Group	Number	Name (use BLOCK LETTERS)	H2
	 Z	ST. ANDREW'S JUNIOR COLLEGE 2017 JC2 BT2	
H2 BIOL	_OGY		9744/1
Paper	1: Multi	ple Choice Mark Scheme	
Tuesday	/	19 th September 2017	1 hour
Addition	al Materials:	Multiple Choice Answer Sheet	

READ THESE INSTRUCTIONS FIRST

Do not open this booklet until you are told to do so.

Write your name, civics group and index number on the multiple choice answer sheet in the spaces provided.

Soft clean eraser (not supplied)

Soft pencil (type B or HB is recommended)

There are **30** questions in this paper. Answer all questions. For each question, there are four possible answers, A, B, C and D.

Choose the one you consider correct and record your choice in soft pencil on the separate multiple choice answer sheet.

INFORMATION TO CANDIDATES

Each correct answer will score one mark. A mark will not be deducted for wrong answer. Any rough working should be done in this booklet.

At the end of the examination, submit <u>both</u> question paper and multiple choice answer sheet.

This document consists of **16** printed pages.

[Turn over

1 The figure below shows an electron micrograph of an eukaryotic cell.



Which of the following option correctly matches the structures \mathbf{R} , \mathbf{S} and \mathbf{T} to their respective functions?

	R	S	т
Α	Involved in proteins glycosylation	Site of lipid synthesis	To convert light energy to chemical energy
В	Site of protein synthesis	Site of detoxification reaction	Supplying cellular energy
С	Site of detoxification reaction	Involved in protein glycosylation	Remove worn out organelles
D	Site of protein synthesis	Contains proteins to be secreted	Storage of starch

- 2 Which comparative statement(s) concerning biological molecules is/are correct?
 - 1 A collagen molecule is a fibrous protein that contains many amino acids with hydrophobic R-groups whereas a haemoglobin molecule is a globular protein with no amino acids with hydrophobic R-groups.
 - 2 Sucrose hydrolysis results in glycosidic bond breakage and the production of equal proportions of fructose and α-glucose molecules, whereas cellulose hydrolysis results in only β-glucose molecules.
 - 3 The glycosidic bonds of glycogen are formed between two α -glucose molecules, whereas in amylopectin, the bonds are formed between an α -glucose molecule and a β -glucose molecule.
 - A 2 only
 - **B** 3 only
 - **C** 1 and 2
 - **D** 1 and 3
- **3** The statements below are about bonds found in biological molecules.
 - 1 They are formed by condensation.
 - 2 Oxygen is part of the bond.
 - 3 ATP is hydrolysed to form the bonds.
 - 4 They can be broken at 100°C.

Which statements are correct for the bonds in the primary structure of proteins?

- A 1 and 2 only
- **B** 1 and 3 only
- **C** 3 and 4 only
- **D** 1, 2 and 4 only
- 4 What supports the view that a membrane protein is involved in active transport?
 - A It allows movement of molecules across a membrane if concentration differences exist.
 - **B** It can only function if mitochondria are supplied with sufficient oxygen.
 - **C** It has a tertiary structure with a binding site with a specific shape.
 - **D** It is found in the cell surface membranes and the mitochondrial membranes.

5 The graph shows the course of an enzyme-catalysed reaction at 30 °C.



What is true at time X?

- A Most enzyme molecules will have free active sites.
- **B** The number of available substrate molecules is high.
- **C** The number of enzyme-substrate complexes is low.
- **D** The rate remains the same if more enzyme is added.

6 The diagram shows two homologous chromosomes in early prophase I of meiosis in an animal cell. Two genes, **A**/**a** and **B**/**b**, whose loci occur on the homologous chromosomes are also shown.



Which row of diagrams is a possible representation of these chromosomes as they progress from anaphase I to prophase II? **ANS: D**



7 The diagram shows the molecular structure of a chemical that can inhibit the activity of reverse transcriptase. It is an analogue of a naturally occurring nucleic acid monomer.



Which option is correct?

	Analogue	Naturally occurring monomer
Α	Acts as a competitive inhibitor	Is an activated DNA nucleotide
В	Acts as a non-competitive inhibitor	Is an activated RNA nucleotide
С	Acts as a competitive inhibitor	Is an activated RNA nucleotide
D	Acts as a non-competitive inhibitor	Is an activated DNA nucleotide

8 In dogs, a gene on chromosome 27 is responsible for the curliness of the dog's hair. One form of this gene produces an enzyme with arginine at residues 151, but a mutant allele of the gene produces an enzyme which has cysteine at this point.

This latter form causes kinks in the keratin so that the coat is curlier. In heterozygotes, both alleles are co-dominant so an intermediate 'wavy' coat can be observed in the phenotype.

In this context, what is meant by gene mutation?

