Edexcel International A Level (IAL) Biology

Your notes

Forensics

Contents

Your notes

Polymerase Chain Reaction

- Every person, with the exception of identical twins, has a **unique DNA sequence** which can be used to create a **DNA profile**
  - This is very useful in forensic science as it provides a way to **identify individuals**
  - DNA profiling can also be used to determine the **genetic relationships** between different organisms e.g.
    - Paternity and maternity testing
    - Ancestry kits
    - Determining evolutionary relationships between different species
- DNA profiles can be created using the following steps
  - Isolating a **sample of DNA** e.g. from saliva, skin, hair, or blood
  - Producing more copies of the DNA fragments in the sample using the **polymerase chain reaction** (PCR)
  - Carrying out **gel electrophoresis** on the DNA produced by PCR
  - **Analysing the resulting pattern** of DNA fragments

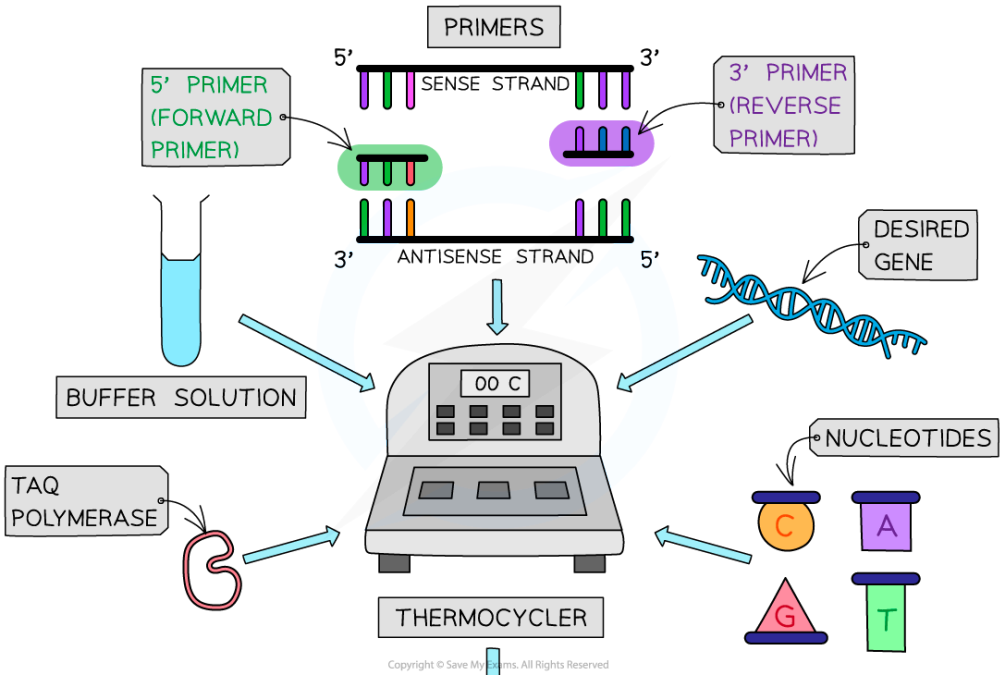
The polymerase chain reaction

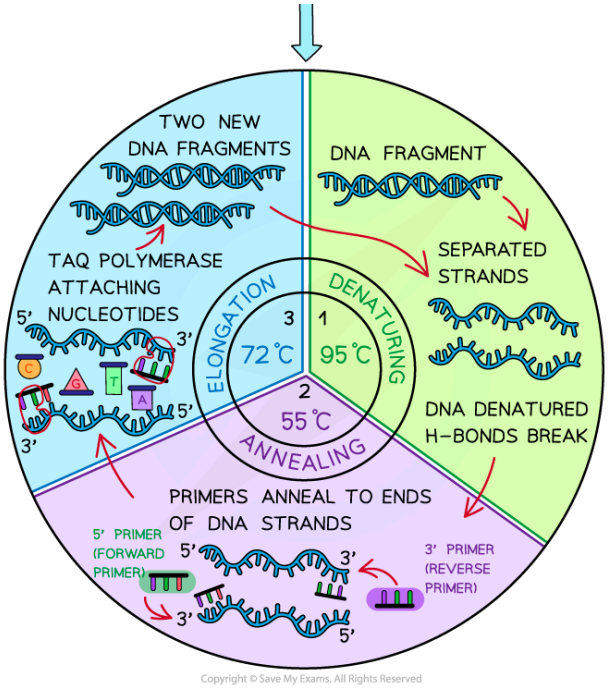
- PCR is a common molecular biology technique used in most applications of gene technology e.g.
  - DNA profiling
  - Genetic engineering
- It can be described as an *in vitro* **method of DNA replication**
- PCR produces many copies of a piece of DNA; this can be referred to as DNA **amplification**
  - It is used to produce **large quantities** of specific fragments of DNA or RNA from very small quantities; even just one molecule of DNA or RNA is enough to run PCR
  - By using PCR scientists can produce billions of **identical copies** of the DNA or RNA sample within a few hours
  - In each PCR cycle the DNA is doubled, so in a standard run of 20 cycles a million DNA molecules are produced.
- The process is carried out in a PCR machine, or **thermal cycler,** which automatically provides the **optimal temperature** for each stage and controls the **length of time** spent at each stage

