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**Knowledge Rich Curriculum Plan**

Biology 3.2 Cells

Year 12



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| **Lesson/Learning Sequence** | **Intended Knowledge:**  *Students will know that…* | **Prior Knowledge:**  *In order to know this, students need to already know that…* | **Tiered Vocabulary and Reading Activity** |
| **Lesson 1:**  **Review of key GCSE content** | NO NEW CONTENT JUST KEY POINTS FROM GCSE | *Students need to already that animal cells and plant cells are eukaryotic cells as they have DNA inside a nucleus. Students will know that an animal cell has a nucleus, cell membrane, cytoplasm, mitochondria and ribosomes and will know the role of each of these parts. Students will know that in addition, plant cells have cell wall, chloroplasts and vacuole and will know the role of these. Students will be able to name some specialised cells. Students will already know that 2 types of microscope are light microscope (we use in the lab) and electron microscope (advantages- higher resolution and magnification)*  *Students need to already know that new body cells form by mitosis and 2 genetically identical daughter cells form. Tumours occur when cells divide uncontrollably.*  *Students will already know that pathogens are microorganisms that cause disease (bacteria, virus, fungi, protist) how the immune system responds by producing antibodies, antitoxins or engulf and digest and that a vaccine is a dead form of a virus which triggers an immune response* | Eukaryotic  Prokaryotic  Daughter cells  Tumour  Antibodies  Antitoxins  Engulf |
| **Lesson 2:**  **Eukaryotic Cells** | Students will know that the: nucleus- A large organelle surrounded by a nuclear envelope (double membrane) which contains many pores. The nucleus contains chromosomes (made form liner DNA and structures called nucleolus  The nucleus controls the cell’s activities. DNA contains information to make proteins. Pores allow substances to move between the nucleus and cytoplasm.  The nucleolus makes ribosomes  Cell membrane- The cell membrane holds a cell together and controls what enters and leaves the cytoplasm, as it is a selectively permeable barrier.  mitochondria- Site of respiration. It is surrounded by two membranes (double membrane). The inner layer folds inwards to form the cristae. The cristae project into a liquid called the matrix.  The inner membrane (matrix) is coated in enzymes, which catalyse the reactions of aerobic respiration to produce ATP.  Chloroplasts- Site of photosynthesis. Chloroplasts use carbon dioxide, water and light energy to build sugars. They are present in all green plants and algal cells. The chloroplast is surrounded by a double membrane. It is filled with a liquid called the stroma, and contains stacks of thylakoid membranes called grana. Grana are linked together by Lamellae.  Golgi apparatus - Processes and packages new lipids and proteins. It also makes lysosomes (which make digestive enzymes)  Golgi vesicles - Stores lipids and proteins made by the golgi apparatus and transports them out of the cell  Lysosome- A round organelle surrounded by a membrane (a bit like a golgi vesicle)  Contains digestive enzymes called lysozymes which digest invading/worn out cells or components of cells  Ribosomes - Site of proteinsynthesis  RER- Membranes surrounding a fluid. Covered in ribosomes.  Folds and processes proteins that have been made by the ribosomes  SER- Like RER no ribosomes attached  Synthesises and processes lipids  Cell wall - gives it support and structure. It is made of the polysaccharide cellulose, and can function as a carbohydrate store by varying the amount of cellulose it holds.  (Plant and algal cell walls are made from cellulose, Fungi cell walls made from chitin) Vacuole- A vacuole consists of a membrane called the tonoplast, filled with cell sap – a watery solution of different substances, including sugars, enzymes and pigments.  The vacuole is important in keeping the cell firm. When the vacuole is full of sap the cell is said to be turgid. | *Students need to already know content checked in prior lesson* | Organelle  Pores  Selectively Permeable  Cristae |
| **Lesson 3:**  **Prokaryotic Cells and Replication** | Students will know that prokaryotic cells are much smaller than eukaryotic cells  Prokaryotic cells do not have membrane bound organelles in the cytoplasm like eukaryotic cells do, they have smaller ribosomes, cell wall made from murein.  Prokaryotic cells also have a slime capsule and flagella  Bacteria reproduce by binary fission which is where the DNA and plasmids replicate and then the cytoplasm divides to form 2 daughter cells, each with a single copy of the circular DNA and a variable number of copies of the plasmids.  Viruses are non living and simply Genetic information surrounded by a protein | Students will already know that prokaryotic cells do not have DNA in a nucleus. It is loose strands in the cytoplasm or plasmids.  Students should already know that viruses are non living | Murein  Glycoprotein  Plasmid  Membrane bound |
