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PEARLS OF LABORATORY MEDICINE

Complete Blood Count (CBC) Basics

Sarah Drawz, MD, PhD
Michael A Linden, MD, PhD

University of Minnesota

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Intro to the CBC

- Blood = plasma (albumin, clotting factors) + cells
- CBC = complete blood count, quantifies 3 types of blood cells
  - Red blood cells (RBCs)
  - White blood cells (WBCs)
  - Platelets

- Gives additional info, e.g.,
  - % of blood composed of RBCs – “hematocrit”
  - Different types of WBCs present – “differential”

Collection

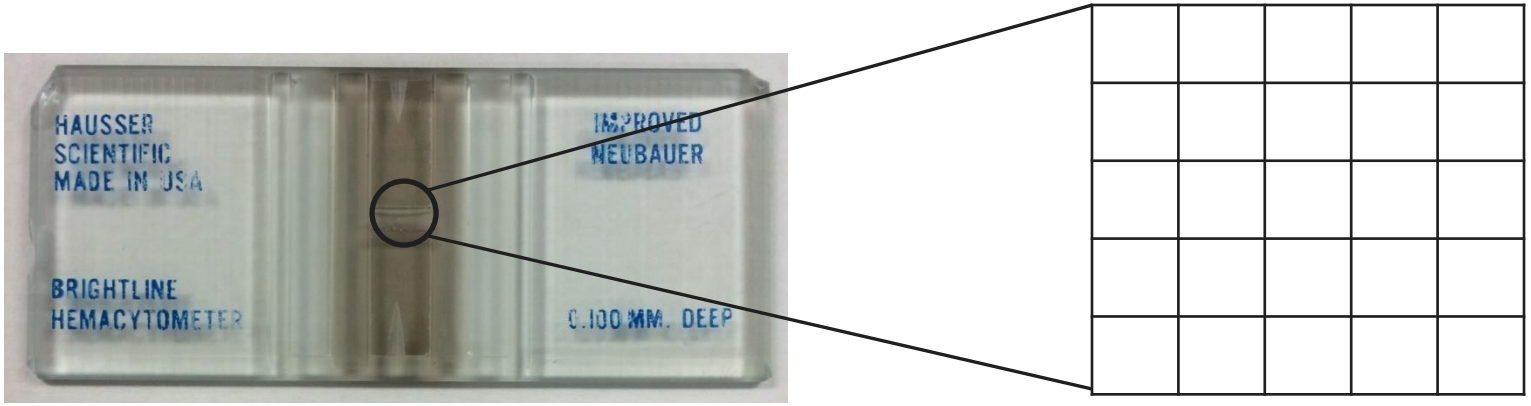
- 3 to 10 ml of whole blood drawn into tube containing anticoagulant
    - EDTA
    - Heparin
    - Citrate
- Most common: purple-top tube with EDTA to chelate Ca2+

Cell count analysis

- Sample dilution and even distribution of cells essential to accurate counts
- Different solution for different cells
  - RBC counts need isotonic solution
  - WBC and platelet counts use RBC-lysis solution
- Performed by manual counts or automated analyzers

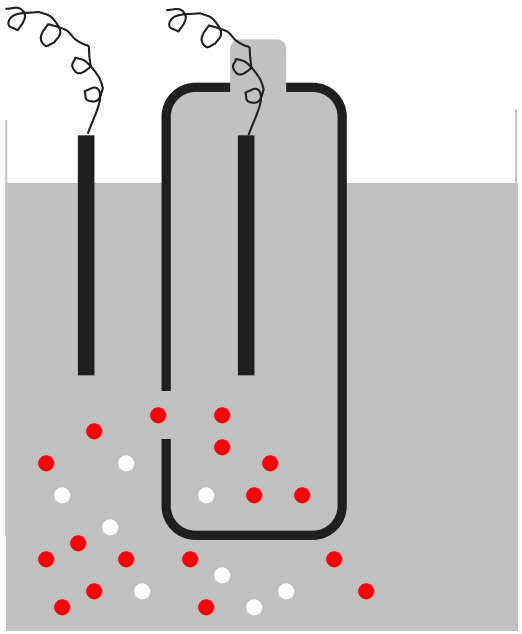
Manual counts

- Rarely used for absolute count
- May use hemocytometer for platelet or low WBC counts
  - Counting chamber with specific volume
  - Viewed with microscope

