# TAMPINES MERIDIAN JUNIOR COLLEGE

## JC2 PRELIMINARY EXAMINATIONS

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# H2 BIOLOGY 9744

Paper 3 Long Structured and Free Response Questions **18 September 2020**

**2 hours**

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| No | Where did I go wrong? ☹ | How can I improve? ☺ |
|---|---|---|
| 1 | I **don't understand** what the question wants from me. | • Identify topic(s) related to the question.<br>• Analyse the preamble and/or diagram carefully. |
| 2 | I don't know / can't remember the **conceptual facts**. | • Review your study techniques to find out what works best for you. |
| 3 | I did not give the **essential keywords** / wrong keyword. | • You must go back to your notes and learn why you missed the essential points in addressing the questions. |
| 4 | My answers are **incomplete** / not of enough depth. | |
| 5 | I **misinterpreted** the questions / data, hence wrote the wrong answer. | • Read all the directions (identify keywords or phrases and register what they mean).<br>• Take the time to paraphrase the question. |
| 6 | I did not **contextualize my answers** to the question. That is, I did not make use of the information in the preamble / stimulus / figure. | • When the question revolves around a specific example, use the contextual information to craft your answers. |
| 7 | I did not **cite data** / I did not include the units for data / did not cite meaningful data for both axes. | • Cite complete data: both x-axis and y-axis, with units.<br>• Examine the trend of the graph. If necessary, divide the graph into ≥ 2 parts for meaningful citation of data. |
| 8 | I did not **organize my answers** properly, especially for comparison questions / essay questions. | • For essay, paragraph your answers for each major idea.<br>• For comparison, number your points. Use comparative words (e.g. but, whereas, while etc.) |
| 9 | I did not manage to attempt the question due to **insufficient time.** | • Look through the whole paper and attempt questions you are confident in.<br>• Be concise. Do not write excessively.<br>• When you are stuck at a question, move on. |
| 10 | I was not able to **apply the conceptual facts** to this kind of 'suggest' questions. | • Identify the topic(s) that the question is related to, draw links to the specific section or concept<br>• Write down keywords or phrases.<br>• Finally see how these would match the question |

# Where did I go wrong….?

(Tick in the appropriate box)

| Structured Qn / No | | CHECKLIST NUMBER | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
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**What was my major weakness(es)…?**

## Section A
Answer all questions in this section.

**QUESTION 1**
The cystic fibrosis transmembrane conductance regulator (CFTR) protein is an ion channel. In the lung, the CFTR ion channel moves chloride ions from inside the cell to outside the cell.

Cystic fibrosis (CF) is a serious genetic condition caused by recessive mutations in the gene for the CFTR protein. One of the most common mutated alleles of this gene is known as ΔF508, a deletion of the amino acid phenylalanine at the 508$^{th}$ amino acid position of the polypeptide. This mutation results in a CFTR protein that does not conduct chloride ions.

Features of CF, which are also observed in heterozygotes, include:

- a reduction in water loss through epithelial cell membranes
- a reduction in sweating.

When there is less water outside the cells, the mucus in the airways becomes dehydrated and thickens, as shown in Fig. 1.1. The cilia becomes unable to sweep properly when the mucus is thick as sticky mucus weighs them down.

Because the cilia are unable to move properly, mucus gets stuck in the airways, making it difficult to breathe. In addition, germs caught in the mucus are no longer expelled from the airway, allowing them to multiply and cause infections. Thick mucus in the lungs and frequent airway infections are some of the most common problems that people with CF face.

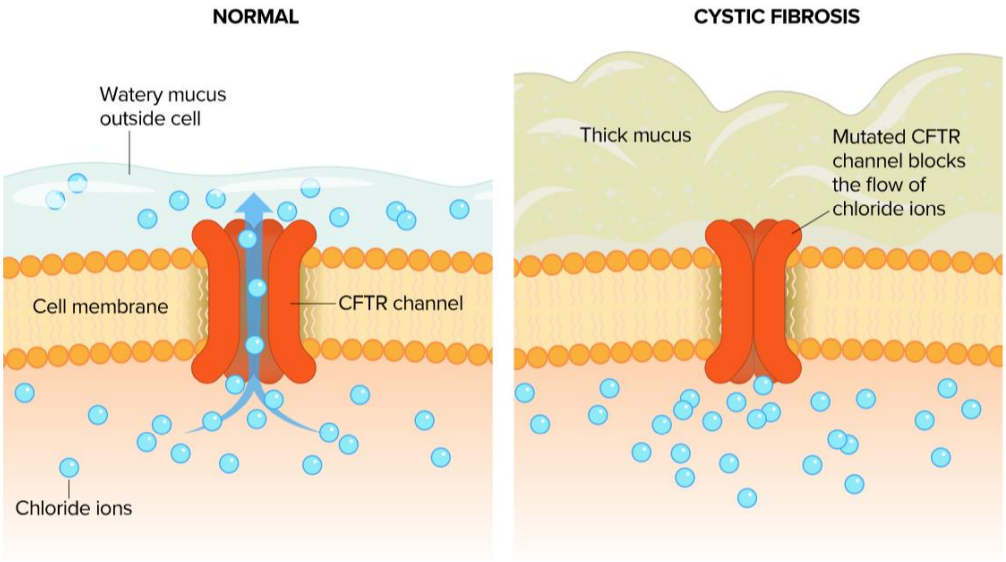



**Fig. 1.1**

**a)** Describe how the ΔF508 mutant CFTR protein may bring about a reduction in water loss through epithelial cell membranes. [3] **[TRANSPORT]**

