# Hematoloji Laboratuvarında Preanalitik Evre

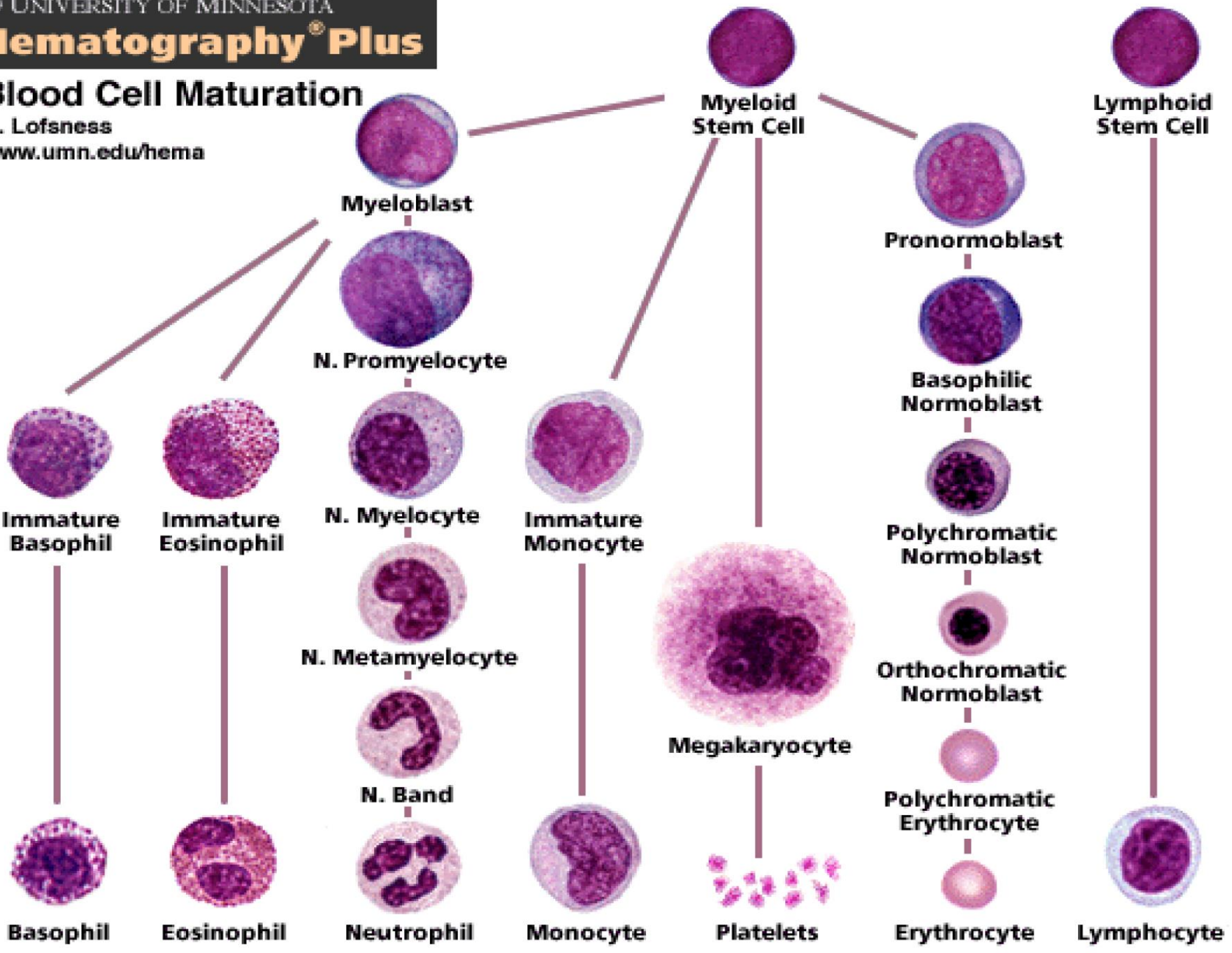
Mesude Falay

Ankara Numune Eğitim ve Araştırma Hastanesi Hematoloji Lab.

**Hematography® Plus**

# Blood Cell Maturation

K. Lofsness
www.umn.edu/hema



# Neden Tam Kan?

| | |
|---|---|
| kan sayımında bening ve sık görülen patolojiler<br>(kliniği?) | • Pre-menapozal Fe eksikliği anemisi<br>• Beta thalassemia minor<br>• Kronik bening nötropeni<br>• Reaktif trombositoz |
| Abnormal / endişe verici kan sayımı<br>(klinikle değerlendir ve takip et) | • Erkek ve postmenapozal Fe eksikliği anemisi<br>• Ilımlı anemi ama tam değerlendirilmemiş?<br>• Açıklanamayan trombositopeni ve trombosiztoz<br>• Üç seride de ılımlı patoloji (red cells, white cells, and platelets), ılımlı pansitopeni |
| Kötü Abnormal tam kan sayımı!! | • Lösemi<br>• Ciddi anemi<br>• Ciddi lökopeni ve pansitopeni |

# Tam Kan Sayım Parametreleri

Lökosit ve Altbirimleri

Eritrosit ve indisleri

Trombosit Ve çapı

| Test Adı | Sonuç | Birim | Referans Değer |
|---|---|---|---|
| Sedimantasyon | 2 | mm/saat | 0 - 20 |
| Tam Kan Sayımı | | | |
| Lökosit (WBC) | 6,89 | x10^9/L | 4,5 - 11 |
| Eritrosit (RBC) | 5,37 | x10^12/L | 4,3 - 5,7 |
| Trombosit (PLT) | 236 | x10^9/L | 150 - 400 |
| Hemoglobin (Hb) | 15,9 | g/dL | 13,2 - 17,3 |
| Hematokrit | 44,5 | % | 39 - 49 |
| Ortalama Eritrosit Hacmi (MCV) | 82,8 | fL | 80 - 99 |
| Ortalama Eritrosit Hemoglobini (MCH) | 29,6 | pg/cell | 27 - 34 |
| Ortalama Eritrosit Hemoglobin Kons.(MCHC) | 35,7 | g/dL | 32 - 37 |
| Eritrosit Dağılım Genişliği (RDW) | 13,8 | % | 11,5 - 14,5 |
| Nötrofil % | 53,6 | % | 40 - 70 |
| Lenfosit % | 34,2 | % | 20 - 45 |
| Monosit % | 5,2 | % | 3 - 9 |
| Eozinofil % | 4,3 | % | 0 - 6 |
| Bazofil % | * 1,3 | % | 0 - 1 |
| LUC % | 1,5 | % | 0 - 4 |
| LUC (Large Unstained Cells - Boyanmamış Büyük Hücreler). Yükselmiş LUC % değeri olması durumunda numunenin periferik yayma yapılarak değerlendirilmesi önerilmektedir. | | | |
| Nötrofil Sayısı | 3,69 | x10^9/L | 1,8 - 7,7 |
| Lenfosit Sayısı | 2,35 | x10^9/L | 1,5 - 4 |
| Monosit Sayısı | 0,36 | x10^9/L | 0,2 - 0,95 |
| Eozinofil Sayısı | 0,29 | x10^9/L | 0 - 0,7 |
| Bazofil Sayısı | 0,09 | x10^9/L | 0 - 0,15 |
| LUC Sayısı | 0,1 | x10^9/L | 0 - 0,4 |
| Ortalama Trombosit Hacmi (MPV) | 10,1 | fL | 6,5 - 10,5 |

# Cihazlar

Tam Kan Sayım Sistemleri ve kullandıkları yöntemler (5 diff)

| Instrument and manufacturer | Technology used for differential count |
|---|---|
| Beckman-Coulter Instrumentation<br><br>(Coulter STKS, GEN-S, LH 700 series) | *VCS Technology (Volume, Conductivity, and Scatter)*<br><br>1. Impedance with low-frequency electromagnetic current<br>2. Impedance with high-frequency electromagnetic current<br>3. Laser light scattering |
| Sysmex Instrumentation<br>(Roche Diagnostics Corporation)<br><br>(SE series, XE2100) | 1. Impedance with low-frequency direct current<br>2. Impedance with radiofrequency current<br>3. Hydrodynamic Focusing |
| Cell Dyne Technology<br>(Abbott Diagnostics Instrumentation)<br>Cell-Dyn 1800<br>Cell-Dyn 3500, 3700 | *The Coulter Principle*<br><br>*Multiple-Angle Polarized Scatter Separation (MAPSS)*<br>1. Four light-scattering parameters: forward light scatter, orthogonal light scatter, narrow-angle light scatter, and depolarized orthogonal light scatter<br>2. Hydrodynamic Focusing<br>3. Use of Flow Cells |
| Cell-Dyn Ruby, Sapphire. | 1. Multiple-Angle Polarized Scatter Separation (MAPSS)<br>2. Hydrodynamic Focusing<br>3. Use of Flow Cells |

