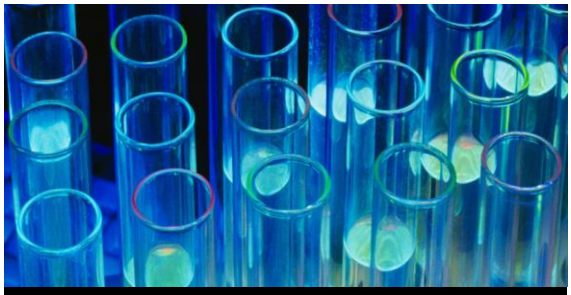


Interpretation of the CBC

Lee Ellen Brunson-Sicilia, MHS, MLS(ASCP)CM

Objectives

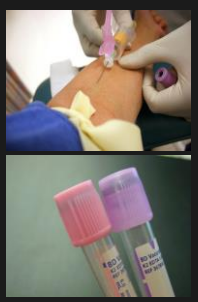
- Discuss the different parts of the complete blood count and how to interpret them.
- Describe factors and conditions that may impact CBC results.
- Describe the roles of peripheral blood cells.

Complete Blood Count (CBC)

- One of the most commonly ordered laboratory tests and the most ordered test in the hematology lab.
- Measure of the cellular components in the blood: WBC, RBC and PLT
- Also provides additional information about properties of RBC.

Specimen Collection

- **EDTA**
- **Mix, Mix, Mix!**
- **Test within 6-8 hours**
- **Room temperature**

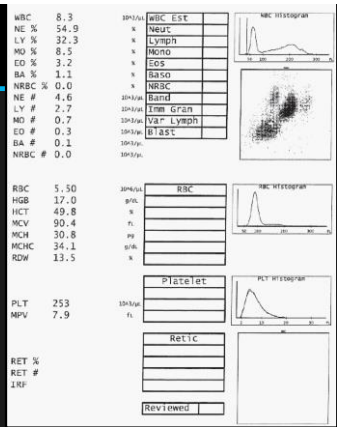


Specimen Processing - Preanalytical

Automated Analysis

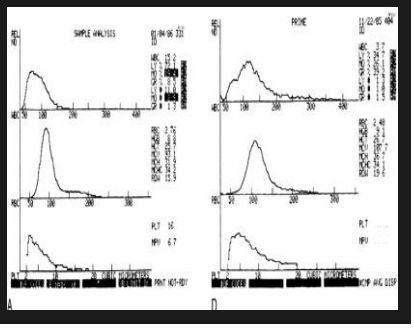
- Specimens are placed on the hematology analyzer to be tested

If we're lucky:



