BIOM9420
CLINICAL LABORATORY SCIENCE

Haematology and antibody-based diagnostics

Sampling

Blood + anticoagulant – heparin, citrated dextran or EDTA

Blood = cells + plasma

Centrifuge cells and remove plasma for analysis

Haematology

Blood

- Organ with functions

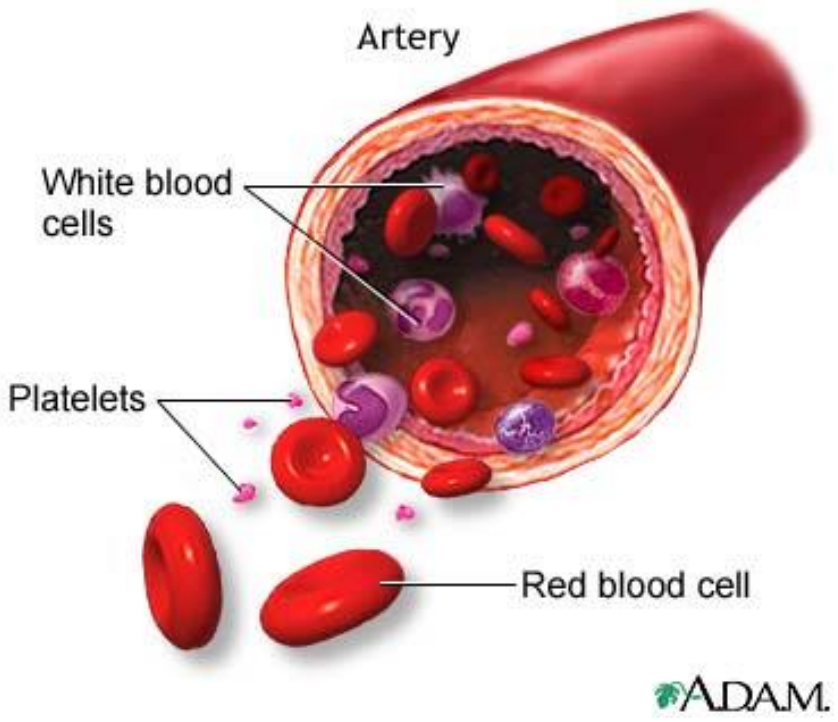
Contains cells, cell fragments (platelets) & matrix