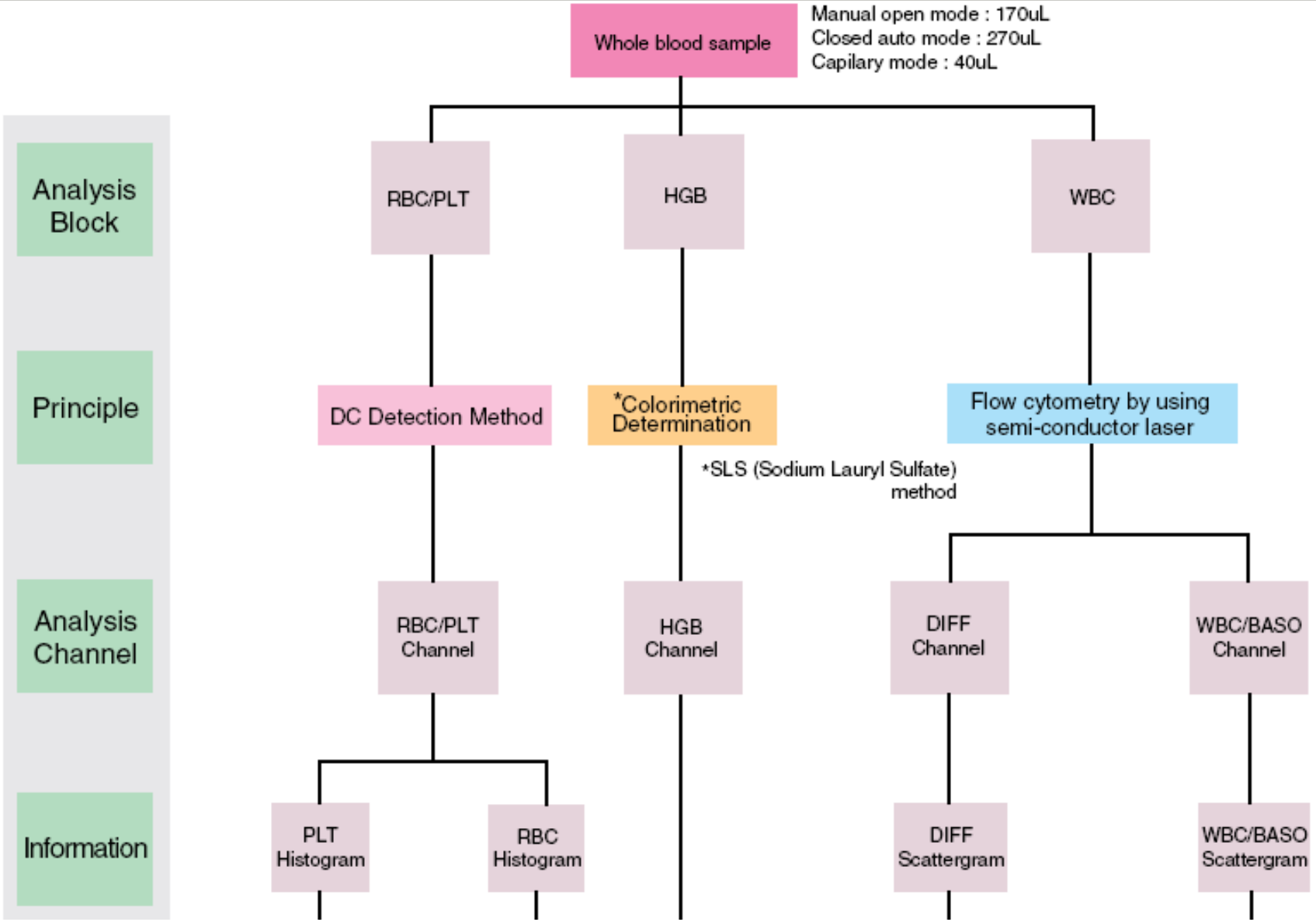

Whole blood sample
Manual open mode : 170uL
Closed auto mode : 270uL
Capilary mode : 40uL
Analysis Block
Principle
Analysis Channel
Information
RBC/PLT
HGB
WBC
DC Detection Method
*Colorimetric Determination
Flow cytometry by using semi-conductor laser
*SLS (Sodium Lauryl Sulfate) method
RBC/PLT Channel
HGB Channel
DIFF Channel
WBC/BASO Channel
PLT Histogram
RBC Histogram
DIFF Scattergram
WBC/BASO Scattergram

**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.




DOI: 10.1309/EK3C7CTDKNVPXVTN

Table 1. Recently introduced haematology parameters and their clinical utility

| Instrument and manufacturer | Parameter | Clinical utility |
|---|---|---|
| Abbott Sapphire<br>Beckman Coulter LH 750<br>Horiba Medical Pentra<br>Siemens Advia<br>Sysmex XE series | Nucleated red blood cell count | Automatic correction of WBC and lymphocyte counts where necessary, fewer manual microscopic counts.<br>Diagnosis of haematological diseases and damage to bone marrow environment |
| Horiba Medical Pentra*<br>Sysmex X series | Immature granulocyte count | Diagnosis of infection and inflammatory states |
| Abbott Cell-Dyn & Sapphire<br>Beckman Coulter LH 750<br>Horiba Medical Pentra<br>Siemens Advia<br>Sysmex XE series | Immature reticulocyte fraction | Monitoring of bone marrow regeneration post transplant or chemotherapy. Classification of anaemias and monitoring of treatment |
| Siemens Advia | Percentage hypochromic red cells, reticulocyte haemoglobin content | Functional iron deficiency<br>Assessment of the availability of iron for erythropoiesis |
| Sysmex XE series | Percentage hypochromic red cells, reticulocyte haemoglobin concentration | |
| Abbott Sapphire | Percentage hypochromic red cells*, reticulocyte haemoglobin content*, mean reticulocyte volume* | |
| Beckman Coulter LH 750 | Mean reticulocyte volume*<br>Low haemoglobin density*<br>Red cell size factor* | |
| Horiba Medical | Mean reticulocyte volume* | |
| Sysmex XE series | Immature platelet fraction | Differential diagnosis of thrombocytopenia, prediction of platelet recovery post transplant or chemotherapy |
| Siemens Advia*<br>Sysmex XE series* | Fragmented red cell count | Diagnosis and monitoring of microangiopathies |
| Abbott Cell-Dyn & 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<br>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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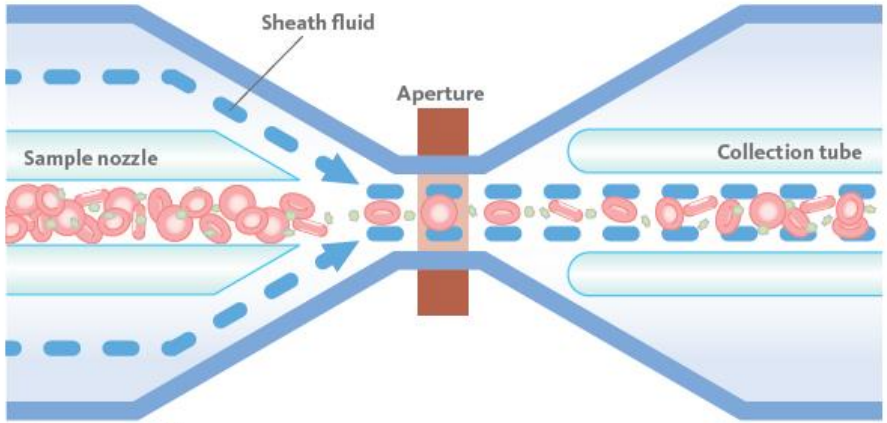
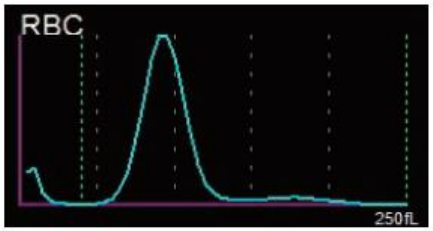
- **STANDARDİZE DEĞİL!!!!!!!!**

# Empedans yöntemi

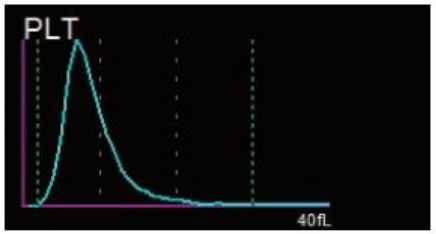



※ This is a conceptual drawing.