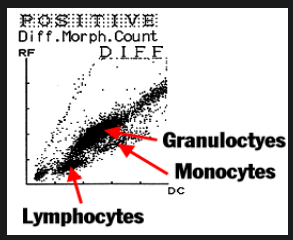
Cell Histogram

- **Number**
- **Size**

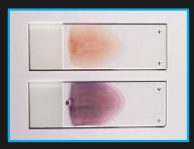


WBC Scattergram

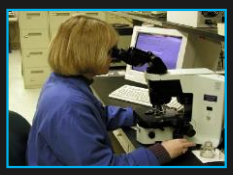
- **Nucleus**
- **Cytoplasm**

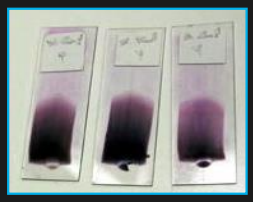


If we're *not* lucky…



- Sometimes blood smears need to be viewed under the microscope

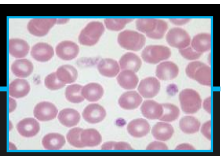




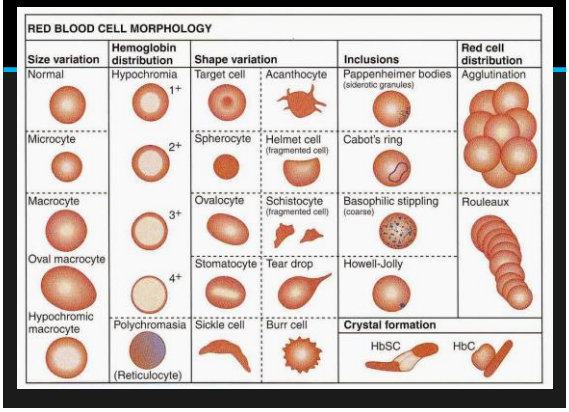
Purpose of manual microscopy

- Confirm WBC and PLT values from analyzer
- Visualize RBC morphology
  - Size
  - Coloration
  - Shape(s)
- Assess WBC present
  - Individual cell types
  - WBC maturation stages
  - Abnormal appearance

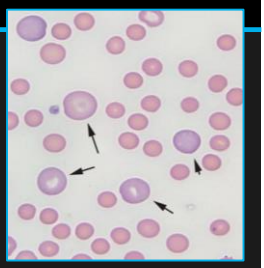
Erythrocytes (RBC)



- Red Blood Cells
- Deliver oxygen throughout body
- RR: 4.5 – 5.5 million/µL
- 120 day lifespan
- Higher in males, newborns
- Diurnal variation

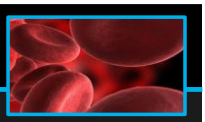


Reticulocytes



- Immature RBCs
- Indicate marrow activity
- ~0.5-2.5% of all RBCs
- Polychromasia
  - Blue = new
- Confirm with analyzer retic count

Hemoglobin (Hgb)

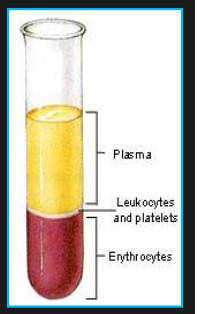


- Carries oxygen within the RBC
- Heme = contains O_2 and iron (red pigment)
- Globin = protein
- Lab value assesses Hgb composition

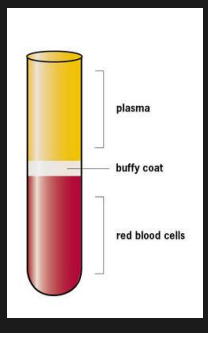
- Males: 14-18g/dL
- Females: 12-16g/dL
- Newborns highest
- Diurnal variation

Hematocrit (Hct)

- Proportion of RBCs to plasma
- Used to assess extent of patient's blood loss
- Reference Range: 36 – 52%
  - Males: 42 – 52%
  - Females: 36 – 47%
- Diurnal variation
- May be unitless (0.42)



Buffy coat



Decreased RBC, HGB, HCT

- **Anemia**
- Chronic blood loss
- Acute hemorrhage

- Hemolysis
- Bone marrow suppression
- Nutrient deficiency (B_{12}, folic acid, iron)

Increased RBC, HGB, HCT

- **Polycythemia**
- Hypoxia
- High altitude
- Smoking

- Cardiovascular disease
- Chronic lung disease
- Congenital heart defects

Rule of Three

RBC X 3 = Hgb

Hgb X 3 = Hct

Note: ± 3

Rule of Three Examples

Patient A	Patient B
RBC: 3.18	RBC: 1.79
Hgb: 8.1	Hgb: 8.7
Hct: 24.6	Hct: 41.0

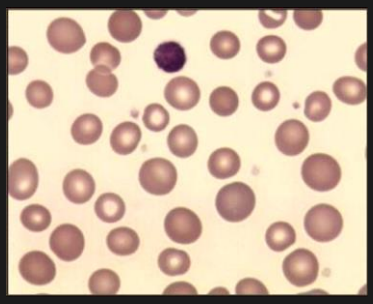
Rule of Three Examples, cont.