Cells

- Red blood cells
- White blood cells
  - Neutrophils
  - Lymphocytes

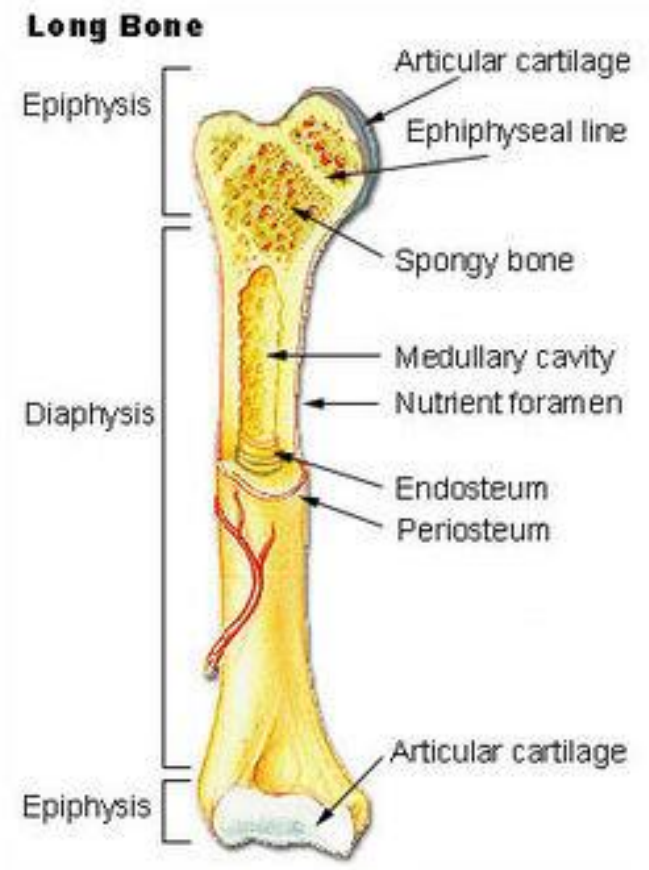
Matrix – fluid, plasma containing

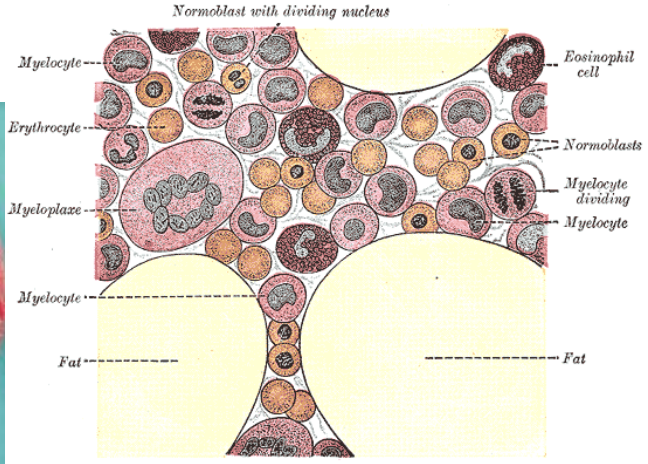
- Proteins
- Electrolytes
- Metabolites

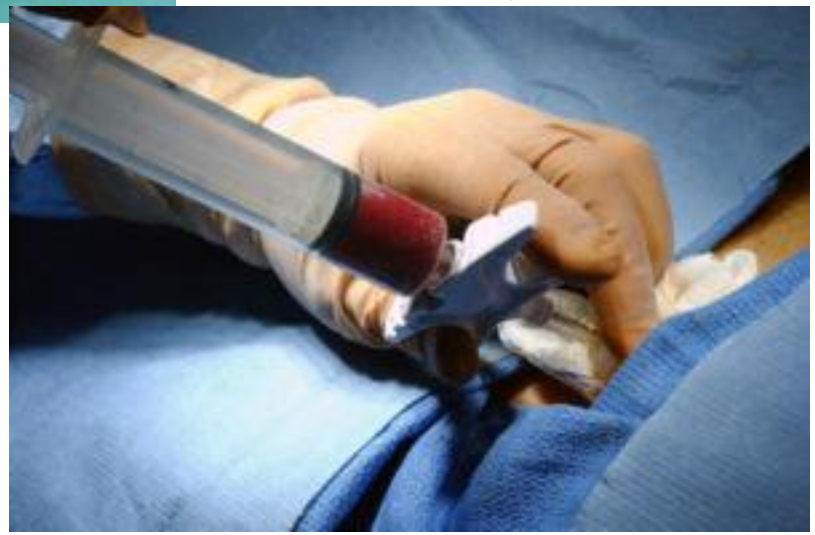


Bone Marrow

- Haematopoietic tissue critical for life
- Factory for blood cells







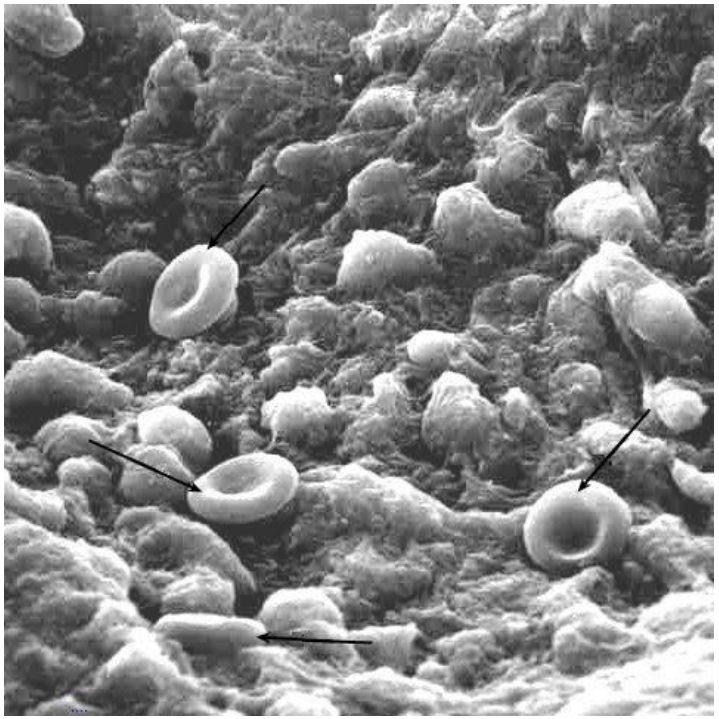
Red Blood Cells

- **Functions**
  - Carries oxygen between lungs, tissues & cells
  - Also carries CO_2, dependent on partial pressures in the tissues
  - Haemoglobin protein,

  haem (iron) globin (protein)
- **Morphology**
  - Disc shape, which

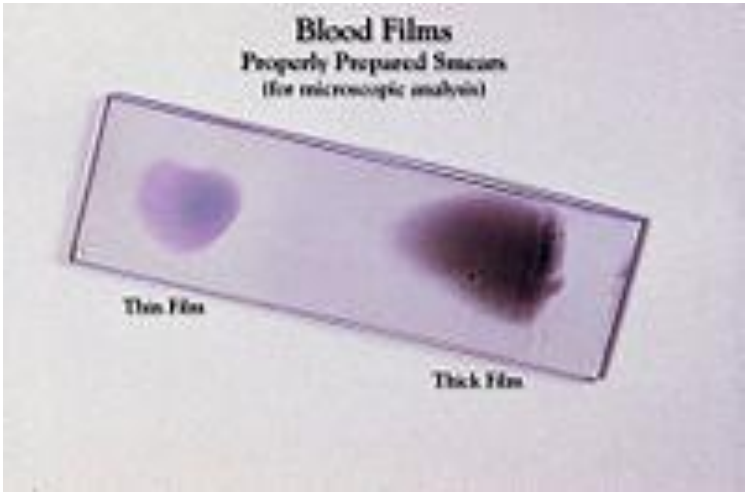
  increases surface area,

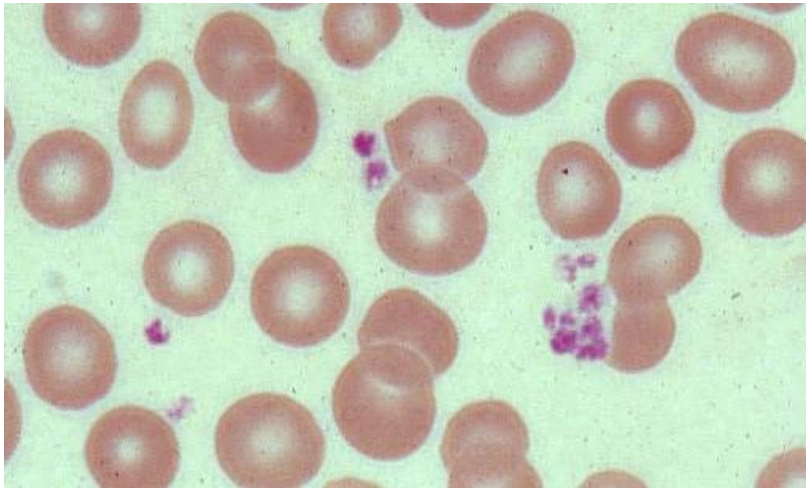
  6 - 8μm diameter
  - No nucleus



Red blood cells

- No nucleus – life cycle = 110 +/- 40 days
  – Must be recycled, by the spleen
- Anaemia: a = (no) haem – low iron – low RBC – low O_2
- Mean diameter = 6.7 – 7.7 μm





