BIOM9420 – CLINICAL LABORATORY SCIENCES

REVISION NOTES

CONTENTS

DIAGNOSTIC ENGINEERING

IDENTIFY WHY CLINICAL TESTS ARE ORDERED

- Clinical tests are ordered for:
  - Screening, diagnosing and monitoring diseases
  - Monitor effectiveness and identify complications of diseases
  - Predict survivability, employability and conduct research

IDENTIFY THE RANGE OF FLUIDS/TISSUES THAT CAN BE ANALYSED BY CLINICAL TESTS

- The main types of fluids and tissues that are tested include:
  - Blood
  - Urine
  - Cerebrospinal fluid (CSF)
  - Saliva
- What is tested is largely dependent on the analyte to be measured and how easily it can be collected.

UNDERSTAND THE REQUIREMENTS OF AN ANALYTICAL TEST AND REASONS FOR VARIANCE IN THE RESULTS

- Practical Requirements
  - Specimen being tested
  - People to do the testing
  - Cost, quality control and safety
- Performance

Accuracy	–	Closeness of the measured value and the "true" value
Range	–	Range of concentration or other quantity in the sample over which the method is applicable
Sensitivity	–	Probability of a positive result
Specificity	–	Probability of a negative result
Blank measurements	–	Measurements due to reagent and sample constituents
Detection limit	–	The smallest concentration or quantity of an analyte that can be detected
Interference	–	Possible external factors affecting the accuracy of measurement
Precision	–	Ability to replicated the test and replicate the measurements
Ruggedness	–	Consistently reliable, despite operators or batches of reagents

UNDERSTAND THE DIFFERENT COMPONENTS OF BIOSENSORS

- A biosensor is a device that uses specific biochemical reactions mediated by isolated enzymes, immune-systems, tissues, organelles or whole cells to detect chemical compounds usually by electrical thermal or optical signals.
- Ideally a biosensor should be :
  - Specific for a certain disease
  - Easily measurable
  - Able to quantify the severity of the disease
  - Allows for early detection
  - Not affected by other biological disturbances
- Biosensor components include:
  - Analyte – substance being measured
  - Sample handling – how is the analyte delivered to the sensitive region
  - Detection and Recognition – how is analyte going to be specifically recognised
  - Output – how to tell if the analyte was detected, how long till results can be determined, how to only detect what is wanted

Understand the Different Types of Biosensors

- Non-contacting sensor
- Contacting sensor
- Invasive contacting sensor
- Sample removal sensor
- Point of care diagnostics
    - Optical:
        - Change in light intensity – colorimetric or photometric
        - Very sensitive and specific as well as produces fast and real-time results
        - Light absorption changes between the reactants and the products of a reaction
        - Calculated using Beer's Law

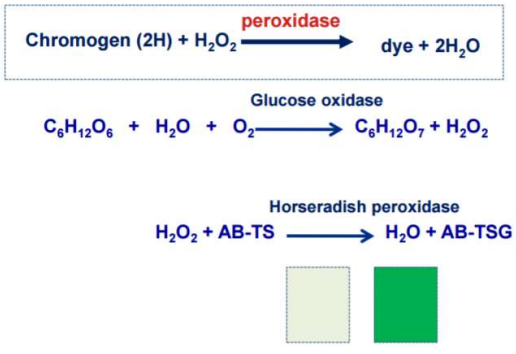
$$A = \varepsilon c l$$

A = absorbance (AU)
E = molar extinction coefficient ($M^{-1}cm^{-1}$ or $L.mol^{-1}cm^{-1}$)
c = concentration (M)
l = path length (cm)

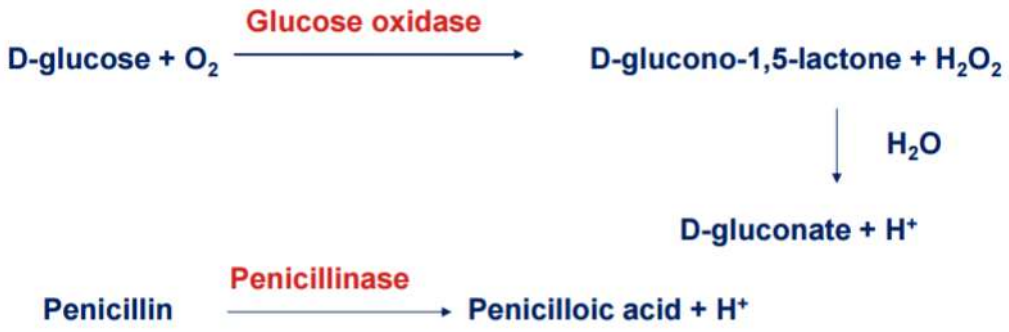
        - Measures the ratio of light intensity entering and passing the material
    - Electrochemical
        - Change in electron distribution – potentiometry and conductivity
        - Converting chemical quantity into an electrical signal
            - Potentiometric sensors – difference in potential (volts)
                - Reaction produces ions with a sensor measuring the potential difference between these two electrodes, with the electrode being selective for one ion
            - Amperometric sensors – difference in current (amperes)
                - Current is proportional to the concentration of the species which are being electrochemically transformed at the electrode
            - Conductometric sensors – difference in resistance (ohms)
    - Piezo-electric
        - Change in mass
        - Uses crystals such as quartz that vibrate under the influence of AC.
            - Added mass causes the crystal to oscillate more slowly.
    - Calorimetric
        - Change in heat
            - If the enzyme catalysed reaction is exothermic, two thermistors may be used to measure the difference in resistance between reactant and product.

Understand the Different Chemical Reactions that Allow the Detection of Glucose in Biological Fluids

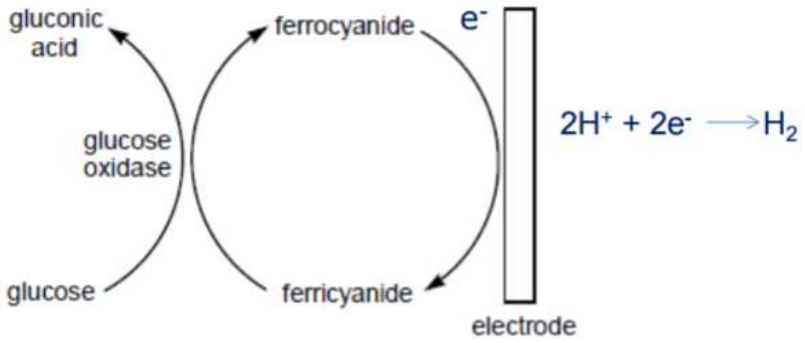
- Colorimetric



- Potentiometric



- Amperometric

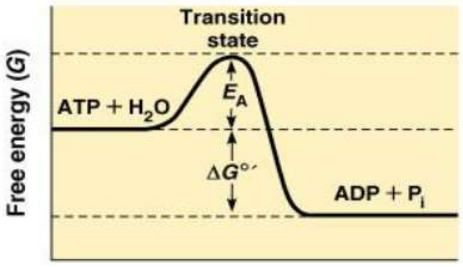


CLINICAL BIOCHEMISTRY (ENZYME LABORATORY, SENSI-GO CLINICAL TRIAL)

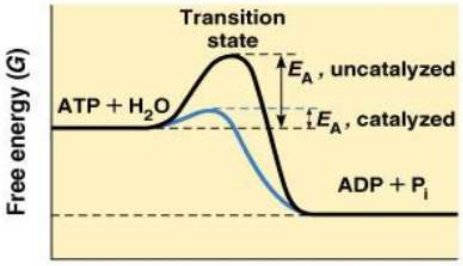
UNDERSTAND HOW ENZYMES CAN BE USED AS DIAGNOSTIC TOOLS

- ALT, AST and GGT all come from the liver, hence are good measures of liver function - damage, viral infections and detoxifications
- Alanine Transaminase (ALT)
    - Transfers an amino group from alanine - mainly in liver but also in skeletal muscle kidney and heart.
- Aspartate Transaminase (AST)
    - Transfers the amino group from aspartic acid
- Gamma Glutamyltransferase (GGT)
    - High concentrations in kidney and pancreas
    - Increases in activity in serum confined to hepatobiliary disease
    - Increases correlates with alcohol intake and other poisons - barbiturates
- Creatine Kinase (CK)
    - Elevated in heart attack - released after death of heart muscle
- Alkaline Phosphatase (ALP)
    - Indicates bone growth and released during pregnancy
- Alpha amylase (AMS)
    - Pancreas

Understand how Enzymes Assist Chemical Reactions to take Place



- Reduce energy required for energy to take place
- Energy of activation is energy required to activate one mole of the substrate.
- Enzymes have an active site
- Lock and key analogy - enzyme is lock, substrate is key - only when bound and fit together do they work
- Some enzymes require certain ions - another factor required - NAD
- Enzymes are proteins, they are modulated by:
    - pH
    - Temperature
    - Competitive inhibitors
    - Concentrations



Understand Michaelis-Menten Enzymes Kinetics and Use the Equation to Solve Problems

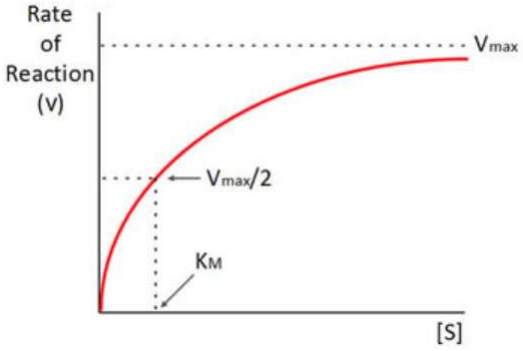
$$E + S \underset{k'_1}{\overset{k_1}{\leftrightarrow}} ES \xrightarrow{k_2} E + P$$

- E = enzyme
- S = substrate
- P = product
- Enzyme and substrates reacts to form ES with a reaction rate constant k1
- The ES driven to form the same enzyme and the product.

- Concentration of enzyme is much less than substrate
- The concentration controls the rate of reaction

Michaelis-Menten Kinetics:

- Several assumptions are made.
    - Simplest case of enzyme kinetics
    - Assumes enzyme and substrate quickly reach steady state in the formation of a complex
    - Assumes the amount of enzyme is much smaller than the amount of substrate, ie [E] is limiting
    - Assumes temperature and other conditions remain constant



- Relates the initial rate of reaction to the substrate concentration.
- Maximum rate of reaction is approached when the substrate concentration is high enough that all enzymes are bound to the substrate
- The MM constant is the substrate concentration in mol/L when the initial velocity is half of Vmax.
- K is a constant for the given enzyme substrate pair under given conditions.
- Relates the initial reaction rate V_0 to the substrate concentration [S].

