Basic Hematology/ Auto Methods

AMY MILLER MLS (ASCP)CM

Today's Discussion

- What is Hematology?
- Common Hematology Test
- Preparing a Peripheral Smear
- Automated Methods
- Quality Assurance in Hematology

What is Hematology

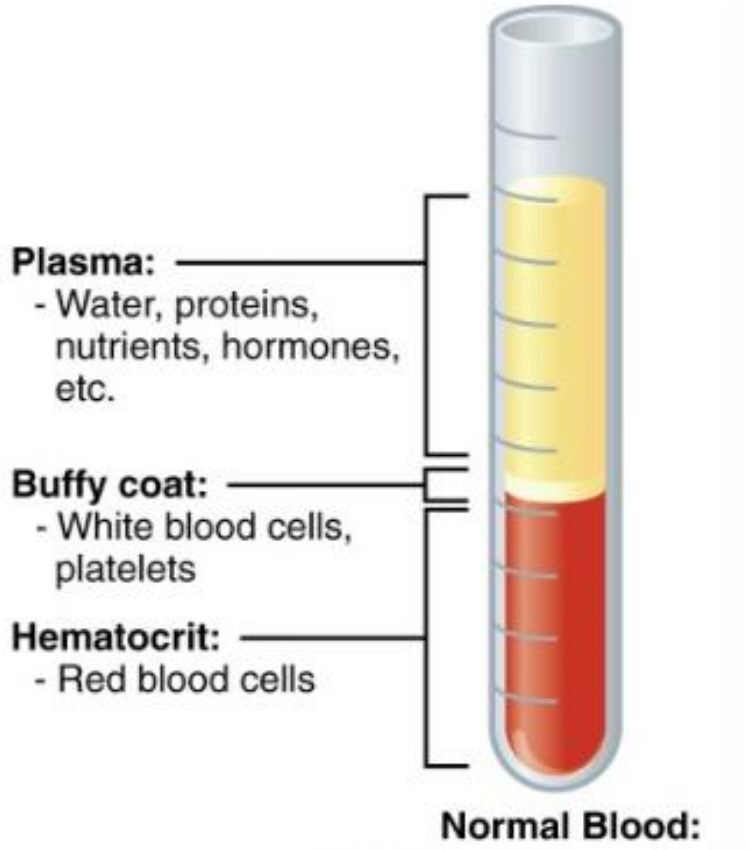
- Study of blood cells and blood disorders

- These are studied in order to predict, detect, and diagnose blood diseases and many systemic diseases that affect blood cells

- 3 categories of blood cells:
  - Red blood cells (RBCs)
  - White blood cells (WBCs)
  - Platelets (PLTs)

Blood Components

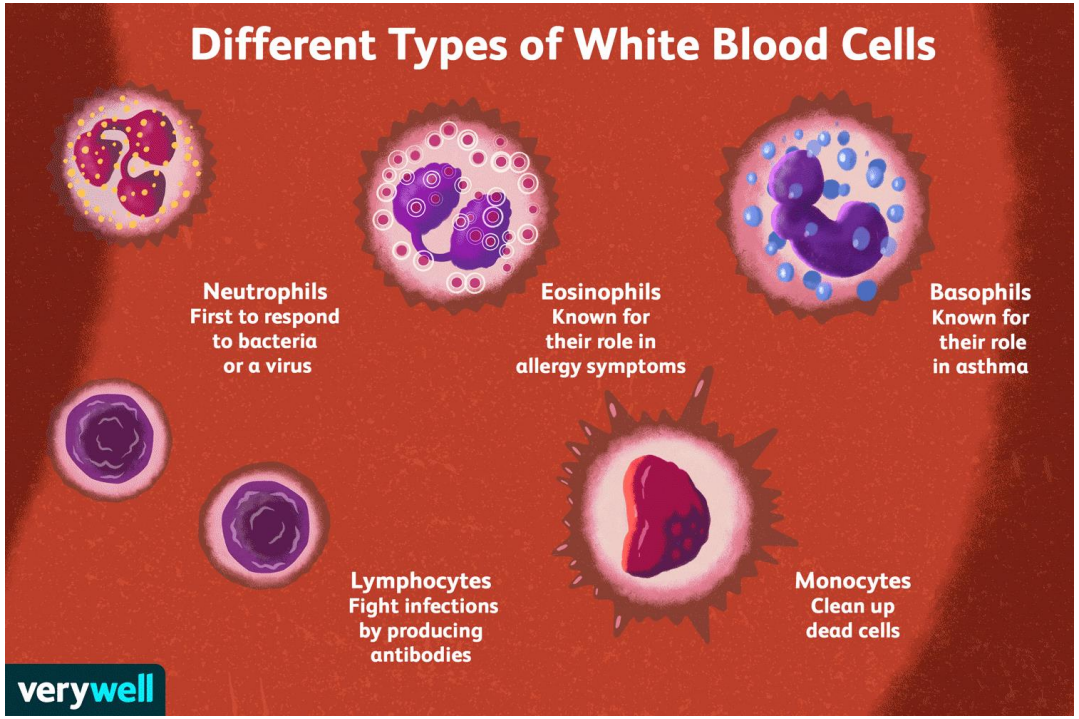
- Blood consists of plasma and formed elements
- 3 distinct layers found in blood when drawn in a tube anticoagulant present
  - **Plasma**
    - Makes up 55% of blood
    - Straw color
    - Contains cellular elements and dissolved substances
  - **Buffy coat**
    - <1% of whole blood sample
    - Contains WBCs and platelets
  - **Erythrocytes**
    - Packed RBCs



https://courses.lumenlearning.com/suny-ap2/chapter/an-overview-of-blood/

White Blood Cell

- Cell type dedicated to protecting the host from infection and injury
- Created in the bone marrow or lymphoid tissue the transported in the blood to the tissue where it is needed
- Types of WBCs found in peripheral blood of a healthy individual
  - Neutrophil
  - Eosinophil
  - Basophil
  - Lymphocyte
  - Monocyte

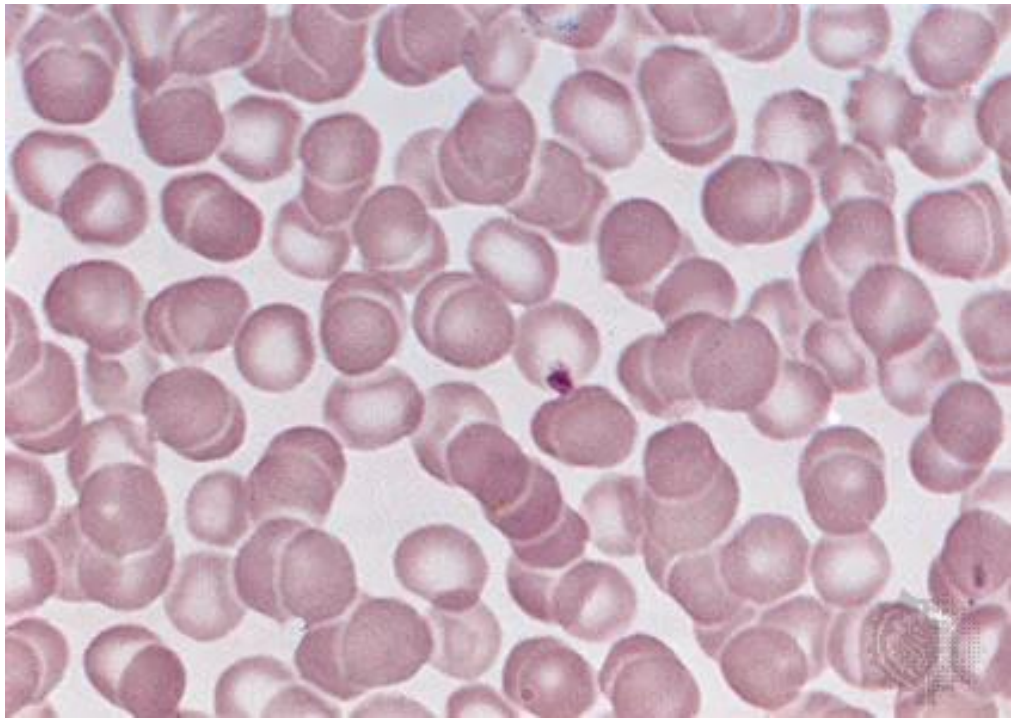


https://www.verywellhealth.com/understanding-white-blood-cells-and-counts-2249217

Red Blood Cell

- Anucleated, biconcave, discoid cells filled with reddish protein, and hemoglobin
- Transports oxygen and carbon dioxide
- Appearance
  - Salmon pink
  - 7-8 µm in diameter
  - Biconcave zone of pallor
    - 2/3 of the center