Red blood cell count

- Red blood cell number:
  - Adults

    (males): 5.5 +/- 1.0 x 10^{12} / L

    (females): 4.8 +/- 1.0 x 10^{12} / L (lower pregnancy)

    ~ 5 million / mL
- Haemoglobin (Hb)

  (males): 15.5 +/- 2.5 g / dL (100mL)

  (females): 14.0 +/- 2.5 g / dL
- Mean cell Hb

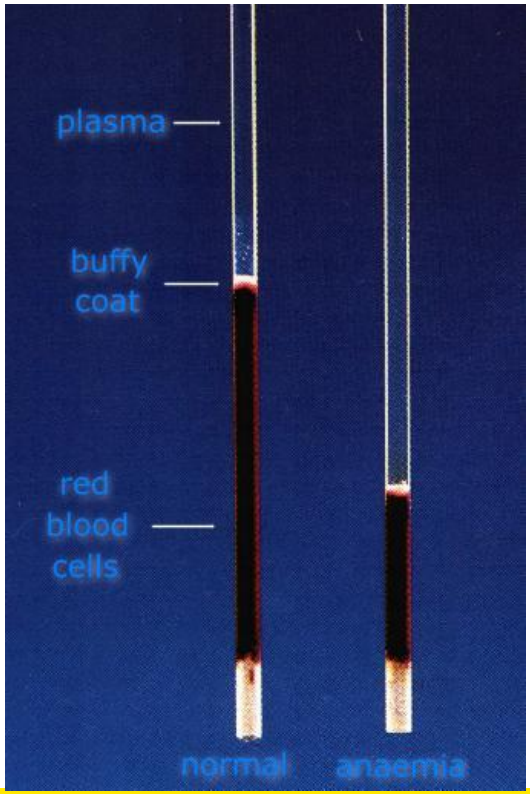
  = 140 – 155g / L / 5.0 x 10^{12} cells / L

  = 28 – 31 x 10^{-12} g / cell

  ~ 30pg

Haematocrit

- **Packed cell volume (PCV) or haematocrit (Hct)**
- **% of red blood cells to the total blood volume.**
- **Normal range**
  - adult males - 0.47 +/- 0.07 (L/L)
  - adult females - 0.42 +/- 0.05 (L/L)
- Low - anaemia, blood loss, bone marrow failure, leukaemia, over-hydration or rheumatoid arthritis.
- High - dehydration (burns or diarrhea), polycythaemia.



Mean cell volume

PCV / cell number

0.47 L/L / 5.5 x 10^{12} /L

= 0.085 x 10^{-12} L

~ 85 x 10^{-15} L

= 85 fL

What is the [Hb] per cell ?

30 pg / 85 fL

= ?g/L

If the MW of Hb = 65000, what is the [M]?

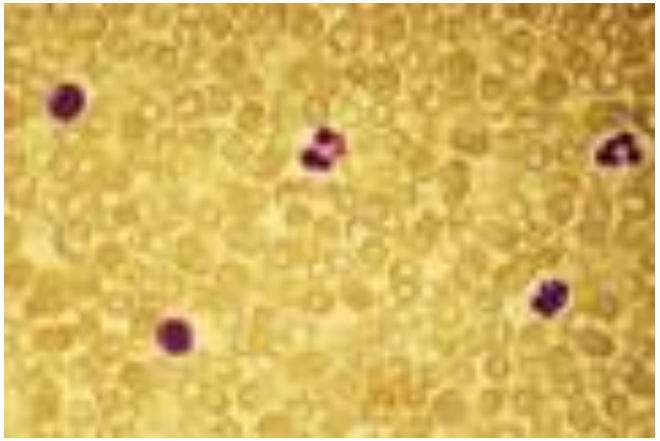
= g/65000 M

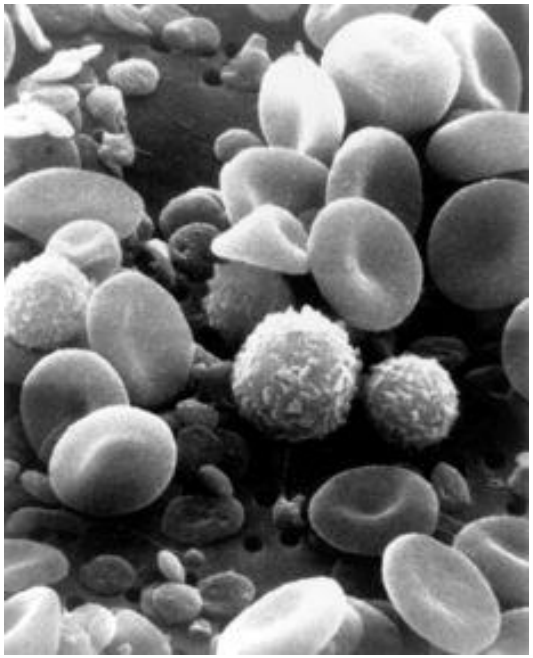
White Blood Cells (WBC)