- The graph is a rectangular hyperbolic function, with the maximum, Vmax being an asymptote.

$$V = \frac{V_{max}[S]}{K_m + [S]}$$

  - Km is the dissociation constant - MM constant
  - Moles/L
  - Indirect measure of the stability of the ES complex
- The lower the values, the higher the affinity between enzyme and substrate, the higher the affinity, the less likely it is to dissociate.
  - Km = $1x10^{-9}$ >> $1x10^{-12}$M
  - MM constant
- Scenarios:
  - As [S] increase:
    - Velocity increases
  - [S] is large compared to Km
    - Vmax is approached
  - [S] = Km
    - V is at Vmax/2

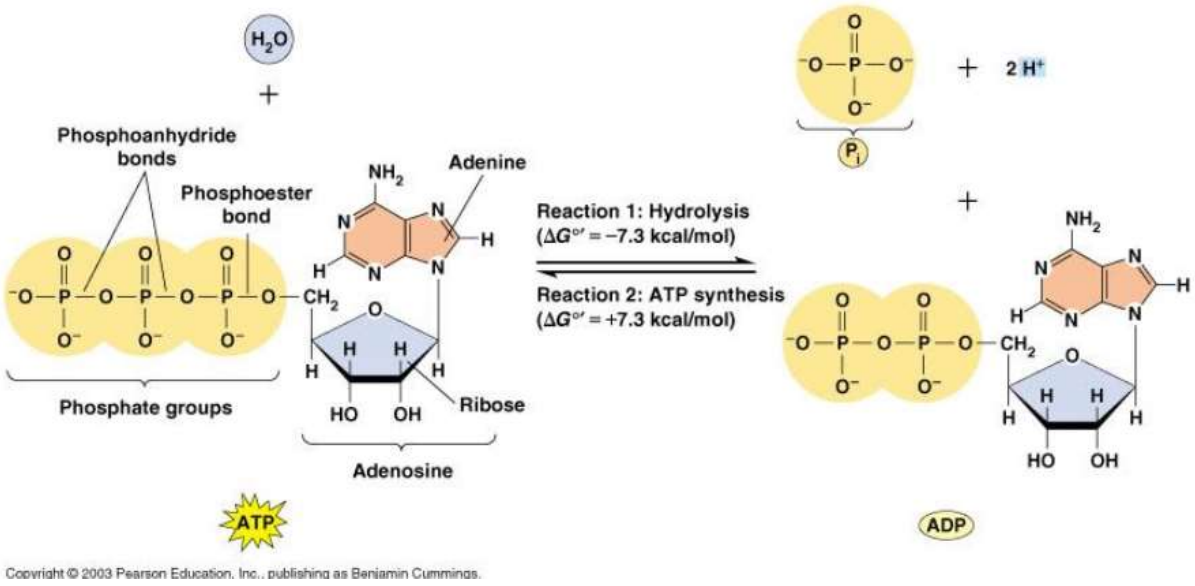
Understand and Apply Beer's Law to Problem Solving

The absorbance of a coloured solution measured at 405nm in a cuvette with a 1cm path length is 0.1. If the product has an $\varepsilon = 9900\ M^{-1}cm^{-1}$, what is the concentration of the product?

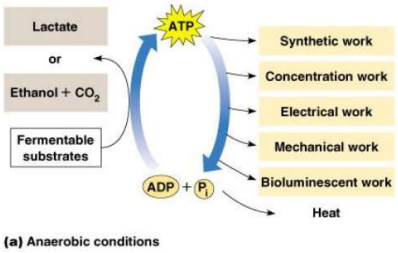
- $A = \varepsilon cl$
- $c = Al/\varepsilon$
- $= 0.1 / 9900\ M^{-1}cm^{-1} \times 1cm$
- $= 1 \times 10^{-5}\ M$

Understand how ATP is Generated and Utilised in the Body and Understand Aerobic and Anaerobic Respiration

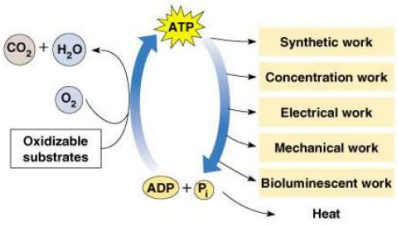
- Hydrolysis and Synthesis
  - ATP is the source of energy for organisms.
  - Energy is released when ATP is hydrolysed and ADP is produced.



- ATP and ADP Equilibrium
  - Concentration is maintained FAR from equilibrium
  - ATP is 1000 times more than ADP
- Synthesis of ATP
  - Anaerobic:
    - Absence of oxygen
    - Substrates are converted to either ethanol and carbon dioxide
  - Aerobic
    - Presence of oxygen
    - Substrates are oxidized to carbon dioxide and water



(a) Anaerobic conditions



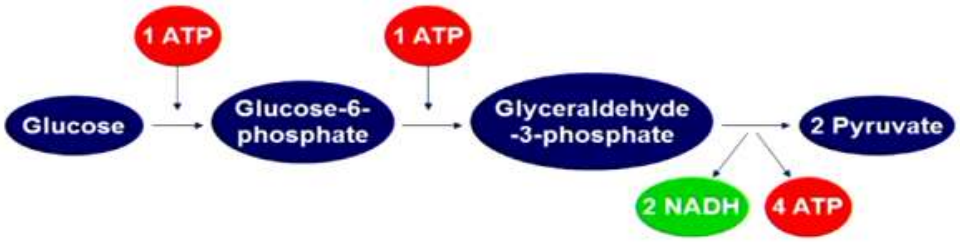
(b) Aerobic conditions

- ATP from Glucose

$$C_6H_{12}O_6 + 6\,O_2 + 36\,ADP + 36\,P \longrightarrow 6\,CO_2 + 6\,H_2O + 36\,ATP$$

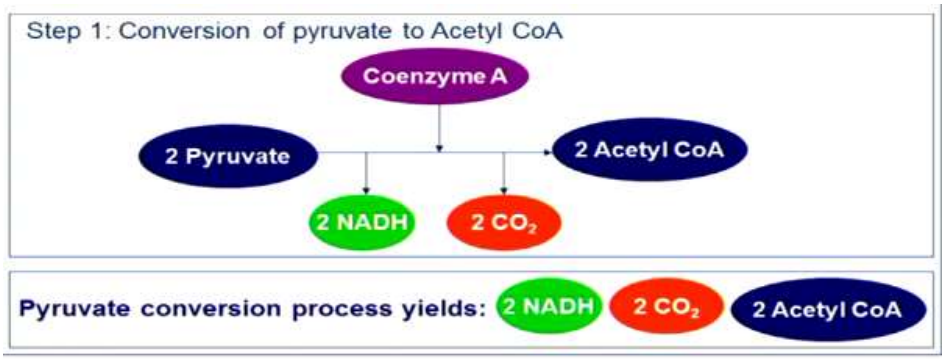
glucose oxygen carbon dioxide water

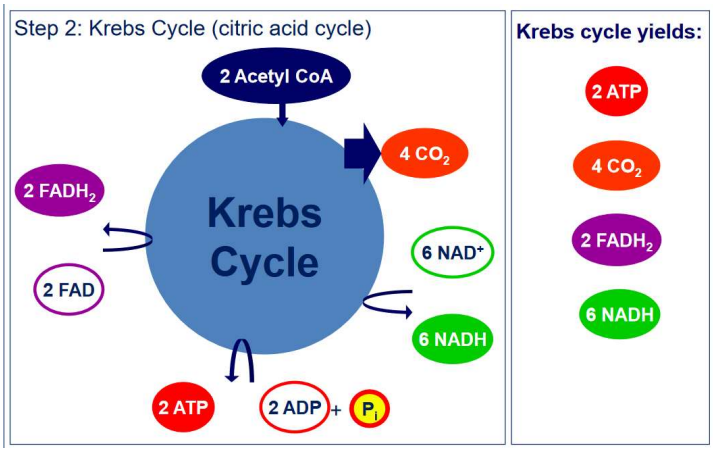
  - The process takes place over 3 major reaction pathways – aerobic energy production
    - Glycolysis – net gain of 2 moles of ATP
      - Glucose is broken down in cytoplasm of cell



Glycolysis process yields: 2 ATP 2 NADH 2 Pyruvate

- Krebs Cycle (Citric Acid) – Cellular Respiration
  - Occurs in the mitochondria





- Electron Transport Chain (ETC)
  - NADH and FADH2 transfer the electrons gained to oxygen
    - This process releases energy to make ATP.
    - Transfer of electrons to oxygen and hydrogen forms water.
    - Driven by amount of ATP available.
- 36 moles of ATP per mole of glucose

HAEMATOLOGY

IDENTIFY THE COMPONENTS OF BLOOD AND UNDERSTAND THEIR FUNCTION AND HOW CHANGES IN THEIR LEVELS ARE RELATED TO DISEASE

- Sampling
  - Anticoagulants prevent the blood from clotting, eg – heparin, citrated dextran or EDTA.
  - Blood is a mixture of cells and plasma.
  - Centrifuge is used to separate the cells and plasma.
- Haematology
  - Blood is an organ with functions, it contains cells, cell fragments (platelets) and matrix.
  - Cells include red blood cells, white blood cells with include neutrophils and lymphocytes.
  - Matrix include fluid, and plasma which includes proteins, electrolytes and metabolites.
- Bone Marrow
  - Blood cells originate from the bone marrow – it is the haematopoietic tissue which is critical for life.

RED BLOOD CELLS

- Functions are to carry oxygen between lungs, tissues and cells. Also helps remove CO_2 dependent on partial pressures. It is a haemoglobin protein (iron protein).
- Disc in shape with a surface area of 5-7 microns in diameter.
- RBC has not nucleus, lasts about 110+-40 days – recycled by the spleen.
- Anaemia – low iron, low RBC count, low O_2.

- RBC count.
  - Number of red blood cell numbers:
    - Adults:
      - Men – 5.5x10 12/L
      - Women – 4.8x10 12/l – much lower during pregnancy.
    - Haemoglobin
      - Males, 15.5g/dL (100ml)
      - Females, 14.0 g/dL
      - Mean cell Hb – 30pg (pictograms)
- Haematocrit – packed cell volume (PCV)
  - Percentage of red blood cells to the total blood volume.
  - The normally ranges for men and female are 0.47 and 0.42 L/L respectively.
  - Low Hct – anaemia, blood loss, bone marrow failure, leukaemia, over hydration or rheumatoid arthritis
  - High Hct – dehydration, polycythaemia
  - Mean cell volume = PCV/cell number

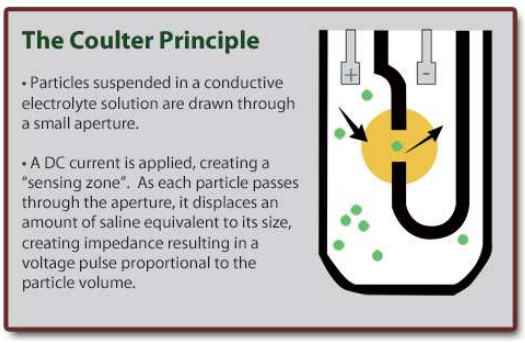
WHITE BLOOD CELLS - GRANULOCYTES

- WBC - two common measurements of WBC are total number per volume and number of each sub-types of WBC expressed as a percentage – also known as a **differential**.
- WBC are also known granulocytes – average lifespan – 15-20 days.
- Morphology – based on nucleus, shape, size and avidity for stains.
- Neutrophils - multi-lobed nuclei held together by strands of chromatin; enzymes in granules - fights infections, bacteria.
- Eosinophils - antibody receptors and histamine; asthma
- Basophils - circulating mast cells, control inflammatory responses.
- WBC - functions:
  - Immune defence - lymphocytes, T cells and B cells (Antibody production)
  - Control response to foreign bodies - monocytes and macrophages

- Monocytes - largest of cells, leave the circulation to become macrophages (scavenger cells)
- Lymphocytes - are the most numerous and increase in response to viral infections - helper cells, subclasses of T cells. B cells produce antibodies also known as plasma cells.
- **Leukocytosis** - increase in WBC count
- **Leukaemia** - WBC cancer, increased in number. Acute - shorter time to appear clinically. Chronic - appears over longer time.

COULTER PRINCIPLE - STILL THE STANDARD METHOD USED IN CELL COUNTING

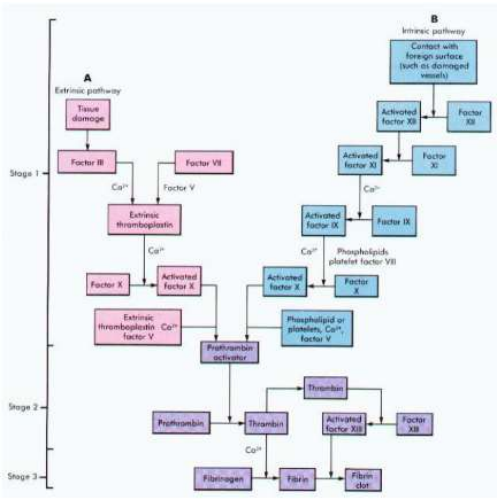
- Wallace Coulter - as particles are pulled through an orifice, and across an electric current, they produce a change in impedance that is proportional to the size of the particle traversing the orifice.



- Voltage pulse generated is directly proportional to particle volume.