- Each PCR reaction requires
  - **DNA** or **RNA** to be amplified
  - **Primers**
    - These are short sequences of single-stranded DNA that have base sequences complementary to the 3' end of the DNA or RNA being copied; they define the region that is to be amplified, identifying where the DNA polymerase enzyme needs to bind
  - **DNA polymerase**
    - The enzyme used to build the new DNA or RNA strand.
    - The most commonly used polymerase is ***Taq* polymerase,** which comes from thermophilic bacterium *Thermus aquaticus*
      - *Taq* polymerase **does not denature** at the **high temperature** required during the first stage of the PCR reaction
      - The optimum temperature of *Taq* polymerase is high enough to prevent annealing of the DNA strands that have not been copied yet
  - **Free nucleotides**
    - Enable the construction of new DNA or RNA strands
  - **Buffer solution**
    - Ensures the optimum pH for the reactions to occur in
- There are three main stages of the PCR reaction
  - **Denaturation**
    - The double-stranded DNA is heated to 95 °C which breaks the hydrogen bonds that hold the two DNA strands together
  - **Annealing**
    - The temperature is decreased to 50–60 °C so that primers can anneal to the ends of the single strands of DNA
  - **Elongation / Extension**
    - The temperature is increased to 72 °C, as this is the optimum temperature for *Taq* polymerase to build the complementary strands of DNA to produce the new identical double-stranded DNA molecules
- These three stages make up a single PCR cycle, and **many cycles can be completed**
  - Each PCR cycle **doubles the amount of DNA**

Your notes

Your notes





PCR can be used to amplify a fragment of DNA. Note that you don't need to know about forward and reverse primers