Patient C	Patient D
RBC: 4.27	RBC: 5.26
Hgb: 13.3	Hgb: 12.8
Hct: 30.7	Hct: 47.8

RDW → RBC Distribution Width

- Measure of anisocytosis → condition which RBCs are unequal in size
- Distinguishes hereditary RBC defect from acquired
- RR: 12% - 15%
  - High RDW → more variation in size

Variation in RBC size: *anisocytosis*



RBC Indices

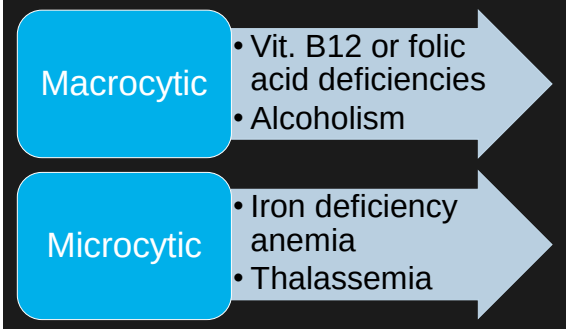
- Description of RBC morphology
- What is the overall size of the RBCs?
- How much Hgb do the RBCs contain?
- Calculated using RBC, Hgb, Hct

MCV → Mean cell volume

- Average RBC volume
- RR: 80 – 100 fL (normocytic)
- ↑ - **macrocytic**
- ↓ - **microcytic**

$$MCV = \frac{Hct \times 10}{RBC}$$

MCV



MCV

- **MCV = Average cell volume**
- **Large + small will average normal!**
- **Refer to the RDW**

MCH → Mean Cell Hemoglobin

- Amount of hemoglobin within RBC
- Correlates with MCV result
  - Smaller the cell → less Hgb → lower MCH
- RR: 28 – 34 pg

$$MCH = \frac{Hgb \times 10}{RBC}$$

MCHC → Mean Cell Hemoglobin Concentration

- Mean Hgb concentration of RBC
- RR: 32 – 36 g/dL
- Normal MCHC → normochromic
- Low MCHC → hypochromic
- High MCHC → hyperchromic

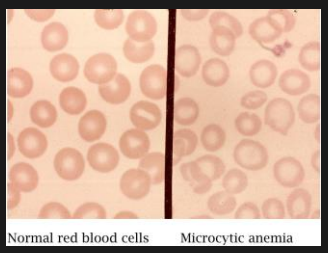
$$MCHC = \frac{Hgb \times 100}{Hct}$$

Normocytic/normochromic anemia

RBC: 0.77
Hgb: 2.2
Hct: 6.6
MCV: 87
MCH: 29
MCHC: 33
RDW: 13.7

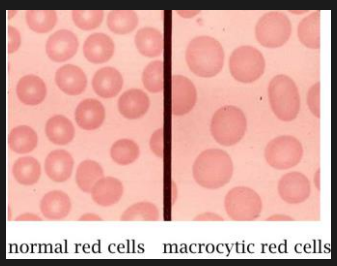
Microcytic/hypochromic anemia

RBC: 5.07
Hgb: 8.9
Hct: 33.5
MCV: 66.0
MCH: 17.6
MCHC: 26.7
RDW: 19.5

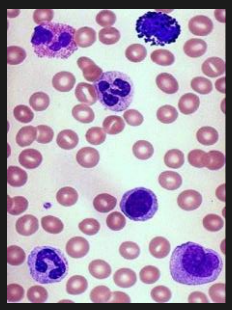


Macrocytic/normochromic anemia

RBC: 1.05
Hgb: 5.9
Hct: 16.7
MCV: 158.7
MCH: 56.2
MCHC: 35.4
RDW: 31.5



WBC (White Blood Cells)



- TOTAL leukocyte count
- 5 subtypes
- All stages of maturity
- RR: 4,500-11,000/µL
- Lifespan: hours to a few days