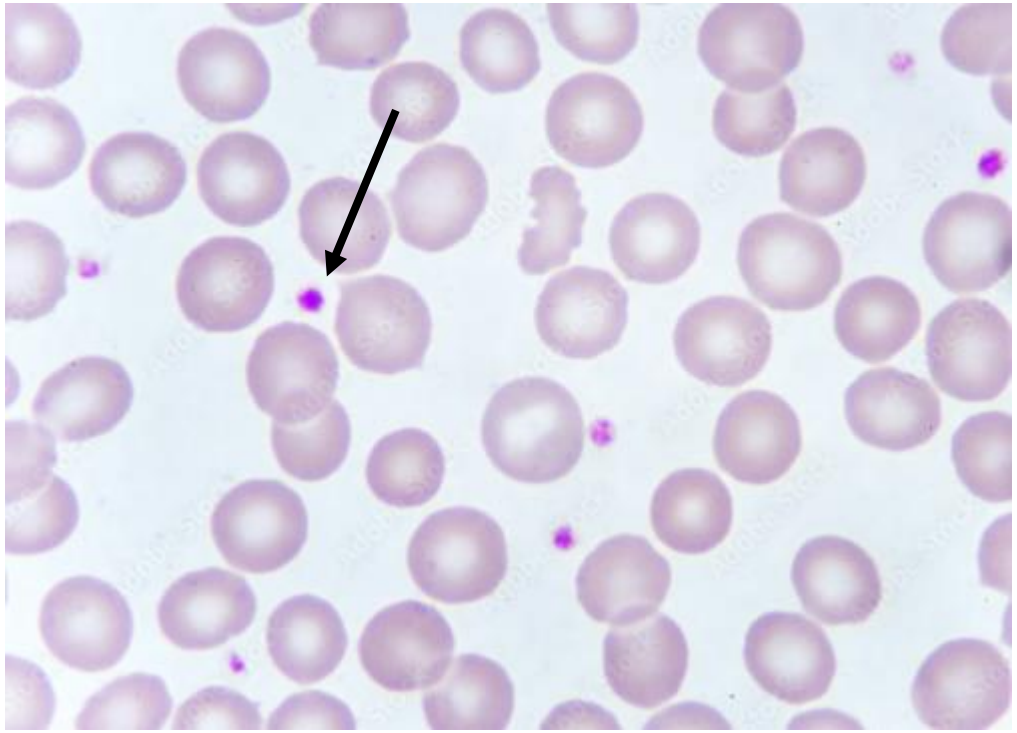
120 days in circulation



https://www.britannica.com/science/red-blood-cell

Platelet

- Thrombocytes
- Small fragments of cytoplasm derived from megakaryocytes
- Blood cells that maintain blood vessel integrity by initiating vessel wall repairs
- Rapidly adhere to damaged blood vessel and forms a platelet plug with neighboring platelets and release small molecules that trigger thrombosis or clot formation
- Appearance:
  - 2-4 µm
  - Round or oval, anucleated
  - “cell fragments”
  - Slightly granular



https://stock.adobe.com/images/red-blood-cells-and-platelet-in-blood-smear-analyze-by-microscope/224379374

Specimen Collection

- Most routine hematology tests require a whole blood collected in tube with an anticoagulant to prevent clotting
  - **Ideal tube: EDTA anticoagulant tube**
    - Preserves the stain and morphology of cells
- Sodium citrate is an alternate for platelet clumping or platelet satellitism on EDTA specimen
- Specimen stability
  - 48 hr. stored at 2-8 C°
  - 24 hr. stored at room temp
    - May exhibit an increase in MCV after 24 hours
- Other tube type collection
  - Sodium citrate: Shrinks cells, used for Platelet and coagulation testing
  - Heparin: natural anticoagulant in the body, may interfere with staining of cells and cause WBC clumping

Specimen Rejection

- Specimens containing clots and/or fibrin strands
  - Visually check specimens prior to testing
- Grossly hemolyzed samples
- Samples drawn above the IV line
- Mislabeled specimens

https://tidsskriftet.no/en/2021/10/images-medicine/brown-serum

Common Tests in Hematology

Complete Blood Count

- Very common test that is ordered to analyze the blood cells
- Includes:
  - Enumeration of cellular elements (RBC, WBC, PLT)
  - Quantitation of hemoglobin (HGB) and hematocrit (HCT)
  - Statistical analyses which provides a snapshot of cell appearances
- Can be tested through automatic or manual methods

BOX 1.1

Basic Complete Blood Count Measurements Generated by Automated Blood Cell Analyzers

RBC Parameters
RBC count
HGB
HCT
MCV
MCH
MCHC
RDW
RETIC
WBC Parameters
WBC count
NEUT count: % and absolute
LYMPH count: % and absolute
MONO count: % and absolute
EO and BASO counts: % and absolute
Platelet Parameters
PLT count
MPV

BASO, Basophil; *EO,* eosinophil; *HGB,* hemoglobin; *HCT,* hematocrit; *LYMPH,* lymphocyte; *MCH,* mean cell hemoglobin; *MCHC,* mean cell hemoglobin concentration; *MCV,* mean cell volume; *MONO,* monocyte; *MPV,* mean platelet volume; *NEUT,* segmented neutrophil; *PLT,* platelet; *RBC,* red blood cell; *RDW,* RBC distribution width; *RETIC,* reticulocyte; *WBC,* white blood cell.

RBC Parameters

- **RBC Count**: can be performed manually or automatically
  - Used to detect anemia and polycythemia
    - Anemia- loss of oxygen-carrying capacity (decreased RBC count or HGB concentration)
    - Polycythemia- increased RBC count (leads to hyperviscosity)
- RBCs are assayed for hemoglobin (HGB) concentration and hematocrit (HCT)
  - **Hemoglobin**
    - Iron containing molecule within the RBC that carry oxygen to and carbon dioxide from the tissues
    - Measured using Drabkin reagent
  - **Hematocrit**
    - Also called packed cell volume (PCV)
    - Ratio of volume of packed RBCs to the volume of whole blood
    - POC testing using the i-STAT and Epoc
    - Normal range 37-52%

RBC Parameters

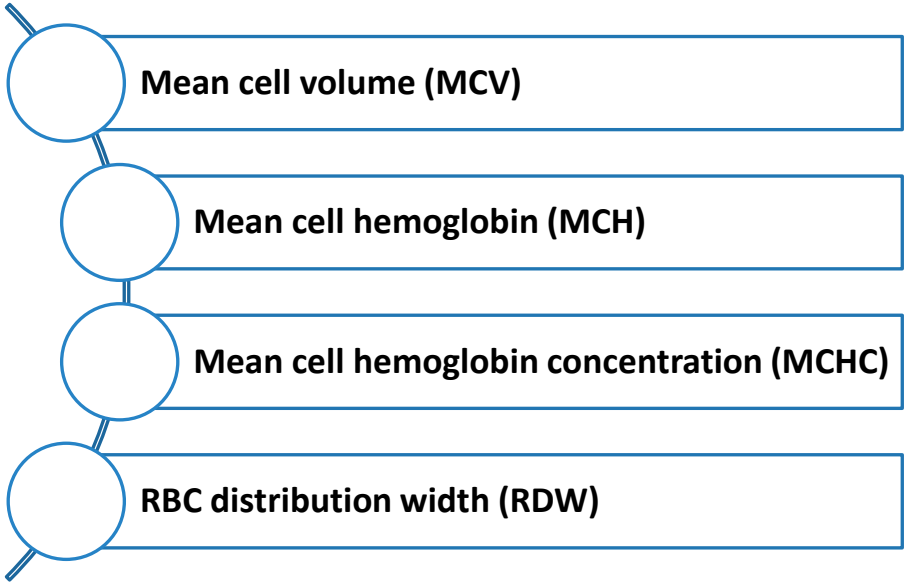
- **Reticulocytes**
  - Last immature RBC stage
    - Normally reticulocyte spends 2 days in the bone marrow then 1 day in the peripheral blood before developing into a mature RBC
  - Also called polychromatophilic erythrocytes
  - Contains remnant cytoplasmic RNA and organelles (mitochondria and ribosomes)
  - Used to assess the erythropoietic activity in the bone marrow
  - Appearance
    - 6-8 um
    - Stain slightly blue gray

RBC Indices

RBC count, HGB, and HCT are used to calculate the RBC indices.