- A change in the gene locus
- B chromosome 27 inversion
- **C** production of a new protein
- **D** structural change in DNA

9 Individuals with the rare Bombay phenotype (i.e. homozygous recessive, hh) do not produce H-antigen. As a result, they cannot make A-antigen or B-antigen on their red blood cells, regardless of what alleles they may have at the gene locus which determines the ABO blood group. This is because A-antigens and B-antigens are synthesised from H-antigens. Individuals with blood group O do not produce A-antigens or B-antigens but produce H-antigens on their red blood cells.

Family member	Blood type
Alan's father	0
Alan's mother	Bombay phenotype
Alan	A
Alan's wife	Bombay phenotype
Alan's wife's father	0
Alan's wife's mother	0

Alan and his family had the following phenotypes in their blood groups:

If Alan and his wife had a child, what is the probability that the child will have Bombay phenotype?

- **A** 0
- **B** ½
- **C** 1⁄4
- **D** ³⁄₄

10 Seven plants of the same species were selected from a field. Three plant cuttings were obtained from each parent plant, each of which were planted at various elevations on a mountain terrain. They were allowed to grow for 3 months. Their heights and flower development were shown in the table below.



Which of the following statements can be deduced from the results of the experiment?

- **A** The height of the plants is genetically determined.
- **B** The development of flowers is affected by environmental factors.
- **C** Genetic variation exists in the population of plants in the field due to genetic drift.
- **D** Plants that did not develop flowers at medium elevation have mutations that inhibit flower development.

- **11** Which of the following is not the consequence of natural selection?
- A The field mustard plant survived a summer drought in southern California because some individuals contained alleles that made them flower earlier. Plants with flowers wilt more easily than plants without flowers. Now, almost all the field mustard plants in California flower in spring.
- **B** In areas with fewer predators of herbivorous insects, plants which produce higher concentrations of alkaloids (which are toxic to insects) dominated the landscape. Most of the herbivorous insects in these areas are found to be able to accumulate alkaloids in their bodies without affecting their metabolism.
- **C** Endemic to New Zealand, the kakapo (a large flightless bird) had no natural predators before the humans arrived. They have evolved to have very few offspring throughout their entire lifespan. This phenomenon is also common for other island species which do not have natural predators in their respective habitats.
- **D** Maple probably has the most variation in bark of any tree species. Japan experiences tornadoes which destroy large trees like the maple. Over the last few decades, it was observed that only the Japanese maple with dark-coloured bark remained.
- 12 Neanderthals evolved from *Homo erectus* via the intermediate *H. heidelbergensis*; this *H. erectus* lineage left Africa about 600kya (kya = 1000 years ago), the descendants made their way to Europe and the earliest *H. heidelbergensis* fossils appeared 500kya. The *H. erectus* line continues in Europe after the *H. heidelbergensis* speciation, with the later fossils being found in France (450kya), Hungary (350kya), Indonesia (250kya) and China (210kya).

In contrast, *H. sapiens* evolved in east Africa from *H. erectus* through the intermediate form of *H. rhodesiensis*. *H. rhodesiensis* fossils range from 600–200kya.

Based solely on the information given above, which of the following statements is least likely to be true?

- A *H. erectus and H. rhodesiensis* are unable to interbreed to produce viable and fertile offspring.
- **B** Geographical isolation resulted in the disruption of gene flow between Neanderthals and *H. sapie*ns.
- **C** The divergence of *H. rhodesiensis* from *H. erectus*, and *H. sapiens* from *H. rhodesiensis* is sympatric speciation.
- **D** The fossils of *H. erectus* found in France share more similarities with those found in China than with the earliest fossils of *H. heidelbergensis*.

13 The plica semilunaris is a small fold of tissue on the inside corner of the eye. It is the vestigial remnant of the nictitating membrane, an organ that is fully functional in some other species of mammals. For example, in diving animals like beavers and manatees, the nictitating membrane is transparent and moves across the eye to protect it while under water.



Which of the following statements least explains the presence and structure of plica semilunaris in humans?

- **A** Early ancestors of humans were not divers.
- **B** Any presence of nictitating membrane in non-diving mammals posed a selective disadvantage for individuals who had it.
- **C** Mutations occurred to reduce the size of nictitating membrane in humans to its present-day vestigial structure as there was no use for it.
- **D** The genes involved in producing the plica semilunaris were inherited from a common ancestor shared by humans, beavers and manatees.

Explanation:

Early ancestors of humans were not divers. That's why our nictitating membrane is now vestigial, rather than a fully functional one.

The fact that we inherited it despite not being divers explains why we still have this vestigial remnant, instead of not having it at all.

14 Which of the following correctly shows the effects of climate change on coral reefs and associated ecosystems?

	Average number of zooxanthellae in each polyp	Mass of basal plate of hard corals	Diversity of catch from nearby fisheries
Α	Decreased	Decreased	Decreased
В	Decreased	Unaffected	Increased
С	Increased	Decreased	Decreased
D	Increased	Unaffected	Increased

15 Which of the following is true of the pathogenicity of the dengue virus?

- A Infected cells release interferons which cause dengue fever.
- **B** Infected cells release toxins which kill cytotoxic T cells and red blood cells.
- **C** Dengue virus infects and kills macrophages, causing dengue shock syndrome.
- **D** Antibodies specifically recognise and neutralise the dengue viruses circulating in the circulatory and lymphatic systems.