PLATELETS:

- Normal count = 150-400x10 9/L
- 3 micron fragments.
- Comes from megakaryocytes - 160 microns residing in bone marrow ~20 platelets
- Contains factorings that control clotting



- Thrombosis:
  - Clot formation
  - Requires activation of proteins in plasma
  - Rope structure from the plasma
- Coagulation cascade:
  - Intrinsic and extrinsic cascade.
  - One activates another and another and another and so on.
  - Enzyme cascade
  - Protein conversion
  - End result is fibrin produced from fibrinogen and stabilised by FXIII.

PLASMA

- Pale yellow fluid, volume is around 2.5 - 3 litres.
- Water makes up around 90% - mainly water and protein
- Contains clotting agents, the clear fluid exuded from clotted whole blood and plasma called **serum**.

Serum = plasma - proteins involved in clotting

- Helps maintain the acid-base balance, clotting and inflammatory response and protection from infection - antibodies.
- Plasma proteins:
  - Major groups include:
    - Albumin - 60%
    - Globulins 34%
- Proportions of plasma proteins vary in certain diseases - therefore they are used in diagnosis of various diseases.
- Most plasma is produced by the liver - therefore also a measure of liver function
- Antibodies are produced by B-lymphocytes
- Albumin can pass through capillary walls and indicate kidney functions. Liver replaces lost albumin.

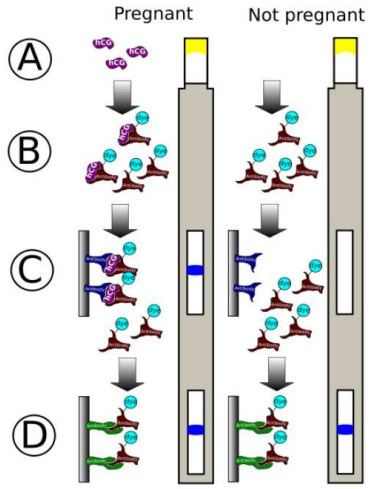
CLINICAL BIOCHEMISTRY

- Measurement of components of plasma that can identify and monitor diseases
- Diagnostic tests developed by engineers - measuring organ functions.
- Measuring proteins:
  - Albumin
  - Immunoglobulins
  - Enzymes
  - Hormones
- Immunoglobulins
  - Synthesised by B lymphocytes
  - There are many different types, with IgG being the most common.

    - Comes together as a protein of multiple chains, has an **antigen binding site** - B lymphocytes helps recognise these antigens.
- Antigens and Antibodies.
    - Most antigens have many epitopes.
    - All antigens binds to different parts of antibodies.

Understand how Monoclonal Antibodies can be used in Diagnostic Tests

- Hormones - follicle stimulating hormone (pregnancy tests)
- Tumour markers - Epidermal growth factor receptor
- Drug monitoring - Gentamicin
- Infectious diseases - Herpes, rubella, HIV
- Antibody as diagnostics:
    - Hormones proteins usually measured by an antibody detection system
    - Beta(HCG) - used in pregnancy detection, made up of 2 protein subunits, alpha and beta, results in placenta maintenance, raised once pregnant.
    - Luteinising hormone (LH) has a similar subunit - causes ovulation and increases post menopause
    - LH and Beta(HCG) control oocyte maturation and release, great indicators of ovulation and pregnancy.
- Pregnancy test strip:
    - Strips are embedded in the membrane
        - Material must go into urine
        - Diffusion must occur in a decent amount of time
    - The material has antibodies - the antibodies recognise HCG (blue in colour)
    - The HCG is broken up by the first antibody - such that the first strip turns blue.
    - More antibodies, in excess, the green is an "anti" antibody. Clinical test, it is a fail safe, tell the user that it is working. Because all urine carries the "brown" antibody. The "purple" HCG is only prevalent during pregnancy.



- Limitations
    - Sensitivity = (positive positive)
    - Specificity = (negative negative)
    - False negatives
    - False positives - some cancers cause elevated levels of HCG

Genetic Testing

Understand where Genetic Information is Stored

- DNA as a diagnostic tool
    - In every cell
    - Small samples are required
    - Minimally invasive
    - Predictive of future outcomes
    - "Unique" to each individual
- DNA basics:
    - Every single cell of the body

- DNA are inside chromosomes
- Once unwound, becomes double stranded helix.
- DNA -> RNA (leaves the nucleus) -> Ribosomes -> Proteins
- DNA tells how protein is made, the protein does the work.
- Cystic Fibrosis:
    - CFTR - mutations in this gene on chromosome 7, small change in that one gene, leading to change in protein.

IDENTIFY THE TYPES OF GENETIC TESTING AND BE ABLE TO GIVE EXAMPLES OF THESE.

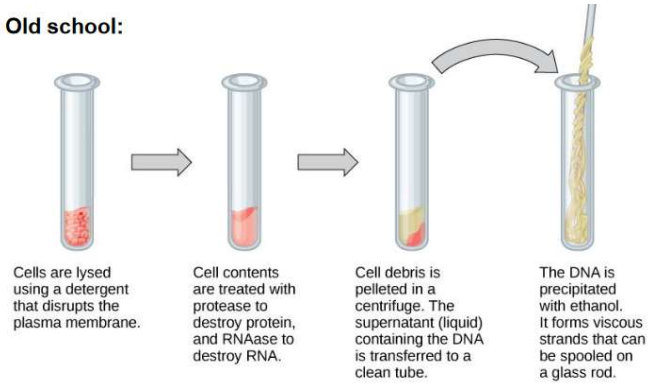
- Diagnostic Testing:
    - Identifies a genetic condition or disease that is making or in the future will make the person ill
    - The results of diagnostic testing can help in treating and managing the disorder.
    - Breast cancer, Parkinson's, Huntington's, Down Syndrome ->Commonly tested for
- Predictive and Pre-Symptomatic Genetic Testing:
    - Finds genetic variations that increase a person's chance of developing specific diseases.
    - This type of testing may help provide information about a person's risk of developing a disease
    - Can help in decisions about lifestyle and healthcare.
    - Commonly done are for Huntington's, Down Syndrome and certain types of cancer
- Carrier Testing:
    - Tell people if they "carry" a genetic change that can cause disease.
    - Carriers usually show no signs of the disorder, however, can be passed down to children, who either become affected or become carriers.
- Prenatal Testing:
    - Offered during pregnancy to help identify foetuses have certain disease.
- Pre-Implantation Genetic Testing:
    - Done in conjunction with IVF to determine the health/state of the embryos.
- Newborn Screening:
    - Is used to test babies after birth - to find out if they have certain diseases that could cause problems with health and development.

AMNIOCENTESIS	CHORIONIC VILLUS SAMPLING (CVS)	MATERNAL BLOOD TEST
1960s	1980s	2011
Identifies chromosomal abnormalities, inherited diseases and defects in the spinal column or brain. A needle through the belly extracts amniotic fluid, which contains fetal cells that are then analyzed	Identifies chromosomal abnormalities and inherited diseases. A needle through the belly or a catheter through the cervix suctions cells from the placenta. Placental tissue contains the same genetic material as the fetus	Allows doctors to assess the risk of chromosomal abnormalities. A blood sample is taken from the mother. Maternal blood contains fetal DNA, which passes through the placenta
15 to 20 weeks	10 to 13 weeks	10 weeks or later
Miscarriage occurs about 1 out of every 1,000 procedures	Miscarriage occurs about 1 out of every 500 procedures	Noninvasive
99%	98% to 99%	Detects 99% of Down-syndrome cases; up to 50% of positive tests are false positives

- Panoramic scans - very good and accurate scans.
    - Bioinformatics component is added - most of DNA in mum's blood is mum's DNA, small proportion of cell free.
    - About 10% is foetal DNA - baby's DNA.
    - Mum's DNA is inside the cell, baby's DNA outside - in most cases.
    - Isolate mum's DNA from the WBC - RBC has no nucleus - compare the cell free DNA, to see what's the baby's and what's the mother's.
- Pharmacogenetic Testing:
    - Give information about how certain medicines are processed in a person's body.
    - This type of testing can help a healthcare provider choose the medicines that work best with a person's genetic makeup
- Research Genetic Testing:
    - Helps scientists learn more about genes contribute to health and disease, as well as develop gene-based treatments.
- Forensic - Identity Testing:
    - Used in paternity test
    - Crime scene investigation

UNDERSTAND HOW GENETIC TESTING IS PERFORMED.

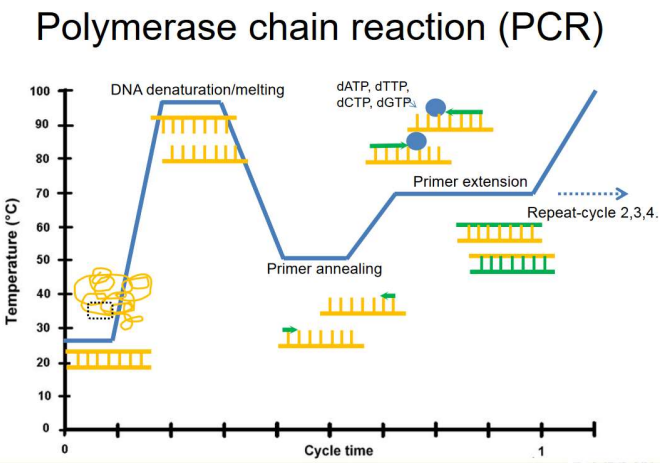
- Genetic Testing
    - To analyse, one or more of the following have to be done:
        - Isolate DNA
        - Cut DNA
        - Amplify DNA
        - Visualise DNA
        - Sequence DNA
- DNA Isolation



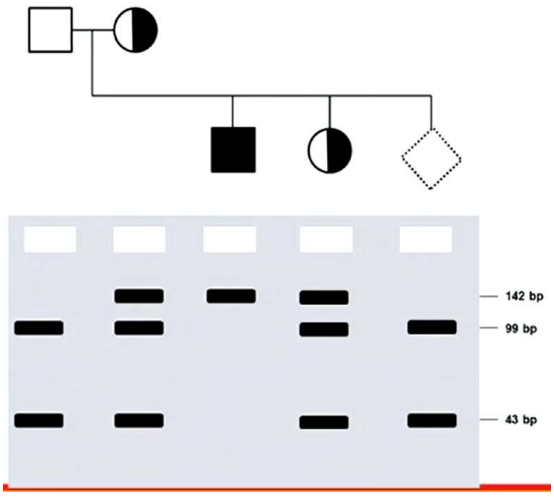
- Cutting DNA: Restriction Endonucleases
    - Restriction endonucleases - Bacterial enzymes that cut dsDNA at specific base pairs.
        - Bacteria share genetic information.
        - Cut DNA at a particular point
        - Each enzyme has a recognition site, search DNA until found the right one.
        - Catalyse hydrolysis of the phosphodiester bonds
- Amplifying DNA: Polymerase Chain Reaction (PCR)
    - Allows the production of a large number of copies of a specific of DNA sequence.
    - Requirements:

- Template DNA - what is going to be copied
- Forward and Reverse Primers - small single strands of DNA that initiate synthesis
- DNA polymerase - enzyme that synthesises DNA
- dNTPs - building blocks of DNA
- Temperature and Buffer conditions

- Simple technique:
  - PCR tube, put components into the tube.
  - Place the tubes into the machine - the temperature inside changes, cycles in a protocol
  - DNA copies increases exponentially.
- PCR Reaction
  - One cycle of PCR reaction.
    1. Small amount of DNA is amplified, the boxed area
    2. Temperature is increased, the DNA is melted, hydrogen bonding, higher temperatures results in two strands of DNA
    3. Temperature is decreased enough such that the strands can find the primers - anneal, whilst the two large remain separate
    4. Temperature increased enough for primer extension, such that DNA polymerase comes in and use substrate to build up the DNA chains



- Visualising DNA: DNA Gel Electrophoresis
  - It is used to identify, quantify and purify nucleic acids
  - A matrix of agarose is made - polymerise agarose with DNA run though, and is separated by size and conformation.
  - DNA goes from negative to positive - DNA has negative charge on backbone.
  - Migration is affected by size, large DNA will be closer to negative.
- Using standards to measure for accuracy, it's good to visualise - dyes that bind DNA, then fluoresce
- Diagnosis
  - Primers to amplify the important gene
  - PCR is undertaken to amplify the factor A gene.
  - Cut with restriction enzyme
  - When cut, 99bp and 43bp - unaffected, 142bp, 99bp and 43bp - affected

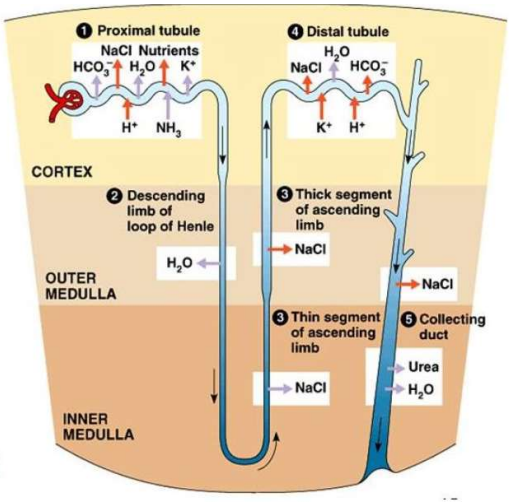


- DNA sequencing
  - Process of determining the exact sequence of nucleotides within a DNA molecule.