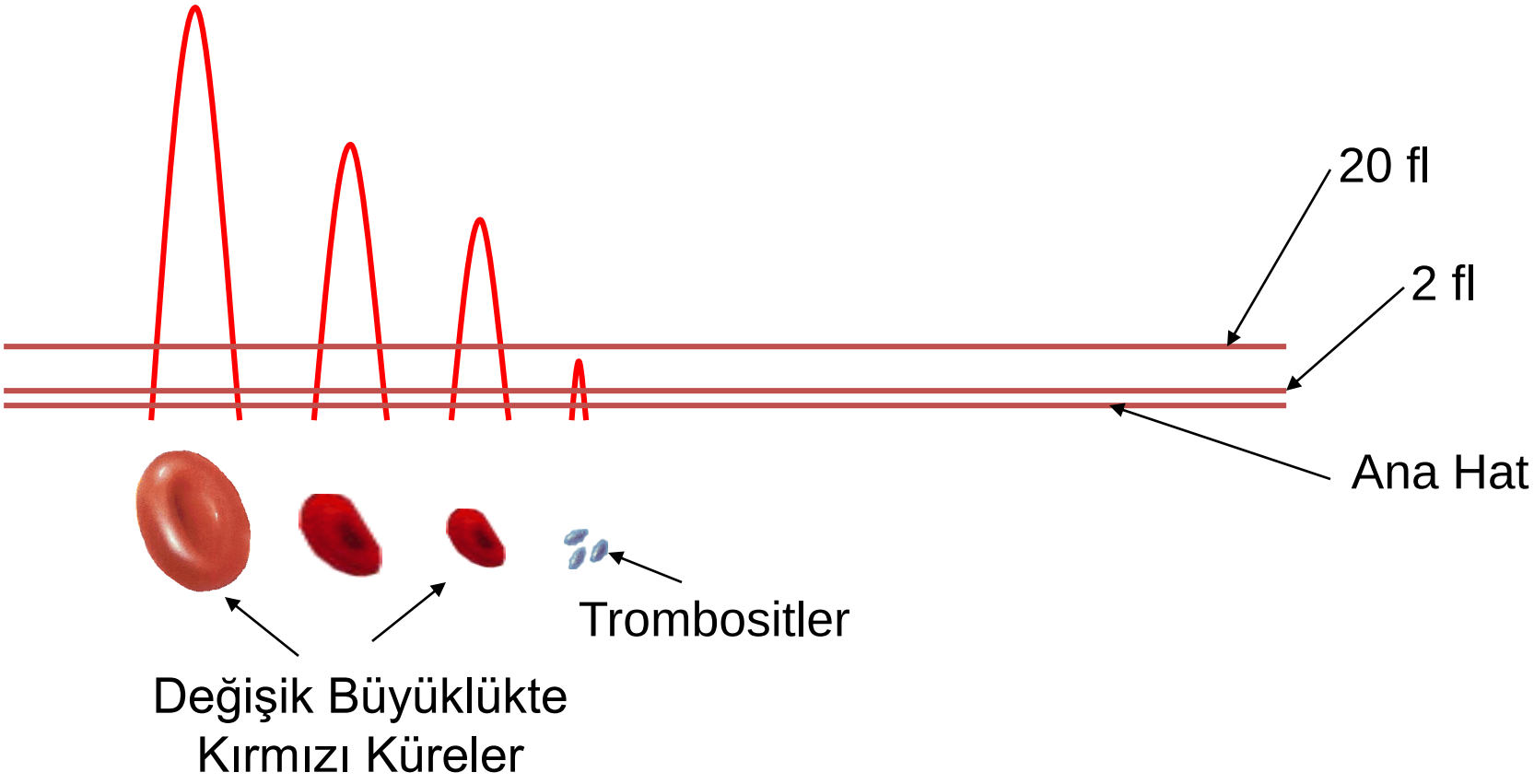
RBC histogram



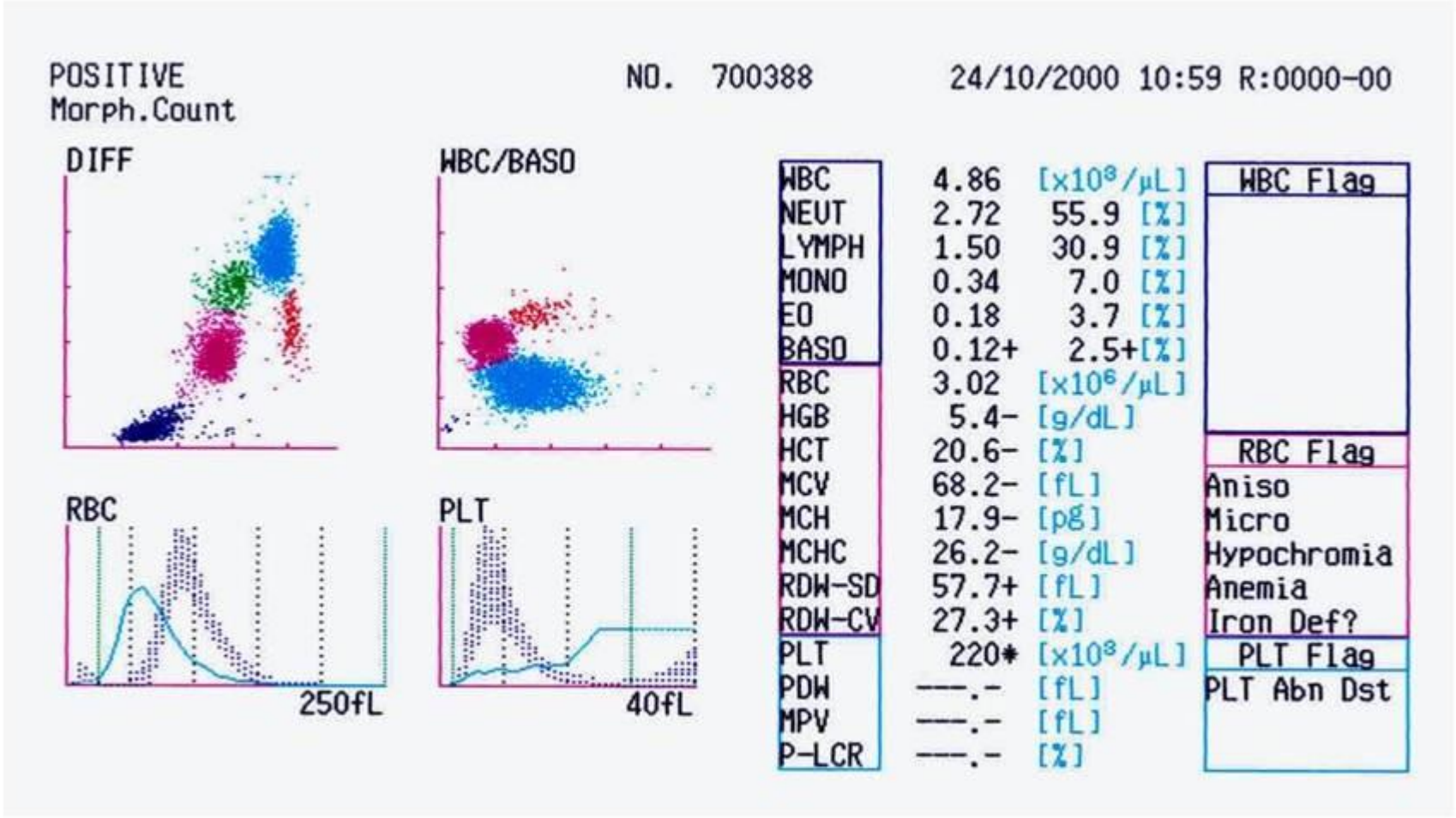

PLT histogram



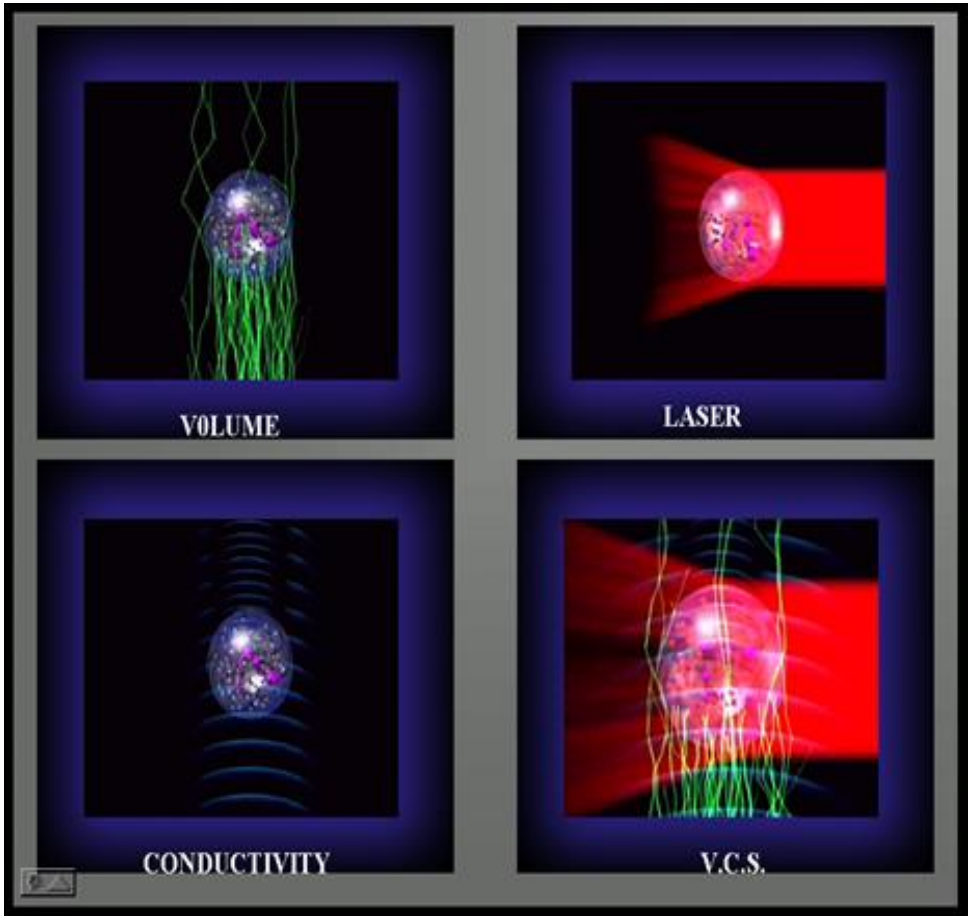
# Trombositlerin Eritrositlerin Arasından Seçilmesi

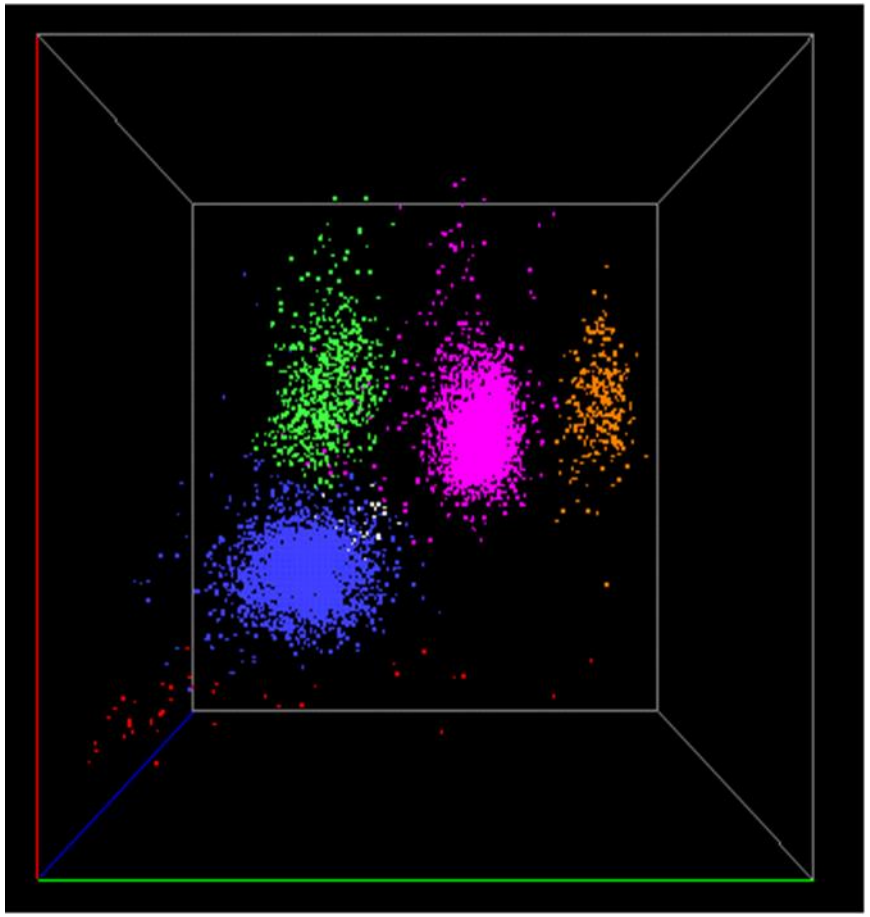


Küçük eritrositler (<50 fL) eritrosit histogramında yer almazlar, trombosit histogramına girebilirler. Bu durumda eritrosit sayısı düşüktür, daha önemlisi trombosit sayısı olduğundan daha yüksek bulunur .PY yapılmalı