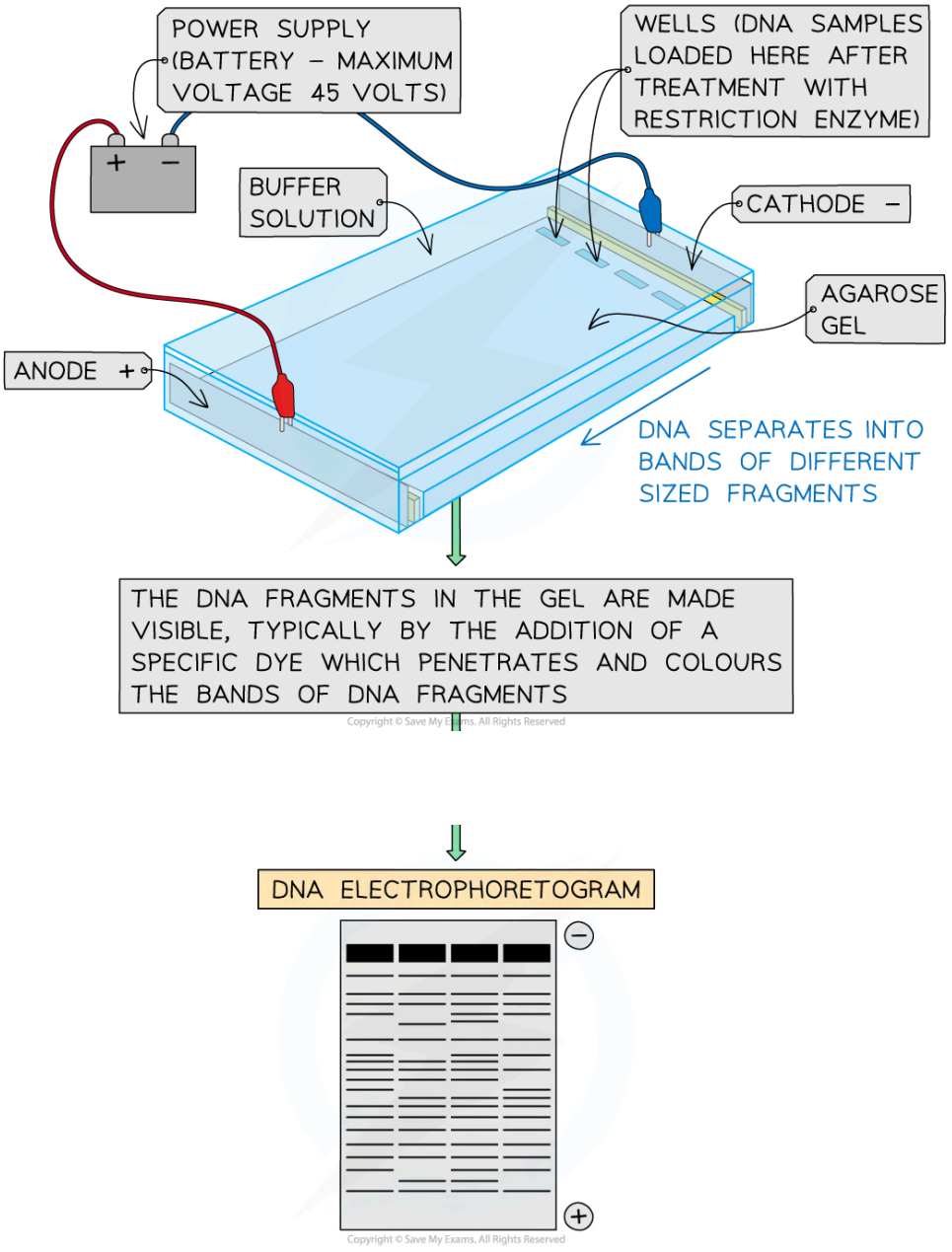
- After PCR is completed the DNA is treated with restriction endonuclease **enzymes** and a **fluorescent tag** can be added; both in preparation for **gel electrophoresis**
  - Restriction endonucleases break the DNA up into fragments of different length
  - Fluorescent tags enable the DNA fragments to be seen under UV light

Your notes

Gel Electrophoresis in Forensics

- Gel electrophoresis is a technique used widely in the analysis of DNA, RNA and proteins
  - DNA fragments are created, e.g. using enzymes known as **restriction endonucleases** that cut DNA at specific restriction sites
  - The resulting fragments are inserted into a well at the end of a piece of agar gel, before a current is passed through the gel
- Molecules move through the agar due to the difference in charge across the gel
  - **Positively charged molecules** will move towards the **cathode** (negative pole) while **negatively charged molecules** will move towards the **anode** (positive pole)
  - DNA is negatively charged due to the phosphate groups and so when placed in an electric field the molecules **move towards the anode**
- The **molecules are separated** according to their **size / mass**
  - **Different sized molecules** move through the gel at different rates
  - The tiny pores in the gel allow smaller molecules to move quickly, whereas larger molecules move more slowly
- The process of gel electrophoresis involves the following stages
  - An **agarose gel plate** is created and **wells** are cut into the gel at one end
  - The gel is submerged in a tank containing **electrolyte solution;** this is a salt solution that conducts electricity
  - **The DNA samples are transferred into the wells** using a micropipette, ensuring that a sample of DNA standard is loaded into the first well
    - The purpose of the standard is to produce a set of known results with which to **compare** any new results
  - The negative electrode is connected to the end of the plate with the wells and the positive anode is connected at the far end
    - The **DNA fragments** move towards the **anode** due to the attraction between the negatively charged phosphates of DNA and the anode
    - The **smaller mass / shorter pieces** of DNA fragments move **faster and therefore further** from the wells than the larger fragments
  - **Probes are then added**, after which an **X-ray image** is taken or **UV-light is shone** onto the paper producing a **pattern of bands** which can be compared to the control, or standard, fragments of DNA
    - Probes are single-stranded DNA sequences that are complementary to the regions of interest; they can be