1. A deletion of phenylalanine **changes the conformation** of the CFTR protein
2. **Unable to transport chloride ions**, leading to **an increase of ion concentration inside the cell**
3. **Decreases** the **water potential** **inside the cell**
4. **Less water** movement **out** of cell by **osmosis** / ***reverse argument***

**[Any 3]**

**b)** Explain why inherited diseases, in general, have low occurrences in the general population. [3] **[EVOLUTION]**

1. ***Ref. to*** affected individuals at selective disadvantage / reduced chance of survival
2. Do not survive to reproductive age to pass on the mutant allele to offspring
3. Most inherited diseases are caused by **recessive alleles**
4. ***Idea of*** Low chance of two carriers mating
5. Allele is expressed only in individuals who are **homozygous recessive**

**[Any 3]**

**c)** CF is one of the most common inherited diseases. This unusually high occurrence has given rise to the suggestion that carriers of the ΔF508 allele may have a selective advantage over non-carriers.

Approximately 1 in 30 people in the European and North American populations are carriers of the defective allele ΔF508.

Within the European and North American populations, calculate the probability that a newly-born baby will be homozygous for this allele.

You should show your working. [2] **[INHERITANCE]**

Probability of two carriers being the parents of a newly-born child
= 1/30 × 1/30
= 1/900 **[1]**

Probability of such a child being homozygous recessive = 1/4

Therefore, overall probability = 1/4 × 1/900 = **1/3600** **[1]** **[Accept: 0.00028 / 0.028%]**

**d)** There are at least 1500 different mutated alleles of the CFTR gene. These alleles have been classified into six classes according to the mechanism by which they disrupt the synthesis, trafficking and function of CFTR protein.

The six classes are described in Table 1.1.

**Table 1.1**

| class of mutation | effect of CFTR mutation |
|---|---|
| class I | complete lack CFTR protein |
| class II | misfolded CFTR protein not transported to the cell surface |
| class III | reduced or lack of CFTR channel opening ('gating' defect) |
| class IV | a 'misshaped' CFTR pore that restricts the movement of chloride ions through the channel ('conductance' defect) |
| class V | splicing defect with a great reduction in normal CFTR proteins |
| class VI | decreased CFTR protein stability in the cell surface that leads to its removal and degradation |

**i)** State the most likely class of mutation to which ΔF508 belongs. [1] **[MUTATION]**

- **Class III** / **class IV**

**ii)** With reference to Table 1.1, explain why in the population of individuals who carry two recessive alleles, the symptoms of CF may range from mild to severe. [3] **[INHERITANCE]**

1. ***Idea that*** Different mutations results in different versions/variants of CFTR proteins that functions to different extents

2. ***Ref. to*** class I/II/III being severe mutations, and class IV/V/VI being mild mutation
**OR** ***Ref. to*** class I/II being severe, III/IV being intermediate, class IV/V/VI being mild

3. ***Ref. to*** a mild mutation results in a CFTR protein that can still transport some chlorides, hence the mild symptoms / ***reverse argument*** *for severe mutations*

4. ***Ref. to*** the individual can contain two alleles from the same/different mild classes, resulting in mild symptoms / ***reverse argument*** *for severe mutations*

**[Any 3]**

**iii)** Suggest how, in the class VI mutation, the unstable CFTR protein is removed from the cell surface membrane **and** subsequently degraded. [4] **[OCGE in Euk]**

**[How it is removed]**
1. Cytoplasmic domain of CFTR tagged with **ubiquitin** proteins
2. Triggers **endocytosis** to form CFTR-containing vesicle…
3. …which **invaginates** to form **multi-vesicular bodies** (MVB)

**[How it is degraded]**
4. **Fusion** of MVB with **lysosomes**
5. **Hydrolytic enzymes** in the lysosomes degrades the CFTR

**[Any 4]**

Cholera is caused by a toxin secreted by the bacterium *Vibrio cholerae* in the gut. This toxin enters the epithelial cells and activates an intracellular G-protein that leads to an excessive amount of cyclic AMP (cAMP). cAMP binds to CFTR to facilitate abnormally high movement of chloride out of the cell, increasing water loss from the gut epithelial cells. This results in severe diarrhea and may lead to death if untreated.

In an experiment carried out in 1994, it was shown that when mice that were heterozygous for ΔF508 were exposed to cholera toxin, they lost 50% less water than homozygous dominant mice also exposed to cholera toxin.

This supported a suggestion that the selective advantage of carrying the ΔF508 allele may be protection from the effects of cholera.

**e)** Suggest how the ΔF508 allele might be expected to convey a selective advantage in areas of the world where cholera is common. [4] **[TRANSPORT, EVOLUTION]**

1. water loss depends on extent of activity of CFTR protein
2. **carriers** of ΔF508 allele have **fewer functional CFTR proteins**
3. fewer functional CFTR for cAMP to bind
4. reduced chloride ion movement **out** of cell
5. hence, **less water is lost** from gut epithelial cells
6. **heterozygote advantage**
7. **carriers** of ΔF508 allele **more likely to survive cholera**

**[Any 4]**

**f)** In 2000, a further experiment to investigate the possible link between the ΔF508 allele and the severity of cholera in humans was conducted. To do this, the effect of prostaglandin was measured in human subjects including:

- some who had cystic fibrosis (homozygous for ΔF508)
- some who were carriers (heterozygous for ΔF508)
- a control group who did not have cystic fibrosis and did not carry ΔF508.