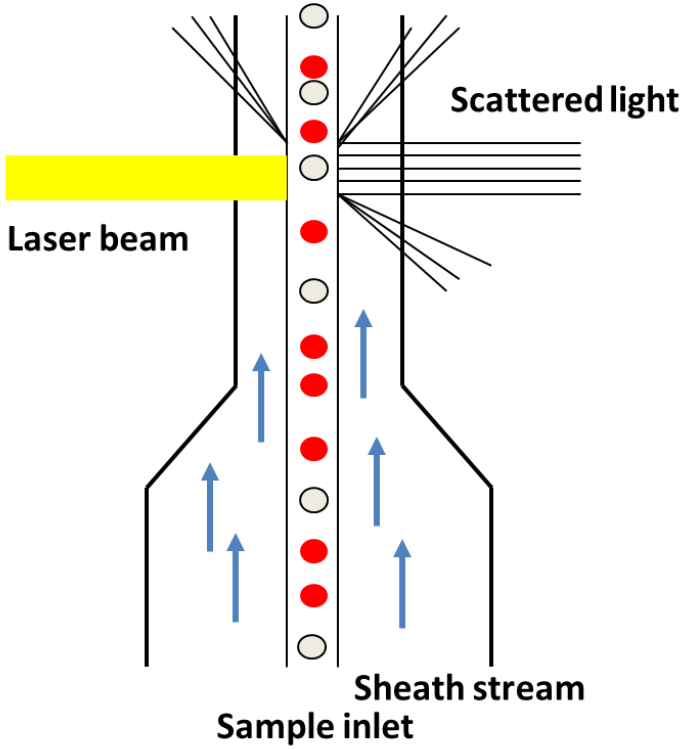


Automated analyzers

- Increased accuracy and speed
- Based on electrical impedance, light scattering, radiofrequency conductivity, or cytochemical reactions
- Electrical impedance
  - Change of voltage when cells pass through aperture

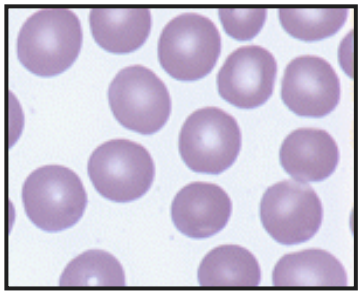


Automated analyzers



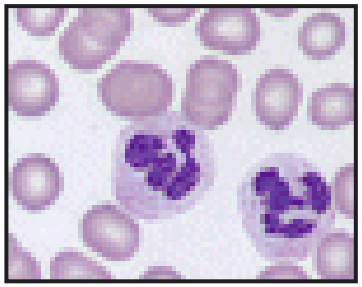
- Flow cytometry and light scatter
- Laser hits single stream of cells, light scatter interpreted into info on size, structure, granularity

CBC parameters



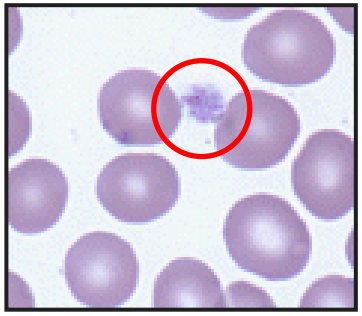
RBC

- Oxygen carrying cells
- Anucleate (unless immature)



WBC

- Immune function



Platelets

- Clotting
- Anucleate cell “fragments”

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Minimizing error

- All laboratory processes are subject to error
- Examples of sources of error in the CBC will be discussed under each cell type
- Automated analyzer computers have multiple programs to detect possible error
- If data meets possible error criteria, data is “flagged” for operator review prior to release

RBC parameters

- Quantitative
  - Hemoglobin (Hgb, g/dL)
  - Hematocrit (Hct, %)
  - RBC count (per µL)
- Qualitative (averages)
  - Mean corpuscular volume (MCV, fL)
  - Mean corpuscular hemoglobin (MCH, pg)
  - Mean corpuscular hemoglobin concentration (MCHC, g/dL)
  - Red cell distribution width (RDW, %)
- Sometimes – reticulocyte count

RBC parameters

Hemoglobin (Hgb, g/dL)

- Colored protein, measured by absorbance at 540nm

Hematocrit (Hct, %)

- Proportion of volume occupied by RBCs
- Manual – height of column after centrifugation
- Automated – RBC number/RBC volume

RBC count (cells per µL)

- Obtained via electrical impedance and/or light scatter
- RBCs and WBCs counted together by analyzer
  - RBC outnumber WBC ~500:1, negligible error