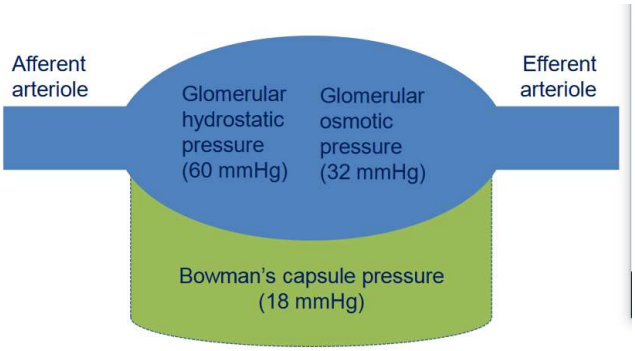
RENAL FUNCTION

UNDERSTAND KIDNEY FUNCTIONS

- Kidneys
  - Filter 1.3L/min of blood
  - Urine output of ~ 1mL/min
  - Filters out nitrogenous waste products
  - Maintaining fluid and electrolyte balance:
    - Acids
    - Salts
    - Regulation of extracellular fluid - blood pressure
  - Endocrine
  - Drug and hormone elimination
    - Insulin and drug clearance
- Kidney Structure
  - Filtration via renal artery
  - Medulla and cortex filters
  - The renal pelvis collects waste
- Nephron
  - Situated in the Bowmans capsule
  - Filtration occurs through the long loopy structures where water and salts are balanced.
- Filtration
  - GFR = 125mL/min [Alb] = 4mg/L
  - Qp = 700 mL/min [Alb] = 40g/L
  - The differences between the flow of the plasma
- Filtration and Reabsorption



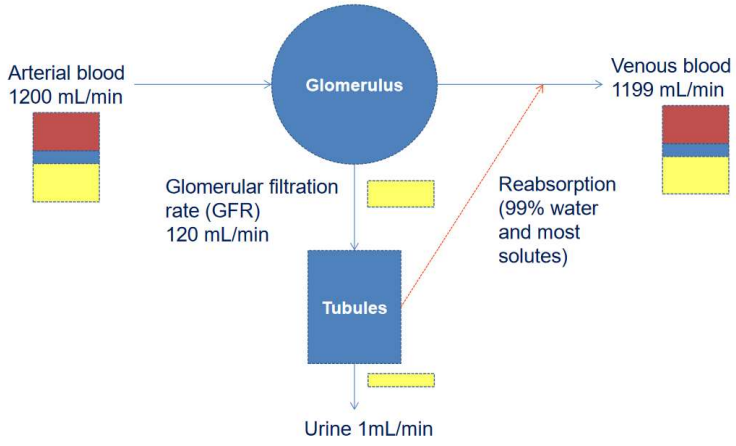
  - Water and salt is lost all alone
  - The process is driven by the concentration and pressure difference



Net filtration pressure (10 mmHg) = Glomerular hydrostatic pressure (62 mmHg) - Bowman's capsule pressure (18 mmHg) - Glomerular oncotic pressure (32 mmHg)

  - Albumin is maintained, along with the cells and other proteins and platelets.
  - The small molecules - salt, water and glucose - needs to be balanced.
  - Glomerulus filtration are based on size and charge.

        - Charge on the selective filtration membrane is anionic - negative.
        - Made up of endothelial surface layer, made up of hyaluronic acid - negatively charged - along with other carbohydrates and sulphates.
- Tubule - Reabsorption and Secretion
    - Reabsorption:
        - Sodium - Chloride and water will follow
        - Potassium and bicarbonate ions
        - Amino acids
        - Glucose
    - Mechanisms:
        - Passive diffusion
        - Facilitated diffusion
        - Active transport of sodium ions
        - Co-transport - bound to carrier, passing out protein break-down products
        - Counter-transport
        - Osmotic flow
- Osmolarity
    - Water will flow between membranes such that the osmotic pressure is the same in both
    - Hypertonic - more concentrated
    - Hypotonic - less concentrated
- Oncotic Pressure
    - The semipermeable membrane - pressure difference is maintained to help drive the filtration of plasma proteins.
- Body Fluids and Compartments
    - Body fluids can be:
        - Intracellular/Extracellular - inside or outside cells
        - Intravascular/Extravascular - inside or outside blood vessels
        - Interstitial - extravascular and extracellular
    - Proteins are osmotically active - only large solutes are because the barrier is porous
        - Osmotically active is when solutes can't pass through the membrane to help equilibrate osmotic pressure.
- Kidney Function:



Identify and Understand Disease of the Kidneys

- Kidney Diseases - can be detected by either protein or WBC in urine
    - Nephritis - inflammation of the kidney
    - Nephrotic syndrome - the membrane starts leaking
    - Nephritic syndrome - cause by bigger leaks in the membrane
    - Glomerulonephritis - inflammation of glomeruli
    - Diabetic nephropathy - disease of the capillaries of the glomeruli - need for dialysis
    - Interstitial nephritis - due to infection or reaction to drugs. Affects the tubules
    - Pyelonephritis - urinary tract infection that have moved upstream - detected by WBC in urine.
- Acute Renal Failure
    - Rapid lose of renal function that maybe reversible depending.
    - Signs include:
        - Acid base balance
        - Potassium and fluid retention - filtration not working properly
    - Diagnosis - blood test to test serum creatinine and urea - high levels indicate issue
    - Causes:
        - Pre-renal - related to blood supply - dehydration, hypotension due to shock
        - Intrinsic - damage to the kidney
        - Post-renal - obstruction of the urinary tract.
- Chronic Renal Failure
    - Progressive loss of renal function indicated by low levels of GFR
    - Causes include diabetic nephropathy, hypertension, glomerulonephritis
    - Can be detected via an increase in serum creatinine and urea as well as potassium levels, with fluid volumes also increased.
    - Severity is classified as following:
        - GFR - 15-30ml/min - ready for dialysis
        - GFR < 15ml/min - requires dialysis

Understand Parameters Used to Measure Kidney Function and able to Use these in Problem Solving

- Renal Clearance
    - Clearance is the rate at which it is excreted divided by concentration
    - Markers for GFR:
        - Inulin - filtered only - most accurate
            - Inert sugar - ideal tracer
            - Tracer - no absorption, no production:
                - Input = output - no absorption
        - Creatinine - Filtered, some secreted
            - Released from skeletal muscle
            - Inversely proportional to GFR
            - Poor indicator, good tracker
            - Creatinine clearance formula

$$Cr\ clearance = \frac{Cr\frac{excreted}{unit\_time}}{[Cr]_{serum}} = \frac{[Cr]_{urine} \times V}{[Cr]_{serum}}$$

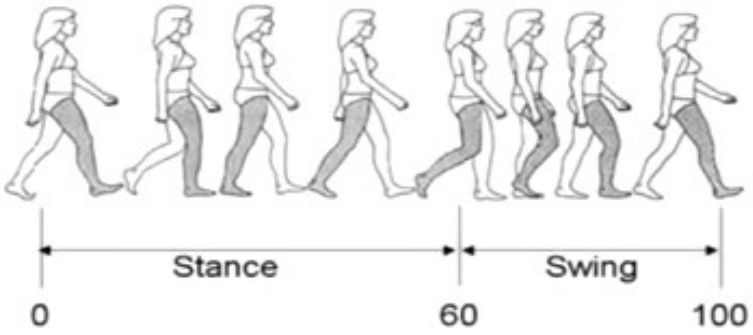
$$Creatinine\ clearance = \frac{(140 - age) \times mass\ (kg)}{72\ \times serum\ creatinine\ (\frac{mg}{dL})} \times 0.85\ (if\ female)$$

- Urea - varies with diet
    - Easy to detect
    - Because reabsorption, hard to obtain accurate measurement

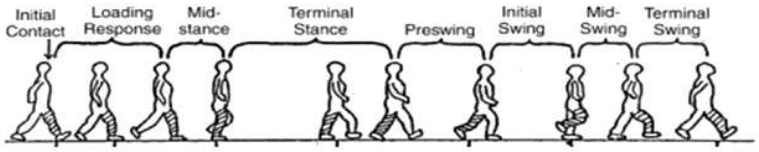
UNDERSTAND TECHNOLOGIES USED TO TREAT KIDNEY DISEASES

CLINICAL GAIT ANALYSIS

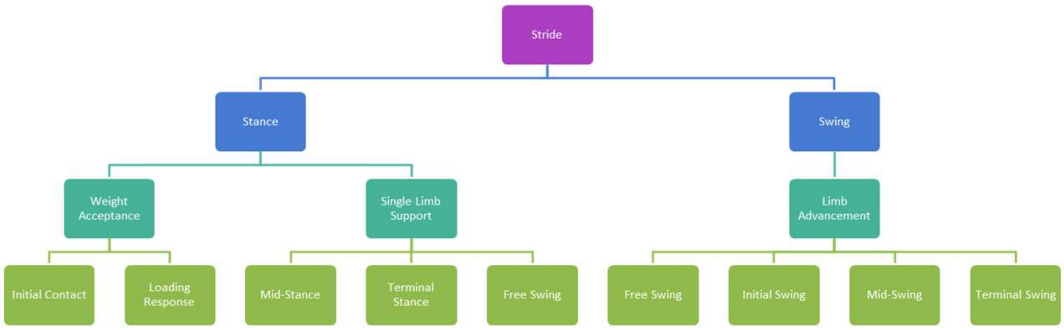
- Gait is a person's manner of walking
- Gait analysis is used for (quantitative):
    - Inform and assess medical interventions:
        - How much load should the implant withstand?
    - Inform technology and development
- Applications:
    - Orthopedics
    - Injury mechanics
    - Rehabilitative and assistive technology - exoskeletons, crutches
    - Prosthetics and orthotics - artificial legs and arms, leg braces
    - Surgical planning and assessment
- In crouch gate, the hamstrings are tight - botox to release?
- Convention to describe gait:
    - Gait Cycle:
        - Step and strides are different
        - Stride - is one cycle:
            - Beings with initial contact
            - Ends when the same foot contacts the ground
        - One stride = 2 steps



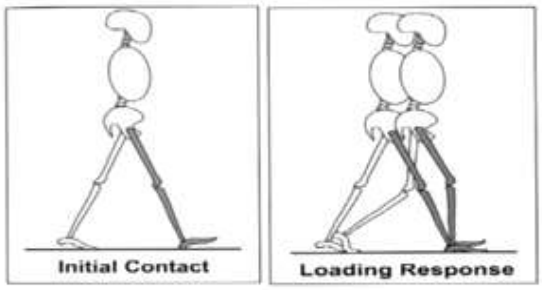
    - Stance:
        - Reference limb in contact with ground, 60%
            - Double Support - both feet on ground
                - Preparing the leg to take the weight of the body
            - Single support - one feet on ground
                - Single lab support
    - Swing:
        - Reference limb no in contact with ground, 40%