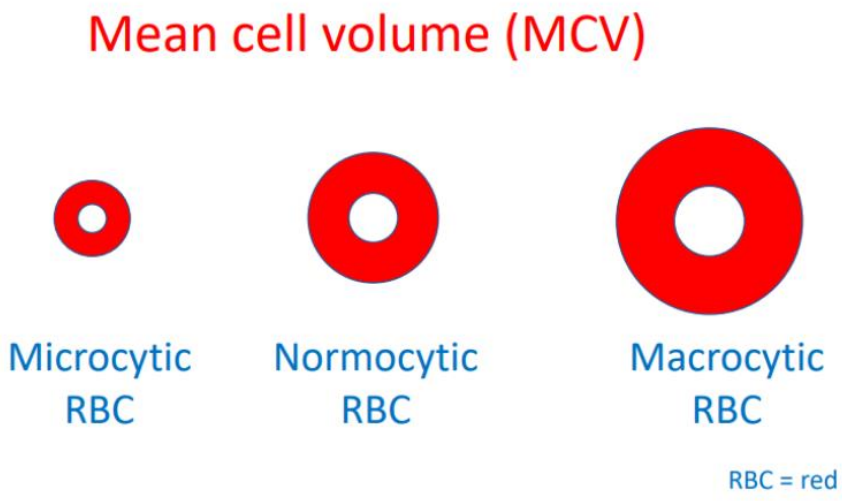
MCV, MCH, MCHC are used to determine the average volume and hemoglobin content and concentrations of the RBCs in the specimen

Serves as a quality control check and for initial classification of anemia's



RBC Indices

- **Mean cell volume (MCV)**
  - Average volume of the RBC
    - RBC diameter on a wright-stained blood film
  - Measured in femtoliters (fL)
  - $MCV = \frac{HCT\ (\%) x\ 10}{RBC\ count}$
    - RBC count (x10^{12}/L)
  - Reference interval 76 to 100 fL



https://www.thebloodproject.com/ufaq/what-is-the-mean-cell-volume-mcv/

RBC Indices

- **Mean Cell Hemoglobin (MCH)**
  - Average weight of hemoglobin in an RBC
    - Mass of hemoglobin per cell
  - Measured in pictograms (pg)
  - Parallels the MCHC
  - MCH= $\frac{HGB\ x\ 10}{RBC\ count}$
    - HGB (g/dL)
    - RBC count (x10^{12}/L)
  - Reference interval for adults is 26-34 pg

RBC Indices

- **Mean Cell Hemoglobin Concentration (MCHC)**
  - Average concentration of hemoglobin in each individual RBC
    - RBC staining intensity and the amount of central pallor
  - Measured in grams per deciliter (g/dL)
  - MCHC=$\frac{HGB\ x100}{HCT\ (\%)}$
  - Reference Interval: 32-36 g/dL
  - Range
    - Normochromic RBC: 32-36 g/dL
    - Hypochromic RBC: <32 g/dL
    - Hyperchromic RBC: >36 g/dL

Hypochromic RBC
Low MCHC

Normochromic RBC
Normal MCHC

Hyperchromic RBC
High MCHC

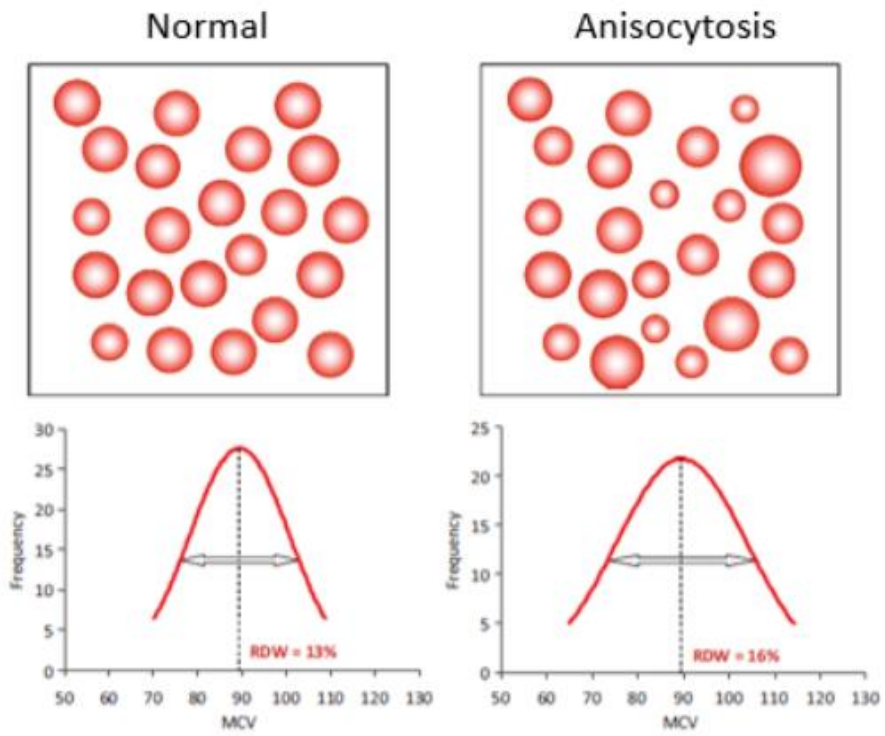
The central white color represents the central pallor observed in RBCs on a Wright-Giemsa stained peripheral smear.

https://www.thebloodproject.com/ufaq/what-is-the-mean-cell-hemoglobin-concentration-mchc/

RBC Indices

RBC distribution width (RDW)

- Expresses the degree of variation RBC volume/size
- Used to help classify anemia
- RDW=$\frac{SD\ of\ RBC\ Volume\ x\ 100}{MCV}$
- Normal range= 11.5-14.5%
  - Anisocytosis= increased RDW
- Routinely reported by automatic analyzers



https://www.thebloodproject.com/cases-archive/29-year-old-man-with-longstanding-history-of-anemia-and-gallstones/one-lab-value/

Platelet Parameters

Platelet Count

- Can be counted manually or automatically

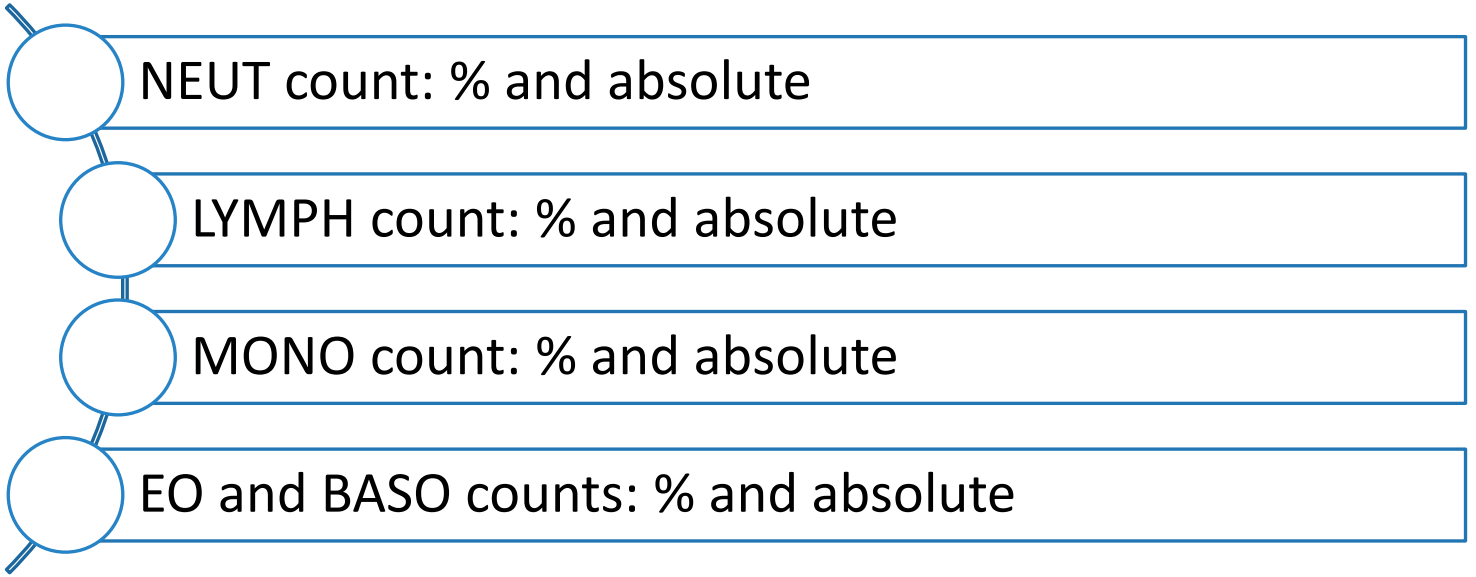
Mean Platelet Volume (MPV)

- Calculated by a automated blood cell analyzer
  - Unable to calculate through manual/visual methods
- Elevated MPV value= larger platelets