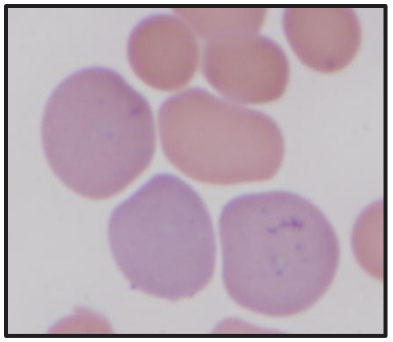
Manual hematocrit tube

RBC parameters

- Mean corpuscular volume (MCV, fL)
  - Average volume of each RBC
  - RBC volumes/RBC count
- Mean corpuscular hemoglobin (MCH, pg)
  - Average Hgb in each RBC
  - Hgb/RBC count
- Mean corpuscular hemoglobin concentration (MCHC, g/dL)
  - Average Hgb concentration in each RBC
  - Hgb/Hct
- These three are calculated averages – may not accurately describe mixed population of cells

RBC parameters

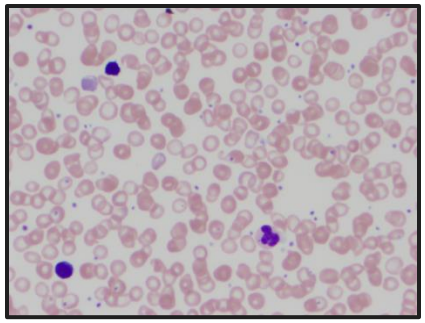
- Red cell distribution width (RDW, %)
  - Range of RBC sizes



- Reticulocytes
  - Immature, anucleated cells containing RNA
  - Reflect bone marrow's ability to make new RBCs
  - Manual – "supravital" stain of ppt RNA
  - Automated – fluorescent dye stains RNA

RBC parameters – clinical scenarios

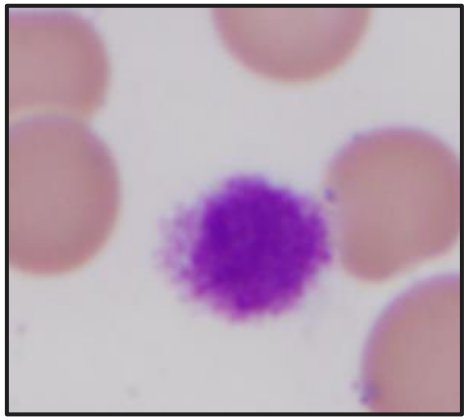
- Decreased Hgb, Hct, or RBC count – **anemia**
  - 1° - iron deficiency anemia
  - 2° - acute blood loss



- Increased Hgb, Hct, or RBC count – **polycythemia**
  - 1° – bone marrow proliferative disease
  - 2° – compensation due to chronically low oxygen from smoking, sleep apnea, high altitude

RBC parameters – examples of error

Parameter	Falsely increased	Falsely decreased
Total RBC count	High WBC count	Hemolysis (in vitro), clotting
Hct	Giant platelets	Hemolysis (in vitro), clotting
MCV	Cell clumping	Giant platelets



Giant platelet

Hemolyzed

Non-hemolyzed

WBC parameters

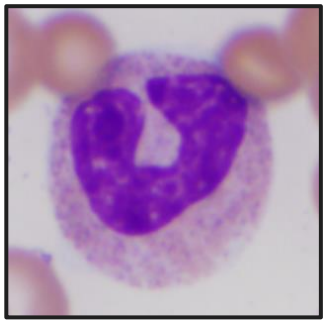
- Quantitative cell count (cells per µL)
- Differential (cells per µL and % of total WBCs)

WBC parameters

- Quantitative cell count (cells per µL)
  - Dilution of blood in RBC lysis buffer, usually acid or detergent
  - Total count of nucleated cells obtained by electrical impedance or flow cytometry (automated) or hemocytometer (manual)

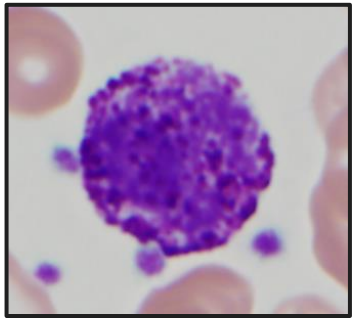
WBC parameters

- Differential (cells per µL and % of total WBCs)

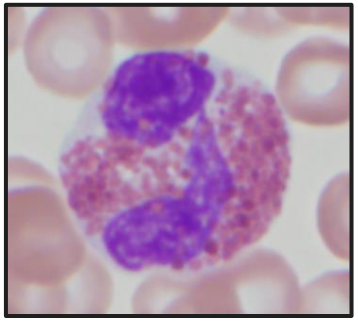


Immature neutrophil
"band form"