Prostaglandin is a chemical that increases water loss from epithelial cells by increasing chloride secretion through the CFTR protein. The results are shown in Fig. 1.1.

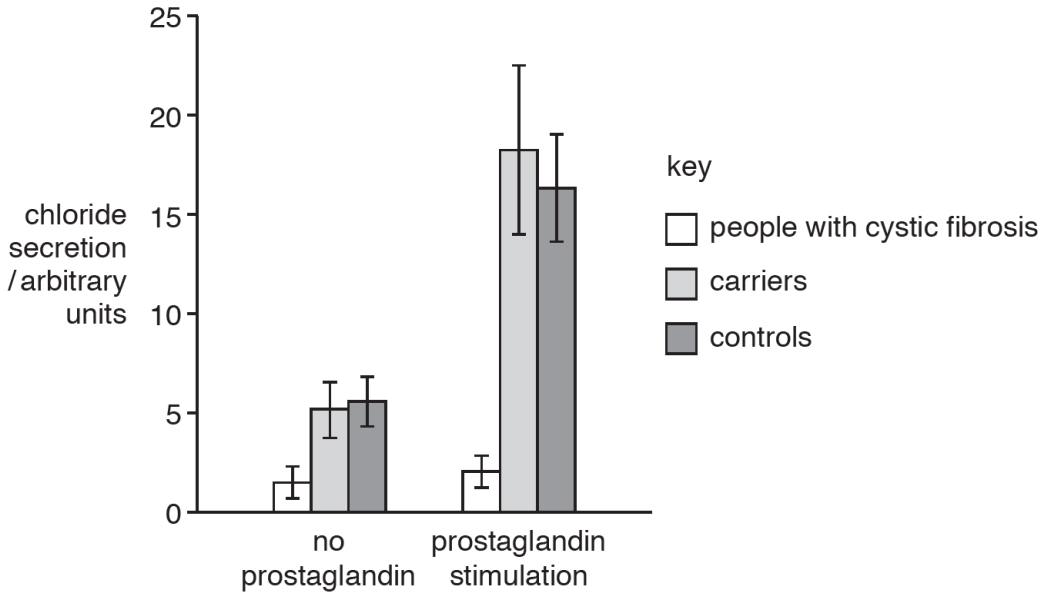



**Fig. 1.1**

**i)** Describe the results obtained in the study carried out in 2000. [3] **[DATA RESPONSE]**

1. prostaglandin **increased chloride secretion** in both **carriers** (**5 a.u. VS 18 a.u.**) and **controls** (**6 a.u. VS 16 a.u.**) **[Accept: ± 1 a.u.]**

2. **CF patients unaffected** (**2 a.u. VS 2.5 a.u.**)

3. ***Ref. to*** statistical significance e.g. in CF patients, error bars overlaps hence no difference

4. (significantly) more chloride secretion in carriers and controls than in CF patients (with and without prostaglandins)

***[Any 3]***

**ii)** Suggest why a chemical (prostaglandin) was used to increase water loss from epithelial cells in the 2000 study. [2] **[POS]**

1. ***Idea that*** To mimic the effect of cholera

2. ***Idea that*** dangerous / unethical to give cholera toxin to human subjects

3. ***Idea that*** prostaglandin concentration can be controlled

4. ***(Idea that*** *Unethical to conduct trials on cholera patients****)***

***[Any 2]***

**iii)** The studies carried out in 1994 and 2000 differ in various aspects, such as the results obtained, methodology and conclusions. One such difference is the lack of statistical data for 1994.

Comment on other differences between the studies carried out in 1994 and 2000. [3] **[DATA RESPONSE]**

| | **1994** | **2000** |
|---|---|---|
| 1. | measures **water loss** | measures **chloride secretion** |
| 2. | no data for homozygous recessive | homozygous recessive included |
| 3a. | **mice** were subjects | **humans** were subjects |
| 3b. | **[evaluate]** ***idea that*** may not be able to compare mice with humans, since they are different species | |
| 4a | **cholera toxin** used | **prostaglandins** used |
| 4b. | **[evaluate]** ***idea that*** prostaglandins may not act in same way as cholera toxin | |
| 5 | supports heterozygous advantage | shows no heterozygous advantage |
| 6. | Lack of a control group<br>(e.g. replace toxin with boiled toxin) | Control group included |

***[Any 3]***

**g)** In Europe and North America, the frequency of the Δ*F508* allele is relatively high, although there are now very few cases of cholera. In the past, cholera was very common throughout Europe.

Suggest how this may explain the present day frequency of the Δ*F508* allele in Europe. [2] **[EVOLUTION]**

1. In the past, Δ*F508* allele was selected for / heterozygotes were at selective advantage, hence its allele frequency increased

2. since it is a recessive allele, heterozygotes do not have CF, hence allele preserved

3. ***Idea that*** insufficient time since eradication of cholera to eliminate recessive allele

***[Any 2]***

**h)** In parts of Asia and Africa with hot climates, there are many cases of cholera but the frequency of the Δ*F508* allele is relatively low. Some scientists have therefore suggested that the distribution of the Δ*F508* allele is related to temperature, as well as the incidence of cholera.