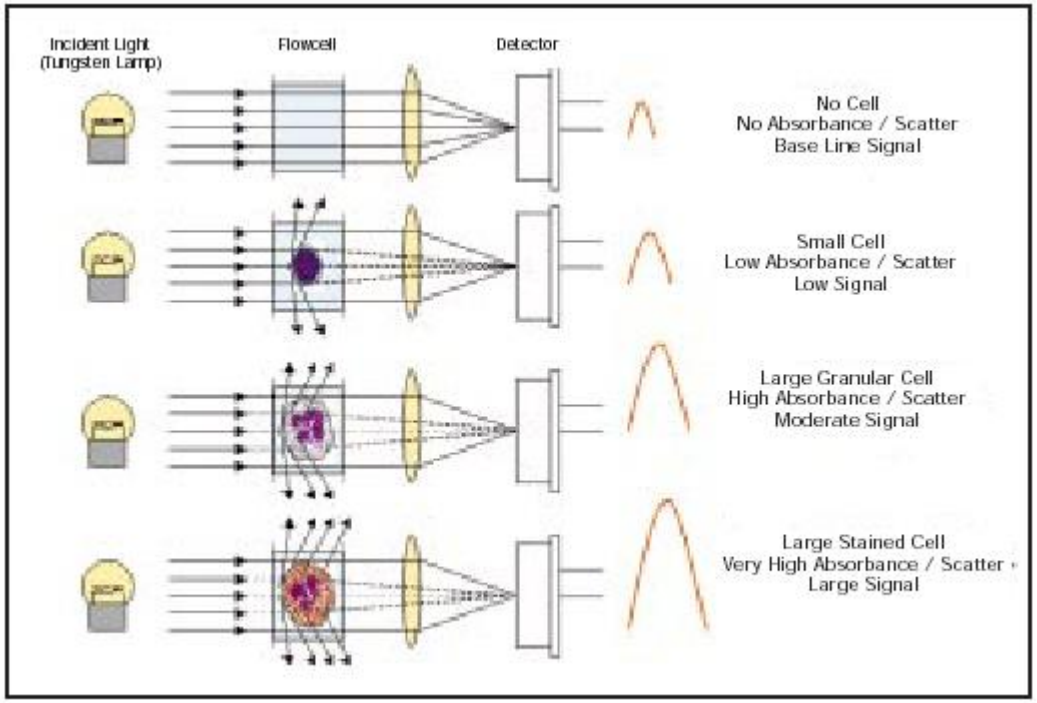


# VCS Teknolojisi



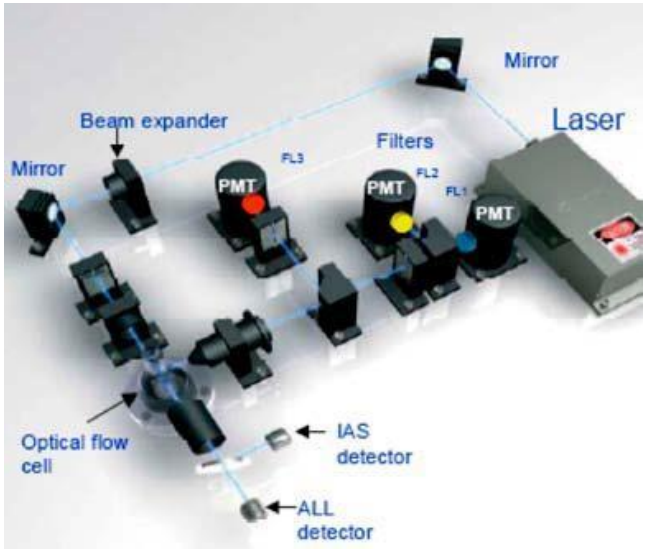



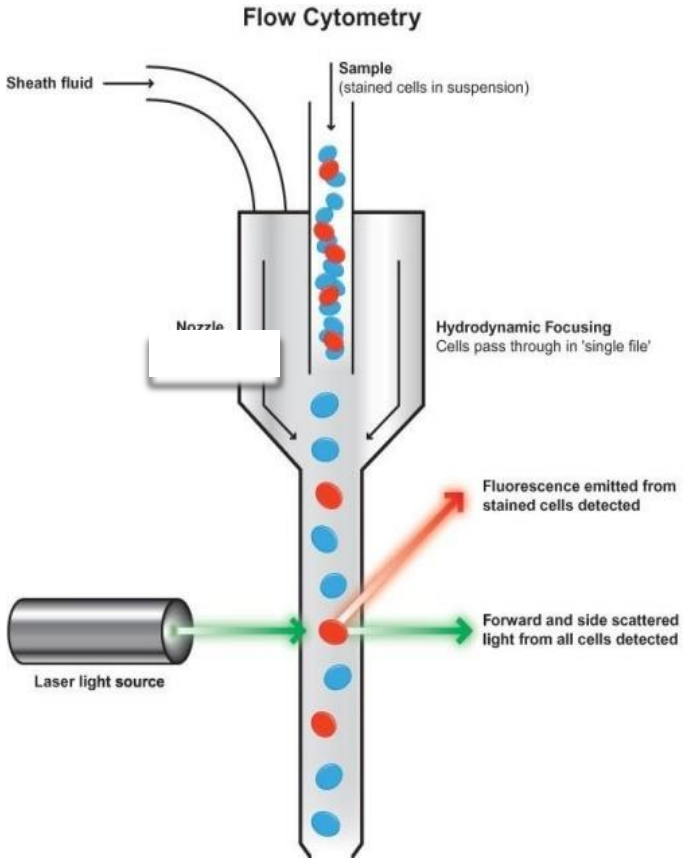
# Optik Sistem



# Optical light scatter and Fluorescence

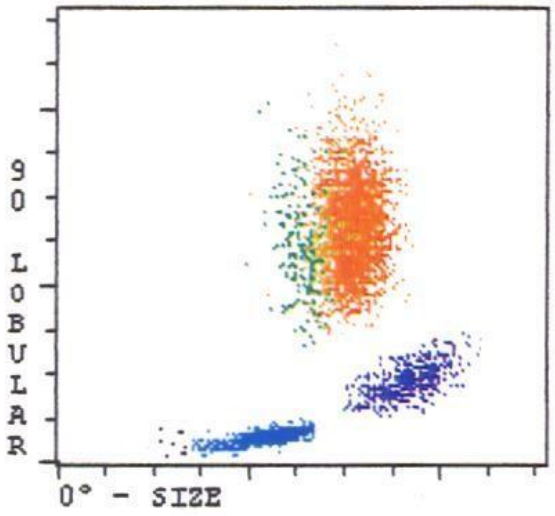
- WBC ,WBC diff, PLT , RBC .
- Laser light (farklı dalga boylarında) :flow cell' den gecerken hücreler büyüklük ve granülaritesine göre seçilir
- Floresan boyalarda kullanılabilir(e.g CD61, CD4, CD8, CD34)

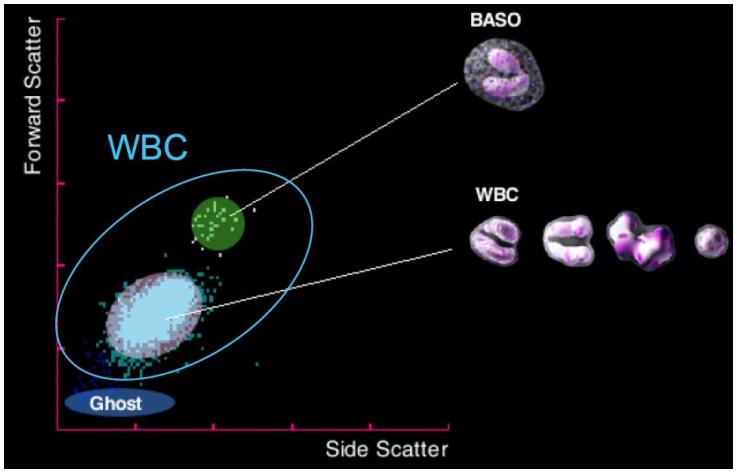


# Tam kan sayımı:

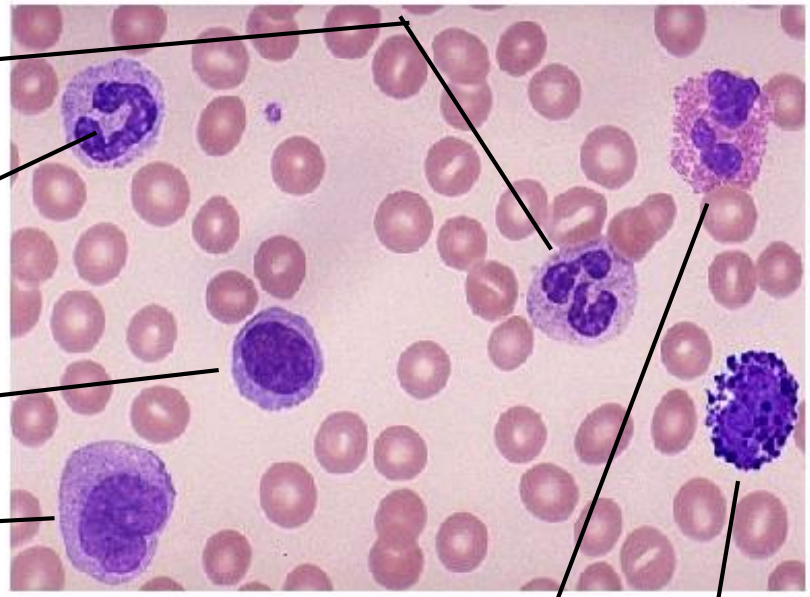
- Optical light scatter and Fluorescence
  - forward scatter (FSC) : büyüklük
  - side scatter (SCC): granülarite



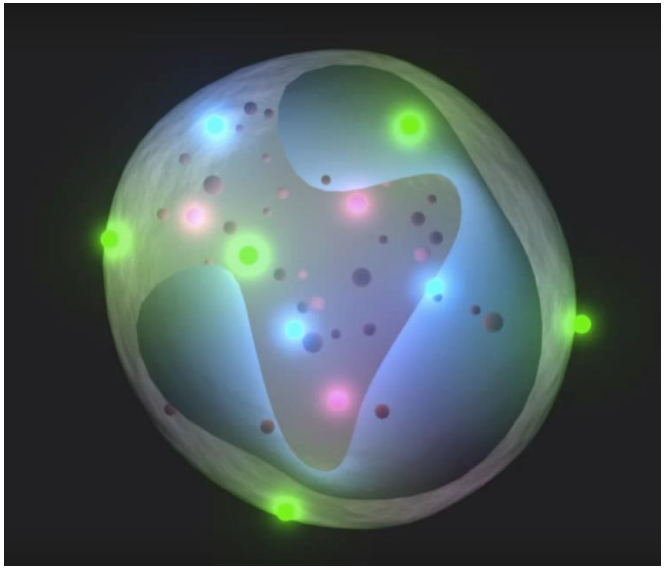



# Diff Ne?

- Nötrofiller – diğer isimleri
  - PMNs
  - granülosit
  - segs
  - bands or stabs (immatür nötrofil akut enfeksiyonu gösterir)
- Lenfositler
- Monositler
- Eozinofil
- Bazofil