| **Lesson 4:**  **Microscopes** | Students will know that light microscopes are used in school and electron microscopes are often used in industry as they have different features. Students will learn that light microscopes have a lower magnification, lower resolution. Light microscopes use light rays. Live specimens can be seen with a light microscope, they are less expensive than electron microscope and no specialist training is needed when using them.  Electron microscopes allow us to see subcellular structures which we cannot see with an electron microscope. They are more expensive and require specialist training. Electron microscopes use electron beams and produce a black and white image on a screen. The specimen must be prepared in a vacuum (area of no air particles) as this affects the image produced. Only dead specimens can be viewed. Students will learn that Transmission Electron Microscopes (TEM) and Scanning Electron Microscopes (SEM). TEM allows beams to pass through to observe inner structures. SEM gives information about the surface of the sample. SEMs can be 3D images and samples can be thicker.  Students will learn the method of preparing a slide (also called a temporary mount) and using the microscope. They will learn that the steps involved: Place a drop of water on the centre of a clean dry slide  (This is suspending the specimen between the slide and cover slip) The refractive index of water also improves the quality of the image)  2. Using the tweezers, place the specimen in the middle of the drop.  (The specimen needs to be thin to let light through)  3. Add a drop of stain  (Stains highlight the organelles) To use the microscope: Clip the slide on the stage, Select the lowest power objective lens Look through the eye piece, Use the course adjustment wheel to move the stage, Use the fine focus until you get a clear image  Increase the magnification if necessary | *Students need to already know that light microscopes and electron microscopes are different and have various advantages and disadvantages depending on the sample they want to view. Students will have prepared a slide and used a light microscope to view onion cells at GCSE. Students will already know that magnification = Image/actual* | Objective lens  Focus wheel  TEM  SEM |
| **Lesson 5:**  **Cell Fractionation** | Students will know that organelles can be separated using fractionation. The 3 main steps are homogenisation (breaking up the cells) this is done by grinding up the cells with a blender. This breaks up the plasma membrane and releases the organelles into the solution. The solution must be ice cold to reduce the activity of the enzymes that break down the organelles. The solution must be isotonic (same conc as the cells being broken down to prevent damage by osmosis) A buffer solution is added to maintain pH. Stage 2 is filtration- getting rid of the large insoluble pieces. A gauze is used for filtration. Stage 3 Ultracentrifugation- separating the organelles. After filtration, the mixture contains a range of organelles. These are poured into a tube and put into a centrifuge (a machine that spins at a range of high speeds) The heaviest organelles come off first as sediment at the bottom (pellet) at low speeds. The supernatant is drained off and spun again at a higher speed. The process is repeated. Order: Nuclei, chloroplasts, mitochondria, lysosomes, ER, Ribosomes. |  | Homogenisation  Buffer  Ultracentrifugation  Filtration  Supernatant  Sediment  Pellet |
| **Lesson 6:**  **Cell Cycle and Mitosis** | The cell cycle consists of a period of cell growth and DNA replication called interphase. Mitosis happens after interphase.  Interphase is divided into separate growth stages called G1, S1, G2  G1= Gap Phase 1  Cell grows in size, new organelles and proteins are made  S= Synthesis  Cell replicates its DNA ready to divide by mitosis  G2= Gap Phase 2  Cell keeps growing and proteins are needed for cell division  Mitosis- Cell cycle ends. Students should interpret this in a pie chart.  Chromosomes are double armed structures, The centre is called the centromere, the 2 separate strands are called chromatids. There are 2 strands because an identical copy is made during interphase. When mitosis is over they appear as single strands again in the daughter cells.  Mitosis is split into a series of stages; Prophase, Metaphase, Anaphase, Telophase.  During prophase the chromosomes condense getting shorter and fatter. Centrioles move to opposite poles forming protein fibres called spindles. Nuclear envelope breaks down.  During metaphase the chromosomes line up in the middle of the cell and attach to the spindles by the centromere  During Anaphase the centromeres divide, separating each pair of chromatids. The spindles contract and pull the chromatids to opposite poles (by the centromere) This makes them appear V shaped.  