- Two common measurements of WBC are:

  - Total number per volume

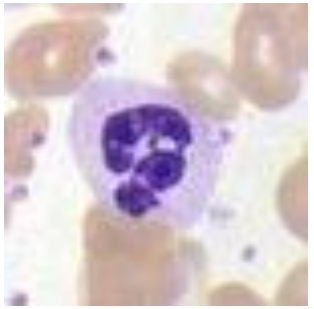
  - Number of each of five sub-types of WBC expressed as % known as a **differential** or "diff“

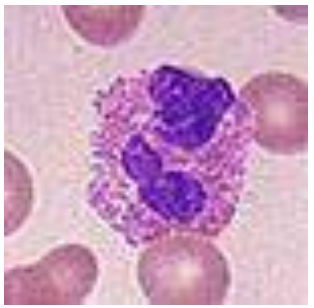




WBC – aka granulocytes

- **Average Lifespan – 15 – 20 days**
- **Morphology**
  - identity based on nucleus, shape, size, and avidity for stains
- **Neutrophils – multi-lobed nuclei held together by strands of chromatin; enzymes in granules - fight infections, bacteria**
- **Eosinophils – Antibody receptors and histamine; asthma**
- **Basophils – circulating mast cells, control inflammatory response**



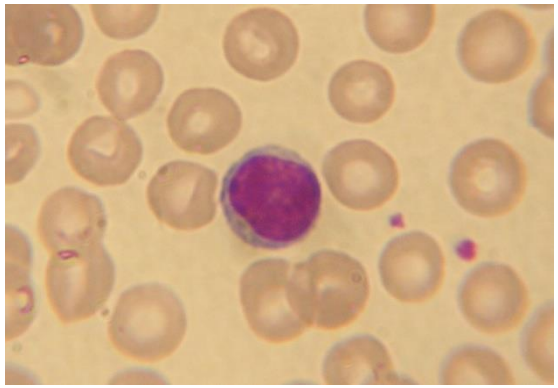


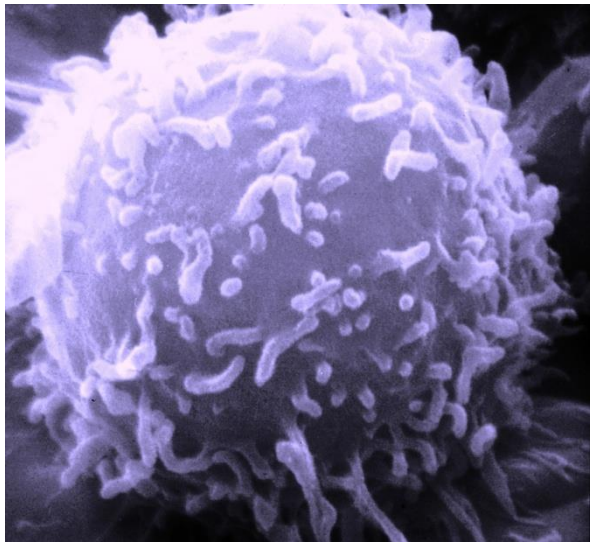
Function

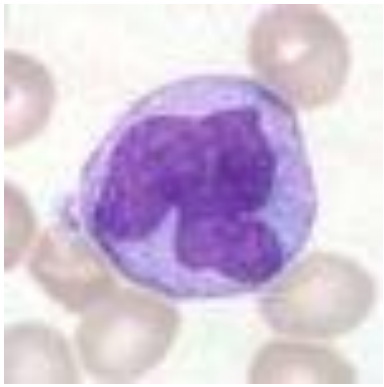
http://www.youtube.com/watch?v=JnlULOjUhSQ

White Blood Cells - other

- Functions
  - Immune defence – **lymphocytes**, T cells & B cells (Antibody production)
  - Control response to foreign bodies – **monocytes** & macrophages

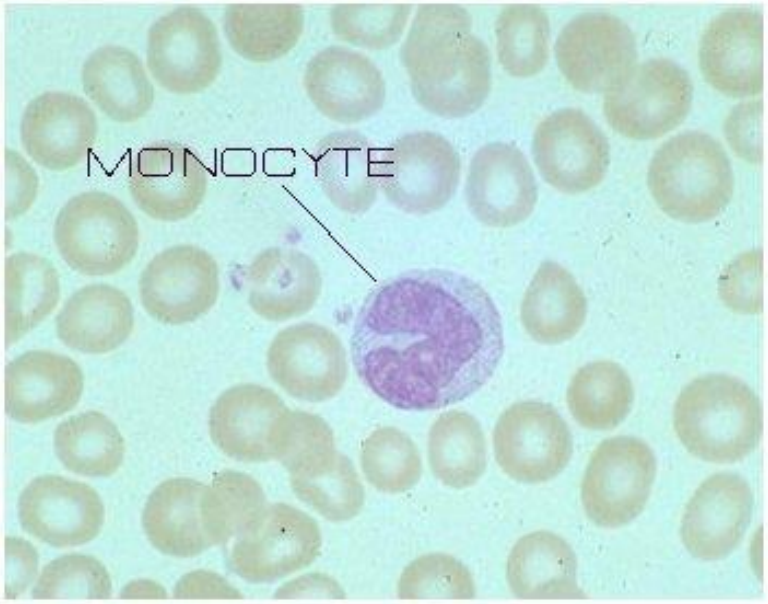






Monocytes

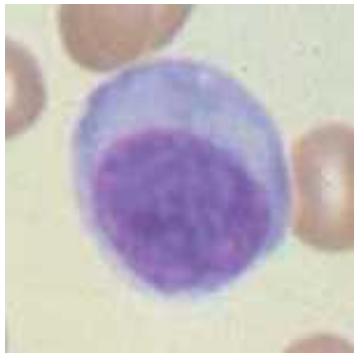
- Monocytes are the largest cells
- Leave the circulation to become macrophages (scavenger cells)