Leukocytes

Neutrophils | **Lymphocytes** | **Monocytes**

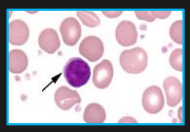
Eosinophils | **Basophils**

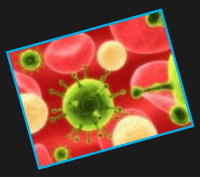
Leukocytosis

- Increased WBCs
- Full term newborns
- Leukemic condition
- Bacterial infection
- Tissue damage
- Inflammation

Neutrophils

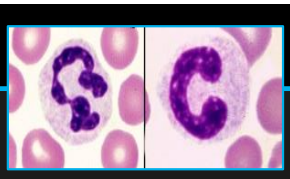
Leukopenia



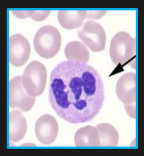


- Decreased WBCs
- Viral infection
- Chemotherapy
- Severe infection
- Diurnal variation

Neutrophils

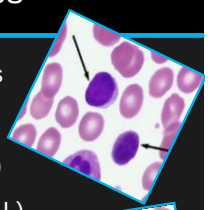


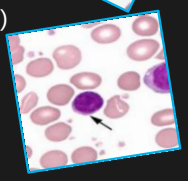
- Predominant WBC in adults
- Phagocytosis
- Bacterial infections
- "Left shift"



Lymphocytes

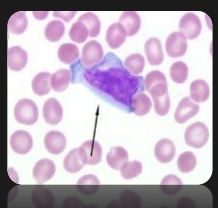
- Predominant WBC in pediatrics
- Viral infections
- Acute lymphoid leukemia (ALL)
- Chronic lymphoid leukemia (CLL)
- Inversion (reverse diff)
  - Adults with lymphs>neutrophils





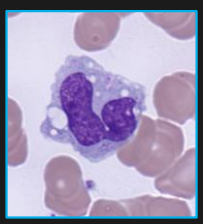
Lymphocytes

Atypical (reactive) lymphs are indicative of anti-viral activity.



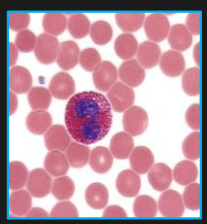
Monocytes

- Tissue macrophage
- Phagocytic
- Increase with neutrophils in infections
- Chronic inflammation
- Malignancies



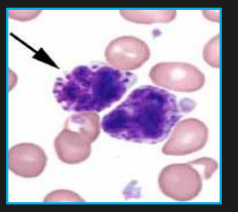
Eosinophils

- Higher levels in newborns
- Allergic reactions
- Asthma
- Parasitic infections
- Chronic myeloid leukemia (CML)



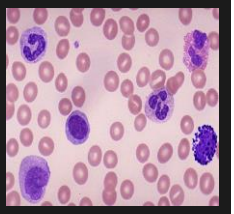
Basophils

- Least numerous of leukocytes
- Inflammatory response → IgE
- Contribute to severity of allergic reactions
- CML



Leukocyte Differential

- Automated or manual: 5-part diffs
- Relative – expressed in %
- Absolute – expressed in cell # per volume
- Use WBC and relative counts to obtain absolute values



Leukocyte Differential

WBC	**7.1 (x10³/µL)**
NE	**66.0%**
LY	**25.6%**
MO	**7.5%**
EO	**0.6%**
BA	**0.3%**

Relative values

Leukocyte Differential

- Absolute counts reflect true increase or decrease of each specific WBC

Ex: Absolute lymph = % lymph x total WBC

Absolute vs. Relative

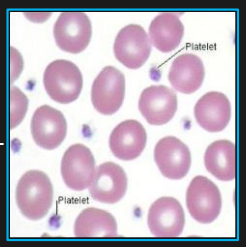
Patient A	**Patient B**
WBC: 3.0 x 10³/µL	**WBC: 9.0 x 10³/µL**
60% lymphs	**60% lymphs**
1,800/µL	**5,400/µL**

Leukocyte Differential Reference Ranges

	Relative RR %	Absolute RR #/µL
Total WBC		4,500 - 11,000
Neutrophils	40 - 80	1,800 – 8,800
Lymphocytes	25 - 45	1,125 - 4,950
Monocytes	2-10	90 - 1,100
Eosinophils	0 - 5	0 - 550
Basophils	0 - 2	0 - 220

Platelets

- Thrombocytes
- Role in cessation of bleeding
- RR: 150,000 - 450,000/µL
- 1-2 week lifespan
- Easily miscounted (manual and automated)



Platelets, cont.