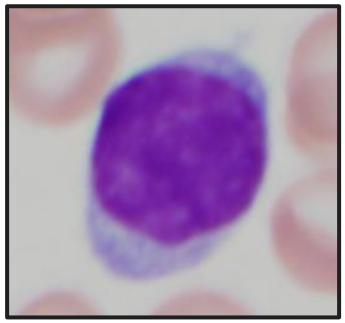
Neutrophil



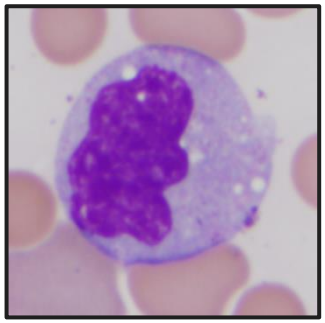
Basophil



Eosinophil



Lymphocyte



Monocyte

WBC differential

- Automated
  - Individual cells analyzed by flow cytometry
  - Light scatter
    - Forward (cell size)
    - Side (complexity, granularity)
  - Cells identified based on expected profile
    - E.g., neutrophils – larger than lymphocytes with granular complexity, monocytes – fewer granules than neutrophils and therefore, less SSC

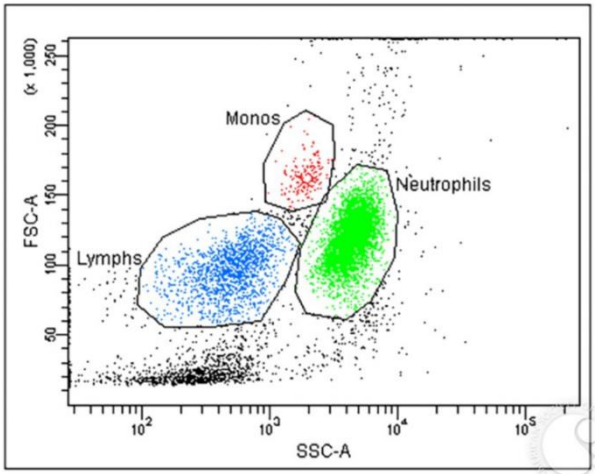
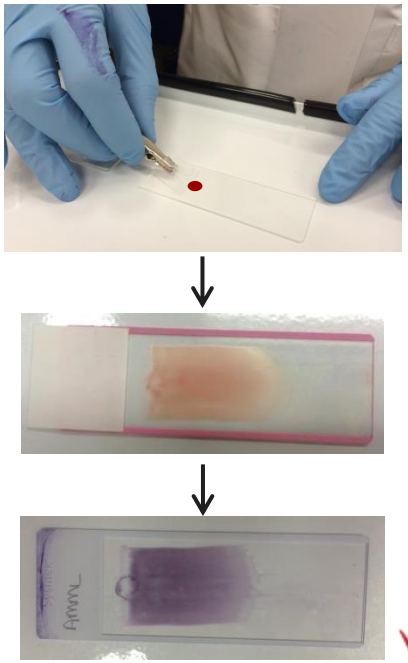


Image was originally published in ASH Image Bank. Maslak and Rose. White cell differential -1. ASH Image Bank. 2008; 00003658. © the American Society of Hematology.

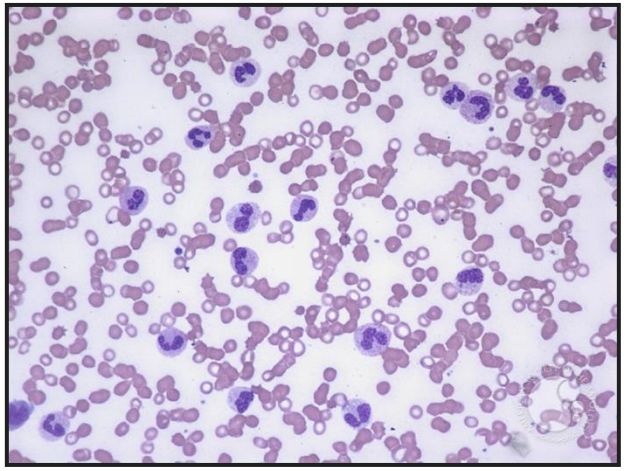
WBC differential

- Abnormal cells that do not identify as WBCs are tagged for manual review
  - E.g., atypical lymphocytes, immature blasts
- Manual review
  - Drop of blood smeared on glass slide
  - Dyes
    - Basic (nuclei, basophilic)
    - Acidic (eosinophilic)