Suggest an explanation for the relatively low occurrence of the Δ*F508* allele in hot climates. [2] **[EVOLUTION]**

1. ***Idea that*** thermoregulation through sweating needed in hot countries for survival

2. heterozygotes less able to sweat ***(deduced from the very first preamble)***

3. heterozygotes at a selective **dis**advantage / ***ref. to*** over-heating

***[Any 2]***

**[Total: 32]**

**QUESTION 2**

An investigation into a treatment for people who are infected with HIV was carried out involving a large number of people from over 70 countries. In September 1994, the patients began receiving a treatment called HAART (highly active antiretroviral therapy). They were monitored over the following years for helper-T cell counts and clinical outcomes. Data were analyzed within six-month periods.

Fig. 2.1, on the next page, shows the results of this investigation. The combined AIDS and death rate per six-month period is a measure of the number of patients who developed an illness characteristic of AIDS or who died.

**a)** HIV infects helper-T cells. If the infection progresses, the number of helper-T cells decreases. This decrease makes people more susceptible to opportunistic infections.

**i)** Helper-T cells are usually dormant unless activated.

Outline how helper-T cells are activated **and** the roles they play in the immune system. [3] **[IMMUNITY]**

**[How helper-T cells are activated – at least 1]**

1. T-cell antigen receptor binds to **antigen presented on class II MHC complex** on the surface of **antigen-presenting cells**

2. APC secretes **cytokine** to **activate** helper T cell (which triggers helper T cells to secrete cytokine leading to its own proliferation)

**[Roles of helper-T cells – at least 1]**

3. (Secretes other cytokines to) stimulates (proliferation and) **differentiation** of **B cells** into **plasma cells** and **memory B-cells**

4. (Secretes other cytokines to) activate **cytotoxic T cells** to kill (virally) **infected cells**

**ii)** Suggest what is meant by *opportunistic infections*. [1] **[IMMUNITY]**

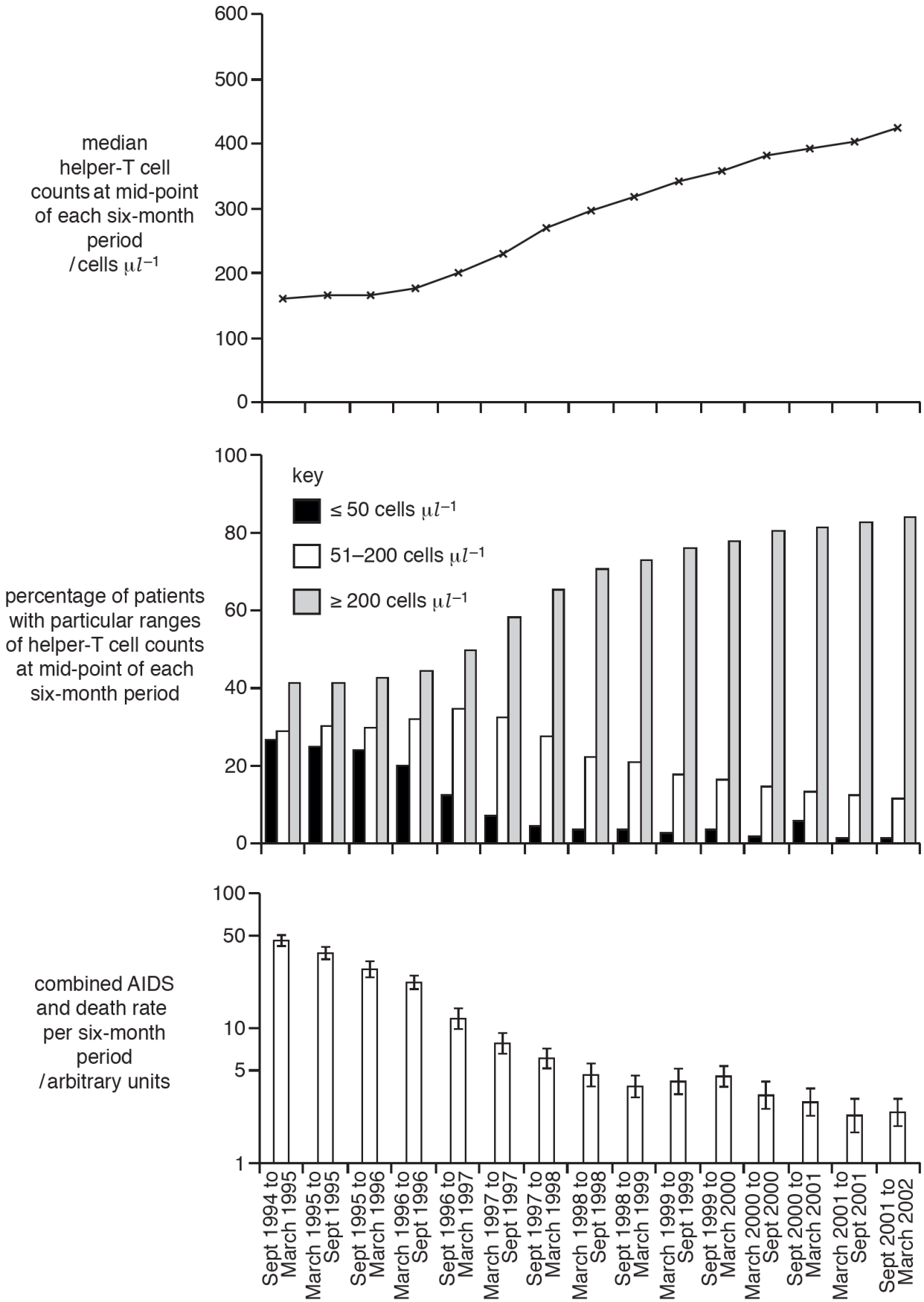
- ***Idea of*** infections that occur more often or are more severe in people with weakened immune systems than in people with healthy immune systems