- Thrombocytopenia – risk of bleeding
- Thrombocytosis – risk of inappropriate clotting
- **Mean platelet volume (MPV)**
  - RR: ~8-12 fL
  - Average volume of circulating PLTs
  - Analogous to MCV
  - Inc MPV – PLT destruction
  - Dec MPV – impaired PLT production

How does the automation work?

Automation in Hematology

- Prior to 1950, hematology testing was comprised of several manual testing methods.
  - Cell counts with hemacytometers
  - Spun hematocrits
  - Spectrophotometric hemoglobin concentration
  - Microscopic blood smear evaluation
- Today, automation provides data with increased reliability, precision, and accuracy.

Automated Hematology Instruments

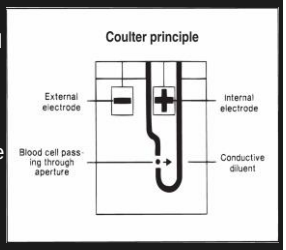
- Primary testing vehicle in hematology lab
- Automated instruments can perform CBC including:
  - RBC count
  - RBC indices (MCV, MCH, MCHC, RDW)
  - Platelet count, MPV
  - WBC count
  - Five-part leukocyte differential
  - Reticulocyte count

Automated Blood Cell Counting Instruments

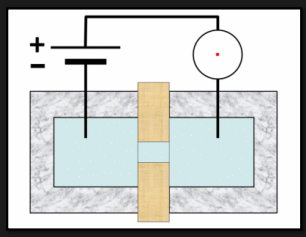
- **Two principles of cell counting currently used:**
  - Impedance – based on the break in current that occurs when a poorly conductive blood cell passes through an electrical field
  - Optical light scattering - based on light scattering measurements obtained as a single blood cell passes through a beam of light (optical or laser)

Coulter/Impedance Analyzers

- A poorly conductive blood cell passes through an aperture.
- Produces an increase in impedance between internal and external electrodes
- Strength of pulse is proportional cell volume/size
- Number of pulses is cell count



Electrical Impedance

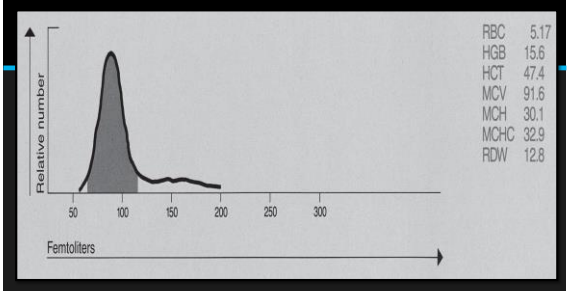


Coulter/Impedance Analyzers, cont.

- Small volume of EDTA blood sample is aspirated and diluted in an electrically conductive diluent.
- Then divided into 4 aliquots:
  - RBC/PLT chamber
  - WBC/Hgb chamber
  - Mixing chamber – differential
  - Reticulocyte dilution chamber
- Within each chamber are internal and external electrodes

Coulter cell counts

- Blood diluted in an electrically conductive diluent
- Charge is created between internal and external electrodes
- Three apertures (in each chamber); three separate counts
- If all agree, average of results reported
- Histogram generated based on cell volume and cell number in aspirated specimen volume



Red blood cell (RBC) histogram and count. The shaded area represents those cells used in the RBC count, MCV and RDW. Excluded cells can represent large platelets, platelet clumps, or electrical interference on the left and RBC doublets, RBC triplets, RBC agglutinates, or rouleaux on the right.