- Stance is the phase before pre-swing
- The three phases after are the swing

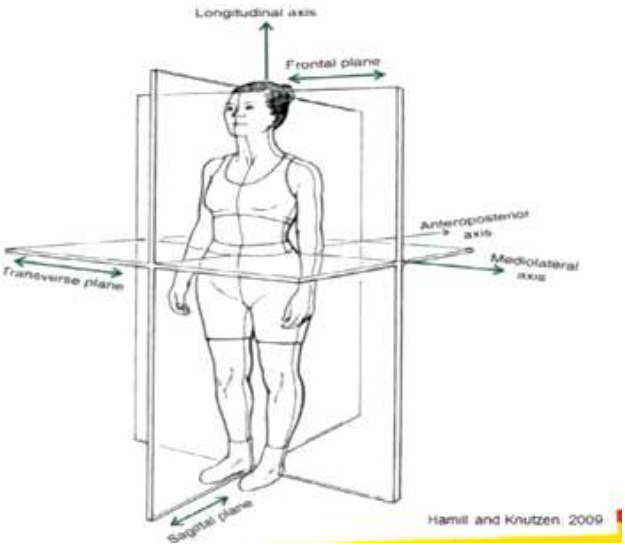


- Gait Tasks
  - Weight Acceptance:
    - 3 functional demands:
      - Shock absorption - eg bending knees when walking
      - Initial limb stability
      - Preservation of progression
    - 2 gait phases:
      - Initial contact
      - Loading response



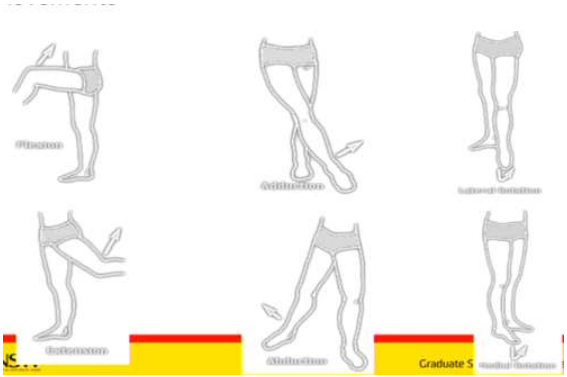
  - Single Limb Support
    - One limb needs to be able to support the weight of the entire body
    - Progression continues
    - 2 phases:
      - Initial contact
      - Loading response
  - Swing Limb Advancement
    - 4 advances:

        - Preswing
        - Initial swing
        - Mid swing
        - Terminal Swing
- To describe gait:
    - One gait cycle is made up of one stride or two steps
    - 3 tasks
        - Accept weight
        - Balance weight
        - Swing leg
- Gait Analysis
    - What do we measure:
        - Temporospatial parameters:
            - Walking speed - how fast one walks over 10 meters
            - Stance/Swing percentage
            - Step length
            - Cadence
        - Joint Kinematics:
            - Joint angles
        - Joint Kinetics:
            - Forces and moments of the movement
        - Energy Expenditure
        - Electromyography (EMG)
            - Muscle firing patterns
    - Temporospatial Parameters
        - Step Length:
            - Generally is heel to heel, same point to same point
            - Symmetrical to centimeters in normal people
        - Cadence:
            - Number of steps per minute
            - Average is around 113 steps/min
        - Walking velocity:
            - Step length x cadence
        - Comfortable Walking speed
            - Minimizes energy expenditure
            - Average is around 80m/min
    - Joint Kinematics - Hard to measure
        - Pelvis
        - Hip
        - Knee
        - Ankle
    - Planes of Movement:
        - Transverse top
        - Frontal front
        - Sagittal side



- Anatomy
    - Pelvis:

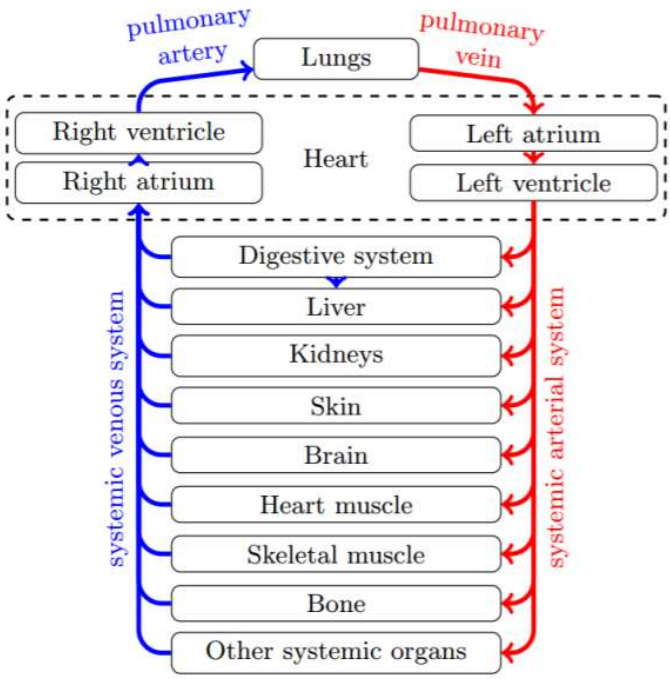
- Global angle - compared to lab coordinates
  - Relative to a fixed frame
  - Relative to x, y, z axis
  - X - pelvic **tilt**
    - forwards is anterior, backwards is posterior
  - Y - pelvic **obliquity**
  - Z - transverse, only **rotation**, internal and external rotation



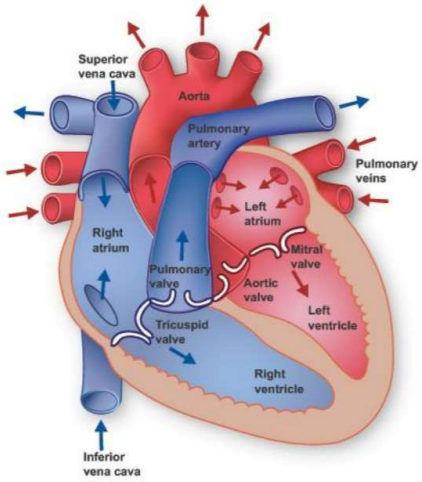
- Hip Joint:
  - Ball and socket
  - Local/relative angle - compared to the moving segment - compared to pelvis
  - Movement in all 3 degrees
    - Sagittal - flexion/extension
    - Frontal - abduction/adduction
    - Rotation
      - No translations
- Knee Joint:
  - Hinge joint
  - Room for rotation
    - 1/2 degrees of freedom
      - Flexion/extension
      - Rotation
    - VARUS (air) - bowlegged - meniscus degrading
    - VALGUS - knocked knee
- Ankle Joint
  - Articulation between the tibia, fibula and talus
  - Hinge Joint
    - Plantar flexion - point
    - Dorsi flexion - back onto heels
  - Hip, knee, ankle are all relative angles.
  - Foot rotation is relative to the direction of travel.

CARDIAC MONITORING

IDENTIFY THE MAIN FUNCTIONS OF THE CIRCULATORY SYSTEM

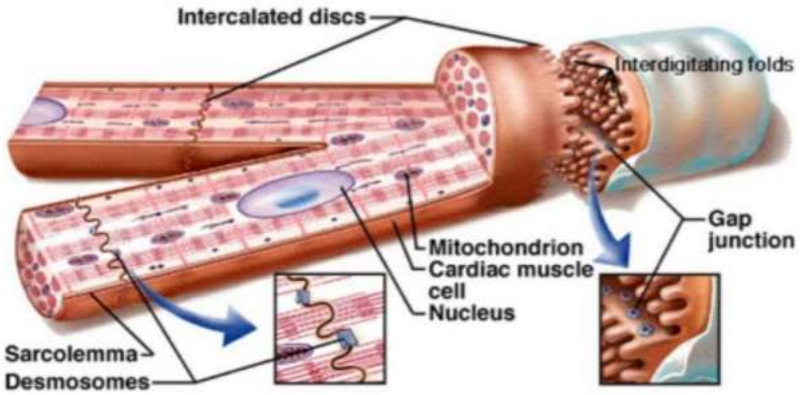


- Functions of the Circulatory System
  - Transportation
    - Takes oxygen to body cells
    - Carries nutrients and hormones
  - Regulation
    - Controls pH, temperature, water content
  - Protection
    - Takes white blood cells (leukocytes), antibodies and interferons to protect against diseases



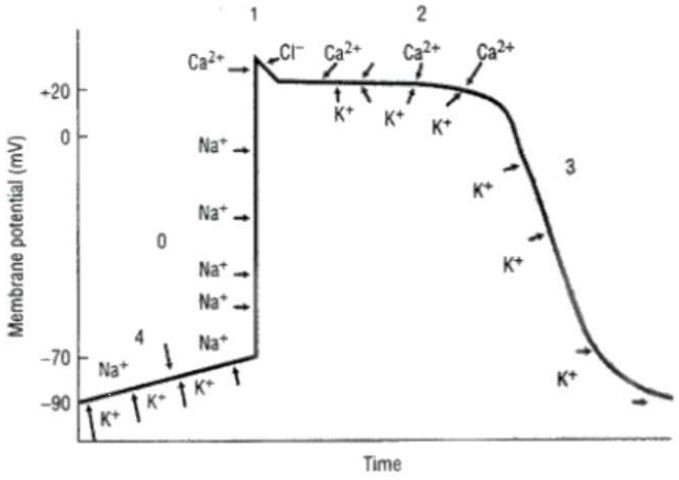
- Atrium are primary pumps - contract a little before ventricles do.
- 4 valves, preventing back flow of blood back into the heart.

- Cardiac muscles are similar to skeletal muscle cells
- Main difference - intercalated disc - helps keep the muscle together, share the electrical charge between the cells



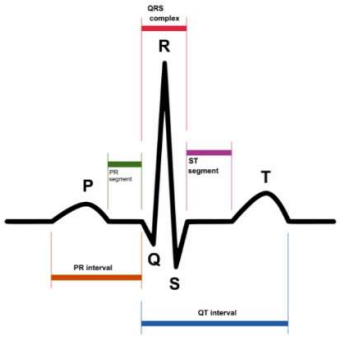
Understand the Electrical Activity of the Heart

- Electrical Activity of the Heart
  - Membrane is semi-permeable to only potassium ions.
  - Chloride ions on one side and potassium on the other - creating a potential difference.
  - Different ions maybe present, but the idea remains the same.



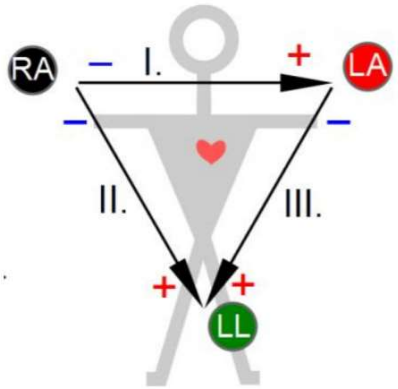
  - 4 - Potassium ions entering through the membrane and once it reaches a certain level, around -70mV, the channels for sodium ions begin to open up, this is the beginning of cardiac muscle contraction, the membrane is leaky for sodium ions initially.
  - 0 - The sodium ions being to enter really quickly, this is when cardiac muscle contraction is going really fast.
  - 1 - The calcium channels are opened up to further built up the contraction then the chlorine ions.
  - 2 - Contracts continuously in the plateau, membrane potential
  - 3 - The membrane will repolarize, potassium ions will leak out, and come back to resting

IDENTIFY FEATURES OF THE ECG AND HOW THEY RELATE TO ACTIVITIES OF THE HEART



- P - wave - represents contraction and electrical activity preceding atrial contraction
- QRS - ventricle contraction
- T - section - repolarisation, membrane potential coming back to baseline

Einthoven's law
If any two of the three bipolar limb ECG signals is known, the third can be mathematically calculated by summing the two known signals.