Telophase- Chromatids reach opposite poles. Uncoil and become long and thin again, chromosomes again. Nuclear envelope forms around each group so there are now 2 nuclei. Cytokinesis completes telophase.  Students will learn how to calculate the length of time spent in a particular stage. | Students will already know that there are 2 types of cell division; mitosis and meiosis. Students should be able to describe the cells produced during mitosis and meiosis  Students should already know that cell division is essential for growth & repair  The cell cycle is a series of stages a cell goes through when it is growing and dividing  Stage 1 is DNA Replication  Stage 2 is mitosis  Stage 3 is cytokinesis  Chromosomes are double armed structures make of DNA | Interphase  Cytokinesis  Centrioles |
| **Lesson 6 b**  **RP 2 Stained Squashes of cells** | Students will use prepare squashes of cells from root tips to observe mitosis and calculate mitotic index  Students will learn that the cover slip is pressed down firmly | Students will already know how to prepare a slide to observe cells under a microscope | Mitotic Index |

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| **Uncontrolled cell division**  **Binary fission and viral replication** | Mitosis and the cell cycle are controlled by genes. Normally when cells divide enough times to make enough new cells they stop. If there is a mutation in the gene that controls cell division then cells will grow out of control to form a tumour  Cancer is a tumour that invades surrounding tissue.  Students will learn that understanding the cell cycle helps scientists to develop treatments for cancer.  Targeting G1 (cell growth and protein production) chemotherapy prevents the synthesis of the enzymes needed for DNA replication. Without these, the cell cannot go into S phase so cell kills itself.  Targeting S phase radiation and some drugs damage the DNA. At several points in the cell cycle DNA checks are carried out, if faulty then the cell will kill itself. Both of these methods prevent further tumour growth.  Prokaryotic cells divide by binary fission. The circular DNA and plasmids replicate, cells increase in size and loops move to opposite poles. Cytoplasm divides. 2 daughter cells each with one copy of the circular DNA and variable numbers of copies of plasmids.  Viruses need a host to replicate. They use attachments proteins to bind to complimentary receptor proteins n the surface of host cells. Some viruses can only infect one type of cell due to the specificity of their proteins. Once attached their RNA/DNA is injected into the host cell. It then uses the DAN from the cell to replicate. The cell bursts releasing new viruses. | Students will already know that cancer is a result of uncontrolled cell division. Students will have heard of radiation and chemotherapy for possible treatments of cancer.  Students will already know that a mutation is a random change in the DNA sequence  Students will already know that bacteria reproduce by binary fission forming 2 identical daughter cells approx. every 20mins. |  | Tumour  Gene  Mutation  Binary Fission  complimentary receptor  Host |
| **Lesson 7 Cell membrane structure** | In eukaryotic cells, organelles are surrounded by membrane too. They are a barrier between the cell and its environment. The have a partially permeable. The main composition is lipids, proteins, carbohydrates.  Cell membranes have a fluid mosaic structure.  Phospholipids form a double layer (bilayer) which is described as fluid as the phospholipids are constantly moving. Phospholipids have a hydrophilic head and a hydrophobic tail. Heads face out towards water and tails face inwards away from water. Water soluble substances are unable to pass through- it acts as a barrier for dissolved substances.  Proteins are scattered through the bi layer like a mosaic including channel proteins and carrier proteins which allow large molecules and ions to pass through. Receptor proteins detect chemicals from other cells which signal for the cell to respond (eg insulin response)  Glycoproteins are proteins with a carbohydrate attached and glycolipids are lipids with a carbohydrate attached.  Cholesterol is a type of lipid and is also present and adds stability/rigidity to the membrane as it fits between the phospholipids binding the tails together. | Student will already know that the cell membrane controls what goes into and out of the cell  Students should know from 3.1 what a phospholipid is, carbohydrates contain CHO, Proteins contain CHON. |  | Fluid Mosaic model  Mosaic  Stability  Ions  Hydrophobic  Hydrophilic |