Cell counting chambers

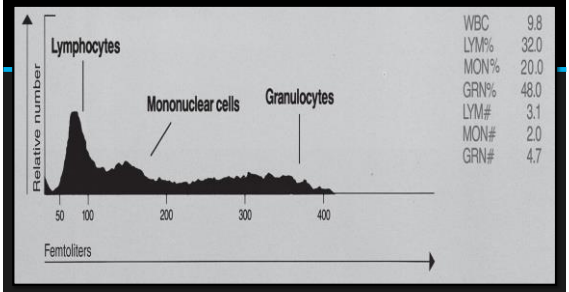
- RBC/PLT chamber – aliquot #1
  - RBC/PLT counted
- WBC/HGB chamber – aliquot #2
  - RBCs lysed
  - Hgb directly measured by spectrophotometry
  - WBCs counted

Coulter cell counts

- **RBC count:** > 35 fL counted as RBCs
- **PLT count:** Particles between 2–20 fL are counted
- **WBC count:** >35 fL counted as WBCs

WBC histogram

- WBC count histogram – 3-part differential – Allows visualization of subpopulations of cells based on WBC relative **sizes**
  - Lymphocytes 35-90 fL
  - Monocytes 90-160 fL
  - Granulocytes 160-450 fL
    - Neutrophils
    - Eosinophils
    - Basophils
    - Immatures



WBC histogram and count. In a normal patient, the lymphocyte region represents lymphocytes, the mononuclear region represents monocytes, and the granulocyte region represents neutrophils, eosinophils, and basophils.

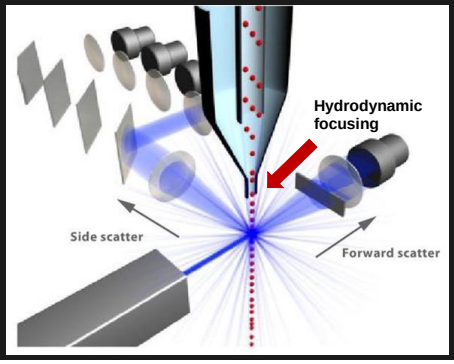
Coulter/Impedance Analyzers

- **RBC count** – directly measured
- **HCT** – directly measured
  - **MCV and RDW** – derived from RBC histogram
  - **HCT** – calculated from MCV and RBC count
  - **MCH** – calculated from RBC count and Hgb
  - **MCHC** – calculated from Hgb and HCT
- **WBC count** – directly measured
  - **% of WBC types** – derived from WBC histogram
- **PLT count** – directly measured

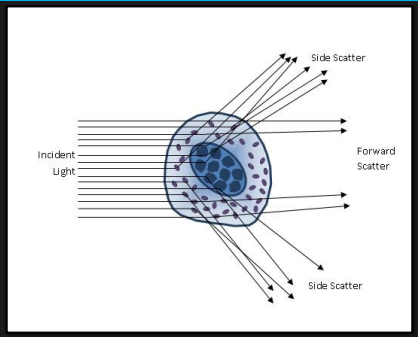
Mixing chamber – 5-part diff

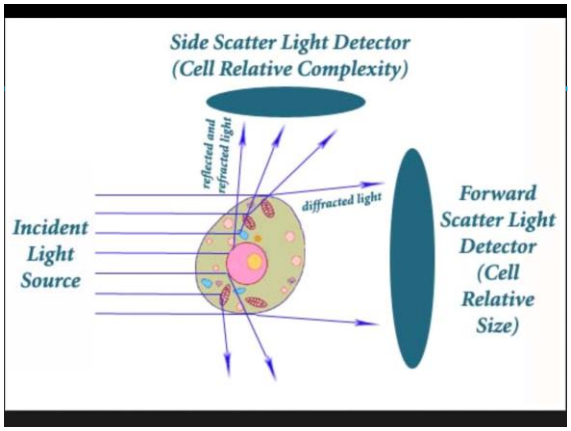
- **Aliquot #3** mixed gently with a lysing reagent to lyse RBC
- Stabilizing reagent added to preserve WBC integrity
- Cells pass one-by-one through a laser beam
- Light shines through cell, is measured by detectors
- Light scatter characteristics give information about cell type
  - Cell surface
  - Cell shape and size
  - Cell granularity
- Cells are counted and classified – WBC differential