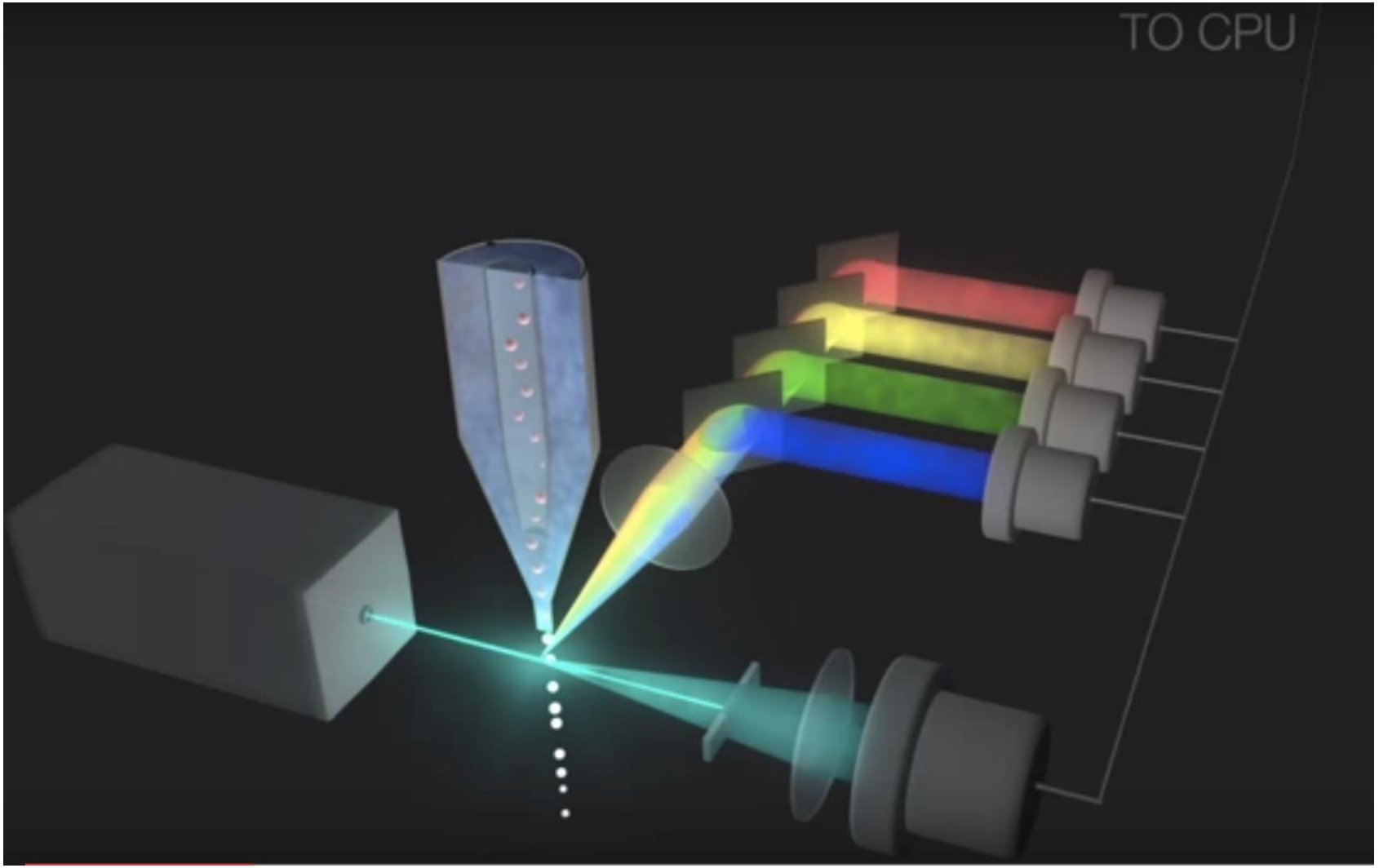


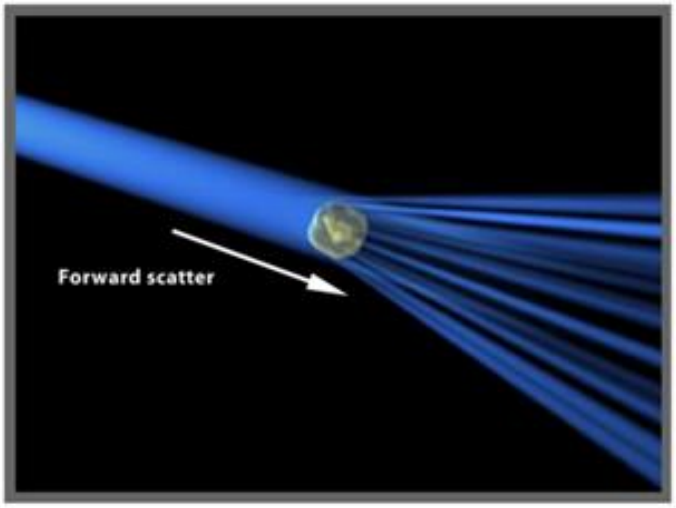
# Flow Cytometry

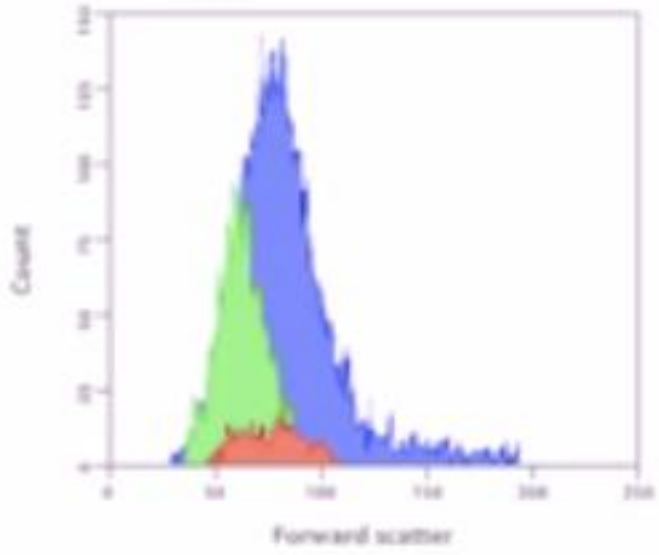


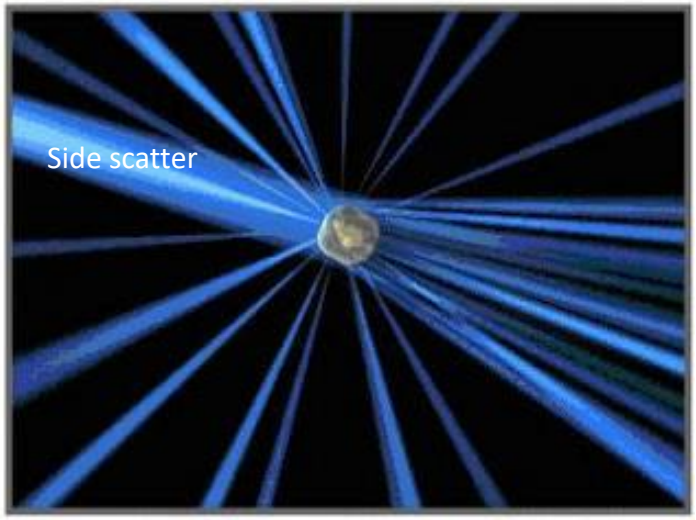

RICH!

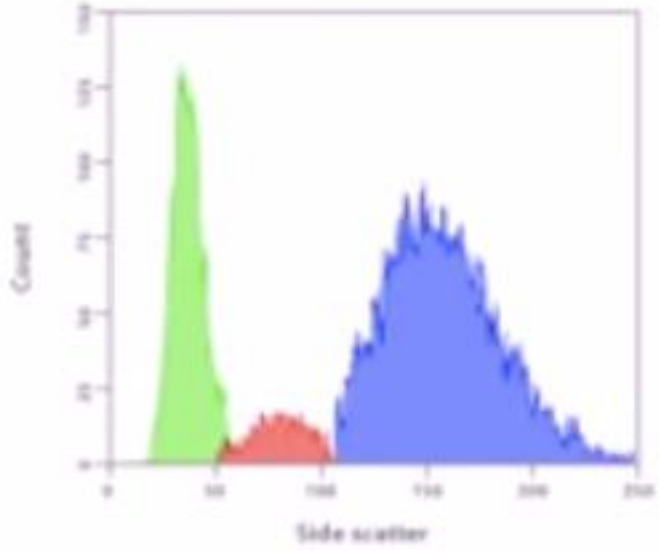
# KARAKUTU TEKNOLOJİSİ






- Büyüklük
- Granülarite
- Fenotip
- Sağlıklı mı?

# Klinik Kullanımı

Table 2. Clinical Applications of Flow Cytometry Immunophenotyping

| Disease | Diagnosis | Classification | Prognosis and/or Staging | Disease Monitoring |
|---|---|---|---|---|
| Acute Leukemias | Yes | Yes | Controversial | Yes |
| Chronic Lymphoproliferative Disorder (CLPD) | Yes | Yes | Yes | Yes |
| Myelodysplasia (MDS) | To be established | No | To be established | No |
| Paroxysmal Nocturnal Hemoglobinuria (PNH) | Yes | Yes | No | Yes |
| Mastocytosis | Yes | Yes | No | Yes |
| Primary Thrombocytopathies | Yes | Controversial | Yes | No |
| Primary Immunodeficiencies | Yes | Yes | No | No |
| HIV Infection | No | Yes | Yes | Yes |
| Transplantation Outcome | Yes | Yes | Yes | Yes |

# Hematoloji Laboratuvarı – Kalite programı

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


preanalitik değişkenler
kontrol edilemeyenler
fizyolojik
dolaşan antikorlar
ilaçlar
kontrol edilebilenler
hangi tüp?
örnek nasıl ve
nerden alınıcak?
turnike?
volüm?
taşıma?
stabilite?
santrifüj?
saklama?.....

CLINICAL AND LABORATORY STANDARDS INSTITUTE®

May 2007

# H42-A2

## Enumeration of Immunologically Defined Cell Populations by Flow Cytometry; Approved Guideline—Second Edition



This document provides guidance for the immunophenotypic analysis of non-neoplastic lymphocytes by immunofluorescence-based flow cytometry; sample and instrument quality control; and precautions for acquisition of data from lymphocytes.

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

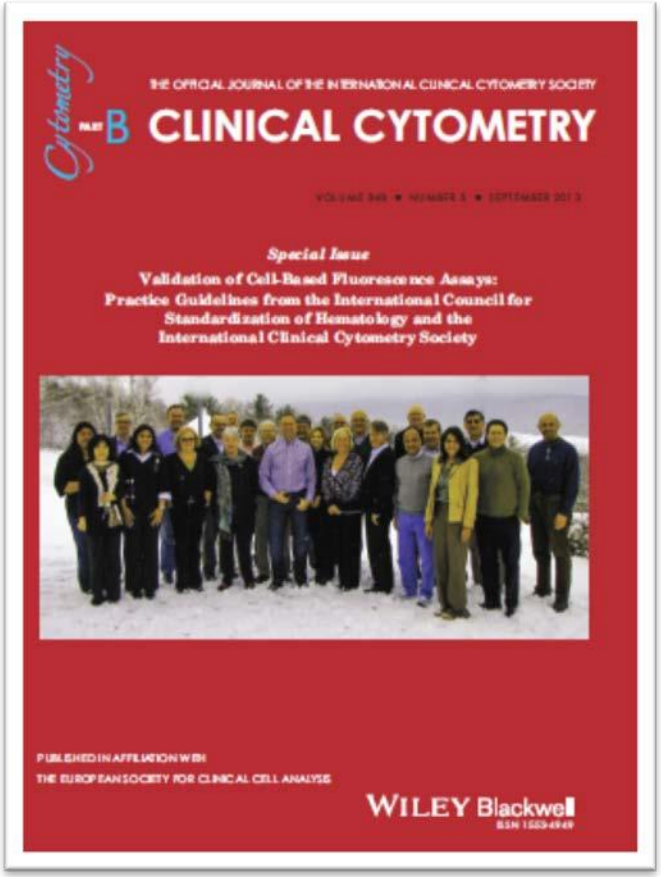
# ICSH/ICCS Recommendations

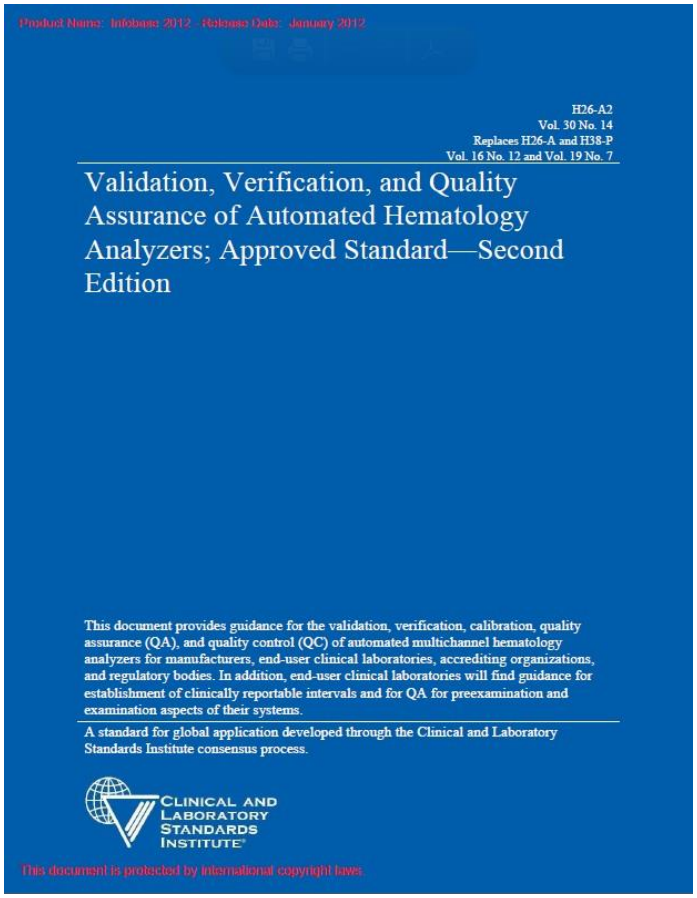
Cytometry Part B (Clinical Cytometry) 84B, 2013