RA = -0.2 mV
LA = +0.3 mV
LL = +1.0 mV

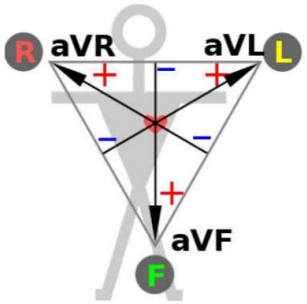
Lead I = 0.5 mV
Lead II = 1.2 mV
Lead III = 0.7mV

- What can we learn from ECG:
  - Increased voltages - increase cardiac hypertrophy, more muscle tissue than normal, normally with extreme athletes.
  - Decreased voltages - cardiac myopathy, some muscle not being activated - fluid in pericardium in the gap, the increased resistance to ECG.
  - Prolonged QRS - longer for action potential, cardiac hypertrophy, cardiac dilation
  - Cardial Infarction = heart attack
  - Atrial fibrillation - action potential are not even, out of syncronisation - inefficient filling of left ventricle

A three lead ECG is used and at a certain point in time: the right arm is at +0.2 mV; the left arm is at -0.1 mV; and lead II is reading -0.1 mV. Calculate the LL voltage, and the aVF voltage.

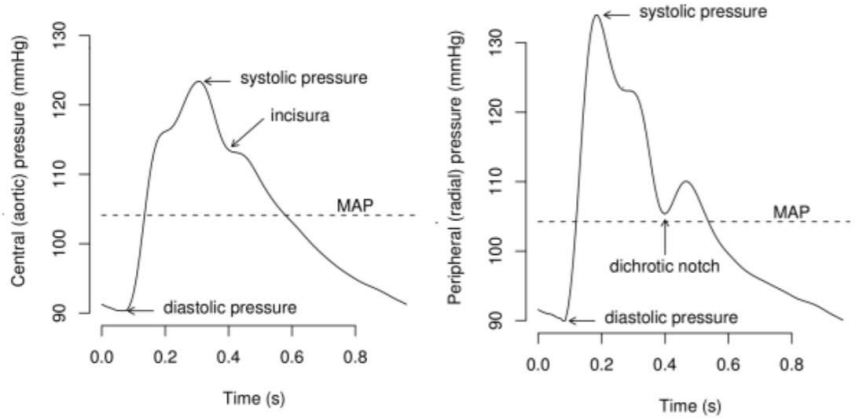
$$Lead\ II = LL - RA$$
$$LL = Lead\ II + RA$$
$$LL = \ 0.1 + 0.2 = 0.1\ mV$$

$$aVF = LL \ \frac{LA + RA}{2}$$
$$aVF = 0.1 \ \frac{0.1 + 0.2}{2}$$
$$aVF = +0.05\ mV$$



Understand Blood Pressure Measurements

- Where do we measure blood pressure?
  - Pressure depends on where the pressure is measured
  - The systemic arterial system pressure is measured.
  - Greatest resistance - therefore highest pressure.



Pulsepressure = systolic − diastolic
Mean pressure = area under the curve
≈ diastolic pressure + 1/3 *pulse pressure*

- Systolic - highest pressure
  - Higher near the wrist due to wave reflection
- Diastolic - lowest pressure
- Piezo-electric pressure sensor enters the artery
  - Used in acute and critical times - ICU
- Korotkoff Technique:
  - Gives systolic and diastolic pressure
  - Values vary, because of users issue (experience and inexperience), hearing issue.
  - Golden standard - compared to pretty much everything.
  - Downstream of left ventricle

Korotkoff sounds

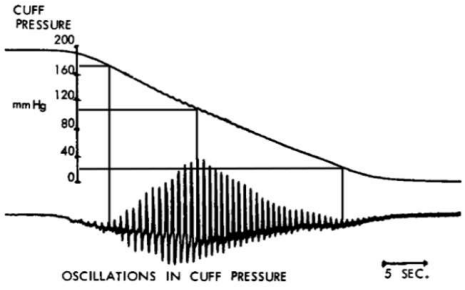
Phase I: The appearance of the sound with a snapping characteristic.
Phase II: Continuous persisting murmurs.
Phase III: Increasing of sound intensity and sharpness above that of Phase II.
Phase IV: The muffling of sounds.
Phase V: The cessation of sounds

- Definitions and classification of blood pressure levels.

Category	Systolic		Diastolic
Optimal	<120	and	<80
Normal	120–129	and/or	80–84
High normal	130–139	and/or	85–89
Grade 1 hypertension	140–159	and/or	90–99
Grade 2 hypertension	160–179	and/or	100–109
Grade 3 hypertension	≥180	and/or	≥110
Isolated systolic hypertension	≥140	and	<90

- Invasive central blood pressure measurement

Using the following oscillometric test, give values for systolic, diastolic, and mean arterial pressure and diagnose with stage of hypertension.



$$DP = \frac{3 \times MAP - SP}{2}$$

$$DP = \frac{3 \times 100 - 170}{2}$$

$$DP = 65\ mmHg$$

$$MAP = DP + \frac{SP - DP}{3}$$

Grade 2 hypertension
(Systolic > 160 mmHg)

- DP too low when extrapolation from the graph ~20 mmHg - you'd be dead. Hence, calculate mathematically because MAP and SP and values within reason.

Understand Techniques Used to Diagnose Cardiac Diseases

- Coronary Artery
  - Branch from the root of the aorta, they perfuse throughout the heart - operate under pressure
  - Supplies cardiac muscle with blood (oxygen)

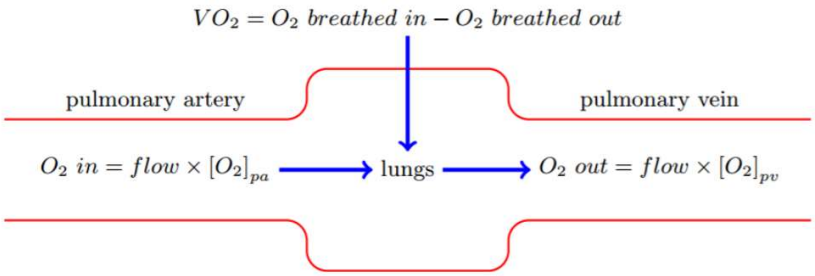
- Prone to blockage
  - Bypass the blocked artery with a vein from the leg
  - Stent - wire mesh holds the blood vessel open
  - Angioplasty - similar to stent, like a balloon then expanded.
- Small diameter
- Detecting and diagnosing coronary artery blockage - Angiogram
  - X-Rays
    - Can't pick up soft tissue
    - Insert a radio opaque dye, the dye is detectable by the x-ray.
    - Catheter is pushed through the leg, with the dye is then pushed through
    - X-ray gives an idea of how "thick" it is
    - X-rays, 2D, machine has to be rotated.
- Computed Tomography (CT)
  - CT scans allow for many pictures taken simultaneously, pieced together, then 3D image can be made for the patient - limitation in resolution - radio opaque dye
- Magnetic Resonance Imaging
  - Non-invasive, high resolution

Understand Cardiac Output Measurements

- Cardiac Output Measurement and Estimation

$$CO = SV \times HR$$

- Cardiac output - rate of blood being ejected by the left ventricle into the aorta
- Stroke volume - volume of blood being ejected into a single beat
- Heart rate - number of beats of the heart per unit time



$$\begin{aligned} VO_2 &= O_2\ out - O_2\ in \\ &= flow \times [O_2]_{pv} - flow \times [O_2]_{pa} \end{aligned}$$

$$CO = flow = \frac{VO_2}{[O_2]_{pv} - [O_2]_{pa}} = \frac{ml/min}{ml/L} = L/min$$

If a person breathes in 1000 ml/min $O2$ and breaths out 800 ml/min, and we assume that $O2$ in the venous system is 160 ml/L and in the arterial system 200 ml/L, calculate the cardiac output.

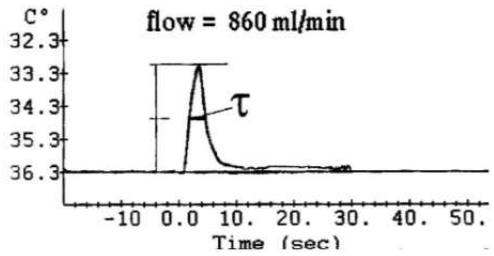
$$VO_2 = O_2\ breathed\ in\ - O_2\ breathed\ out$$
$$VO_2 = 1000\ - 800$$
$$VO_2 = 200ml/min$$

$$\begin{aligned} arteriovenous\ O_2\ difference &= [O_2]_{pa} - [O_2]_{pv} \\ &= 200 - 160 \\ &= 40\ ml/L \end{aligned}$$

$$\begin{aligned} CO &= \frac{VO_2}{arteriovenous\ O_2\ difference} \\ &= \frac{200}{40} \\ &= 5000\ ml/min \end{aligned}$$

- Dye or Thermodilution Technique
  - Catheter goes into the body, the dye is released or heated fluid and measure how long it takes to travel around the body.

$$\begin{aligned} CO &= \frac{amount\ of\ dye\ injected}{\int concentration\ curve \cdot dt} \\ &= \frac{amount\ of\ dye\ injected(mg)}{area\ under\ the\ curve\ (mg/ml \times seconds)} \\ &= ml/sec \end{aligned}$$



20 mg of dye injected in the inferior vena cava. In the radial artery, an average concentration of 0.025 mg/ml is measured over 12 seconds. What is the cardiac output?

$$\begin{aligned} CO &= \frac{amount\ of\ dye\ injected}{\int concentration\ curve \cdot dt} \\ &= \frac{20}{0.025 * 12} \\ &= 67ml/sec \\ &= 4L/min \end{aligned}$$

- Doppler Ultrasound
  - Measure the velocity of the moving object

- Cheap and inexpensive

$$CO = Stroke\ Volume \times Heart\ Rate$$
$$= (average\ flow\ velocity \times cross\ sectional\ area) \times HR$$

- Use for measuring from ultrasound

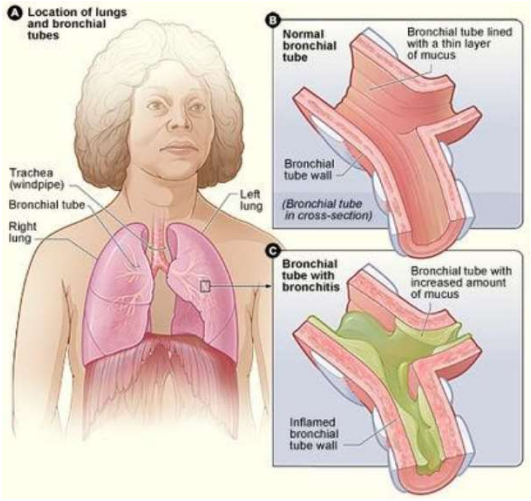
PULMONARY FUNCTION

Identify Variables that Affect Pulmonary Function Tests

- Pulmonary Tests
  - Indicates:
  - Lung volume
  - Movement of air in lungs
  - Stiffness of lungs and chest wall
  - Diffusion characteristics of the membrane
  - Response of lung to therapy
- Used for:
  - Older people, 60+
  - Pulmonary diseases
  - Pathologically obese
  - History of smoking, cough or wheezing
  - Under anesthesia
  - Undergoing abdominal or thoracic operations
  - Monitoring for known diseases
    - Obstructive lung disease
    - Restrictive lung disease
- Factors that affect the results include:
  - Age - older the lower
  - Gender - men greater than women
  - Body height and size - the bigger the better
  - Race

Identify Major Classes of Lung Disease, Give Examples of Diseases in Each of these Categories