| **Lesson 8**  **RP 4 Permeability** | Students will learn how to use equipment to investigate the permeability of cell membranes.  Permeability is affected by:  Temperature & solvent concentration  Low temperature <0 phospholipids don’t have enough energy to move. They are packed closely together and the membrane is rigid. Channel proteins and carrier proteins deform increasing permeability. Ice crystals pierce the membrane further increasing permeability when it thaws.  Temp 0-45 phospholipids can move. Membrane partially permeable. Increasing temp increases permeability as phospholipids have more energy to move more.  Temp +45 phospholipid layer starts to melt increasing permeability. Water in cell expands putting pressure on membrane. CPs and CPs deform so permeability increase further.  Solvent concentration affects permeability as the solvent dissolves the lipids causing the membrane to lose structure. | Students will already know that increasing temperature increases the kinetic energy od particles. | ***Interpreting graphs***  ***Calibration of equipment*** | Permeability  Deform  Solvent  Colorimeter  Absorbency |
| **Lesson 8b**  **Movement across membranes**  **Osmosis**  **RP 3 Dilution series and water potential** | Students will learn that Osmosis is the movement of water from an area of high water potential to low water potential across a partially permeable membrane,  Water potential is the likelihood of water molecules to diffuse out of or into a solution. Pure water has a water potential of 0.  The 3 factors that affect the rate of osmosis are gradient, thickness of exchange surface, surface area of exchange surface.  Students will learn how to make up serial dilutions of known concentrations of solute.  Students will learn how to interpret a calibration curve and use it to find the isotonic point and explain why mass increases or decreases in terms of osmosis | Students will already be able to define osmosis as the movement of water from a dilute to concentrated solution across a partially permeable.  Students will have already carried out the GCSE required practical and will recall the names of the equipment involved.  Students will already know that water balance is important in cells. If too much water enters animal cells the burst (lysis). If too little water is in the cells that crenation occurs.  Plant cells don’t under go lysis due to having a cell wall. Instead they become turgid or flaccid or plasmolysed | ***MS 3.2 Plotting data in an appropriate format***  ***MS 3.4 Determining isotonic point on a graph of water potential against change in mass***  ***Calculating percentage change in mass*** | Osmosis  Water potential  Partially permeable  Isotonic  Hypotonic  Hypertonic  Lysis  Crenation  Turgid  Plasmolysed |
| **Lesson 8c**  **Facilitated diffusion** | Some large molecules (amino acids, Glucose) would not pass easily through a phospholipid bilayer – would be too slow.  Charged particles (ions and polar molecules) are water soluble so would pass slowly through the hydrophobic part of the membrane. Instead these diffuse through channel proteins or carrier proteins which is called facilitated diffusion. Occurs down a gradient and is passive so no energy required.  Carrier proteins attach to the large molecules  The channel protein changes shape  The channel protein releases the large molecule on the other side of the membrane.  Channel proteins form pores for charged particles to diffuse through. Different channel proteins facilitate the diffusion of different charged particles.  Recap factors that affect the rate of diffusion. Facilitated diffusion depends on the number of channel or carrier proteins.  Aquaporins are special channel proteins that allow the facilitated diffusion of water through the membrane. Kidney cells have lots of aquaporins to reabsorb water rather than it be excreted. | Students will already know that diffusion is the net movement of particles from high to low concentration. Diffusion occurs both ways but net movement will be to a lower concentration.  Diffusion occurs down a concentration gradient. | *Finding the gradient of a line /using a tangent on a curve to find rate of diffusion* | Polar  Channel protein  Carrier protein  Ions  Facilitated  Pores  Aquaporins |
| **Active transport & Co transport in illeum** | Carrier proteins are involved as per previous lesson.  ATP is a common source of energy released during respiration. ATP undergoes a hydrolysis reaction splitting ATP not ADP and Pi an inorganic phosphate. This releases energy so solutes can be transported. Co-transporters are a type of carrier protein. They bind 2 molecules at a time. The concentration gradient of one is used to move the other.  Students will learn that Sodium ions move into the cell down a concentration gradient which moves glucose into the cell against a gradient.  Factors affecting rate are:  Speed of carrier proteins  Number of carrier proteins  Rate of respiration and so the availability of ATP  Students will learn that cotransport occurs in the mammalian ileum to absorb glucose and involve the sodium potassium pump to create a concentration gradient. | Students will already know from GCSE that active transport is the movement of particles against a gradient and requires a carrier protein and ATP. This occurs in root hairs. |  | Hydrolysis  Cotransporters |