*Special Issue*

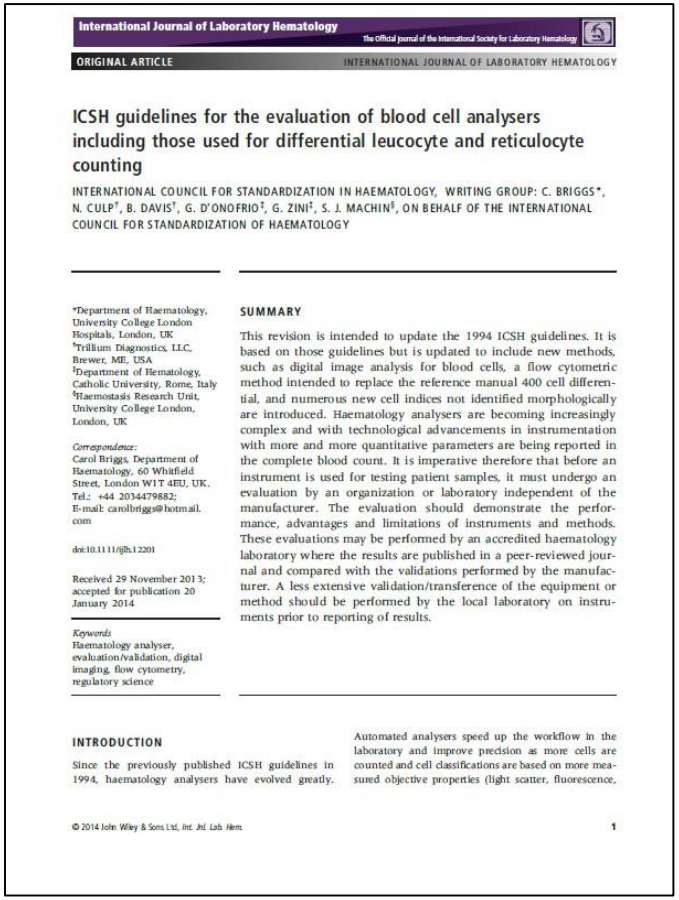
Validation of Cell-Based Fluorescence Assays: Practice Guidelines from the International Council for Standardization of Haematology and International Clinical Cytometry Society

Written by an ICSH/ICCS Workgroup






CLSI




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

Am J Clin Pathol. 1993 Oct;100(4):371-2.

# Recommendations of the International Council for Standardization in Haematology for Ethylenediaminetetraacetic Acid Anticoagulation of Blood for Blood Cell Counting and Sizing. International Council for Standardization in Haematology: Expert Panel on Cytometry.

[No authors listed]

**Abstract**

Of the three ethylenediaminetetraacetic acid (EDTA) salts used for anticoagulation of blood specimens for hematologic testing, potassium salts are the most readily soluble. Tripotassium EDTA is dispensed as a liquid and thus causes a slight dilution of the specimen. This salt also has been shown to affect the red blood cell size more at increased concentrations and on storage than the dipotassium salt. Therefore, dipotassium EDTA is recommended as the anticoagulant of choice in specimen collection for blood cell counting and sizing. The amount of dipotassium EDTA used is 1.5-2.2 mg (3.7-5.4 mumol) per milliliter of blood.

PMID: 8213631

[Indexed for MEDLINE]

**ICSH Kan Sayım : $K_2$EDTA**

EDTA tuzları (K3, K2, and Na3) PH ya bağlı olarak RBC etkiliyor

# FC örnekleri?

- Periferik Kan (tam kan sayımı; kapiller, venöz, arteryal)
- Kemik iliği
- Vücut sıvıları (BOS, periton sıvısı, BAL vb)
- Lenf nodu
- Taze doku (dalak vb.)
- Dondurulup çözündürülmüş hücreler
- Parafin blok
- Aferez ürün

# Hasta bilgileri

Kimlik bilgileri,

Ön tanı, yaş, cinsiyet,

Tedavi bilgileri (kemoterapi vb)

Örneğin alındığı tarih ve saat, ve ne örneği???

Doktor  adı

periferik kan/ kemik iliği aspirasyon örneği yayma sonucu!!! (FC için)

# Hangi antikoagülanlar :

(EDTA), heparin, asid sitrat dextroz (ACD) : periferik kan ve kemik iliği aspirasyon örneği için güvenilir

**EDTA**

Perifer kan örnekleri için uygun (morfoloji ve FC)

Dezavantajı kan bekledikçe ışık saçılım ozelliklerininin (FSC, SSC) bozulması,

Avantajı olgun miyeloid hücrelerin tüpün çeperine daha az yapışmasına neden olmasıdır. EDTA' li örnekler en geç 12-24 saat çalışılmalıdır!!

**HEPARİN**

Periferik yada Kİ yayma kalitesini etkilediği için tercih edilmez. Beyaz küre sayımlarının örnek alındıktan sonraki ilk 6 saat içinde yapılması gerekir.

heparinli örnekler ise en geç 48-72 saat içinde çalışılmalıdır.

# Uygun örnek alımı ve saklama şartları

- Örneğin alınış zamanı, kalitesi mutlaka değerlendirilmeli
- Analiz öncesi mutlaka morfolojik değerlendirme yapılmalı, pıhtılı örnek reddedilmeli
- Dokular SF içinde muhafaza edilmeli
- BOS, BAL antikoagülansız tüpe alınır
- Örnek saklanacaksa BOS +4 $^{o}$C ‘ de transfix’ le 24-48 h yada oda ısısında saklanabilir, dondurulmaz!!
- Diğer vücut sıvıları için saklama yada transfix önerilmez
- Doku örneği +4 $^{o}$C ‘ de, SF ile 24h saklanabilir
- PB/Kİ: LPD için oda ısısında 48h, B bücre klonalite takibi için +4 $^{o}$C ‘’72 h

  myeloid, monositik parametre için 48 h

High grade lenfomalarda bekletilmeden analiz

# Örneklerin taşınması:

Polipropilen tüp/enjektör  kullanılmalı!

hücrelerin tüplerin çeperine yapışabilmesi nedeniyle polisteren malzemenin kullanılmaması gerekir.

Örnekler oda ısısında taşınmalıdır. Beklenmesi zorunlu olan durumlarda örnekler +4°C de saklanabilir (tercihan yatay durumda).

**Table 1:** Pre-analytical variables influencing flow cytometric tests.

| Influencing factor | Variable | Problem |
|---|---|---|
| Pre-analytics | | |
| Indications | Antibody panel | Correct question regarding present health problem? |
| Sample collection | | |
| Sample material | Such as peripheral blood, bone marrow, CSF, puncture fluids | Proper and sufficient material collected for the question? Is the material representative? |
| Anticoagulant | EDTA[a], heparin, citrate, ACD[b] | Loss of vitality (EDTA), cell aggregation (heparin), dilution artifacts (ACD). |
| Sample transport and sample storage | Duration, temperature, vibration, sample preparation prior to analysis, e.g. sample-stabilizing reagents | Decreased vitality of the cells, selective cell loss, increased autofluorescence, cell aggregation. |
| Sample preparation | Such as centrifugation, temperature, transferring, processing of solid tissues to cell suspensions | Decreased vitality of the cells, selective cell loss, increased autofluorescence, cell aggregation, cell contamination. |

[a]EDTA, Ethylene diamine tetra-acetic acid; [b]ACD, Acid Citrate Dextrose.

Klinik bilgi ?
Örnek uygun alınıp taşınmış mı?
Örnek red ve kabul kriterleri net değil!
Uygun olmayan örnek?

J Lab Med 2016; aop

# FC Örnek Kabulü:

- Tüm örnekler gözle değerlendirirlmelidir.
- Hemoliz: eritrositlere hasar veren bir işlemin lökositlere de zarar vermiş olma olasılığı yüksektir.
- Pıhtılı kan/Kİ aspirasyon örneği: Ufak pıhtılar hücre kaybına sebep olsa da hedef hücrelerin yeterince bulunması ve yeni örnek almanın mümkün olmaması durumunda çalışma sürdürülebilir. Gözle görülür belirgin pıhtıların bulunması durumunda örnek red edilmelidir.
- Eksik miktarda örnek alınması: antikoagülan miktarının istenenden fazla olması hücrelerde hipertonik koşullardan kaynaklanan hasara sebep olabilir. Değerlendirirken bunu gözönünde tutmak gerekir.
- Laboratuvara gelen örneklerin mutlaka ısısı kontrol edilmelidir(donma/aşırı ısınma?). Anormal bir durum varsa mutlaka kaydedilmelidir. Gelen örnekten mutlaka morfolojik değerlendirme için yayma da yapılmalıdır.

# Tam Kan Örnek kabulü

- Laboratuvar kabul kontrolü (SOP’ de tanımlanmış)
  - kantite,
  - kalite
  - barkod,
  - istem,
  - Pıhtı, eksik , hemoliz, fazla vb.
  - Uygun sürede (2 saat) içinde lab’ a gelmemiş, aşırı ısınmış/donmuş örnek

**Uygun olmayan örnek reddedilir**

# Kan antikoagülanla çok iyi karıştırılmalı

Pıhtılı örnekten kaçınılmalı

Antikoagülanlı tüpler

(K2EDTA)

Tüp iyice karıştırılmalı
8-10 defa alt üst edilmeli

- Örneğe bağlı Preanalitik hatalar

**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 | | | |
| Monoctyosis | | | |
| 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 | | |

Preanalitik evre!!!!!!!!

