dyn 3000, 3500, 3700, 4000 & Sapphire
D 5 population diff	Siemens ADVIA 120, ADVIA 2120
E 5 population diff	Beckman Coulter StkS, MaxM, GenS & LH series
G 5 population diff	ABX Pentra series, ABX Argos, Coulter Ac•T 5 Diff
J 5 population diff	Sysmex XE2100, XE5000, XT1800, XT2000, XS1000i

Full Blood Count

Scheme Details

Contact Details

Further Info

Analytes or Clinical Applications Covered

Hb, RBC, PCV, MCV, MCH, MCHC, WBC, Platelets

Material Distributed

Partially fixed human whole blood

Distributions per annum

12

Samples per Distribution

2

Scheme Started

1968

Accreditation Status

Accredited to CPA Standards for EQA schemes in laboratory medicine, centre No. 022

Available to laboratories in the following categories

UK clinical
UK non-clinical
Non UK

Partially Fixed Human Whole Blood

☐ All Methods
☒ Your Method

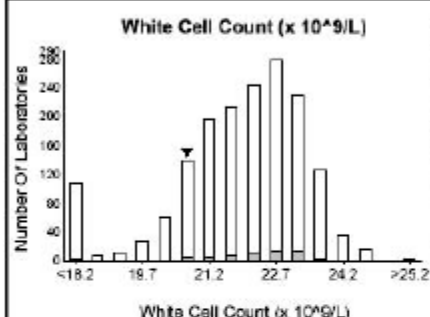
Sample Quality

You reported : Satisfactory

Overall

Satisfactory % : 99.3

Unsatisfactory % : 0.7



Your instrument is
Sysmex K4500

Your Result : 20.6

Target Value : 22.01

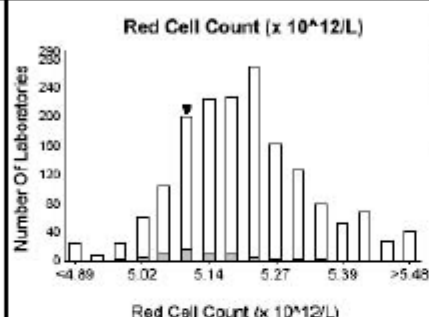
DI : -1.17

CV : 5.79

N : 66

N(trimmed) : 60

Perf Score : 22.5



Your instrument is
Sysmex K4500

Your Result : 5.07

Target Value : 5.115

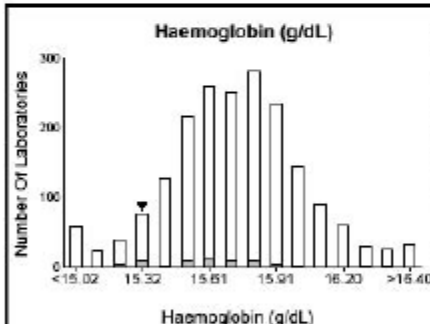
DI : -0.50

CV : 1.75

N : 86

N(trimmed) : 80

Perf Score : 17.8



Your instrument is
Sysmex K4500

Your Result : 15.3

Target Value : 15.61

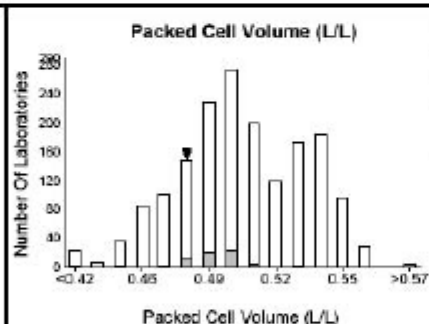
DI : -1.18

CV : 1.69

N : 66

N(trimmed) : 60

Perf Score : 38.9



Your instrument is
Sysmex K4500

Your Result : 0.480

Target Value : 0.4897

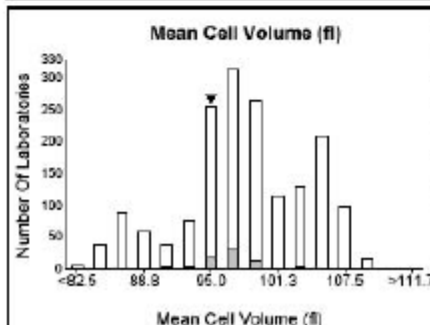
DI : -0.77

CV : 2.84

N : 86

N(trimmed) : 80

Perf Score : 21.0



Your instrument is
Sysmex K4500

Your Result : 94.7

Target Value : 95.76

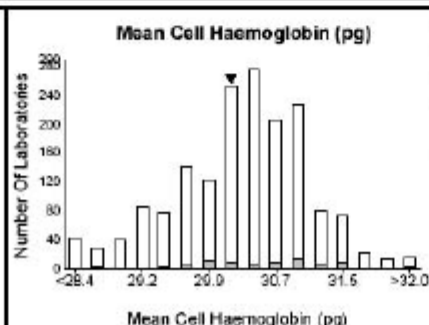
DI : -0.56

CV : 2.02

N : 66

N(trimmed) : 60

Perf Score : 12.9



Your instrument is
Sysmex K4500

Your Result : 30.2

Target Value : 30.55

DI : -0.54

CV : 2.14

N : 66

N(trimmed) : 60

Perf Score : 26.0

POST ANALİTİK AŞAMA

Rapor Örneği

Reference : ISO 15189:2007 Pg 23 & 24

5.8.3 Results shall be legible, without mistakes in transcription and reported to persons authorized to receive and use medical information. The report shall also include, but not be limited to, the following:

- a) clear, unambiguous identification of the examination including, where appropriate, the measurement procedure;
- b) the identification of the laboratory that issued the report;
- c) unique identification and location of the patient, where possible, and destination of the report;
- d) name or other unique identifier of the requester and the requester's address;
- e) date and time of primary sample collection, when available and relevant to patient care, and time of receipt by the laboratory;
- f) date and time of release of report, which, if not on the report, shall be readily accessible when needed;
- g) source and system (or primary sample type);
- h) results of the examination reported in SI units or units traceable to SI units (see ISO 31), where applicable;
- i) biological reference intervals, where applicable;
- j) interpretation of results, where appropriate;
- k) other comments (e.g. quality or adequacy of primary sample which may have compromised the result, results/interpretations from referral laboratories, use of developmental procedure); the report should identify examinations undertaken as part of a development programme and for which no specific claims on measurement performance are made, and, where applicable, information on detection limit and uncertainty of measurement should be provided upon request;
- l) identification of the person authorizing the release of the report;
- m) if relevant, original and corrected results;
- n) signature or authorization of the person checking or releasing the report, where possible.

Delta Kontrol

- Hastanın eski sonucu varsa `.
- Kan sayım parametrelerinde 2-3 haftadaki deęişim oranları

Hb ve RBC	WBC	Platelet
10 %	20-25 %	50 %

Reference: Lewis: Dacie and Lewis Practical Haematology, 10th ed.,Pg 665

PANİK DEĞERLER

Parametre	Panik aralık
WBC (K/mm ³)	≤ 1.0 or ≥ 30.0
HGB (g/dL)	≤ 6.5 or ≥ 19.0
HCT (%)	≤ 20.0 or ≥ 60.0
PLT (K/mm ³)	≤ 30.0 or ≥ 1000

Kan örnekleri

- Tekrar kontrol
- İlave inceleme??

özet...

- **Pre-analitik aşama**
- **Analitik aşama**
- **Post-analitik aşama**

Laboratuvar test sonucu



Klinik Tanı



**Klinik karar
Hasta**

Kalite

**İyi laboratuvar
uygulaması**

TEŞEKÜRLER...