| **Lesson 9**  **Phagocytosis** | Pathogens & abnormal body cells have antigens on their surface.  Phagocytes carry out phagocytosis. They are found in the blood and are the first cells to respond to an immune system trigger.  The stages involved are:   * Pathogen recognised as foreign by phagocyte * Pathogen attached to phagocyte by antibody and surface receptors * Phagocyte engulfs the pathogen * The phagocyte is contained in a phagocytic Vacuole (Phagosome) in the cytoplasm * The phagosome fuses with the Lysosome (phagolysosome) which then release enzymes to break down the pathogen * The Phagocyte displays the antigen on external surface of plasma cell membrane (antigen presentation) to start immune response (T & B cells)   Phagocytes activate T lymphocytes which are also white blood cells by presenting the pathogen’s antigens. | Student will already know examples of non specific defence mechanisms. Students will already know that white blood cells act in 3 ways to destroy pathogens. Engulf & digest, produce antibodies, produce antitoxins. Pathogens produce toxins and damage cells. Students will know that antigens are on the surface of cells and can trigger an immune response. |  | Pathogens  Antigen  Phagocyte  Lymphocyte |
| **Lesson 10**  **T & B Lymphocytes** | T Lymphocytes are activated by phagocytes when they present the antigens of the pathogen. T lymphocytes have receptor proteins which attach to the complimentary antigen. **T- Lymphocytes-** Cells produced in the bone marrow but mature in the **T**hymus Gland  TH are helper cells that release chemical signals that activate and stimulate phagocytes and TC (cytotoxic T Cells) which kill abnormal cells. These also activate B Lymphocytes. B Lymphocytes have antibodies that bind to antigens to form an antigen-antibody complex. When the B lymphocyte meets the complimentary shaped antigens, it binds to it. This further activates the B cell to begin a process called clonal selection. The B cell divides into plasma cells.  Clonal Selection  Only Specific complimentary antibody is selected  Specific B cells reproduce by mitosis  Plasma Cells  All produce mono clonal antibodies  Memory cells  For secondary response  Destroy  Agglutinate  Opsonin's- Antitoxins  Students will learn about antibody structure. Antibodies are proteins. The specificity depends on the variable regions. The binding region has a unique tertiary structure (due to the sequence of amino acids). Antibodies have 2 binding sites so can bind to 2 antigens at the same time. This results in agglutination. Phagocytes can then bind to antibodies and phagocytose the pathogens. This destroys the pathogen. | Students will already know that lymphocytes produce antibodies which are protein that attach to the antigens on the surface of the pathogens |  | Complimentary  Clonal selection  Opsonins  Agglutination  Monoclonal antibodies |
| **Cellular, humoral, primary and secondary response** | T cells and other immune system cells are part of the cellular response  B cells and clonal selection form the humoral response  The primary response occurs when the pathogen enter for the first time. This is slow because it takes time for B cells to produce new antibodies. Eventually enough of the correct antibodies will be produced to overcome the infection. Symptoms occur whilst B cells are making the antibodies and this is why a person feels ill. After exposure, T and B cells produce memory cells which stay in the body for a long time. Memory cells will detect the same pathogen second time round and respond more quickly the second time. The person is no immune- the immune system has the ability to respond quickly to a second infection.  The secondary response is quicker and stronger. Clonal selection happens faster. Memory B cells are activated and divide into plasma cells.  Students will be able interpret and annotate an immune response graph. | Students will have heard the work immune/immunity | ***Interpreting graphs*** | Cellular  Humoral  Memory cells  Symptoms |
| **Vaccinations** | Vaccines help to avoid the period of time where B cells are dividing and during this time a person feels ill.  Vaccines contain antigens that cause the body to produce memory cells against a certain pathogen. This means you become immune without any symptoms.  Vaccines are effective because they reduce the occurrence of the disease and those who are not vaccinated because there are fewer people to catch it from. This is herd immunity.  Antigenic variation- sometimes the antigens on a specific pathogen change which means the memory cells are no longer specific to the antigens so people become ill again. This makes it difficult to develop vaccines.  Active immunity is when your immune system makes its own antibodies after being stimulated by an antigen; natural- immunity after catching a disease, Artificial is where you become immune after having a vaccine.  Passive immunity is when you are given antibodies from a different organism. Natural- From mum to baby, Artificial – immunity after having an injection containing antibodies. | Students will know that a vaccination contains a dead of harmless form on the pathogen which triggers an immune response. | ***Interpreting data on vaccines and antibodies*** | Herd immunity  Antigenic variation  Active immunity  Passive Immunity |