WBC Parameters

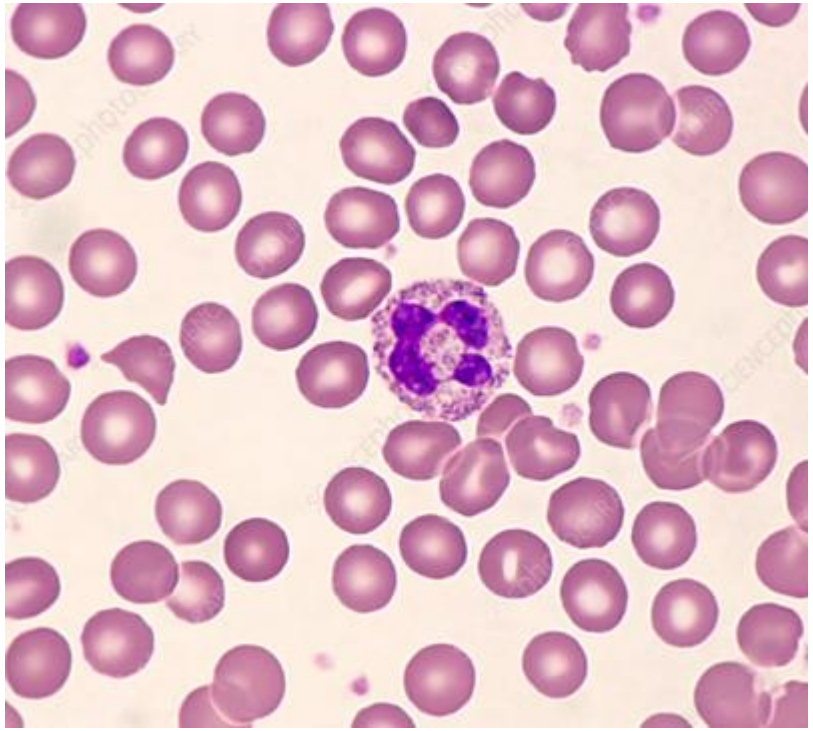
- WBC Count
  - Counted manually or automatically
  - Leukocytosis- increase WBC count

Absolute value: number of each cell in each class per microliter of blood
  - Determined by multiplying the % of the cell type by the total amount of WBCs present



WBC Parameters

- **Neutrophil**
  - Phagocytic cells whose major purpose is to engulf and destroy microorganisms and foreign material
  - Segmented
  - Cytoplasm is pink- or lavender-staining with granules containing bactericidal substance
  - Neutrophilia- Elevated neutrophil count
    - Can be caused by Infection, acute stress, burn, leukemia, steroid use, and RA
  - Neutropenia- decrease neutrophil count
    - Caused by folate, B12 deficiency, aplastic anemia, chemo, and some prescription drugs

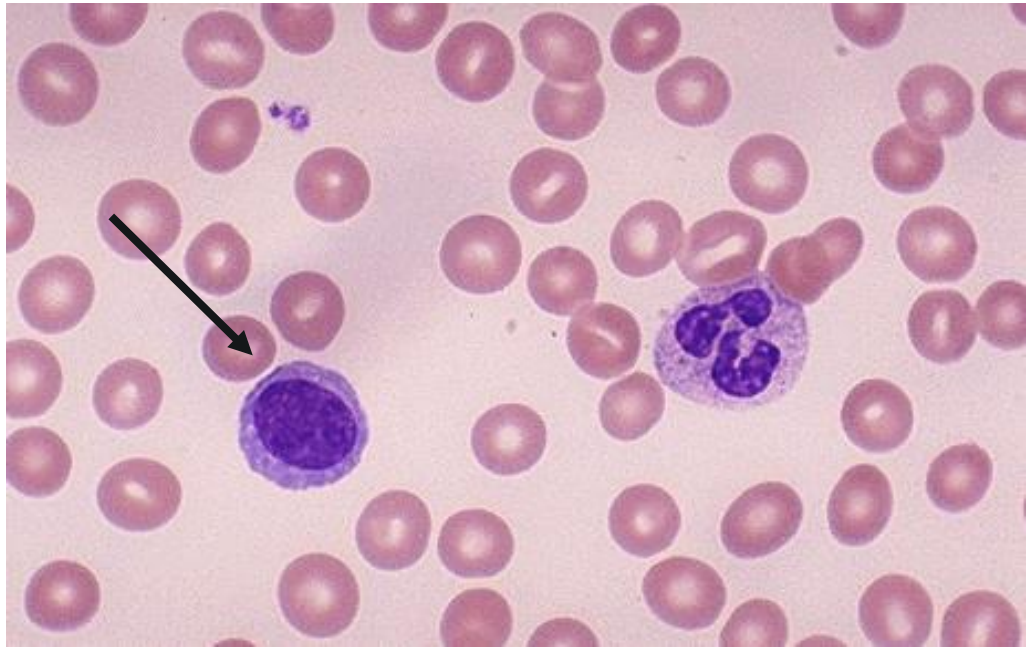


https://www.sciencephoto.com/media/855622/view/neutrophil-light-micrograph

- **Band neutrophils**
  - Slightly less mature neutrophil
  - Nonsegemented nucleus in a U or S shape
  - Signifies a bacteria infection
  - “Left shift”

WBC Parameters

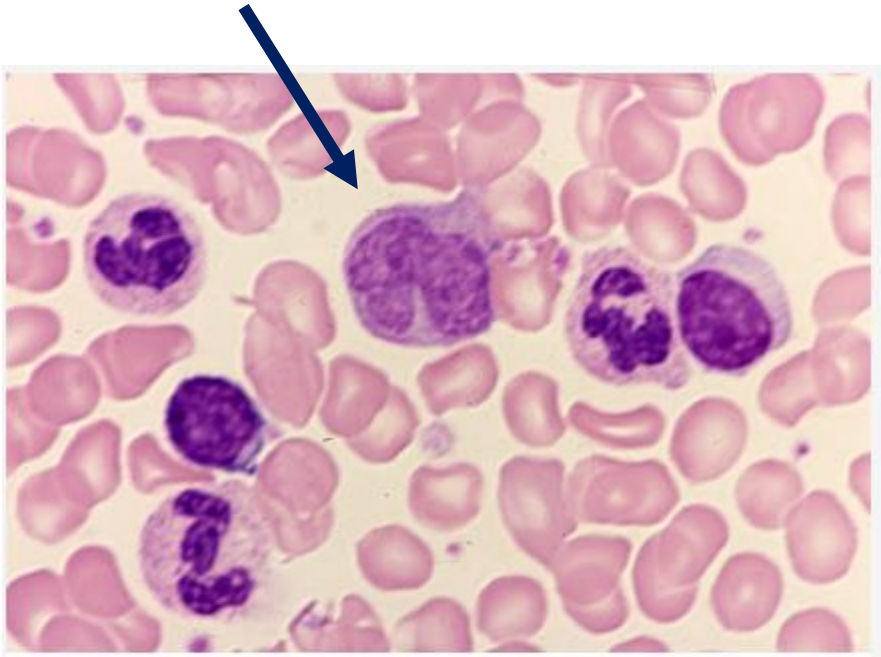
- **Lymphocyte**
  - Cells that provide immunity for the host
  - Recognize foreign antigens
  - Appearance
    - Nearly round, slightly larger than RBCs
    - Round featureless nuclei
    - Thin rim of nongranular cytoplasm
  - Lymphocytosis- Increase in lymphocyte count
    - Associated with viral infections and adrenal insufficiency
    - Seen with reactive lymphocytes
  - Lymphopenia- Decrease in lymphocyte count
    - Seen with drug therapy or immunodeficiency (AIDS, RA, lupus)
  - T cells, B cells, and NK Cells



http://www.medical-labs.net/normal-white-blood-cells-on-a-smear-91/

WBC Parameters

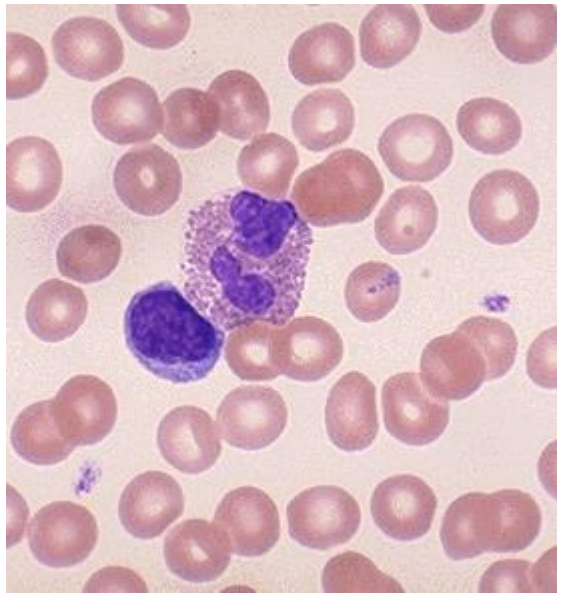
- **Monocyte**
  - Immature macrophage passing through the blood from the point of origin to a targeted tissue location (1-3 days)
  - Appearance
    - Slightly larger diameter than other WBCs
    - Blue-gray cytoplasm with fine azul granules
    - Nucleus that is usually indented or folded
  - Monocytosis- increased monocyte count
    - Seen with infection, inflammation, and stress
  - Monocytopenia- decrease monocyte count
    - Seen with aplastic anemia, AML, and some drugs
  - Macrophage
    - Most abundant cell type in the body
    - Identify and phagocytize foreign particles and assist lymphs in mounting an immune response