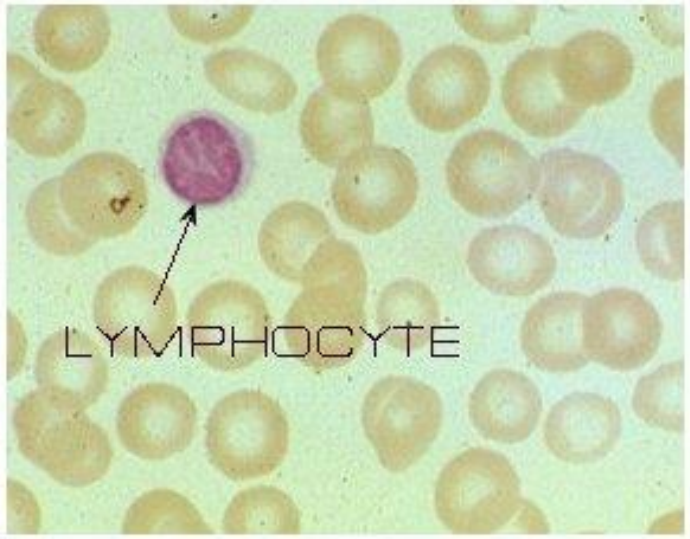


http://greenfield.fortunecity.com/rattler/46/blood3.htm

Lymphocytes

- Lymphocytes are the most numerous and increase in response to viral infections
- Sub-classes of T cells – T_4:T_8 ratio; “helper” / “cytotoxic” : “suppressor” & AIDS
- B cells – produce antibodies also known as plasma cells

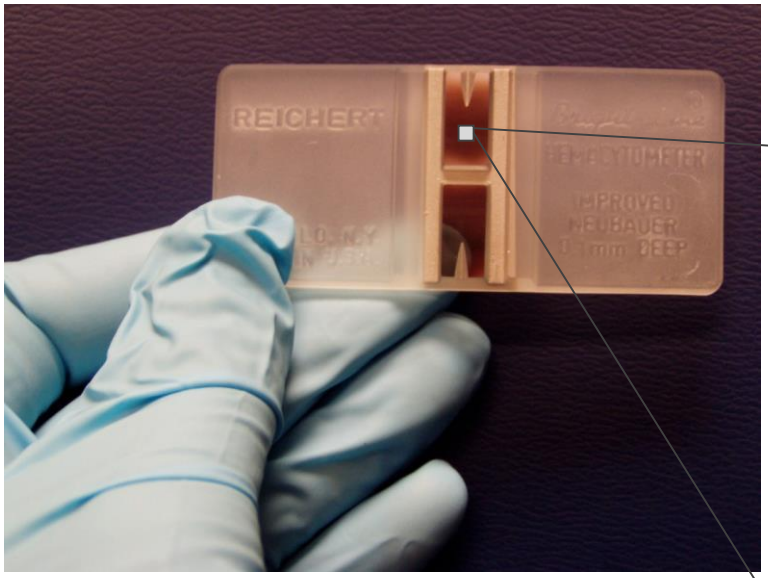




Differential = “diff”

- Blood smear stained to identify cell types
- Relative proportion of each of the white blood cell types expressed as a %
- Haemocytometer, cells / volume
- May Grunwald Giemsa stained smear
  - Count at least 100 cells