**b)** Explain why HIV-infected individuals are usually also put on oral antibiotic treatment, despite the fact that antibiotics have no effect on viruses. [1] **[IMMUNITY]**

1. To eradicate/kill **bacteria** that cause the opportunistic infections in HIV-infected individuals.

2. To **prevent** opportunistic infections caused by **bacteria**

***[Any 1]***


median helper-T cell counts at mid-point of each six-month period /cells μl⁻¹
600
500
400
300
200
100
0
percentage of patients with particular ranges of helper-T cell counts at mid-point of each six-month period
100
80
60
40
20
0
key
≤ 50 cells μl⁻¹
51–200 cells μl⁻¹
≥ 200 cells μl⁻¹
combined AIDS and death rate per six-month period /arbitrary units
100
50
10
5
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Sept 1994 to March 1995
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March 1997 to Sept 1997
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March 1999 to Sept 1999
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March 2000 to Sept 2000
Sept 2000 to March 2001
March 2001 to Sept 2001
Sept 2001 to March 2002

**Fig. 2.1**

**c)** Suggest what might be concluded from the data in Fig. 2.1 about HAART as a means of controlling the consequences of HIV infection **and** comment on the validity and limitations of the data. [5] **[DATA RESPONSE]**

**[Conclusion about HAART as a mean – at least 1]**

1. **[Graph 1]** Dec1994 to Dec2002, median cell count increases from 160 to 420 cells $\mu l^{-1}$
2. **[Graph 2]** Patients with **≤50 helper-T cells $\mu l^{-1}$** **decreased** from **27% to 2%**
3. **[Graph 2]** Patients with **≥200 helper-T cells $\mu l^{-1}$** **increased** from **41% to 83%**
4. **[Graph 3]** Dec1994 to Dec2002, **combined AIDS & death rate** decrease from **49 to 3 a.u**.
5. **Median cell count** trend **continue to increase**, while **T-cell count** and **combined AIDS & death rate stabilized** towards the end of investigation
6. **[Conclusion]** ***Idea of*** Promising results which will alleviate the susceptibility to secondary infections / cancers *(that are consequences of HIV infection)*

**[Validity of the data – at least 1]**

7. Data is from a **large sample from 70 countries** therefore adds to validity of conclusions
8. Graph 3: the use of the **error bars** allows for **statistical comparison**
9. e.g. Mar97-Sep97 higher than Sep97-Mar98, but not statistically significant since error bars overlap

**[Limitations of the data – at least 1]**

10. **Lack of control group** (patients **not receiving HAART**) for comparison
11. No information about normal range of helper-T cells
12. The 3rd graph does not distinguish between AIDS and deaths
13. Dosage of drug not stated

**d)** Nucleotide Analogue Reverse Transcriptase Inhibitor (NtARTi) drugs are used to treat HIV infection. These molecules resemble the four deoxyribonucleotides used to make DNA. However, the 3' carbon of the sugar in NtARTi is linked to two hydrogen atoms.

State the role of reverse transcriptase in HIV **and** explain how NtARTi drugs interfere with the role of reverse transcriptase. [5] **[ENZYMES]**

**[Role of RTase – at least 1]**

1. Uses the **HIV (+)RNA** as **template** to synthesize (complementary) **DNA**

**[How NtARTi drugs interfere with RTase – at least 1]**

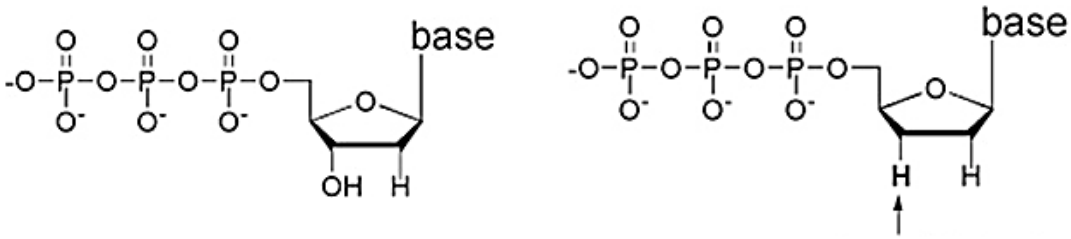
2. act as **competitive** inhibitors of RTase

3. **complementary in shape** and binds to **active site** of RTase

4. once added to the growing polynucleotide chain, the next nucleotide cannot be joined…

5. …due to the **lack of hydroxyl**/–OH group on the **3' carbon**

6. Hence, **phosphodiester bond** cannot form (hence cDNA strand cannot be formed)



**e)** A technique called RNA interference has also been shown to interfere with the replication of HIV. Double-stranded RNA molecules, 21 to 23 nucleotides long, were added to a culture of helper-T cells infected with HIV. The sequence of this small interfering RNA (siRNA) matches part of the HIV protease gene. Once inside the helper-T cell, the two strands of the siRNA separate into single strands. One of the strands is identical in sequence to part of the mRNA of the HIV protease.