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

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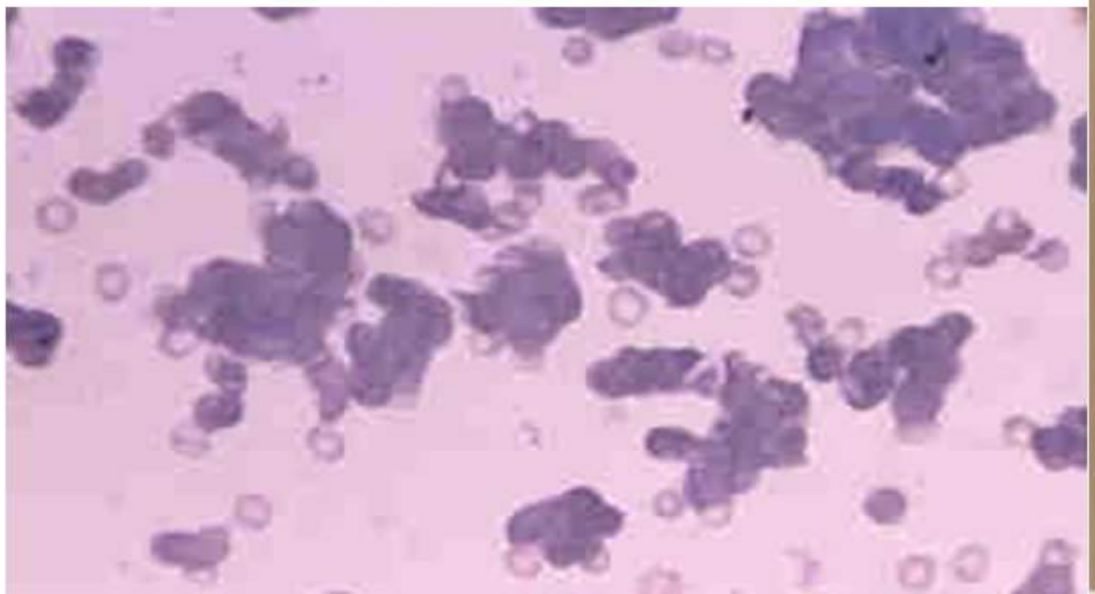
# Soğuk aglutinin

Abnormal RBC Pop

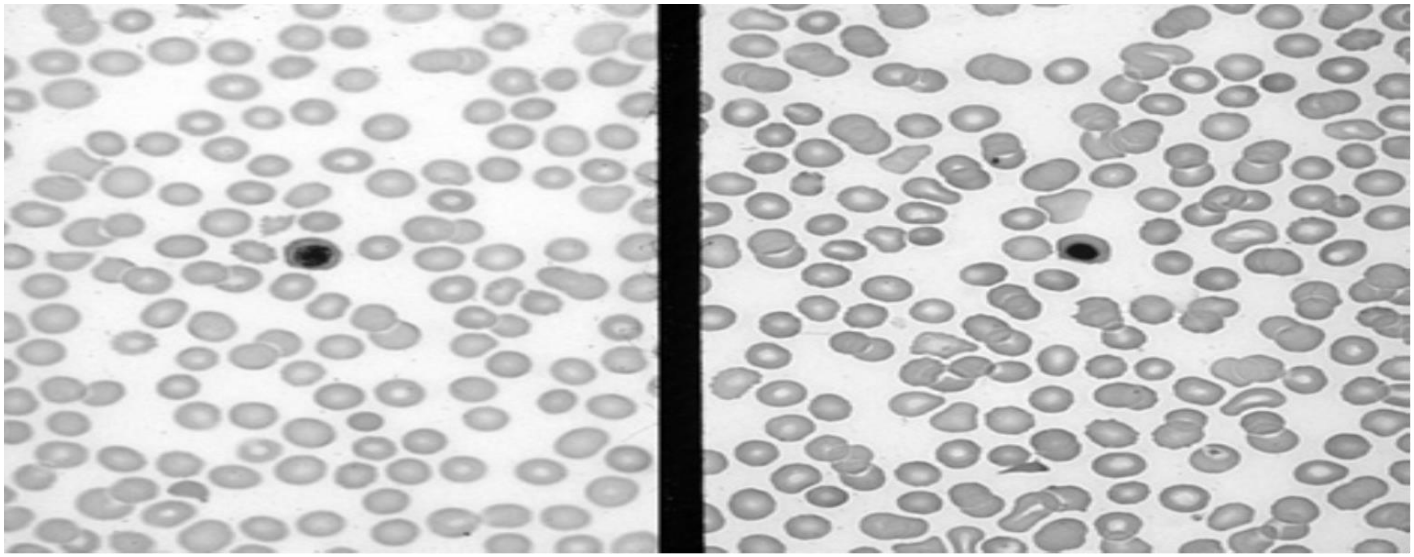
| | | |
|---|---|---|
| RBC | 2.23 | *V |
| HGB | 9.9 | L |
| HCT | 21.8 | VL |
| MCV | 97.9 | H |
| MCH | 44.4 | VH |
| MCHC | 45.4 | VH |
| RDW | 20.5 | RH |

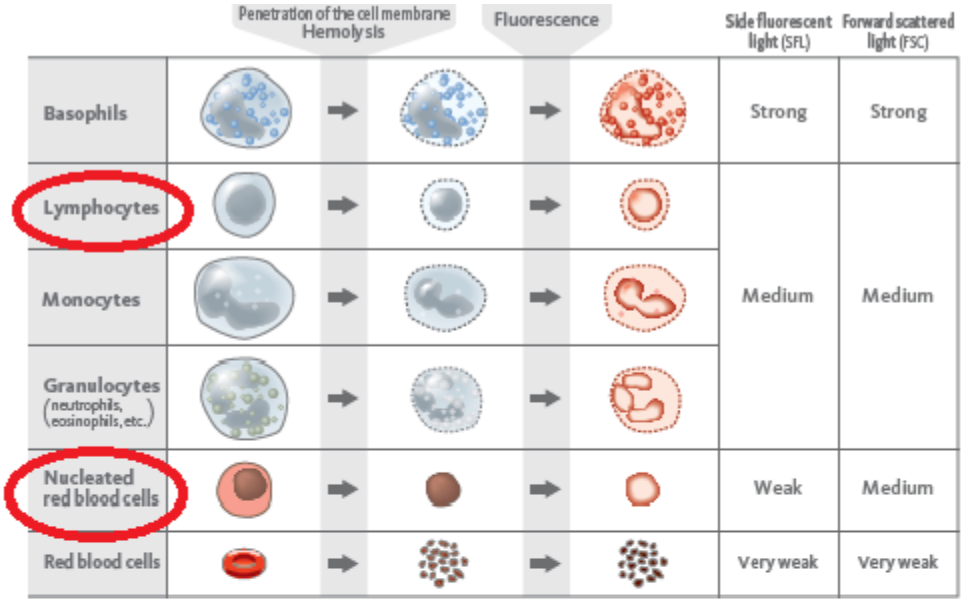
SUSPECT FLAGS:
NRBCs
Dimorphic RBC Pop



# **NRBC:** Tanı ???

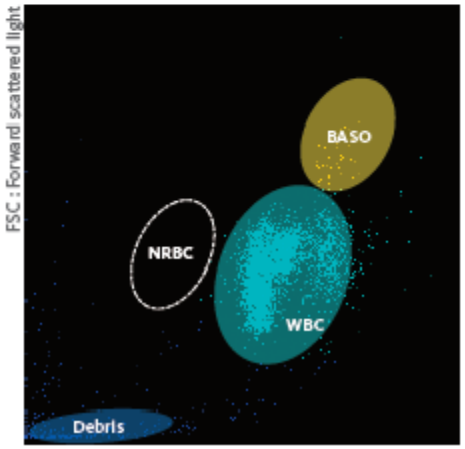
WBC yalancı yükseklik/ lenfositoz






※ This is a conceptual drawing.

## WNR scattergram



# Kan Sayım

- **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

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# Kan Sayım

- **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

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# Kan Sayım

- **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

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# 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 x 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 ?, anlaşamıyorlarsa, 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

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EDITORIAL
INTERNATIONAL JOURNAL OF LABORATORY HEMATOLOGY

# Stability of complete blood count parameters with storage: toward defined specifications for different diagnostic applications

RBC, WBC, PLT , Hb, MCH, MCHC için K2 EDTA 4 mg/dL den az olmalı 24 saat saklanabilir

RBC oda ısısında 6 saat stabil (fragmantasyon)

WBC sayımı için 4 °C en az 24 saat stabil / kan sayım cihazına bağlı olarak 72 saate kadar uzayabilir? Monosit ↑, eozinofil, lenfosit ↓ nötrofil stabil (apopitoz)

PLT' ler 4 °C ' de 24-72 ? hafif ↓

MCV 1-3 saat içinde etkilenir

24 saatten fazla bekleyen örnekte NRBC yok olur

**Beklemiş örnekten çalışıldı ise raporda mutlaka belirtilmeli!!!!**

PLT parametreleri EDTA dan dolayı beklemiş örnekte sağlıklı değerlendirilemez

Periferik yayma için oda ısısında (18–25 °C) 1 saatten fazla beklememeli hemen yapılmalı (apopitoz). EDTA konsantrasyonuna bağlı 12-18 saatte morfolojik değişim?

4 °C de 8 saat

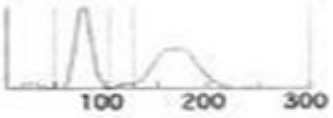
2008 World Health Organization Hematolojik malignensilerin tanısı için 2 saatten fazla antikoagülana maruz kalan PY displazileri değerlendirmek için uygun değil. Taze PY!!!!!!!!!!!!