Cell counting



Haemocytometer or Neubauer chamber

Volume = Dimensions Area at 0.1mm depth

1 x 1 mm = 1 mm^2 x 0.1 mm

= 0.1 mm^3

= 0.1 μL

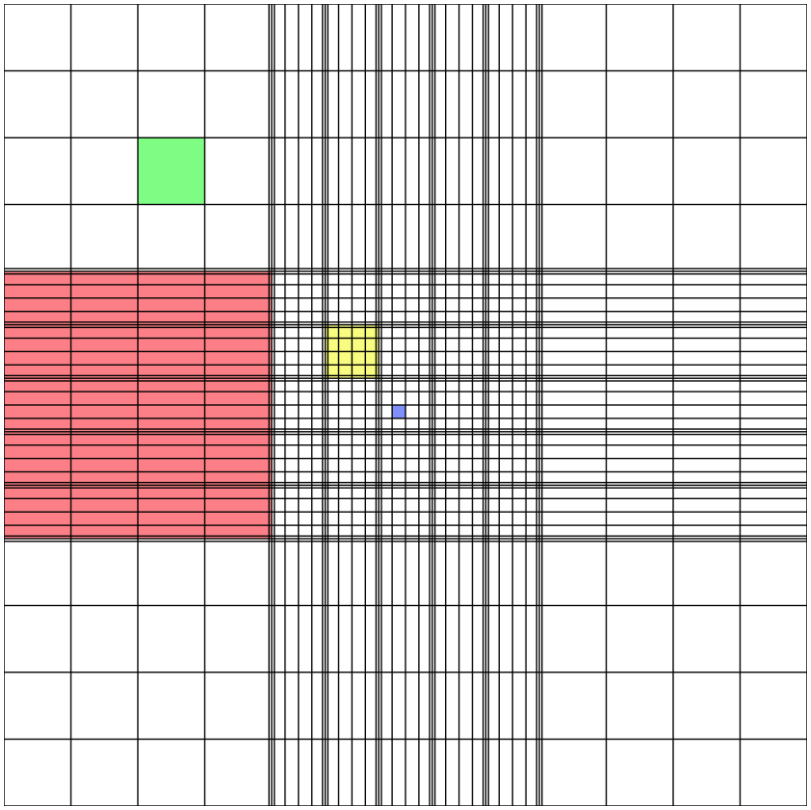
= 100 nl

Therefore,

0.25 x 0.25 mm 0.0625 mm^2 6.25 nl

0.20 x 0.20 mm 0.04 mm^2 4 nl

0.05 x 0.05 mm 0.0025 mm^2 0.25 nl



Average count of cells in red square

Cells / 0.1 μL x 10^7

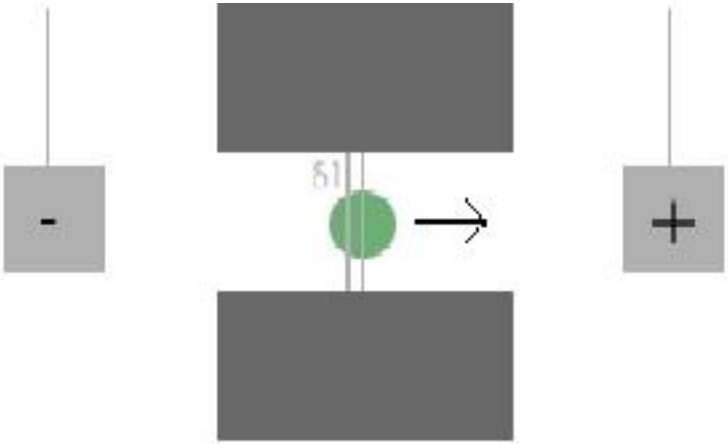
= cells / L

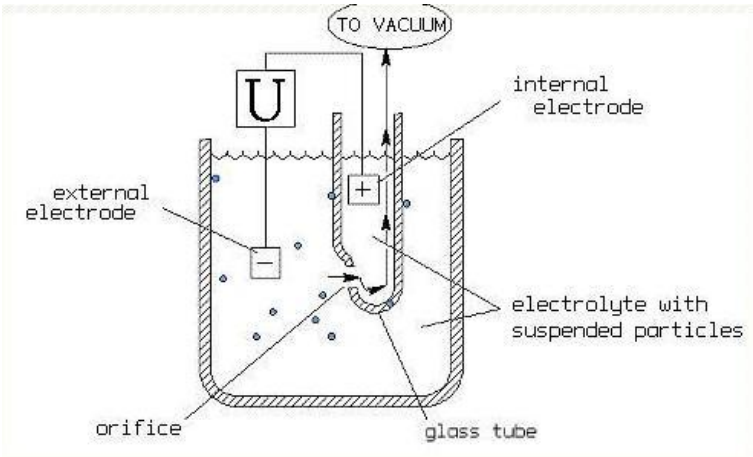
Dilution factors?

How do you work out cells / mL?

Coulter principle

- Wallace Coulter - one of the most influential inventors of the twentieth century, studied electronics at Georgia Tech in the early 1930s.
- “As particles are pulled through an orifice, and across an electric current, they produce a change in impedance that is proportional to the size of the particle traversing the orifice”.
- **voltage pulse** generated is directly proportional to **particle volume**

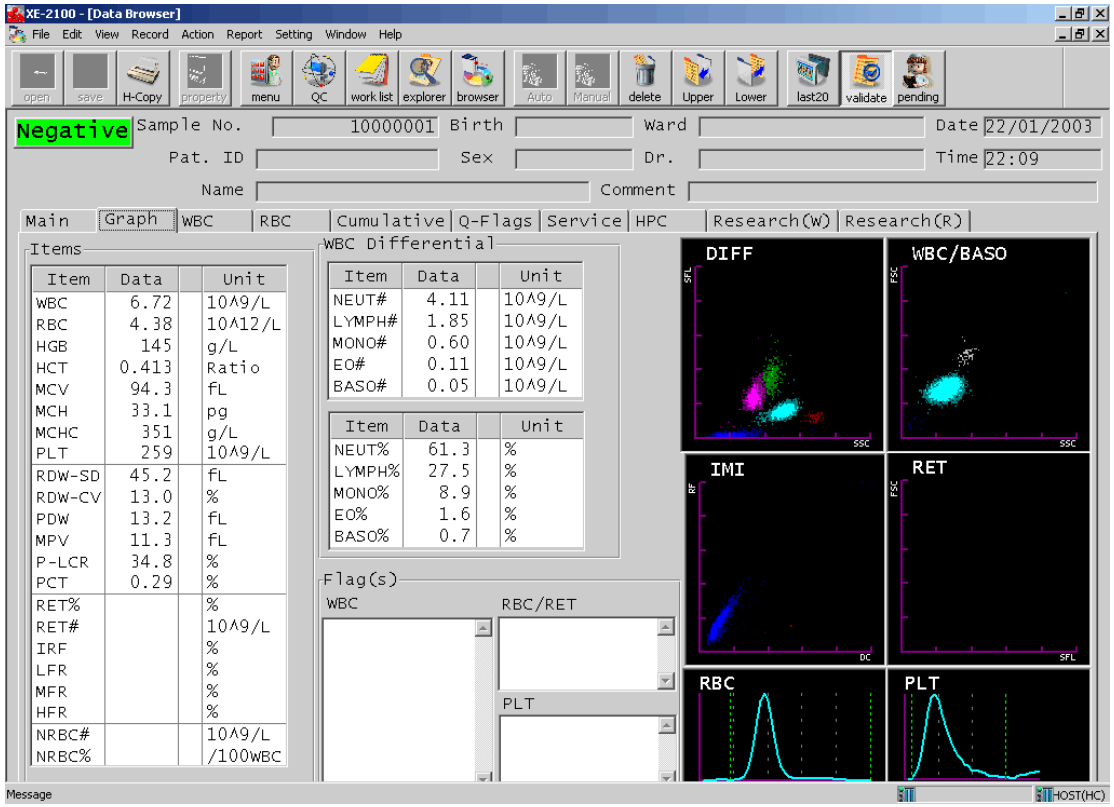




http://www.whcf.org
http://openchemist.net/chemistry/coulter/coulter.html
http://en.wikipedia.org/wiki/Coulter_principle