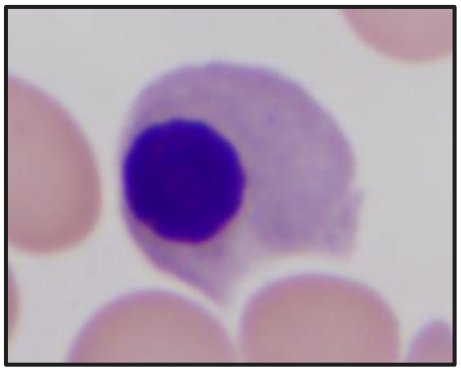
WBC parameters – clinical scenarios

- Decreased total WBC count – **leukopenia**
  - 1° - HIV, bone marrow disease, e.g., aplastic anemia
  - 2° - immunosuppressants
- Increased total WBC count – **leukocytosis**
  - 1° - acute leukemia
  - 2° - neutrophilia and lymphocytosis from infection

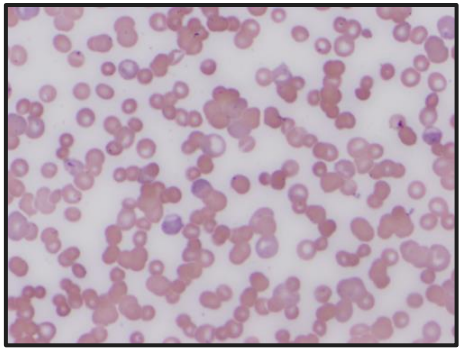


WBC parameters – examples of error

- Total WBC count – falsely increased
  - Nucleated RBCs (counted as WBCs)
  - Antibodies that cause RBC clumping



- Differential
  - Automated –
    - Abnormal cells incorrectly identified
  - Manual –
    - Poor staining leading to cell recognition errors
    - Larger cells pushed to edge, missed in count



Platelet parameters

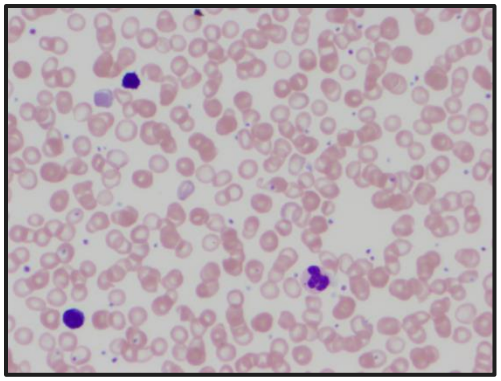
- Platelet count
  - Obtained by electrical impedance or light scatter (automated) or hemocytometer (manual)

Platelet parameters – clinical scenarios

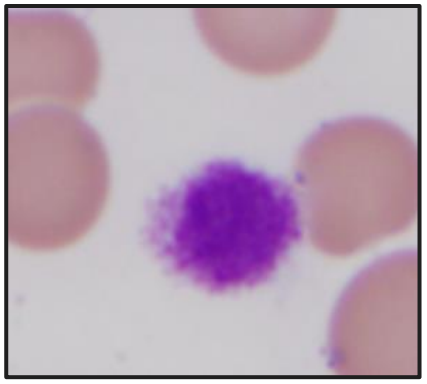
- Decreased platelet count – **thrombocytopenia**
  - 1° - decreased bone marrow production
  - 2° - immune-mediated destruction/sequestration
- Increased platelet count – **thrombocytosis**
  - 1° - proliferative bone marrow disease
  - 2° - acute phase reactant, e.g., inflammation

Platelet parameters – examples of error

Parameter	Falsely increased	Falsely decreased
Platelet count	Hemolysis (in vitro), microcytic (small) red cells	Giant platelets, platelet clumping



Microcytes



Giant platelet

Summary

- CBC is an important screening and diagnostic tool
  - Majority of specimens run on automated analyzers which use impedance and flow cytometry for cell identification and enumeration
  - Manual review and count reserved for abnormal specimens or patients with clinical history
- Knowledge of error sources essential to accurate interpretation

References

1. Perkins SL. Examination of the Blood and Bone Marrow. In: Wintrobe's Clinical Hematology. 12th ed. Philadelphia: Lippincott, Williams & Wilkins; 2009, p. 1-20.

2. Vajpayee N, Graham SS, Bem S. Basic Examination of Blood and Bone Marrow. In: Henry's Clinical Diagnosis and Management. 22nd ed. Philadelphia: Elsevier/Saunders; 2011, p. 509-535.

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