# Taze ve 24 saat oda ısısında beklemiş örnek

ID.
1RT
Date
Time
Mode WB

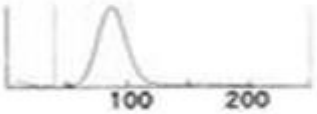
| | | | |
|---|---|---|---|
| WBC | | 4.4 | ×10³/μL |
| RBC | | 5.38 | ×10⁶/μL |
| HGB | | 15.8 | g/dL |
| HCT | | 45.7 | % |
| MCV | - | 84.9 | fL |
| MCH | | 29.4 | pg |
| MCHC | | 34.6 | g/dL |
| PLT | | 287 | ×10³/μL |

WBC



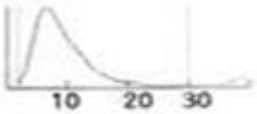

| | | | |
|---|---|---|---|
| LYM% | + | 42.2 | % |
| MXD% | - | 2.3 | % |
| NEUT% | | 55.5 | % |
| LYM# | | 1.9 | ×10³/μL |
| MXD# | - | 0.1 | ×10³/μL |
| NEUT# | | 2.4 | ×10³/μL |

RBC




| | | |
|---|---|---|
| RDW-SD | 38.8 | fL |
| RDW-CV | 12.6 | % |

PLT



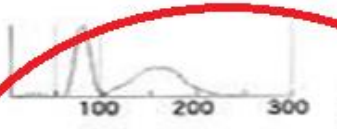

| | | |
|---|---|---|
| PDW | 12.0 | fL |
| MPV | 10.6 | fL |
| P-LCR | 28.7 | % |

Operator

ID.
1-2RT
Date
Time
Mode WB

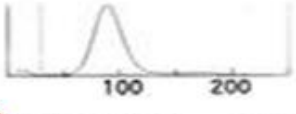
| | | | |
|---|---|---|---|
| WBC | | 4.3 | ×10³/μL |
| RBC | | 5.32 | ×10⁶/μL |
| HGB | | 15.5 | g/dL |
| HCT | | 46.3 | % |
| MCV | | 87.0 | fL |
| MCH | | 29.1 | pg |
| MCHC | | 33.5 | g/dL |
| PLT | | 277 | ×10³/μL |

WBC



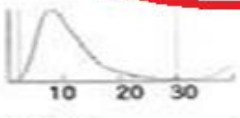

| | | | |
|---|---|---|---|
| LYM% | + | 41.6 | % |
| MXD% | T2 | ----.- | % |
| NEUT% | T2 | ----.- | % |
| LYM# | | 1.8 | ×10³/μL |
| MXD# | T2 | ----.- | ×10³/μL |
| NEUT# | T2 | ----.- | ×10³/μL |

RBC




| | | |
|---|---|---|
| RDW-SD | 41.9 | fL |
| RDW-CV | 13.2 | % |

PLT




| | | |
|---|---|---|
| PDW | 13.7 | fL |
| MPV | 12.1 | fL |
| P-LCR | + 41.2 | % |

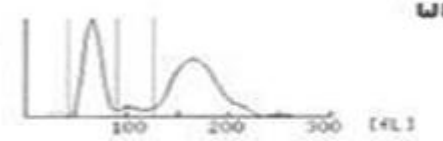
Operator

# İyi karışmış ve karışmamış örnek

No. 3
Date
Time
Mode WB

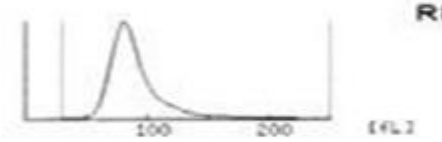
| | |
|---|---|
| WBC | 4.1×10³/μL |
| RBC | 5.11×10⁶/μL |
| HGB | 15.7g/dL |
| HCT | 43.6% |
| MCV | 85.3fL |
| MCH | 30.7pg |
| MCHC | 36.0g/dL |
| PLT | 267×10³/μL |

WBC




| | |
|---|---|
| LYM% | 37.6% |
| MXD% | 5.1% |
| NEUT% | 57.3% |
| LYM# | 1.5×10³/μL |
| MXD# | 0.2×10³/μL |
| NEUT# | 2.4×10³/μL |

RBC




| | |
|---|---|
| RDW_SD | 37.9fL |
| RDW_CV | 12.2% |

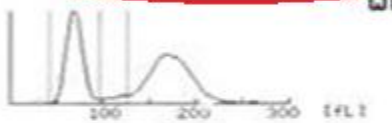
PLT


| | |
|---|---|
| PDW | 12.6fL |
| MPV | 9.7fL |
| P_LCR | 24.1% |

No. 3
Date
Time
Mode WB

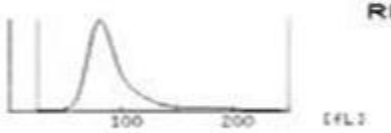
| | | |
|---|---|---|
| WBC | | 4.2×10³/μL |
| RBC | ! | 8.11×10⁶/μL |
| HGB | | +++.+g/dL |
| HCT | ! | 69.4% |
| MCV | | 85.6fL |
| MCH | | 31.2pg |
| MCHC | + | 36.5g/dL |
| PLT | - | 83×10³/μL |

WBC




| | | |
|---|---|---|
| LYM% | + | 40.9% |
| MXD% | | 3.7% |
| NEUT% | | 55.4% |
| LYM# | | 1.7×10³/μL |
| MXD# | | 0.2×10³/μL |
| NEUT# | | 2.3×10³/μL |

RBC

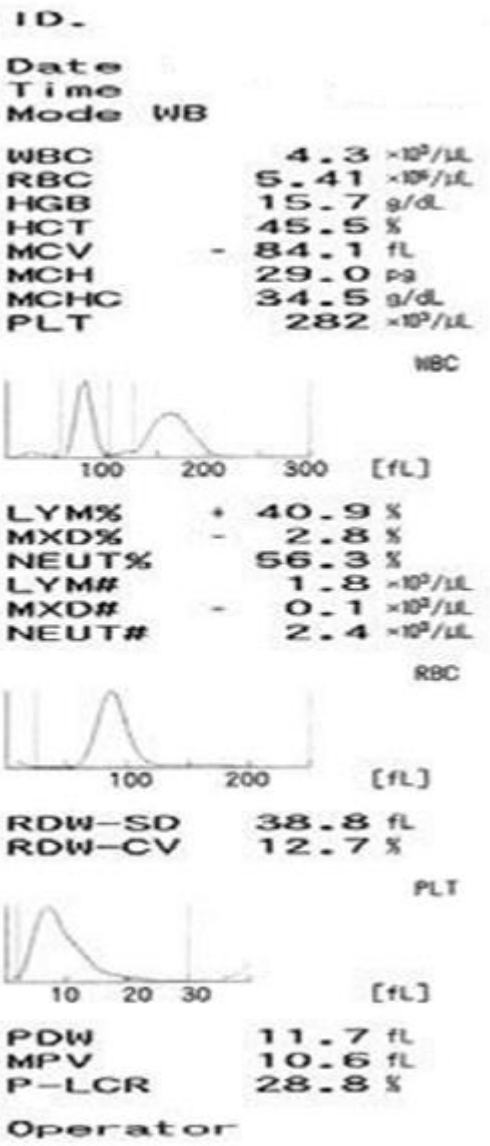


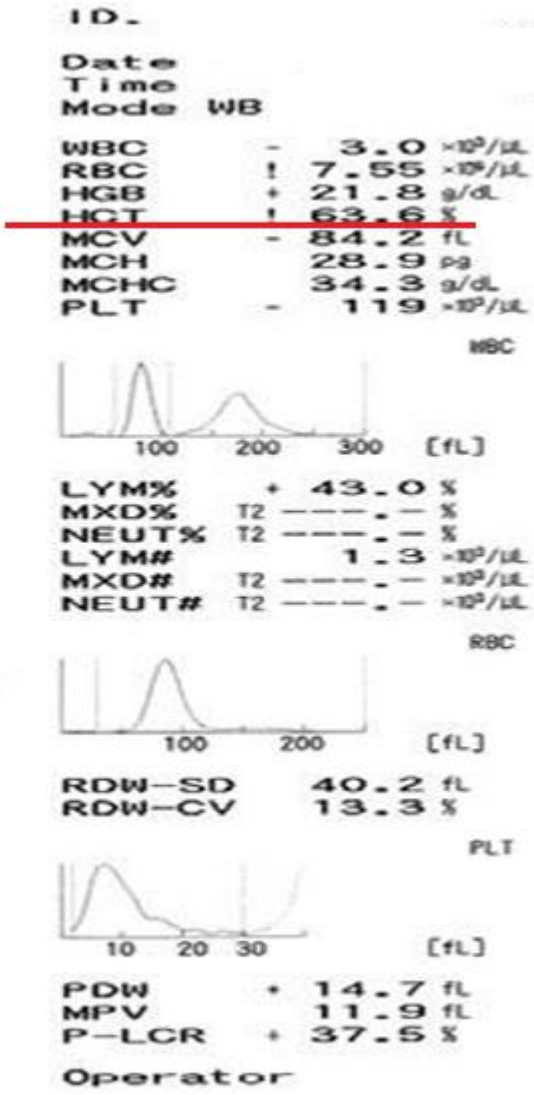

| | |
|---|---|
| RDW_SD | 41.8fL |
| RDW_CV | 13.2% |

PLT


| | | |
|---|---|---|
| PDW | + | 15.4fL |
| MPV | | 11.1fL |
| P_LCR | | 32.9% |

# İyi karışmış ve karışmamış örnek



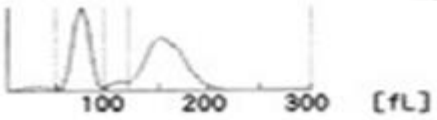
# İyi aspire eddilmemiş örnek

ID. 6

Date
Time
Mode WB

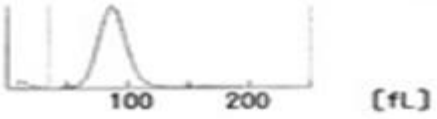
| | | | |
|---|---|---|---|
| WBC | - | 2.2 | ×10³/μL |
| RBC | - | 2.69 | ×10⁶/μL |
| HGB | - | 7.6 | g/dL |
| HCT | - | 23.0 | % |
| MCV | | 85.5 | fL |
| MCH | | 28.3 | pg |
| MCHC | | 33.0 | g/dL |
| PLT | - | 129 | ×10³/μL |

WBC




| | | | |
|---|---|---|---|
| LYM% | | 39.3 | % |
| MXD% | | 4.1 | % |
| NEUT% | | 56.6 | % |
| LYM# | - | 0.9 | ×10³/μL |
| MXD# | - | 0.1 | ×10³/μL |
| NEUT# | - | 1.2 | ×10³/μL |

RBC




| | | | |
|---|---|---|---|
| RDW-SD | - | 36.9 | fL |
| RDW-CV | | 12.0 | % |

PLT

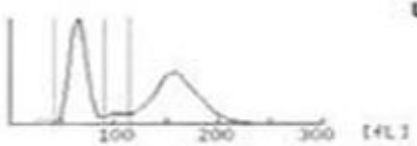

| | | |
|---|---|---|
| PDW | 11.6 | fL |
| MPV | 10.7 | fL |
| P-LCR | 29.6 | % |

Operator

No. 6
Date
Time
Mode WB

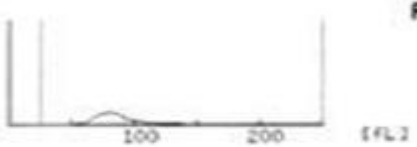
| | | | |
|---|---|---|---|
| WBC | - | 3.5×10³ | /μL |
| RBC | ! | 0.09×10⁶ | /μL |
| HGB | | 13.6 | g/dL |
| HCT | ! | 0.7 | % |
| MCV | | ---.- | fL |
| MCH | | +++.+ | Pg |
| MCHC | | +++.+ | g/dL |
| PLT | ! | 1×10³ | /μL |

WBC




| | | | |
|---|---|---|---|
| LYM% | + | 42.0 | % |
| MXD% | | 4.4 | % |
| NEUT% | | 53.6 | % |
| LYM# | | 1.5×10³ | /μL |
| MXD# | | 0.2×10³ | /μL |
| NEUT# | - | 1.8×10³ | /μL |

RBC




| | |
|---|---|
| RDW_SD | ---.- fL |
| RDW_CV | ---.- % |

PLT


| | |
|---|---|
| PDW | ---.- fL |
| MPV | ---.- fL |
| P_LCR | ---.- % |