Flow cytometry

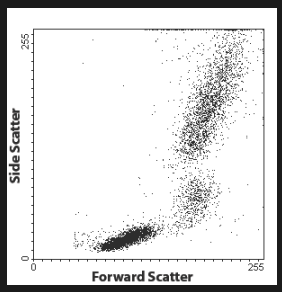


Optical light scattering





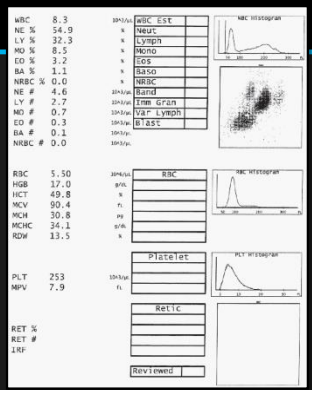
Scattergram/Dot Plot



Reticulocyte counts

- **Aliquot #4** goes to the reticulocyte dilution chamber
- Is stained with reticulocyte stain – **new methylene blue**
- Residual RNA precipitates within reticulocytes
- Cells go through flow cytometer similar to WBC
- RNA aggregates, if present, scatter light
- Cells counted one-by-one

What the results look like:



Troubleshooting - Factors that may lead to abnormal values:

<u>Hemoglobin</u>

- False inc:
  - Tourniquet on too long
  - High altitude
  - Smokers
  - Lipemia

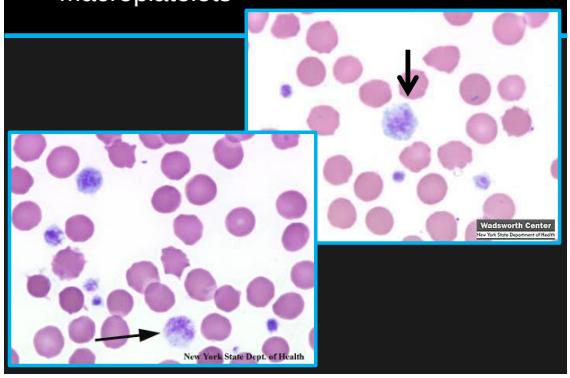
<u>Hematocrit</u>

- False inc:
  - Tourniquet on too long
  - High altitude
  - Smokers
  - Dehydration
- False dec:
  - Collection from IV arm
  - Pregnancy

Sources of error on the CBC

- Preanalytical errors
- Nucleated RBC
- Cold agglutinin disease
- Platelet satellitism
- Macroplatelets
- Microplatelets

Macroplatelets



Reference Ranges - CBC

WBC count:	**4.5 – 11.0 (x10^3/µL)**
RBC count:	**4.0 – 5.5 (x10^6/µL)**
Hgb:	**12 – 18 g/dL**
Hct:	**36 – 52%**
MCH:	**28 – 34 pg**
MCHC:	**32 – 36 g/dL**
MCV:	**80 – 100 fL**
RDW:	**12 – 15%**
PLT:	**150 – 450 (x10^3/µL)**
Relative retic count:	**0.5 – 2.5%**

Reference Ranges - CBC

Differential

Segmented neutrophils (Polys):	**40 – 80%**
Band Neutrophils:	**0 – 5%**
Lymphocytes:	**25 – 45%**
Monocytes:	**2 – 10%**
Eosinophils:	**0 – 5%**
Basophils:	**0 – 2%**

Note: These ranges are a percentage of the total WBC count.

Any questions?