

ROUTINE HEMATOLOGY PROCEDURES

SPECIMEN COLLECTION



- Collection tubes are color coded
- Hematology uses the following
 - EDTA (ethylenediaminetetraacetic acid) – chelates calcium - purple
 - Sodium Citrate – binds calcium – light blue
 - Lithium Heparin – interacts with antithrombin III - green

PHLEBOTOMY COLLECTION EQUIPMENT

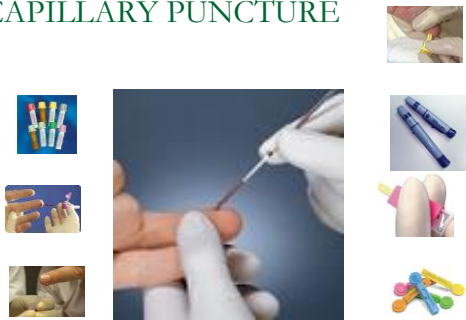
- Specimen Collection Tubes
- Needles
- Tourniquet
- Other – gloves, alcohol, bandages, sharps containers



VENIPUNCTURE

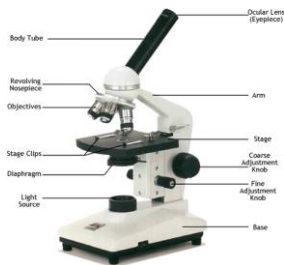


CAPILLARY PUNCTURE



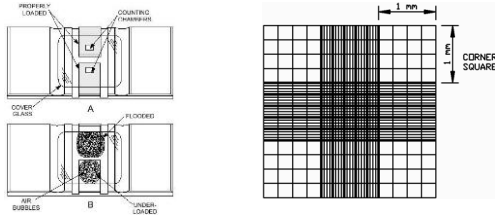
MICROSCOPE

- Resolution
- Optical Defects



HEMACYTOMETER

Manual Counts



MANUAL WHITE CELL COUNTS

- 3% acetic Acid 1:100
- Total number of cells counted in 9 squares + 10% X 100 = cells/mm³
- 100 cells + 10 X 100 = 11,000 cu mm or 11 X10⁹/L

- Note: using LeukoChek
- and neubauer hemacytometer



MANUAL RED CELL COUNTS

- Saline 0.85% 1:200
- Count 5 smaller squares within the center square
- Number of cells counted X dilution factor X 1/volumn = cells / mm³ μL
- 100 cells x 200 x 1/0.04 = 500000 mm³
500000mm³ = 5 x10¹²/L



MANUAL PLATELET COUNTS

- 1% ammonium oxalate or acetic acid 1:100
- Count all areas in large center square
- Multiply the number of platelets counted times 1000

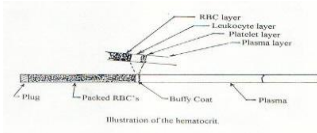
- Note: Medix* Thrombotic Pure Plus Test Kit and Neubauer hemacytometer

HEMOGLOBIN

- Cyanomethemoglobin
- 540 nm
- Lambert-Beer's Law
- Errors
 - ↑WBC Counts
 - Hgb S & C
 - Lipemia
 - Abnormal Globulins

HEMATOCRIT

- Packed Cell Volume
 - Capillary tube
 - Centrifuge 10,000-15,000 g for 5 min
- $Hgb \times 3 = Hct$



MEAN CELL VOLUME = MCV

- $\text{HCT (\%)} / \text{RBC (x } 10^{12}/\text{L)} \times 10 = \text{MCV}$
 - $33/4.0 \times 10 = 82.5 \text{ fl}$
-

MEAN CELL HEMOGLOBIN = MCH

- $\text{Hgb (g/dl)} / \text{RBC (x } 10^{12}/\text{L)} \times 10 = \text{MCH}$
 - $11/4 \times 10 = 27.5 \text{ pg}$
-

MEAN CELL HEMOGLOBIN
CONCENTRATION = MCHC

- $\text{Hgb(g/dl)} / \text{Hct(\%)} \times 100 = \text{MCHC}$
 - $11/33 \times 100 = 33.3 \text{ g/dl}$
-

SEDIMENTATION RATE

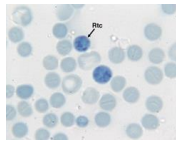
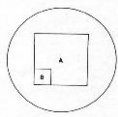
- Rate at which red cells settle from plasma
- Presence of inflammation
- Westergren or Wintrobe
- Things that effect sed rates
 - Protein composition of plasma
 - Size and shape of RBC
 - RBC concentration