| **Lesson 12**  **HIV** | HIV (Human Immunodeficiency Virus) affects the immune system. It eventually causes AIDS (Acquired immune deficiency syndrome)  The immune system deteriorates which makes sufferers more prone to other infections.  HIV kills TH cells which act as host cells. AIDS is confirmed when TH cells reach a critically low level.  Students will learn about the structure of HIV. It contains RNA, and reverse transcriptase (enzyme involved in virus replication). The outer coating, capsid, is made of protein then the envelope which is created from the ‘stolen’ membrane of the host cell. The surface also contains attachment proteins that help the HIV attach to the host helper T cell.  Students will learn how HIV replicates inside helper T cells.  1. The **attachment proteins** attached to the CD4 proteins on the surface of the Helper T Cell  2. The capsid is released into the Helper T Cell and releases its RNA  3. **Reverse transcriptase enzyme** uses the viral (HIV) RNA to make copies of DNA  4. This (Viral)DNA is inserted into the human DNA  5. The host cell enzymes are used to make HIV proteins from the viral DNA found in the human DNA  6. The viral proteins are **assembled** into new viruses which **bud off** the cell and can go on and infect other Helper T Cells  Antiviral drugs can be used to slow the progression of HIV. | Students will know that HIV is a virus that causes AIDS. It is an STI for which there is not yet a treatment |  | Reverse transcriptase  Attachment proteins  Capsid |
| **Lesson 13**  **Monoclonal Antibodies and ELISA TEST** | Students will learn that monoclonal antibodies are produced from one single group of genetically identical B cells (plasma cells).  They are specific because their binding site has a unique tertiary structure what fits with one type of antigen.  Monoclonal antibodies can be used to treat cancer. Cancer cells have tumour markers on their surface which are not found on body cells. Monoclonal antibodies can be made which bind to the markers. Anti cancer drugs can also be attached to monoclonal antibodies and the drugs can accumulate where there are cancer cells as they only attach to those specific cells.  Monoclonal antibodies are used in pregnancy tests. The urine of pregnant women contains a hormone (protein) HCG (Human chorionic gonadotropin).   * 1. hCG (Human Chorionic gonadotropin) hormone is detected during a pregnancy test. hCG hormone is found in pregnant women's urine.   2. Antibodies for hCG are bound to a coloured bead (blue)   3. When urine is applied to the specified area any hCG will bind to the antibody on the beads, forming an antigen-antibody complex.   4. Urine then moves up the stick to the test strip carrying any beads with it   5. The test strip contains antibodies to hCG that are stuck in place   6. The strip turns blue if hCG is present because the immobilised antibody binds to any hCG. If no hCG is present, the beads will pass through the test area without binding to anything, and so it won’t go blue.   Students will learn that the ELISA (Enzyme-Linked immunosorbent assay) can be used to diagnose if a patient has a specific antibody to a certain antigen.  Direct ELISA Method:  One antibody to find one antigen  Place blood sample from patient in spotting tile  **Detection antibody** is added  If antigen is present, it will be immobilised on the wall of the well and the detection antibody will bind to it.  WASH to remove any unbound antibodies  Substrate solution added. If enzyme is present and attaches to substrate then colour change will be evident. (positive result)  Indirect Elisa Method:  HIV antigen bound to wall of well  Add blood sample from patient (might contain HIV + several other antibodies  **HIV specific Antibodies (primary) will bind to HIV antigen if present**  WASH to remove any unbound antibodies  Second antibody (Secondary) with enzyme attached is added.  This can bind to the HIV specific antibodies  WASH to remove any unbound secondary antibodies (no primary=no secondary  can bind so all washed away)  Substrate solution added. If secondary antibody with enzyme is present then it attaches to substrate then colour change will be evident. (positive result) | Students who studies triple science will know that monoclonal antibodies are identical copies of one type of antibody which are produced in a lab and used for medical purposes.  Students will know that pregnancy tests use monoclonal antibodies. | ***Ethical Issues***  ***Evaluating Data*** | hCG hormone  Monoclonal antibody  Tumour markers  ELISA test |