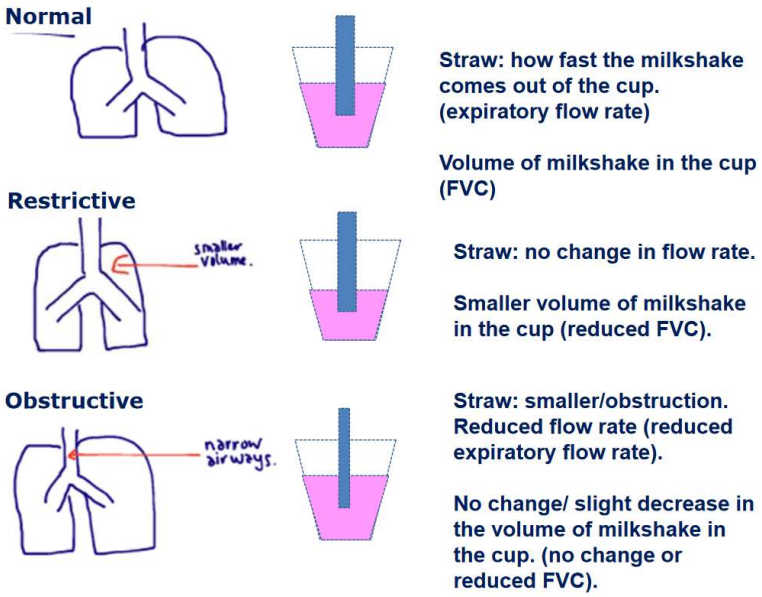
- Obstructive Lung Disease
  - Narrowing of the airways
    - Inflammation
    - Swelling
  - Material inside the bronchial passageways
  - Destruction of lung tissue (tumors)
  - External compression of the airways
  - Characterised by a limitation of expiratory airflow so that airways cannot empty efficiently
    - Bronchitis
    - Emphaysema



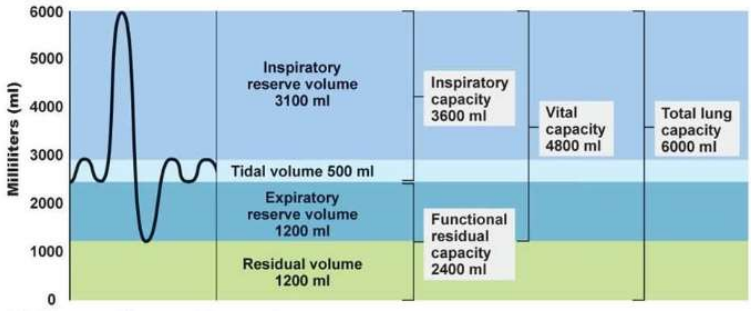
        - Asthma - inflammation, reversible airflow obstruction
        - Bronchiectasis - enlargement of airways, scarring of airways
        - Cystic fibrosis - enlargement of airways, mucus build up
- Restrictive Lung disease - restrict inspiration
    - Interstitial lung disease
        - Pulmonary fibrosis, sacoidosis
    - Chest wall pathology
        - Kyphosis
        - Scoliosis
- Obesity
- Neuromuscular disease
    - Motor neuron disease - death of neurons that control voluntary muscles
        - One loses control of the muscles during breathing, hence making it very difficult
    - Muscular dystrophy
        - Progressive muscle weakness, defects in muscle proteins and death of muscles

Define key Spirometry and Lung Volume Measurements

- Spirometry - main lung function test:
    - Provides most information
    - Most common instrument
    - Spirometers with electronic signal outputs also measure flow
    - Measures changes in volume
    - The measurements include:
        - FVC (Forced vital capacity) - deepest possible breath - max volume of lungs
        - FEV1 (Forced expiratory volume in one second) - volume of air expired in one second.
        - FEV1/FVC
        - PEFR(Peak expiratory flow rate) - max speed of expiration



  - Restrictive has a lower FVC - FEV1 is generally not affected
  - Obstructive has a lower FEV1 - FVC is generally not affected
- Spirometry
  - Spirometry measures the volume of air inhaled or exhaled as a function of time
  - Flow volume spirometers measure: FVC (Lowest point, largest value of volume), PEFR (the highest point on the graph
  - Typically:
    - FEV1/FVC = >80% is normal
    - FEV1/FVC = >79% abnormal, >70% obstruction
- Lung Volumes:
  - Tidal volume (TV) - volume of gas inspired or expired with each normal breath (500 ml)
  - Inspiratory reserve volume (IRV) - maximum volume of additional air that can be inspired from the end of a normal inspiration (3.1L)
  - Inspiratory Capacity (IC) = max volume of air that can be inspired from the end of expiration position
  - Expiratory reserve volume (ERV) - maximum volume of additional air that can be expired from the end of a normal inspiration (1.2L)
  - Residual volume (RV) - volume of air in the lung remaining after a maximal expiration, can't be measured
  - Functional Residual Capacity (FRC) - volume of air remaining in the lung at the end of normal expiration
  - Vital Capacity (VC) - maximum volume of air that can be forcefully expelled from the lungs following maximal inspiration
  - Total Lung Capacity (TLC) -volume of air contained in the lungs.

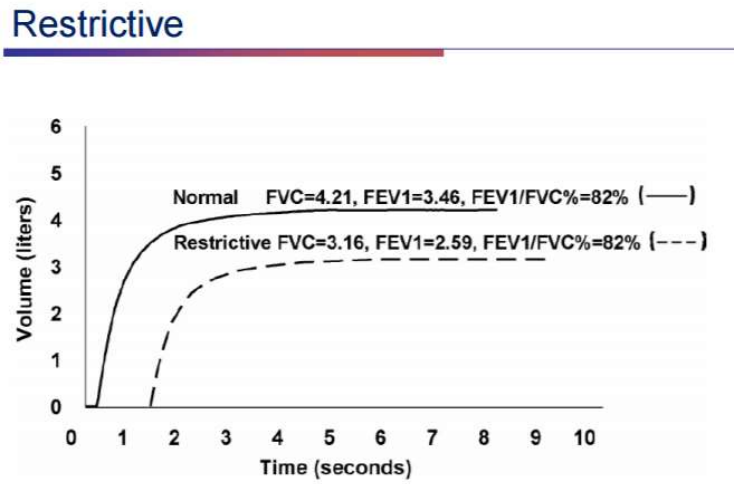


- Spirometry Interpretation

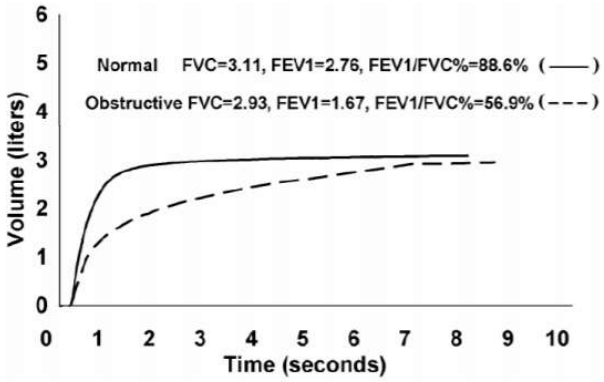
	Obstructive	Restrictive
FVC	Decreased or Normal	Decreased
FEV1	Decreased	Decreased
FEV1/FVC	Decreased	Normal or increased

- Interpretation of % predicted FVC
  - 80 – 120% normal
  - 70-79% mild reduction
  - 50-69 % moderate reduction
  - <50% severe reduction
- Interpretation of % predicted FEV1
  - >75 % normal
  - 60-75% mild obstruction
  - 50-59% moderate obstruction
  - <49% severe obstruction
- Interpretation of % predicted FEV1/FVC
  - 80 or higher = normal
  - 79 or lower = abnormal

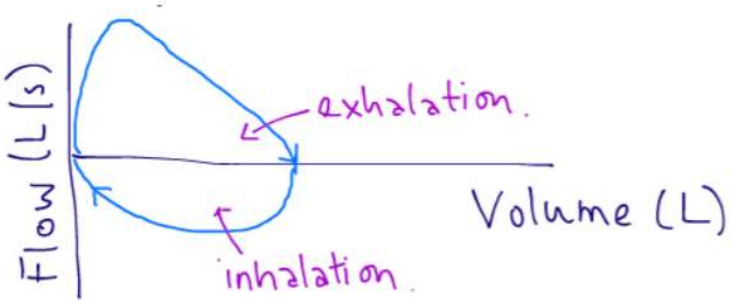
Interpret Flow vs Time and Flow vs Volume Curves



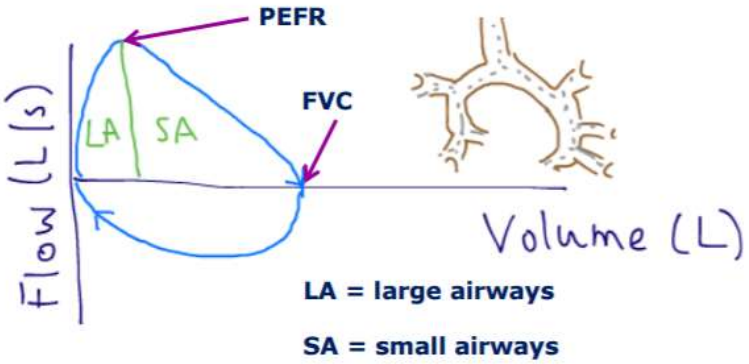
Obstructive



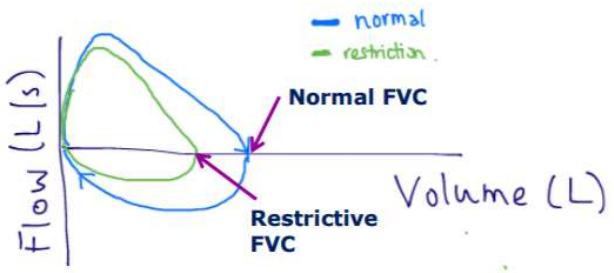
Flow volume loop



Flow volume loop

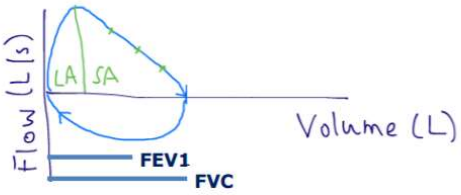


Flow volume loop



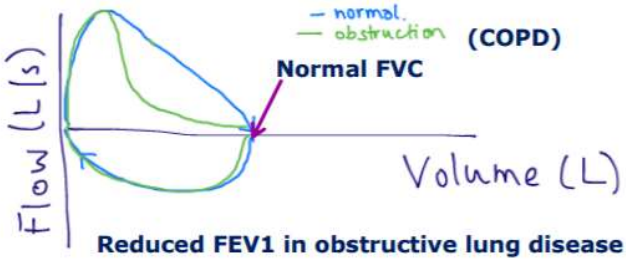
Reduced FVC in restrictive lung disease

Forced expiratory volume in 1 second



FEV1/FVC interpretation
80 % or higher = normal
79 % or lower = abnormal
70 % or lower = obstruction

Flow volume loop

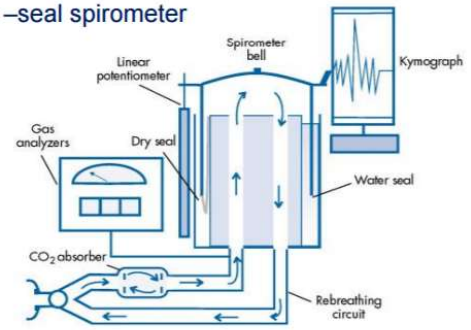


Reduced FEV1 in obstructive lung disease

UNDERSTAND HOW PULMONARY FUNCTION TEST EQUIPMENT OBTAINS MEASUREMENTS

Volume displacement spirometers

- Water –seal spirometer

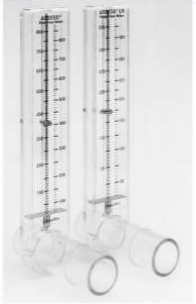


Breath pushes the bell that displaces a pen that records the trace on moving paper. Also shown is a CO_2 absorber and a gas analyser.

Bed side PFT

Wright peak flow meter.

- Air flow moves needle along the gauge.
- Measures peak expiratory flow rate
- Normal males 450 – 700 L/min
- Normal Females 350 – 500 L/min
- < 200 L/min = inadequate cough efficiency.