Source: Lichtman MA, Shafer MS, Felgar RE, Wang N: *Lichtman's Atlas of Hematology*: http://www.accessmedicine.com

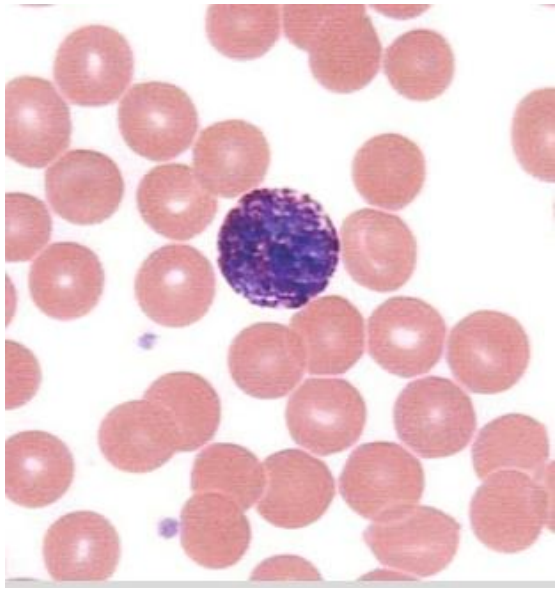
WBC Parameters

- **Eosinophil**
  - Cells with round, bright orange-red cytoplasmic granules filled with proteins involved in immune system regulation
  - Eosinophilia- elevated eosinophil count
    - Seen in allergic reactions or parasitic infections
- **Basophil**
  - Cells with dark purple, irregular cytoplasmic granules that obscure the nucleus
  - Basophil granules contain histamines and various other proteins
  - Participate in allergic reactions
  - Basophilia- elevated basophil count
    - Rare
    - Signals a hematologic disease



https://webpath.med.utah.edu/HEMEHTML/HEME004.html

Eosinophil



http://microanatomy.net/blood/more_basophils.htm

Basophil

Normal Values

REFERENCE RANGES (COMBINED MALE AND FEMALE)

Analyte	Conventional Units
RBC	4.0 – 6.0 x 10^6/µL
HGB	12.0 – 18.0 g/dL
HCT	35% – 50%
MCV	76 – 100 fL
MCH	26 – 34 pg
MCHC	32 – 36 g/dL
Reticulocytes (absolute)	20 – 115 x 10^3/µL
Reticulocytes (relative)	0.5 – 2.5%
nRBCs	0 nRBC/100 WBC
Platelets	150 – 450 x 10^3/µL

https://www.ascp.org/content/docs/default-source/boc-pdfs/boc-us-guidelines/h_ih_sh_ish_content_guideline.pdf?sfvrsn=20

Normal Values

REFERENCE RANGES (COMBINED MALE AND FEMALE)

Analyte	Conventional Units
WBC (total):	3.6 – 10.6 x 10^3/µL
Neutrophils (absolute)	1.7 – 7.5 x 10^3/µL
Neutrophils (relative)	50 – 70%
Lymphocytes (absolute)	1.0 – 3.2 x 10^3/µL
Lymphocytes (relative)	18 – 42%
Monocytes (absolute)	0.1 – 1.3 x 10^3/µL
Monocytes (relative)	2 – 11%
Eosinophils (absolute)	0 – 0.3 x 10^3/µL
Eosinophils (relative)	1 – 3%
Basophils (absolute)	0 – 0.2 X 10^3/µL
Basophils (relative)	0 – 2%

https://www.ascp.org/content/docs/default-source/boc-pdfs/boc-us-guidelines/h_ih_sh_ish_content_guideline.pdf?sfvrsn=20

Erythrocyte Sedimentation Rate (ESR)

- Measures the distance in millimeter that RBCs fall in 1 hour
  - Rate of sedimentation is determined by plasma proteins
- Ordered to detect and monitor the course of inflammatory conditions
  - RA, infections, or certain malignancies
  - Used in diagnosis of temporal arteritis and polymyalgia rheumatica
- Not a specific test for inflammatory diseases
  - Elevated in plasma cell myeloma, pregnancy, anemia, and with old age
- Prone to technical errors and has a low specificity and sensitivity
- Automated system measure ESR by intra red light that is measured over a shorter period of time than 1 hr and extrapolated to give WSR
- Most commonly used method is the modified Westergren method

Automated Reticulocytes

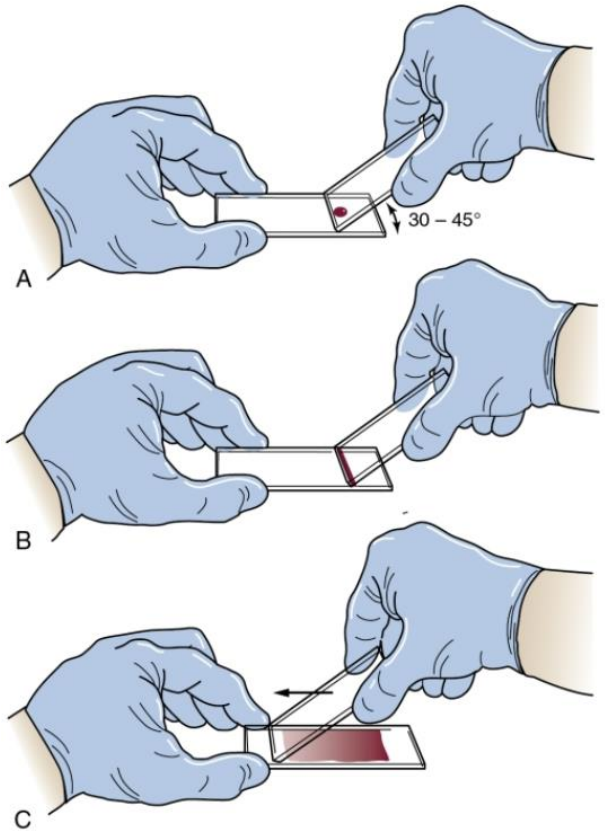
- Uses optical scatter or fluorescence after the RBCs are treated with fluorescent dye nucleic acid stains
  - Stain residual RNA
- The percentage and the absolute count, IRF, and RET-Hb
- The % retic evaluates the bone marrows response to anemia
  - Hemolytic anemia causes retics to ↑
  - Bone marrow disease (impaired RBC production) causes retics to ↓
- Sysmex R-3000/5000
  - Stand-alone retic analyzer that uses auramine O
  - Measures forward scatter and side fluorescence as the cells, in a sheath-stream, pass through a flow cell

Preparing a Peripheral Smear

Technique for preparing blood film

- Manual wedge technique

- Automated slide maker
  - Sysmex SP-10
  - CBC is performed and manual differential slide created if it is required
    - Varies per laboratory and based on patient population
  - Hematocrit reading is used to determine the size of the drop of blood and the angle/speed of the spreader slide in making a wedge preparation
  - Films can be produced approximately every 30 seconds



Staining of blood film- Wright Stain

- Pure Wright stain or Wright-Giemsa stain (Romanowksy stain) is used for staining peripheral blood films and bone marrow
- Purpose is to make the cells more visible and to allow their morphology to be evaluated
- Optimal pH 6.4
- Consists of:
  - Methanol
  - Oxidized methylene blue
  - Azure B and eosin dye
  - Buffer solution
    - Used to control acid-base balance of the stain

Staining of blood film- Wright Stain

- QC of the stain is performed, reviewed daily and recorded in the FVPM book
- Acceptability criteria
  - Smears are more than half the length of the unfrosted portion of the slide
  - Thin, feathered edge without streaks, holes, or tails
  - Even and consistent staining of blood smears

Staining Quality of the Smear

Cell Type	Appropriate Appearance on a Wright-Stained Slide
RBC	Reddish pink. Good differentiation between normochromic, hypochromic, and polychromatophilic cells
Lymphocytes	Dark purple nuclei with varying shades of blue cytoplasm
Neutrophils	Dark purple nuclei with reddish, lilac granules and light pink cytoplasm
Monocytes	Lighter purple nucleus with a gray-blue cytoplasm
Eosinophils	Large bright red/orange granules in the cytoplasm with a blue nuclei
Basophils	Dark purple nuclei and granules in the cytoplasm