$\% \text{ reticulocytes} = \frac{\text{number of reticulocytes counted}}{10}$

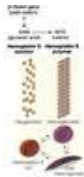
RETICULOCYTE COUNT

- Supravital Stain – new methylene blue
- Miller Disk - This disc consists of 2 squares as shown below in figure 5-2. The area of the smaller square (B) is a tenth that of square A. Therefore, if there are 40 red cells in square A, there should be four red cells present in square B. When employing this method to count reticulocytes, the red cells in square B are counted in successive fields on the slide, until a total of 500 red cells have been counted. At the same time, the reticulocytes in square A are enumerated. At the completion of the count, theoretically, the reticulocytes obtained in this way are divided by 50, in order to obtain the percent reticulocytes present in the blood.
- Count retics in 5 fields of 200 RBC
 $\# \text{ or retic} / 10 = \% \text{ retics}$



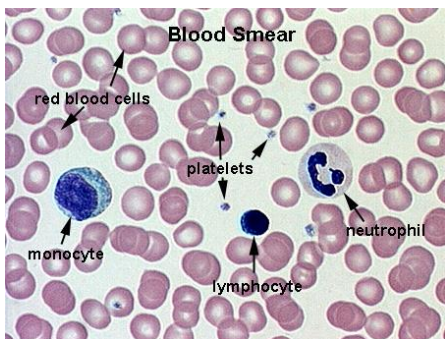
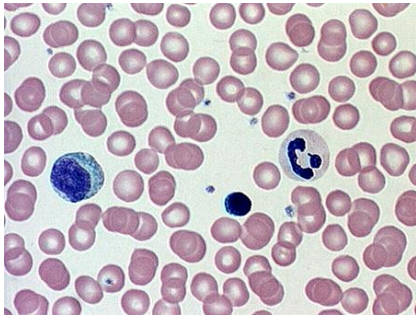
SOLUBILITY TEST FOR HEMOGLOBIN S

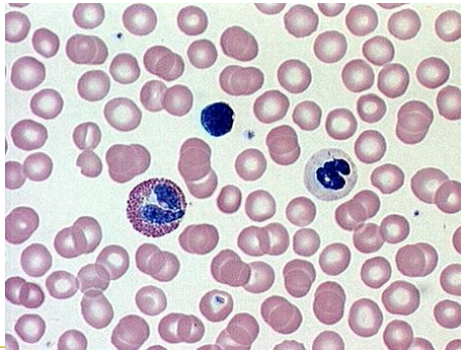
- Sodium dithionite
 - NEGATIVE = Clear Solution
 - POSITIVE = Turbid Solution

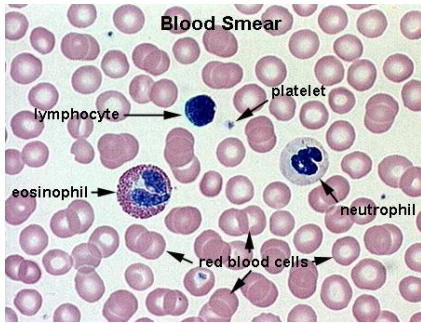


NORMAL PERIPHERAL SMEAR

Microscopic evaluation of stained blood smears.

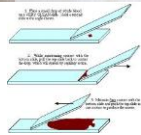






METHODS

- Manual
 - Cover Glass
 - Wedge Smear
- Automated
 - Wedge Smear



4 Simple Steps to Prepare Two Slides at Once



DEMONSTRATION

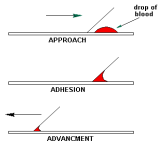
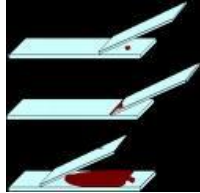
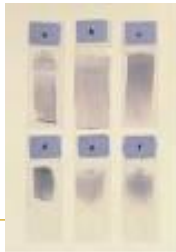


Fig. 13 - How to prepare a blood smear.



OPTIMAL BLOOD SMEAR CHARACTERISTICS

- Minimum Length 2.5 cm
- Gradual Transition from Thick to Thin
- Straight Feather Edge
- Margins Narrower than Slide
- No Streaks, Waves, or Troughs



STAINING - ROMANOWSKY

- Methylene Blue
- Azure B (oxidation product of Methylene Blue)
- Eosin A or B
- Examples – Wright, Wright-Giemsa, Leishman, May-Grunwald, Jenner



GOOD SMEAR

- Macroscopic – Pinkish Purple in Color
- Microscopic
 - Cells Evenly Distributed
 - Areas between cells clear Erythrocytes are orange red
 - Neutrophilic Granules are lilac
 - Eosinophilic Granules are red orange
 - Basophilic Granules are purplish black
 - Lymphocyte cytoplasm is blue
 - Leukocyte nucleoli are purple