- A radioactive label which causes the probes to emit radiation that makes the X-ray film go dark, creating a pattern of dark bands
- A **fluorescent dye** which fluoresces when exposed to UV light, creating a pattern of coloured bands



Gel electrophoresis can be used in DNA profiling

Analysing the results of gel electrophoresis

- Gel electrophoresis produces a **pattern of bands** on the gel that represent DNA fragments of different length
  - The fragments were produced after PCR by cutting the DNA samples into pieces using **restriction endonuclease enzymes**

Your notes

- Restriction endonucleases cut DNA at **specific locations** in the DNA base sequence, so will always cut in between sections of repeated bases known as variable number tandem repeats (VNTRs)
    - VNTRs are known as micro- or mini-satellites depending on the number of repeats that occur; micro-satellites have fewer repeats than mini-satellites
- Different people have different numbers of repeats in their VNTR regions, so the **fragments will differ in length** depending on whether there are few or many repeats

- Different individuals will have **different lengths of DNA fragments**, so a **different pattern of banding** will form on each profile
- Every banding pattern will be **unique to an individual**, so comparisons of DNA from crime scenes with that of suspects is a reliable way of finding out who was present at a crime scene

Examiner Tips and Tricks

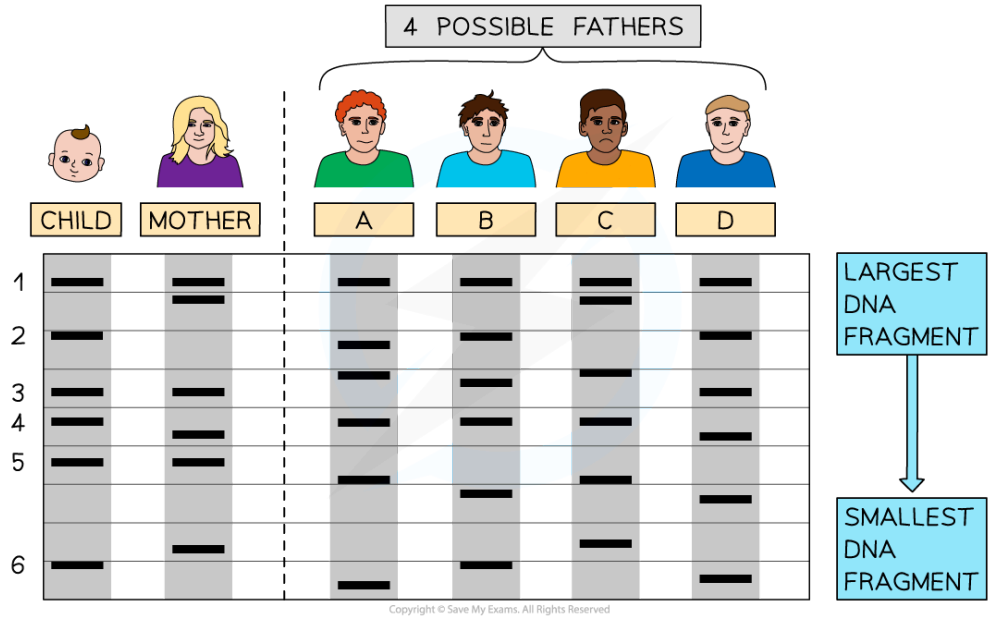
Don't get too bogged down in the details regarding restriction enzymes and VNTRs, but you should be able to explain how gel electrophoresis works to separate DNA fragments of different length.

Your notes

Your notes

DNA Profiling

- A **DNA profile** can be produced using the process of **gel electrophoresis**; the result is a **series of bands of DNA** of different length which can be seen due to the addition of radioactive or fluorescent labels
- DNA profiles can be used to determine the **genetic relationships** between people, e.g. in **paternity tests**
  - During a paternity test the **DNA profile of a child** is compared with a **variety of candidates** that could be the potential father
  - If **many bands** of the child's DNA profile **match with the bands in a paternity candidate's profile**, this could indicate that they are the **most likely** biological father
    - During fertilisation **half** of the DNA comes from each parent, so a child will share **half of their DNA with a parent**
    - When comparing DNA profiles the **more bands that match** between the profiles, the **greater the genetic similarity** between those individuals and the closer the relationship



DNA profiles can be compared to determine relationships in paternity testing. Here the child shares bands 1, 3 and 5 with the mother and bands 2, 4 and 6 with candidate B, so he is the most likely father