Modern cell counters

- Some modern machines also use lasers
- Antibodies & fluorescence
  - Fluorescence activated cell sorters (FACS)



Some normal values (adults)

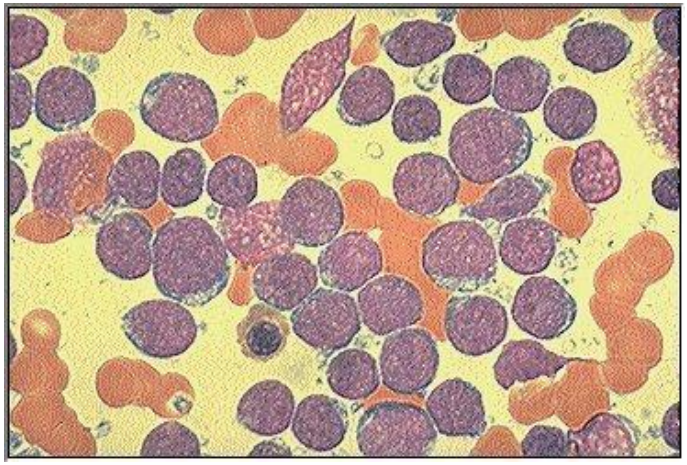
- **Normal Range of WBC concentration**
  - **7.5 +/- 3.5 x 10^9 / L**
- **Granulocytes** (or polymorphonuclears)
  - **Neutrophils:** 40 - 75% relative value
  - **Eosinophils:** 1 - 6% relative value
  - **Basophils:** < 1% relative value
- **Agranulocytes** (or mononuclears)
  - **Lymphocytes:** 20 - 45% relative value
  - **Monocytes:** 2 - 10% relative value
- Each **differential** always adds up to 100%.
- To make an accurate assessment, consider both relative and absolute values.

Leukaemia

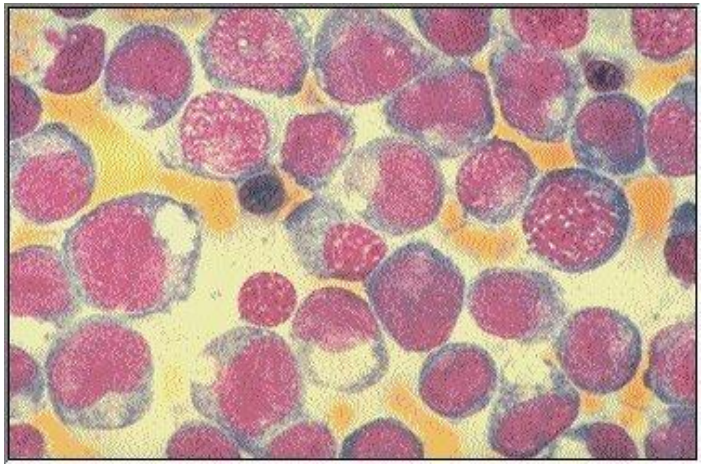
"WBC Cancer" increased number

Acute – shorter time to appear clinically

Chronic – appears over a longer time



ALL



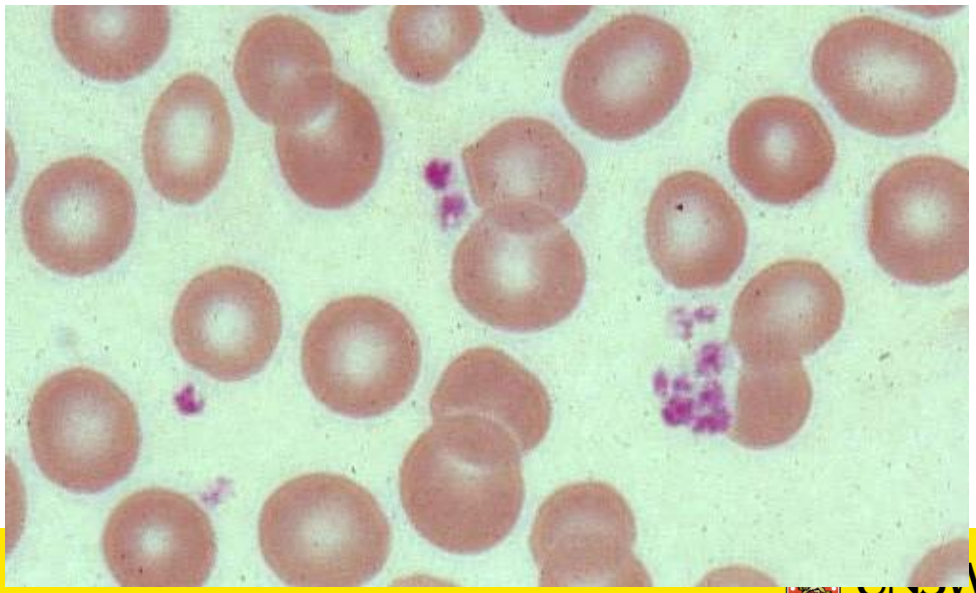
AML

http://greenfield.fortunecity.com/rattler/46/blood3.htm

Platelets

- Normal count = 150 – 400 x 10^9 / L
- 3 μm fragments that bud of the cytoplasm of progenitor cells
- Come from megakaryocytes (160 μm residing in bone marrow – gives rise to ~ 20 platelets
- Contain factors