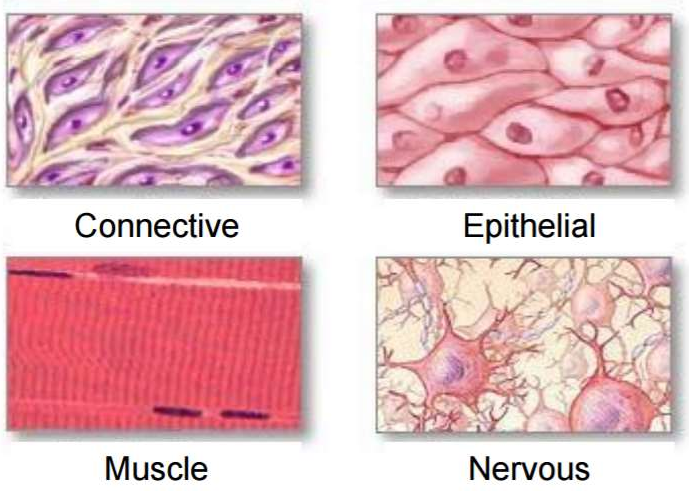
HISTOLOGY

IDENTIFY DIFFERENT CELLULAR ORGANELLES

- Nucleus - brain of the cell
  - DNA is stored, site of mitosis, directs cells activities
  - Controls the shape and feature of the cell
  - RNA produced for protein synthesis
- Ribosomes and Endoplasmic Reticulum
  - Ribosomes:
    - Where protein synthesis
    - Composed of RNA and proteins
    - Found within the cytoplasm and attached to endoplasmic reticulum
  - Endoplasmic reticulum - smooth and rough
    - Transport chemicals between and within cells
    - Provides a medium for the organisation of chemical reactions and synthesis
    - Smooth:
      - Packages proteins for transport - depending on where the packages need to go and what needs to be done before expulsion out of the cell

                - Releases calcium - cell movement
                - Detoxification of foreign substances - mini-liver within the cells
            - Rough:
                - Rough due to ribosomes
                - Membrane protein synthesis
    - Golgi and Lysosomes
        - Golgi:
            - Flattened stacks of interconnected membranes
            - Post translational modifications - depending on what it is used for, it will be modified in such a fashion
            - For packaging and distribution
        - Lysosomes:
            - Similar to rough smooth ER - mini-liver
            - Stomach of the cell - comes off the Golgi
            - Digests food, destroys bacteria
            - Depending on what the bacteria is, the lysosomes will either "spit it out" or "take it in" to deal with the foreign element.
- Cell Mambrane Structure
    - Made up of lipid
    - Hydrophilic on the outside
    - Hydrophobic on the inside
    - This determines what goes in and what comes out
- Membrane Functions
    - Transport barrier
        - Osmosis
        - Diffusion
        - Membrane channels
        - Endocytosis and exocytosis - ribosomes
    - Proteins provide functions
        - Helps the interaction between cells.
        - Regulation of transport detection of signals through receptors
    - Extracellular Structures:
        - Extracellular matrix (ECM)
            - Surrounds the cells
            - Made up of proteins - the proteins depends on where the ECM is.
                - Generally, contains collagen, elastin,
            - Integrin proteins present in the plasma membrane helps to connect to the cytoplasm

IDENTIFY DIFFERENT TISSUE TYPES



- Tissues
  - 4 major types of tissues - structure, morphology
    - Connective
      - Tissue that supports
    - Epithelial
      - Lines and covers
    - Muscle
      - Controls movement
    - Nervous
      - Movement
  - Epithelial
    - Distinguishing features
      - Cells close together
      - Little ECM
      - Apical and basal surface
      - Supported by connected tissue
    - Named based on shapes
      - Simple layer - one layer
      - Stratified - many layers
      - Squamous - squashed
      - Cuboidal - cube shaped
      - Columnar - column like.
  - Connective Tissue
    - Connective tissue proper
      - Loose
        - Fibres, but not packed in tight
      - Dense
        - Regular or irregular - matrix make up
    - Blood
    - Cartilage
    - Bone
    - Adipose - fat tissue
  - Muscle Tissue

        - Skeletal
            - Moving
            - Long, cylindrical cells
            - Striations
            - Not branched
            - Multinucleate
            - Striations
            - Voluntary movement
            - Located to bones
        - Cardiac
            - Heart
            - Striated
            - Branching cells
            - One or two nuclei
            - Intercalated discs
            - Walls of the heart
        - Smooth
            - Lining internal organs
            - Spindle shaped cells with central nuclei
            - Arranged closely to form sheets
            - No striations
            - Propels substances along internal passageways
            - Involuntary control
            - Mostly walls of internal organs
        - Nervous Tissue
            - Transmit electrical signals
            - Located in the brain, spinal cord and nerves
            - Contains two types of cells
                - Neurons
                - Supporting cells - neuroglial cells

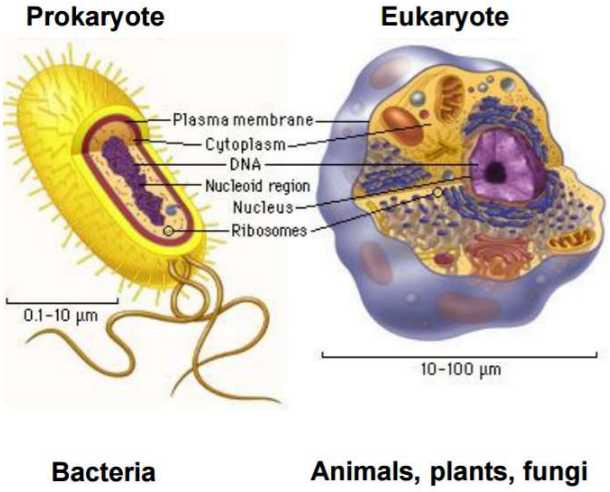
Understand How Different Histology Dyes Localise Different Structures

- Haemotoxylin and Eosin (H&E stain)
    - Most commonly used histological stain
    - Haemotoxylin
        - Stains components blue by reacting with anionic components
        - Nucleus
    - Eosin
        - Stains components pink by reacting with cationic components of the tissue
        - Cytoplasm
        - Intracellular membranous components
        - Extracellular fibres
- Periodic Acid Schiff (PAS)
    - Stains
        - Polysaccharides - sugars
        - Periodic acid bonds with carbon atoms - turns pink in colour
        - Accentuates matrix and basement membrane constituents
        - Used to diagnose polysaccharide diseases

- Masson's Trichrome
    - Distinguish between proteins
    - Collagen stained blue
    - Muscle stained red
    - Nuclei stained dark brown/black
- Azan - stains collagen blue and muscle red - similar to trichrome
- Verhoeff - intensely stains elastic fibres black and lightly stains collagen red.
- Oil Red O - Fat soluble due that stains lipids red
- Alcian blue - stains charged polysaccharides - how charged they are
- Safranin O - nuclei turn red, connective tissue and collagen blue - normally for cartilage
- Immunohistochemistry
    - Diagnosing for epitopes.

Understand the Difference between Prokaryotes and Eukaryotes

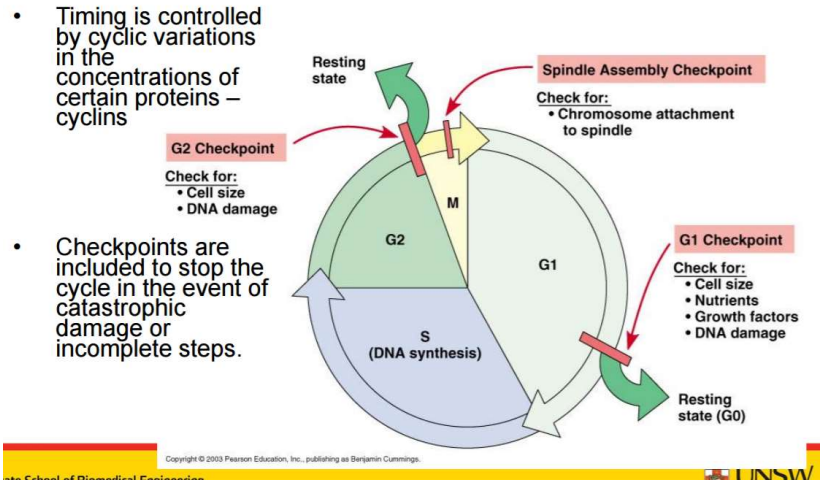
- There are two types of cells:
    - Eukaryotes - animals, plants, fungi
    - Prokaryotes - bacteria
    - Both have nucleus, eukaryotes - more sophisticated in make up of organelles.
    - Both need oxygen



- Eukaryotic Cells
    - Different organelles, all have different jobs
    - Manufacture and transport
        - Nutrients and waste going in and out
        - Nucleus
        - Ribosomes, endoplasmic Golgi apparatus.
    - Breakdown
        - Lysosomes
    - Energy processing
        - Mitochondria
    - Support movement, communication
        - Cytoskeleton - microtubules
        - Extracellular matrix
        - Cell junctions

Understand the Cell Cycle and Cell Proliferation

- Why they divide?
  - Cells specialise for specific functions
  - Cells dies
  - Cell size is limited
- 4 main phases
  - Interphase
    - Synthesis - DNA replication
    - Gap - pasues in the cell cycle
  - Mitosis and cytokinesis
    - Division of nucleus
    - Division of the cell
    - G0 is when the cell replicates then stops - quiescent
- As the cell goes through each phase, they go through checkpoints, important for DNA synthesis, checking for DNA damage - if there is a problem, then the cell will die at that point
- Cell death
  - Apoptosis - programmed cell death - replicate so many times, after a certain number of times, they will die
  - Necrosis - uncontrollable cell death - ionising radiation, virus
- Cell Proliferation
  - Cell numbers double after 24 hours - exponential rate of growth as long as programmed cell death isn't occurring.



X-Ray, CT and MRI

Understand how each Imaging Technique Results in an Image of the Body and can be used to Diagnose Disease

- Medical Imaging
  - Non-invasive visualisation of internal organs and tissues
  - X-Ray are 2D
  - CT and MRI are 3D

X-Rays

- X-rays can be taken after injecting radioactive isotopes
- Mainly used to image bone
- X-rays are part of the natural EM spectrum
- X-rays have high frequency and high energy

- X-Ray Tube
  - Energy converter
  - Receives electrical energy into - x-ray radiation and heat.
  - Radiation produced at the anode in a small area of the surface is call the focal point - smaller the focal point, the more detail.
- X-Rays
  - Can pass all the way through the body
  - Can be deflected or scattered - black
  - Can be absorbed - white
- How do they pass through
  - Depends on energy and atomic number of tissue
  - More energy - the more they pass through
  - Higher the atomic number - the more absorbance
  - Cartilage shows up as soft tissue
- Contrast
  - Inject a substance into the area, such that soft tissue can be x-rayed.
  - Substance should be:
    - Inert
    - Non-toxic
    - Easily excreted
    - Not retained

X-Ray Computed (Axial) Tomography (CAT)

- CT Imaging
  - CT image taken as plain image or with contrast medium introduced
  - Bone is white, soft tissue is black
  - Contrast and resolution is better than CAT
  - Density and intensity of pixels can also be registered
- CT Angiography
  - Visualise blood vessels
  - Detect size
  - More accurate than MRI of ultrasound
  - Contrast agent injected into vein
- CT
  - A lot of information is collected
  - Very detailed and very clear

Magnetic Resonance Imaging

- Works due to the presence of polar hydrogen water molecules
- Random to aligned releases energy that can be collected
- Magnetic nuclei are abundant in the human body and spin randomly
- Place in static magnetic fiend
- Radio frequency (RF) pules trigger the magnetic fields.
- Tissues are distinguished based on density

- Uses:
    - Brain imaging - anatomy, bleeding, swelling
- Types:
    - Interventional MRI
    - Real Time MRI
    - Functional MRI - measures signal changes in the brain due to changing neural activity.