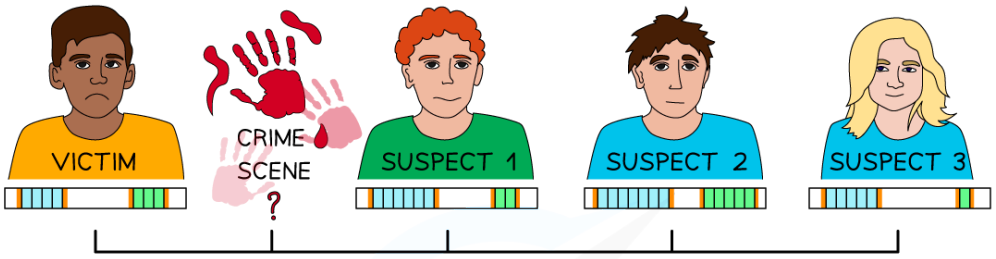
- DNA profiling is a useful tool in **forensic science** where it can be used to link possible suspects to a crime scene
  - Regions of DNA, known as **short tandem repeats**, are examined

Your notes

- These short tandem repeats are a type of non-coding, repeated sequence of bases known as a variable number tandem repeat, or VNTR; short tandem repeats consist of **short** repeating sections, and are also known as micro-satellites

- The greater the number of these regions examined, the more reliable the evidence provided

  - If only a few regions are analysed then there is a greater chance that closely related individuals will have an identical profile; an analysis of 11 or more sites is considered to be reliable evidence in a law court



KNOWN LENGTHS

Control DNA	Victim	Crime scene	Suspects		
			1	2	3

DNA profiles can be created using DNA samples found at crime scenes and then compared with the profiles of suspects to show who was present at a crime scene. VNTRs are shown beneath each individual in blue and green; different individuals have different numbers of VNTRS. In this example the DNA profile of suspect 3 is the closest match with the DNA found at the crime scene, though none of the suspects is a perfect match.

- DNA profiling can also be useful in **selective or captive breeding programmes** of animals or **cultivation of plants**

  - DNA profiles of the particular organisms can be **compared** to determine which are **genetically the most different** from each other

  - These organisms will then be crossbred, ensuring that the individuals that breed together are **not** closely related

    - Breeding between closely related individuals is known as **inbreeding**, and can cause genetic problems at an individual and population level

- In individuals there can be an accumulation of harmful recessive alleles that might otherwise have been masked by healthy dominant alleles
- Inbreeding leads to a **smaller gene pool** within a population, which can reduce a population's ability to adapt to change

Your notes

Your notes

Useful Data Provided by Forensic Analysis

- **Forensics** is the use of science in the **investigation of criminal activities**
  - It involves the **collection and analysis of evidence** from a potential crime scene e.g. the scene at which a dead body is found
- Determining the **time of death** (TOD) of a body is a very important component of **forensic science**
  - The TOD can be used during a police investigation to provide information about the **circumstances surrounding the death** of a person
- In order to accurately estimate TOD, there are **several factors** that must be established
  - Extent of decomposition
  - Stage of succession
  - Forensic entomology
  - Body temperature of the deceased
  - The degree of muscle contraction

Extent of decomposition

- The process of decomposition begins soon after death
  - Decomposition is carried out by organisms known as **decomposers** e.g. bacteria and fungi
    - Enzymes secreted from the cells of these organisms break down biological molecules in dead tissue
- The time since death can often be established visually by looking at the appearance of a body that is decomposing
  - Decomposers break down **cells and tissues** over the course of a few days
    - At this stage in decomposition the appearance of the skin can be a helpful indication of time since death; **skin will often appear greenish in colour**
  - The next stage of decomposition involves the **breakdown of tissues and organs** by micro-organisms over the course of a few days or weeks
    - This process produces gases, such as methane, which will lead to **bloating**
    - The **skin will blister and fall off** the rest of the body
  - A few **weeks after death** the remains of the soft tissues will **turn to liquid** which becomes visible as it **leaves the body**

- This process will continue over the course of **months or years** until only a **skeleton** remain
- After a **few decades or centuries,** the skeleton will **disintegrate** until nothing remains

- The rate of decomposition will be affected by factors such as **temperature** and **availability of oxygen**
  - Decomposition would be slower in anaerobic conditions and at lower temperatures but would be faster at high temperatures