that control clotting

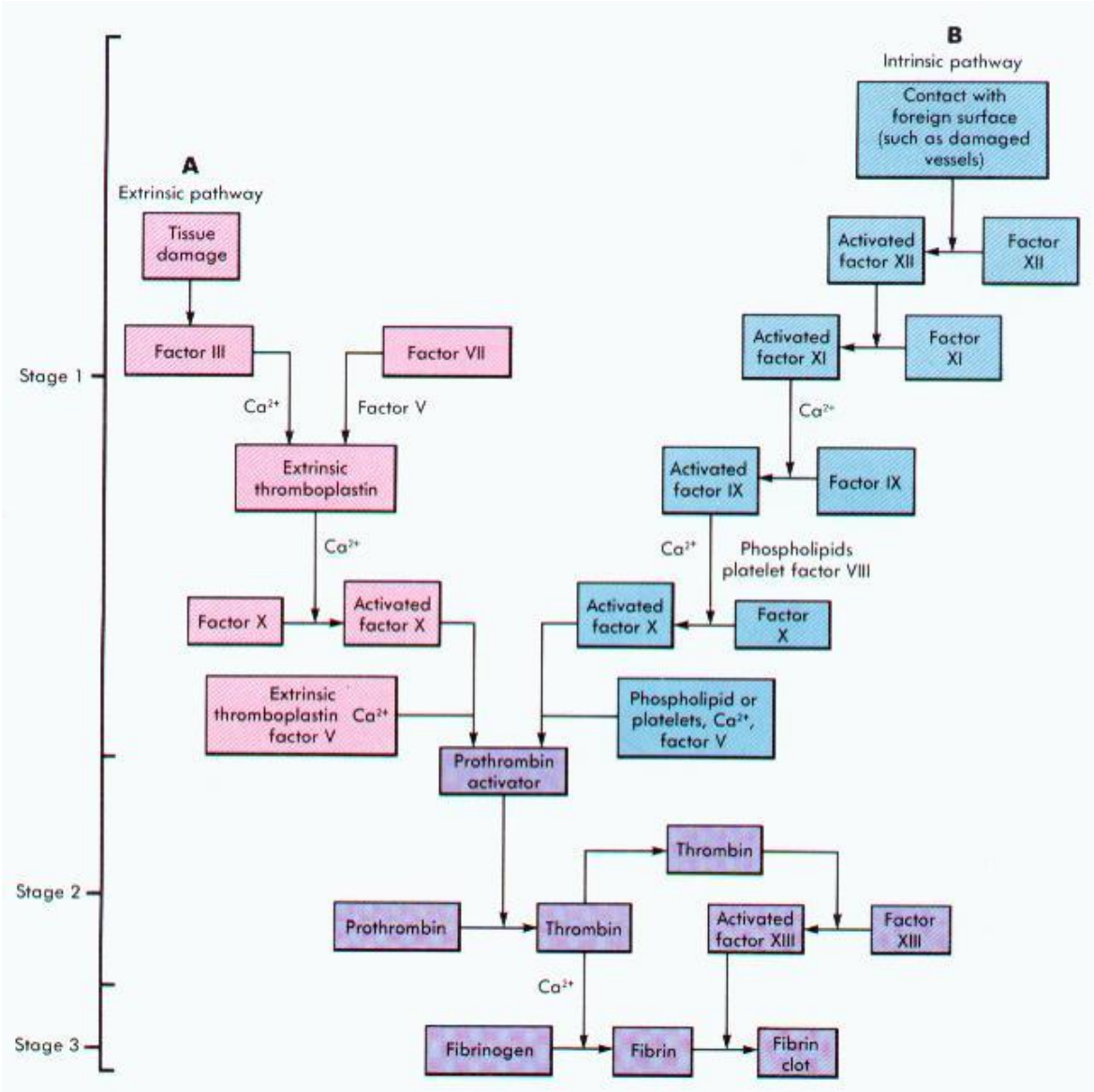


Thrombosis

- “Clot” formation
- Requires activation of proteins in plasma

Coagulation cascade

- Intrinsic and extrinsic pathways
- Enzyme cascade
- Protein conversion
- End result is fibrin produced from fibrinogen and stabilised by FXIII



Plasma

- Pale yellow fluid, volume in a normal adult is around 2.5 to 3 litres.
- Water 90%, Protein 8%, Inorganic ions 0.9% Organic substances1.1%
- Contains clotting agents, the clear fluid exuded from clotted whole blood and plasma is called serum.
- Serum = plasma – proteins involved in clotting (eg. Fibrinogen)
- Functions
  - Maintenance of the acid-base balance.
  - Clotting
  - Inflammatory response and protection from infection - antibodies

Plasma Proteins

- Plasma proteins – major groups
  - **albumin (60%)**
  - **globulins (34%)**
  - fractions (alpha, beta and gamma)
  - **fibrinogen (4%)**
  - **Other (2%)** vitronectin, fibronectin
- Proportions of plasma proteins vary in certain diseases, therefore can be a useful diagnostic aid.
- Most plasma proteins produced by the liver
- Antibodies produced by B lymphocytes
- Albumin can pass through capillary walls and indicate basement membrane integrity – kidney function. Liver replaces lost albumin.