Using the above information, explain how this siRNA is able to interfere with HIV replication. [3]
**[VIRUS]**

1. The **other strand** of the siRNA is **complementary** to and **binds** to the **mRNA** of HIV protease

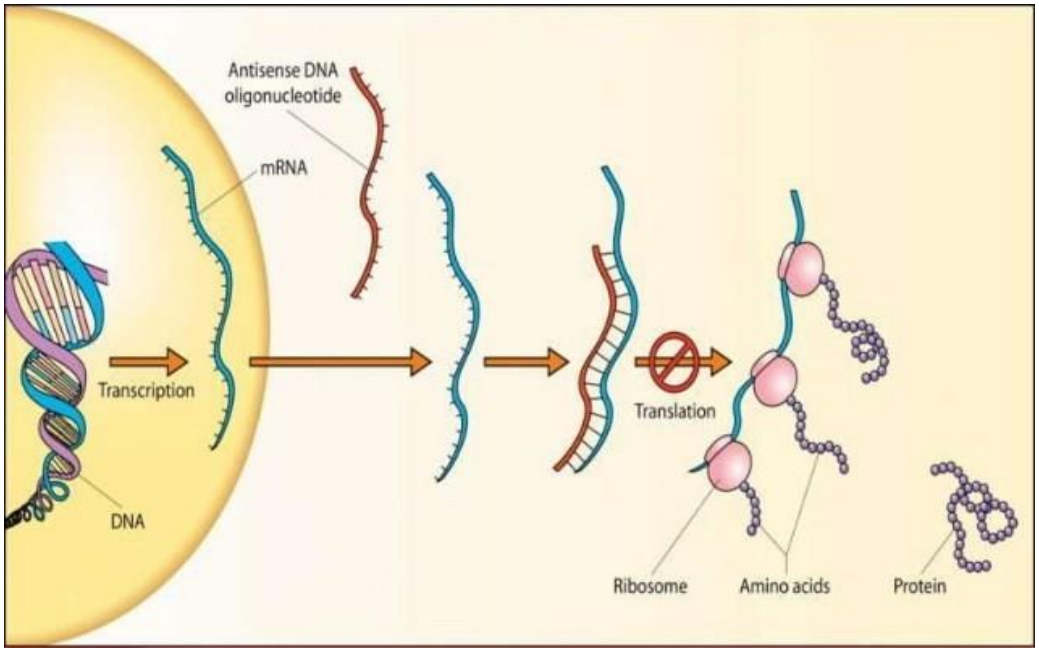
2. **Interferes with translation** of the **HIV protease mRNA** into the enzyme protease…
**OR**
**Blocks the attachment / translocation of ribosome** along the **protease mRNA**

3. …hence **HIV protease** is **not produced and packaged** into new HIV particles.
[Existing protease in the HIV particle is still able to cleave the polyprotein]

4. (In the subsequent infection) without HIV protease, HIV **polyprotein** is **not cleaved** into the functional HIV polypeptides

5. Interferes with **assembly** of new HIV particles.

**[Any 3]**




**[Total: 18]**

**QUESTION 3**

**a)** The Central Dogma of Molecular Biology depicts the unidirectional flow of genetic information to synthesize polypeptides, using DNA as the blueprint.

Outline the processes governed by the Central Dogma of Molecular Biology **and** explain, using specific examples, why the Central Dogma may not always hold true. [13]

**[Transcription] – at least 1**

1. **General transcription factors** and **RNA polymerase** bind to the **promoter**
2. **One** of the DNA strands is used as the **template** to synthesize **mRNA**
3. **Template read** from **3' to 5'**
4. **mRNA synthesized** from **5' to 3'**
5. RNA polymerase add ribonucleotides by **complementary base pairing** via **hydrogen bonds**
6. Catalyzes formation of **phosphodiester bonds** between adjacent ribonucleotides.
7. ***Ref. to*** transcription termination – RNA polymerase dissociates after transcribing past the termination sequence

**[Post-transcriptional modification]**

8. **RNA splicing** – introns excised, exons spliced
9. addition of **7-methylguanosine cap** to **5'** mRNA end
10. addition of **poly(A) tail** to **3'** mRNA end

**[Translation] – at least 1**

11. **Mature mRNA** used as a **template** to synthesize **polypeptide**
12. Small ribosomal subunit binds to the 5' end of mRNA and scans for the start codon (AUG)
13. mRNA read from **5' to 3'**, three bases (**codon**) at a time
14. **Peptidyl transferase** in ribosome catalyzes **peptide bond** between adjacent amino acids
15. **Each codon** codes for **one amino acid**
16. When **stop codon** (UAA/UGA/UAG) is read, **release factor** binds to the A site of large ribosomal subunit
17. **Water** is used to hydrolyze the bond between tRNA and the polypeptide chain

**[Why Central Dogma is not always true] – at least 2**

18. ***Idea that*** Flow may be reverse, from RNA to DNA **+**
    a. ***Ref. to*** HIV: (+)ssRNA reverse transcribed to ssDNA
    b. ***Ref. to*** telomerase: telomerase RNA as the template for reverse transcribed to DNA (telomere)
19. ***Idea that*** Flow start from RNA **+**
    a. ***Ref. to*** HIV, (+)ssRNA is directly used as mRNA

20. ***Idea that*** Flow may be from **RNA to RNA** **+**
    a. ***Ref. to*** influenza: (-)ssRNA as a template to synthesize (+)ssRNA

21. ***Idea that*** Flow may end at RNA **+**
    a. ***Ref. to*** tRNA / rRNA / snRNA / telomerase RNA

**[Any 13]**

**b)** Inorganic ions have an electrical charge, owing to the loss or gain of electrons. Despite playing a diverse role in cells, from regulating a variety of processes to being part of cellular structures, some inorganic ions are toxic to cells.