Stage of succession

- Succession refers to the **change in the types of organisms** found in a habitat over time
  - This is often an ecology term that is applied to a habitat such as a pond or woodland, but in this case the **habitat is the dead body**
    - The difference between succession in ecology and in forensics is that in an ecosystem the early pioneer species are out-competed and disappear as the system matures, while in a dead body **all of the newly arriving species remain as decomposition progresses**
- The **stage of succession** of a body can provide information about the estimated TOD
- **Above ground** the body would undergo the following stages of succession
  - **Bacteria** will be found in and on the dead body immediately after TOD
  - As tissue decomposition sets in it creates ideal conditions for **flies** to lay eggs and their **larvae** to hatch
  - As more soft tissue is consumed by the fly larvae it creates favourable conditions for **beetles** to establish
  - When **tissue dries out** over time **flies will leave** the body as they prefer a moisture-rich environment
  - **Beetles**, however, can decompose dry tissue so they **will remain** on the body
  - Once all tissues have been decomposed most organisms will leave the body
- These **succession stages will differ** depending on where the body is located as the **accessibility to insects** and **availability of oxygen** will be affected e.g.
  - Buried in soil
  - Buried in a coffin
  - Under water

Forensic entomology

- A dead body provides an ideal habitat for many **species of insects;** the study of these insect colonies is known as **forensic entomology**

Your notes

- **Different insect species** will colonise a body at different times after death, providing information about the TOD
  - Flies will be found on the body within a few hours after death, while beetles will only colonise the body later
- Another clue that insects can provide is the **stage of life cycle** they are at
  - E.g. blowfly eggs will hatch after about 24 hours so if larvae are present on the body it indicates that the person died more than 24 hours ago
    - Other insects have longer life cycles, so if **only** blowfly larvae are found it indicates that **only** 24 hours has passed since TOD
  - Factors that might affect the **progression of insect life cycles** include
    - Drugs that may be present in the body
    - Humidity of the surroundings
    - Oxygen availability
    - Temperature

Your notes

Body temperature

- Respiration and other metabolic processes **produce heat** in living organisms
  - This heat is necessary for maintaining our body temperature at around **37 °C** during life
- Once a person dies **metabolic reactions** will eventually **come to an end**
  - Since no more heat is produced the **body temperature drops** until it reaches the temperature of the surrounding environment
  - This process of cooling is known as ***algor mortis***
- Body temperature decreases by **1.5-2.0 °C per hour**, therefore providing forensic scientists with a way to determine the TOD based on the temperature of the body
- Certain conditions will affect the rate at which body heat is lost e.g.
  - Air temperature
  - Surface area : volume ratio
  - Presence of clothing
  - Percentage body fat

Degree of muscle contraction

- **Muscles** in the body begin to **contract** about 4–6 hours after TOD, leading to a general stiffening of the body known as ***rigor mortis***
- *Rigor mortis* comes about as a result of changes to the proteins in muscle cells after death

- Since **no more oxygen** reaches the muscle cells after death they will start to **respire anaerobically,** producing **lactic acid**
- The accumulation of lactic acid **decreases the pH** in the muscle cells, **denaturing the enzymes** that produce ATP
- Without ATP the myosin heads **cannot be released** from the actin filaments, **locking the muscles in a contracted state**
  - Muscles contract due to the action of two protein filaments; myosin and actin
  - The binding of myosin heads to actin proteins followed by the bending of the myosin heads causes muscle contraction
  - ATP is required to allow the myosin heads to detach from the binding sites on actin
- This leads to the stiffness that is the main characteristic of *rigor mortis*

- *Rigor mortis* will begin in the smaller muscles of the head and end in the larger muscles of the lower body, meaning that forensic experts can determine TOD by the progress of *rigor mortis* through the body
  - *Rigor mortis* would have taken place in every muscle between 12 and 18 hours after death, but will wear off again after about 24 to 36 hours from TOD
- The process is affected by the **level of muscle development** and the **temperature of the surroundings**
  - Higher temperatures will speed up the rate of *rigor mortis*

Examiner Tips and Tricks

Remember that the time of death is only an estimate and cannot be pinpointed exactly. There are so many factors that will affect a dead body that it is nearly impossible to provide a time of death that will be precisely accurate; keep this in mind when answering questions on this topic.

Your notes