Characteristics of a Good Blood Smear

- Smear should not touch the edges and should be in the central portion of the slide
- Smear should cover approximately two-thirds the length of the slide
- Smear should be smooth with gradual transition from thick to thin areas, terminating in a straight feathered edge

Characteristics of a Poor Blood Smear

- Drop of blood is too large or too small
- Spreader slide pushed across the slide in an inappropriate manner
- Cold agglutinin- RBC will clump together
- Presence of lipemia
  - Holes in the smear
- Rouleaux- RBC's will form into stacks resembling coins

Sources of Error of the Slide

Problem	Resolution
Presence of crenated RBCs	Dry smear thoroughly
Thin smear due to anemia	Increase spreader slide angle and push speed
Thick smear due to polycythemia	Decrease spreader slide angle and push speed
Presence of agglutinated RBCs	Warm specimen at 37° C for 30 min. Prior to making smear
Increased viscosity associated with myeloma	Lower spreader slide angle and lower push speed

Stain Sources of Error

Stain Deviation	Causes	Correction
Stain too Acidic -RBCs are bright red-orange -WBC nuclei are pale blue	-Buffer or stain too acidic -Excess buffer for stain -Insufficient staining time -Very thin smear or old stain	Correct pH, shorten buffer time/amount, prolong staining time, correct film thickness
Stain too Alkaline -RBCs are blue-green -Eosin granules are gray/blue -WBC nuclei are blue-purple -Lymph cytoplasm is gray	-Buffer or stain too alkaline -Insufficient buffer for stain -Excessive staining time -Blood smear too thick	Correct pH, increase buffer time/amount, decrease staining time, correct film thickness
Precipitation on slides	-Precipitates in stains -Insufficient rinsing	Replace stain, check rinsing time
Vigorous or prolonged washing of cells	-Dislodge of cells, nuclear clumping or under staining	Adjust the washing or rinsing time

Automated Methods

Automated Hematology Analyzer

- Highly specialized equipment for CBC plus a 3-part, 5-part, or 7-part differential
- It analyzes the WBC, RBC, HGB, Indices, PLT, HCT (PCV), RDW, and MPV
- WBC differential count in percentage and absolute values

Types of Automated Analyzers

Three part	**Five part**	**Seven part**
Differentiate cells into three categories	Differentiate cells into five basic leukocytes subtypes	In addition are able to distinguish
1. Granulocytes 2. Lymphocytes 3. Monocytes/mixed cells • Function on the principle of impedance to determine the size and volume of the cells • Differentiate white blood cells into granulocytes, lymphocytes and monocytes/mixed cell count. • Unable to distinguish between monocytes eosinophil and basophils.	1. Neutrophils 2. Lymphocytes 3. Monocytes 4. Eosinophil 5. Basophils • 5-part differential counter uses flow cytometry and volume conductivity scatter to determine granularity, diameter, and inner complexity of blood cells.	1. Nucleated RBC's 2. Atypical cells and immature cells

Automated Analyzer Principle

Basic principles used:

- Electronic Impedance (Coulter principle)
- Radiofrequency
- Optical Scatter
  - Laser light scatter/flow cytometer
  - Fluorescence

Electronic Impedance (Coulter Principle)

- Based on detection and measurement of changes in electrical resistance produced by cells as they traverse a small aperture

- How it works:
  - Blood cells are suspended in a conductive diluent (saline)
  - Diluent is passed through an electric field created between two electrodes
    - Blood cells pass through a small aperture
    - Passage of each particle causes the impedance of the electrical path between the electrodes
    - Increase in impedance creates a pulse that can be measured
  - # pulses = # cells counted

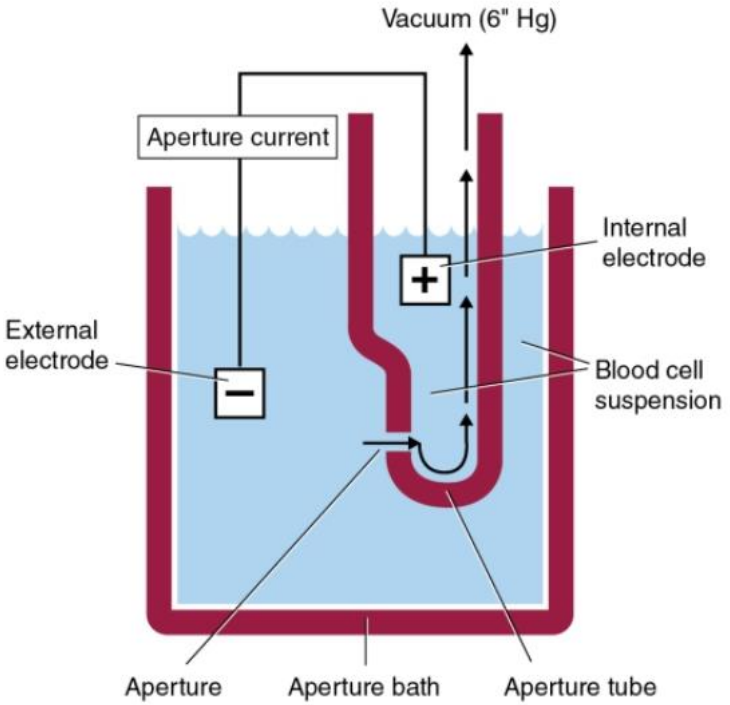


FIGURE 12.1 Coulter Principle of Cell Counting. Source: (From Coulter Electronics. [1988]. *Coulter STKR Product Reference Manual, PN 4235547.* Hialeah, FL: Coulter Electronics.)

Rodak's Hematology 6th edition

Electronic Impedance (Coulter Principle)

- Amplitude of the pulse is proportional to the volume of the cell
  - Allows discrimination and counting of the cells specific volumes through the use of threshold circuits
  - Pulses are collected and sorted
- Data is plotted on a frequency distribution graph or volume distribution histogram
- Performs a three-part differential on one volume distribution histogram

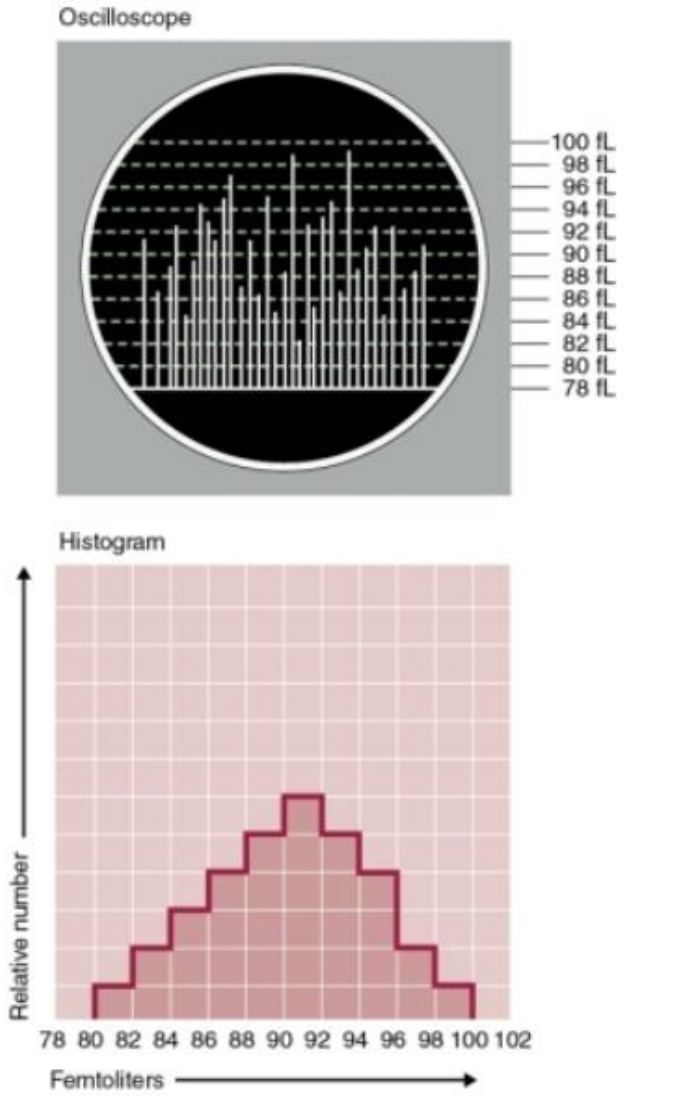


FIGURE 12.2 Oscilloscope Display and Histogram. The construction of a frequency distribution graph is depicted. Source: (Modified from Coulter Electronics. [1983]. *Significant Advances in Hematology: Hematology Education Series*, PN 4206115A. Hialeah, FL: Coulter Electronics.)