Discuss the important roles played by various inorganic ions **and** suggest why some inorganic ions are toxic to cells. [12]

**[Important roles] – at least TWO different ions**

1. Phosphate/$PO_4^{3-}$ **[1m for each sub-point]**
    a. ATP formation
    b. part of phospholipid, which forms membranes
    c. activating enzymes through phosphorylation
    d. forms part of nucleic acid

2. Proton/$H^+$ **[1m for each sub-point]**
    a. proton gradient across membranes (of mitochondria and chloroplast) as an energy store
    b. diffusion of protons through ATP synthase allows phosphorylation of ADP to ATP
    c. maintains acidic pH in lysosomes
    d. combines with NAD/FAD/NADP

3. Calcium/$Ca^{2+}$ **[1m for each sub-point]**
    a. **second messenger** in transduction pathway
    b. stores in high concentration in ER
    c. an increase in cytosolic $Ca^{2+}$ activates protein kinase (C)
    d. **AVP:** role in muscle contraction, blood clotting, etc.

4. Iron/$Fe^{2+}$ **[1m for each sub-point]**
    a. forms part of **haemoglobin** / **myoglobin**
    b. **binds** directly to an **oxygen molecule**

5. Zinc/$Zn^{2+}$ **or** magnesium/$Mg^{2+}$ **[1m for each sub-point]**
    a. cofactors in enzyme activity
    b. helps to mould the shape of active site / directly participate in catalysis

6. Magnesium/$Mg^{2+}$ **[1m for each sub-point]**
    a. forms part of special chlorophyll a
    b. essential for electron excitation and emission

7. **AVP:** Sodium/$Na^+$ – co-transport of glucose into cells (via symporter).
8. **AVP:** Chloride/$Cl^-$ – moves out of cells to provide a low water potential outside cell
9. **AVP:** Sodium/$Na^+$ and potassium/$K^+$ – role in nervous impulse transmission
10. **AVP:** Nitrate/$NO_3^-$ / ammonium/$NH_4^+$ – role in amino acids synthesis
11. **AVP:** Copper/$Cu^{2+}$ - part of a protein involved in electron transport chain

**[Why toxic to cells] – at least 1**

12. Heavy metals such as mercury/lead/silver – (non-competitive) **inhibitors** of enzymes
13. Binds to and alters the 3D conformation of enzymes
14. The altered shape of **active site** no longer **complementary in shape** to substrates
15. ***Idea that*** Interferes with metabolic processes leading to toxicity
16. **Named metabolic pathways**: e.g. glycolysis / Krebs cycle / Link reaction / Oxidative phosphorylation / DNA replication / transcription / translation / **AVP**