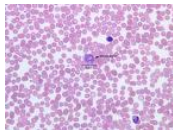


CAUSES OF BAD SLIDES

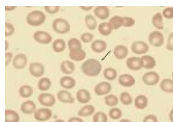
- Excessively Blue or Dark
 - Prolonged Staining
 - Inadequate Washing
 - Too Alkaline Stain or Buffer
 - Thick Blood Smears
- Excessively Pink or Light
 - Insufficient Staining
 - Prolonged Washing
 - Too Acidic Stain or Buffer
- Presence of Precipitate
 - Unclean Slides
 - Drying during Staining Process
 - Inadequate Filtration of Stain

SCANNING THE SLIDE

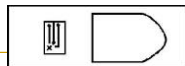
- 10x



- 100x

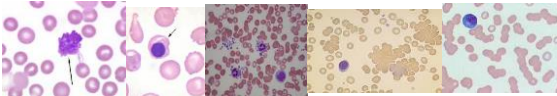


- Pattern for Counting



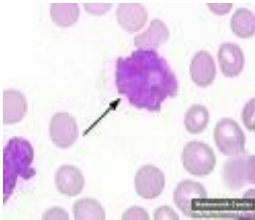
ABNORMALITIES AND EFFECT ON CELL COUNTS

- Smudge Cells- none
- Nucleated RBC – increased WBC counts
- Platelet Clumps – decreased platelet counts
- Platelet Satellitism – decreased platelet counts
- RBC agglutination – decreased RBC count
- Rouleaux - none



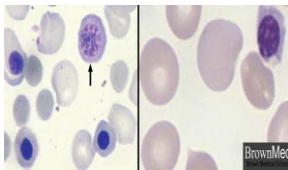
SMUDGE CELL

- Degenerating Leukocytes



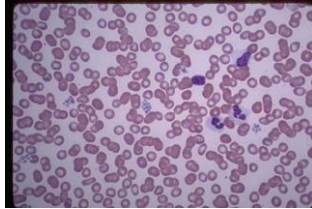
NUCLEATED RBC

- Immature RBC



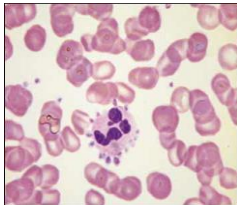
PLATELET CLUMPS

- Clotted Specimen, EDTA induced clumping



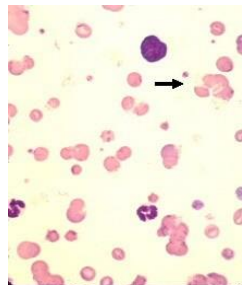
PLATELET SATELLITISM

- Platelets surround leukocytes



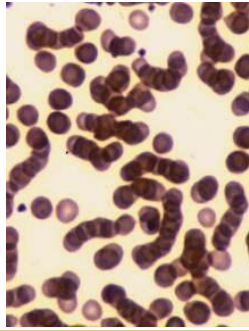
ERYTHROCYTE AGGLUTINATION

- RBC sticking together



ROULEAUX

- Stacking of RBC, looks like coins



ESTIMATING LEUKOCYTE COUNTS

leukocytes counted in 5 fields(10X)/5 = A

A X 0.2 x10⁹/L = leukocytes x 10⁹/L

Example 200 leukocytes counted in 5 fields
 200/5 = 40 x 0.2 = 8 x 10⁹/L

ESTIMATING PLATELET COUNTS

Platelets counted in 5 fields(100X)/5 = A

A X 15 x10⁹/L = platelets x 10⁹/L

Example 150 platelets counted in 5 fields
 150/5 = 30 x 15 = 450 x 10⁹/L

LEUKOCYTE COUNT CORRECTION FOR NUCLEATED RBC

Leukocyte count X 100 / 100 + # NRBC =
Corrected Count

Example Leukocyte Count = $15 \times 10^9/L$
Nucleated RBC per 100 cells counted = 10

$15 \times 10^9/L \times 100 / 100 + 10 = 13.6 \times 10^9/L$

AUTOMATED INSTRUMENTS

- Impedance Instruments
- Light Scatter Instruments



Review

- Blood Collection
 - Manual counts for WBC, RBC, Platelets, HCT
 - Understanding MCV, MCH, MCHC
 - Other testing: sed rates, retic counts, solubility tests
 - Blood Smears: Making, staining, characteristics, counting, abnormalities
 - Instruments
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