Rodak's Hematology 6th edition

Radiofrequency

- Uses a high-voltage electromagnetic current
  - Measures the resistance of a current as it flows between electrodes simultaneously
  - Cell interior density is proportional to the change in this RF signal (pulse height)
- Conductivity that is measured by this high-frequency electromagnetic probe, is decreased by N:C ratio, nuclear density, and cytoplasmic granulation
- Often used with low-voltage DC impedance
  - Separated by two different pulse processing circuits

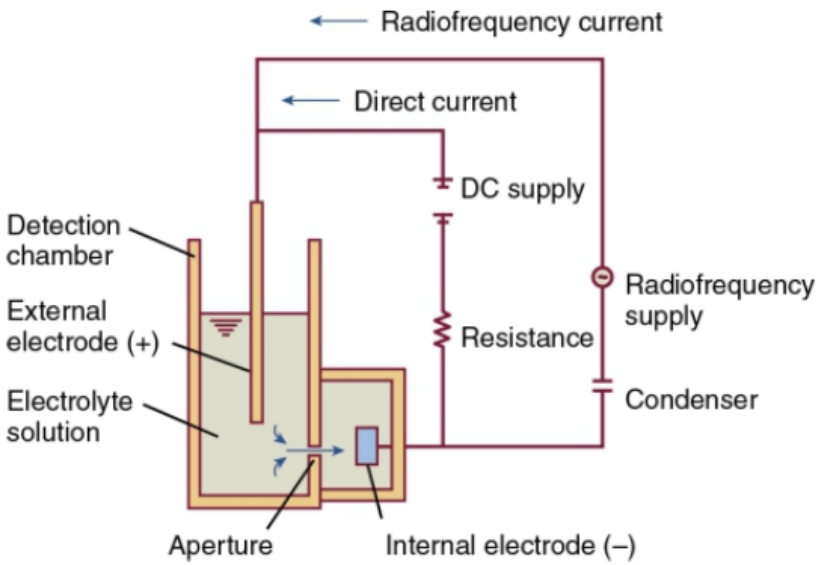


FIGURE 12.3 Radiofrequency/Direct Current (RF/DC) Detection Method. The simultaneous use of DC and RF in one measurement system on the Sysmex SE-9500 is depicted. Source: (From TOA Medical Electronics Company [1997] *Sysmex SE-9500 Operator's Manual [CN 461-2464-2]*. Kobe, Japan: TOA Medical Electronics Co.)

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Radiofrequency

- RF and DC Method together measures the cell in 2 ways:
  - Size
    - Detected via changes in direct current resistance
  - Density
    - Detected by changes in the radio frequency resistance
- DC Impedance (size) and conductivity (density) can be plotted against each other on a 2D distribution cytogram or scatter plot
  - Cell populations are evaluated using cell cluster analysis
- Performs a 5-part differential
- Used by Sysmex analyzers

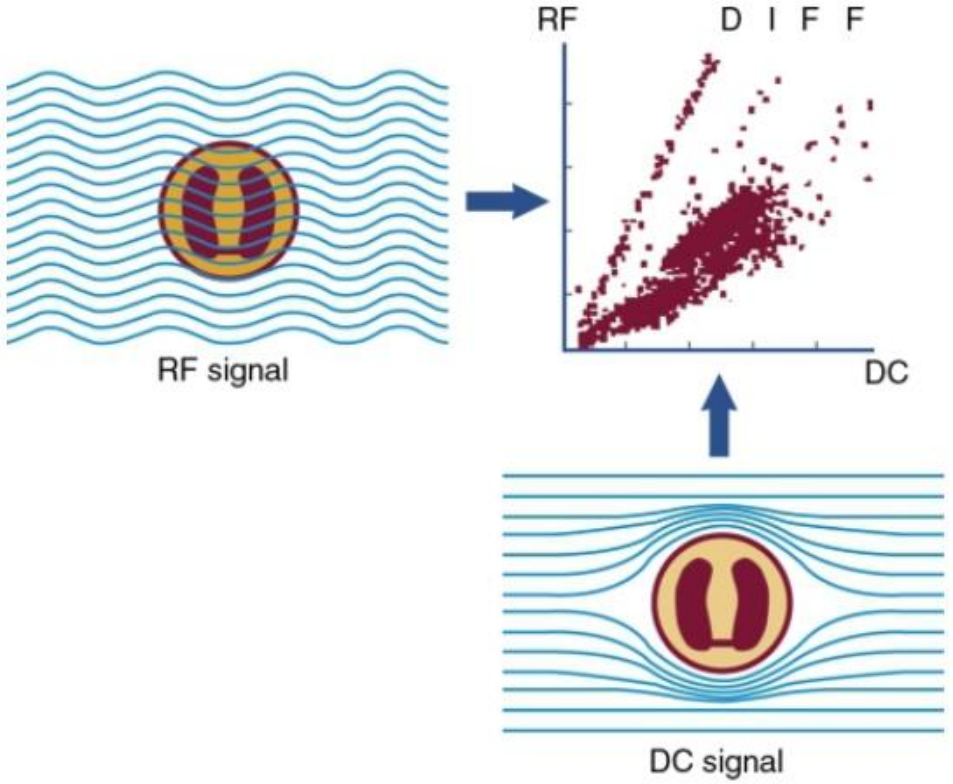


FIGURE 12.4 Two-Dimensional Distribution Scatterplot of Direct Current (DC) versus Radiofrequency (RF) Signals. Low-voltage DC impedance (measurement of cell volume) is plotted against RF resistance (measurement of cell interior density and nuclear volume and complexity) to form a two-dimensional distribution scatterplot. Source: (From TOA Medical Electronics Company. [1997]. *Sysmex SE-9500 Operator's Manual* [CN 461-2464-2], Kobe, Japan: TOA Medical Electronics Co.)

Errors that can occur

- Recirculation error
  - Cells that recirculate through the edge of an electrical field produce an aberrant impulse
- Coincidence error
  - Cells that pass through the aperture simultaneously or almost so are counted and sized as a single large cell
- Non central flow error
  - Cells pass through the aperture off center produce aberrant impulses and appear larger than their actual size

Optical Scatter

- Use detection of interference in a laser beam or light source to differentiate and enumerate cell types

- May be used as the primary methodology or in combination with other methods

- Broken into two parts:
  - Optical scatter systems (flow cytometers)
  - Fluorescent

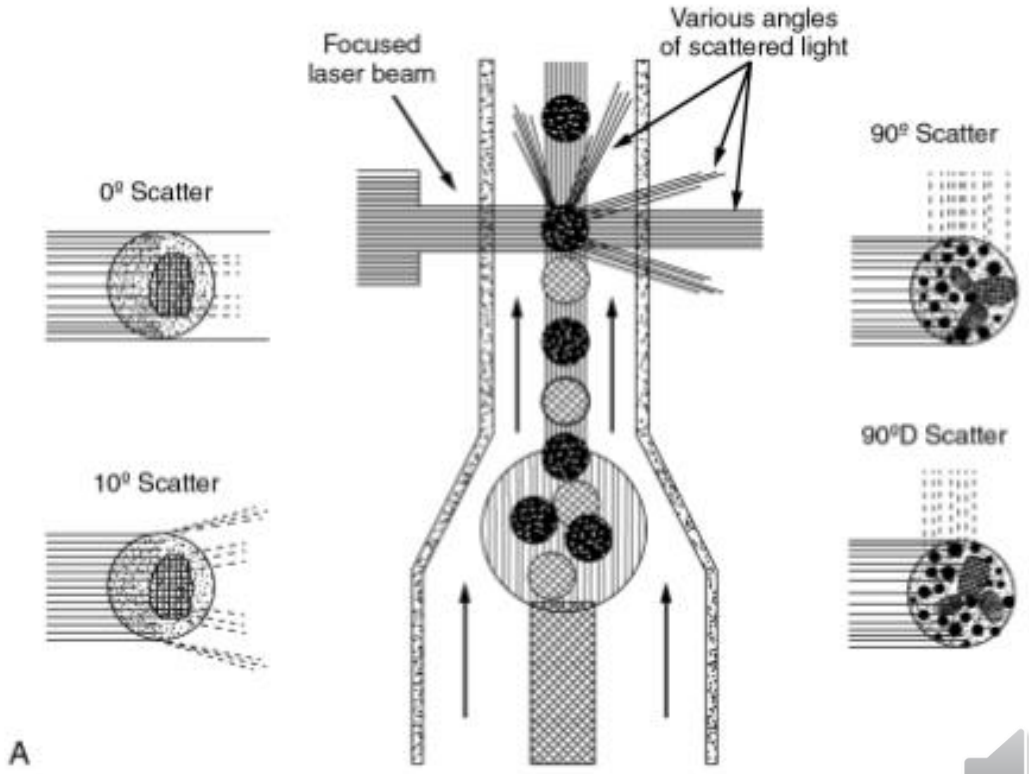
Optical Scatter

Laser light scatter/ flow cytometry

- How it works:
  - In a flow cytometer, a hydrodynamically focused sample stream is directed through a quartz flow cell past a focused light source
    - Light source is either a tungsten-halogen lamp or helium-neon laser (most common)
  - As the laser beam strikes a cell, light is scattered in various directions
  - Absorbance and scattered light generated by each cell is measured at multiple angles
    - Light absorption, diffraction, refraction, and reflection
  - At specific angles photodetectors detect scattered rays which is converted into electrical signals proportional to the amount of scattered light collected
  - Cells are counted cell by cell