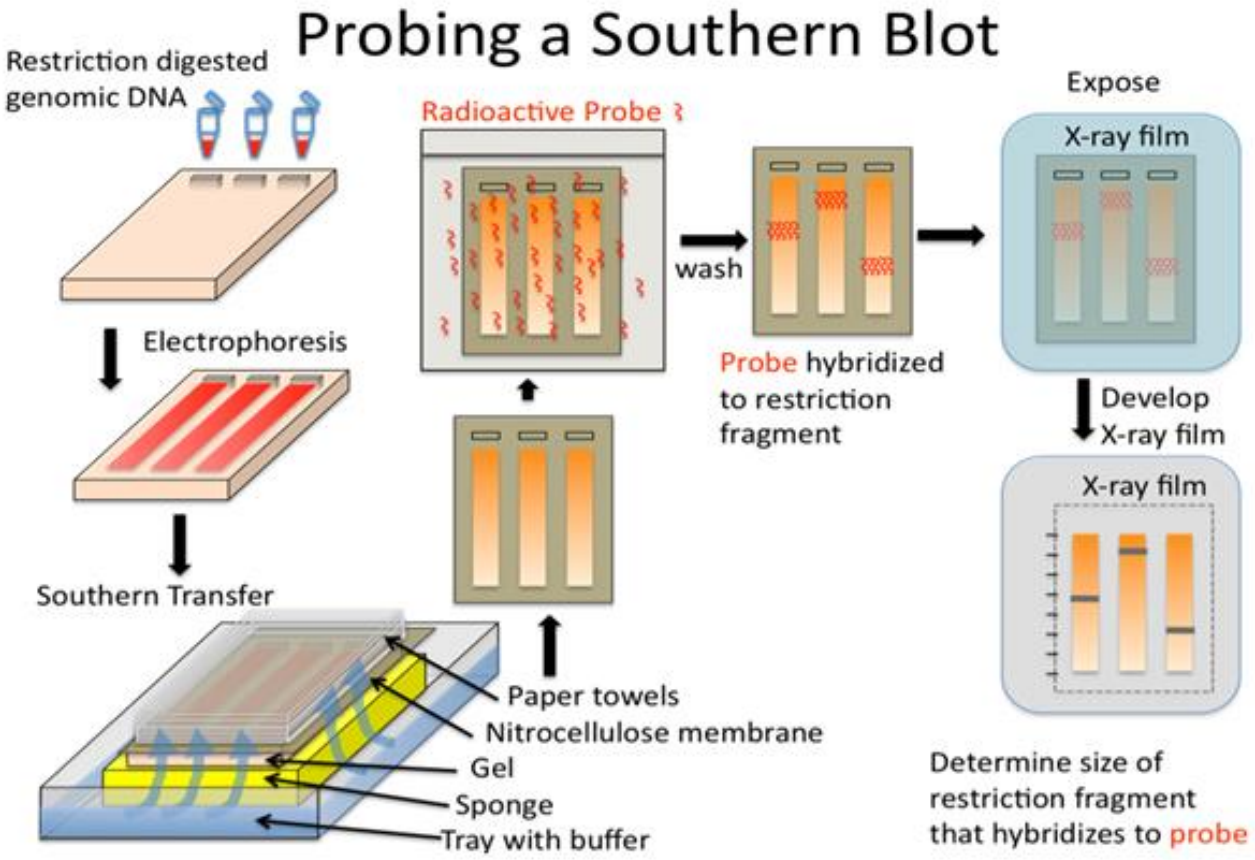
**[Any 12]**

**QUESTION 4**

**a)** Restriction endonucleases are enzymes that cut at specific nucleotide sequences within a DNA molecule. Total DNA isolated from human cells, when digested by restriction endonucleases, produces tens of thousands of DNA fragments of varying length.

Describe how the presence of insulin gene from a human cell can be detected after restriction digestion **and** explain the theoretical basis behind the procedures. [13]

| Description | Theoretical basis |
|---|---|
| ***Gel electrophoresis*** | ***Gel electrophoresis*** |
| **1.** Agarose gel electrophoresis to separate restriction fragments based on molecular size. | **A.** Gel matrix acts as a molecular sieve to separate DNA fragments according to their molecular sizes / larger fragment migrate slower / **reverse argument** |
| **2.** DNA fragments mixed with loading dye comprising of bromophenol blue and glycerol | **B.** ***Ref to.*** role of bromophenol blue – visualize loading and migration |
| | **C.** ***Ref to.*** role of glycerol – added density to allow DNA to sink into well |
| **3.** Mixture loaded into well at one end of the gel nearer to cathode and electricity applied so that DNA migrate towards anode | **D.** ***Ref to.*** role of buffer – conduct electricity |
| | **E.** DNA is negatively charged due to the phosphate group and hence migrate towards anode |
| ***S.B. and N.A.H.*** | ***S.B. and N.A.H.*** |
| **4.** Southern blotting – fragments in gel transferred to nitrocellulose membrane | **F.** ***Ref to.*** Capillary action that pulls DNA fragments out of the gel onto the nitrocellulose membrane. |
| **5.** Addition of sodium hydroxide / NaOH | **G.** NaOH breaks hydrogen bonds between complementary base pairs to denature dsDNA. |
| **6.** Radioactive single-stranded (DNA/RNA) probes binds to (part of) the insulin gene. | **H.** Probe complementary base pairs with part of insulin gene to ensure annealing. |
| **7.** Probe is at least 20 nucleotide long / sufficient length. | **I.** Probe must be long enough to ensure specificity. |
| **8.** Wash membrane to remove unbound probes | **J.** ***Idea of*** Washing step to minimize unbound probe being detected on X-ray film |
| **9.** ***Ref to.*** autoradiography + X-ray film to detect bound probe | **K.** Radioactivity turns X-ray film black which allows revelation of DNA band which probe is bound |
| **Accept:** fluorescent probe + UV light | |


Probing a Southern Blot
Restriction digested genomic DNA
Electrophoresis
Southern Transfer
Paper towels
Nitrocellulose membrane
Gel
Sponge
Tray with buffer
Radioactive Probe
wash
Probe hybridized to restriction fragment
Expose
X-ray film
Develop X-ray film
X-ray film
Determine size of restriction fragment that hybridizes to probe

**b)** Lactose catabolism and tryptophan synthesis are regulated in similar manner in bacterial cells.

Describe how the metabolism of lactose in bacterial cells is regulated **and** explain the advantages of such a regulation system in the context of tryptophan synthesis. [12]

**[How lactose metabolism is regulated] – at least 1**

[Lac operon]
1. ***Lac* operon**

2. *LacZYA* under the control of **one promoter** sequence

3. These genes code for **enzymes/proteins** that **break down / hydrolyze lactose**

[*LacI* regulatory gene]
4. ***LacI*** (regulatory) **gene** codes for an **active repressor** protein

5. Active repressor is **complementary in shape** and **binds** to **operator** sequence

[When lactose is present, glucose absent]
6. Lactose is an **inducer**

7. **Binds** to and changes the **conformation** of active repressor, thereby **inactivating** it

8. Inactive repressor not **complementary in shape** **[mark once]** to operator, thus **dissociates**.

9. Allows binding of **RNA polymerase** to **promoter**

10. **ATP** converted to **cAMP** by **adenyl cyclase**

11. cAMP binds to CRP protein to form **cAMP-CRP complex**

12. cAMP-CRP complex binds to **CRP-binding site** **upstream of promoter**

13. **Increases expression** of Lac operon

[When glucose is present]
14. Glucose **inhibits adenyl cyclase**, thus cAMP not formed.

15. Lack of cAMP-CRP complex, thus *Lac* expression at **basal level**.

**[Advantages in the context of tryptophan synthesis] – at least 1**

16. **Genes** that code for **enzymes that synthesize tryptophan** are **regulated together**.

17. Organizing their genome into operons can allow them to **respond quickly** to the **presence** and **absence** of **tryptophan**.

18. Operons ensure that the cell **does not waste energy** synthesizing these enzymes **when tryptophan is present** / **reverse argument**

**☺ End of Paper 3 ☺**