Hydrodynamic focusing

- Sample stream is surrounded by a sheath fluid as it passes through the aperture
  - Makes the cells pass through in a straight line
- Improves cell count accuracy and reproducibility
- Creates laminar flow
  - Prevents generation of abnormal blood cell pulses
  - Minimize protein build up and plugs
- Eliminates recirculation error, coincidence error, and non-central flow error



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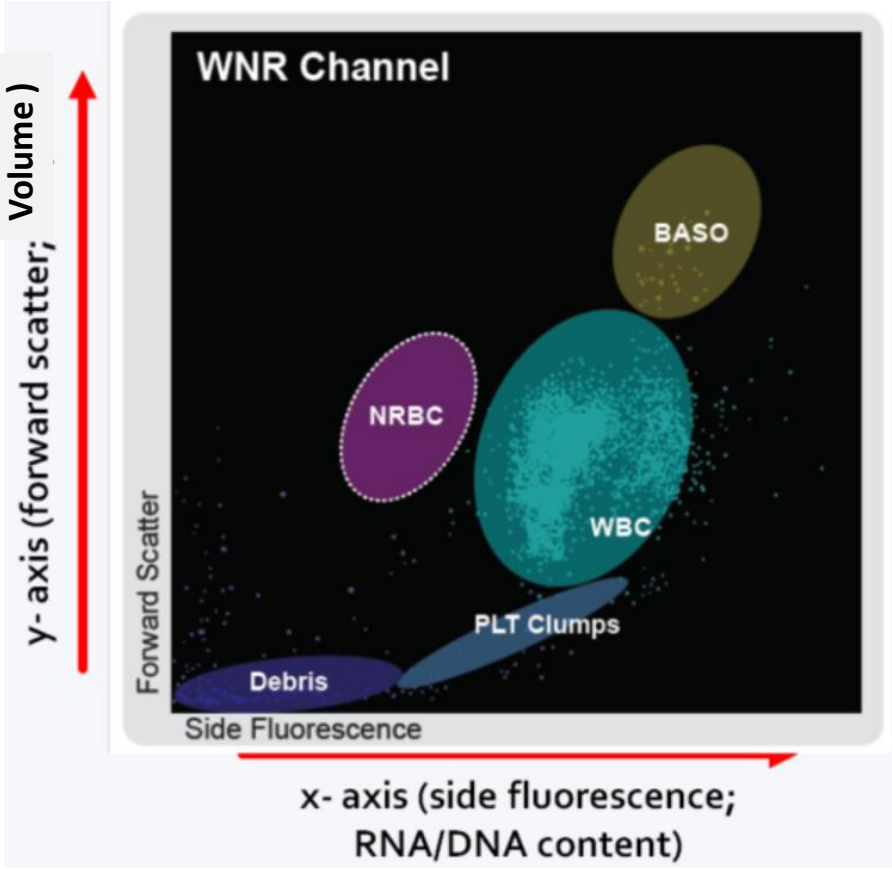
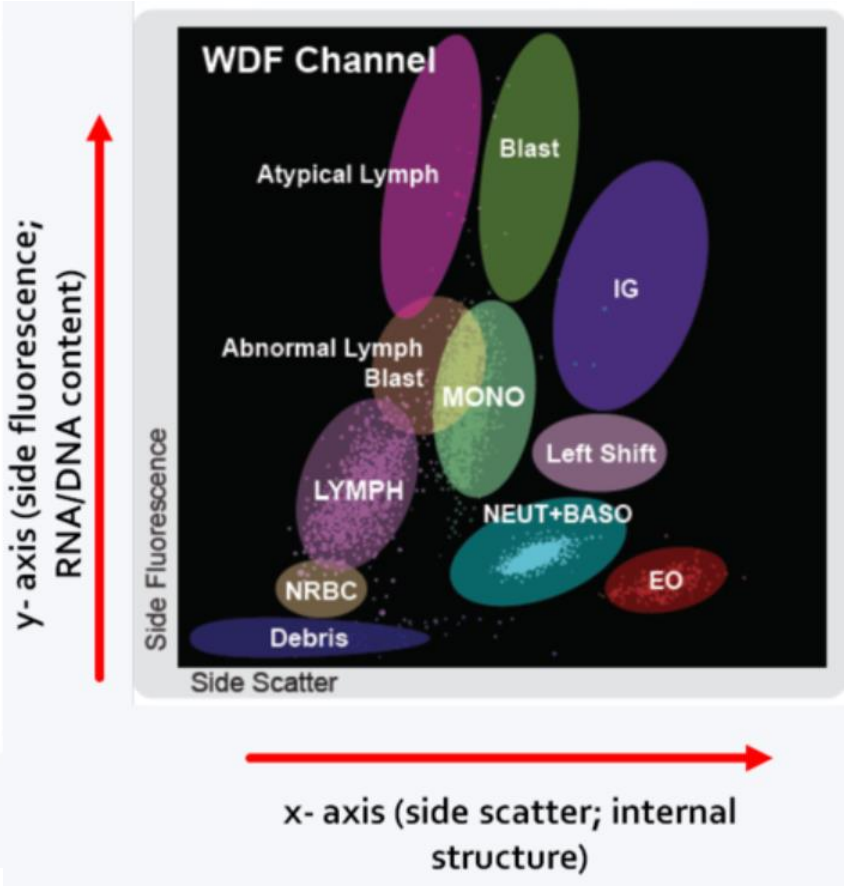
Optical Scatter

Fluorescent:

- Laser technology can be further enhanced with fluorescent dye's or labeled antibodies to measure specific cell populations
- Photo detectors collect and measure the light in different wavelength ranges and scatter wavelengths by the use of specific optical filters
- Cells are characterized by their side-scattered light and fluorescence-intensity which provide additional information on:
  - Count, type, molecular expression, and activation and maturity levels of the cell population
- Useful for the analysis of platelets, nucleated RBCs, and reticulocytes

Flow Cytometry





2 different kinds of scatter:

- WNR= white Cell Nucleated
- WDF= WBC Differential channel by Fluorescence

Sysmex XN-9000 brochure

Quality Assurance in Hematology

Quality Assurance

- Quality- implies the ability to provide accurate, reproducible assay results that offer clinically useful information
- Results must be reliable
  - Reliability requires vigilance and effort on the part of all laboratory professionals
- Quality control and quality assurance encompass preanalytical and postanalytical variables

Preanalytical variables	Analytical variables	Postanalytical variables
• Blood specimen collection and transportation • Stat orders and timeliness	• Laboratory staff competency • Assay and instrument selection • Quality control	• Publication of reports • Timeliness • Patient satisfaction

Quality Control

- Control and monitoring of testing process to ensure that the results are valid and reproducible
- Quality controls provide known values and are sampled alongside patient specimens
- Two controls are required per test run
  - Normal and abnormal
- Purpose of controls
  - Assure proper functionality of instrument
  - Means of assuring accuracy of unknowns
  - Monitoring the integrity of the calibration
    - Controls can begin to show evidence of unusual trends
    - Controls exceed the manufacturer's defined acceptable limits
  - Control results must fall within predetermined dispersion limits, typically +/- 2 SD

Primary and Secondary Standards

- Used during calibration of an instrument to establish accuracy
- **Primary standard**
  - Material of known, fixed composition that is prepared in pure form
    - Weigh out the standard and dissolve it in an aqueous solution. Using this dissolved standard, prepare suitable dilutions and calculate the anticipated concentration for each dilution. Assign the calculated concentrations to assay outcomes
  - Used to create a standard curve
    - Patient values plotted on this curve to create a result
  - Examples:
    - Cyanmethemoglobin (Hemoglobin), fibrinogen, factor VIII, etc.
- **Secondary standard**
  - Preserved material in which the analyte concentration has been assigned by reference to a primary standard
  - May be a preserved plasma preparation at a certified known concentration
    - Will either be thawed or reconstituted

References

- Rodak's Hematology 6th Edition
- Pinal Patel Laboratory Education Specialist