16 The enzyme DNase is added to samples taken from the same chromosomal region from three different cell types. After 20 minutes, the remaining DNA samples are weighed and the following results are obtained.

Cell	Mass of DNA before adding	Mass of DNA after adding
type	DNase/ µg	DNase/ µg
Р	5.0	5.0
Q	5.0	3.4
R	5.0	4.1

Which of the following statements correctly suggests about the genes and their expressions in the three different cell types?

- A Some regions of the cell type **P** chromosomes contain DNA coding for rRNA.
- **B** The mass of DNA in cell type **P** did not decrease because the histone tails are acetylated.
- **C** There are more phosphodiester bonds in the DNA of cell type **Q** than those in cell types **P** and **R**.
- **D** The genes found in the same chromosomal region in the three cell types are expressed the most in cell type Q.

- **17** The following statements are about eukaryotic control elements:
 - 1 Attachment of RNA polymerase II at the TATA box is achieved with the help of a series of specific transcription factors.
 - 2 The DNA binding site on general transcription factors and specific transcription factors is different in DNA sequence.
 - 3 When the histones found in part of a chromosome are acetylated, the control elements of a gene are easily accessed.
 - 4 Repressors bind to regions of DNA to repress transcription.

Which of the above statement(s) is/are true?

- A 3 only
- **B** 1 and 2
- **C** 3 and 4
- **D** 2, 3 and 4

18 Transcription in eukaryotic cells results in the formation of pre-mRNA, which is made up of exons and introns.

Which of the following statements correctly describes what happens during the formation of mature mRNA from the pre-mRNA?

- **A** The 5' of the intron is cut, and joined to the branch-point sequence, followed by the cutting of the 3' end to form the lariat loop.
- **B** RNA splicing occurs, where all introns are recognised as they share highly similar sequences and are excised.
- **C** RNA splicing occurs, where all the introns are excised and some of the exons joined together so that they can be transcribed.
- **D** The addition of the 5' cap and the 3' poly-A tail occurs, followed by RNA splicing.
- **19** Regulation of gene expression occurs in both prokaryotes and eukaryotes.

Which of the following process(es) is/are involved in both prokaryotes and eukaryotes?

- 1 extension of telomeres by telomerase
- 2 down-regulation by repressor molecules
- 3 post-translational modification
- 4 alternative splicing
- A 2 only
- **B** 1 and 3 only
- **C** 2 and 4 only
- **D** 2, 3 and 4 only

20 With reference to the diagram below, relate processes P, Q, R, S, T to statements (1), (2) and (3).



- (1) NAD is regenerated without the use of the electron transport system
- (2) ATP is synthesised via substrate level phosphorylation
- (3) It can take place under anaerobic conditions.

	(1)	(2)	(3)
Α	T only	R only	Q,R,T only
В	T only	R,S only	Q,R,T only
С	S,T only	R only	Q,R,S,T
D	S,T only	R,S only	Q,R,S,T

21 The diagram summarises the process of photosynthesis.





Which row identifies the reactants 1,2,3, 4 and 5?

	1	2	3	4	5
Α	Carbon dioxide	ADP + phosphate	reduced NAD	NAD	water
В	Carbon dioxide	reduced NAD	ADP + phosphate	NADP	water
С	water	NAD	reduced NAD	ADP + phosphate	Carbon dioxide
D	water	NADP	ADP + phosphate	reduced NADP	Carbon dioxide

22 Young maize and wheat plants were grown to maturity at high and low temperatures. The rate of photosynthesis in each of these mature plants was measured at different temperatures. The rate of photosynthesis was measured as the amount of CO₂ used per dm³ of leaf per hour. The results are shown in the graph below



What information can be concluded from the graph above?

- 1 For plants grown at high temperature, the rate of photosynthesis is optimum at 25°C in maize and 18°C in wheat.
- 2 For plants grown at high temperature, maize had a greater increase in rate of photosynthesis compared to wheat until optimum temperature was reached.
- 3 The rate of photosynthesis was affected more significantly in maize plants than in wheat plants when grown at low temperatures.
- 4 Low temperatures slowed down the formation of membranes in maize plants but not in wheat plants which caused a decrease in lamellae formation.
- A 2 and 3 only
- **B** 1 and 4 only
- **C** 1, 2 and 3 only
- D All of the above

23 Which of the following pairs of statements is **true** of transduction and conjugation?

	Transduction	Conjugation
^	Bacterial DNA is transferred from	Bacterial DNA is transferred from
	donor cell to recipient cell	donor cell to recipient cell
	Only host DNA adjacent to prophage	F plasmid is exchanged between
D	is transferred from donor cell to	donor cell and recipient cell
D	recipient cell in specialised	
	transduction	
C	Lambda lysogenic phage is involved	T4 lytic phage is involved
C	in generalised transduction	
	Viral DNA is replicated via rolling-	DNA on F plasmid is replicated via
D	circle mechanism in the donor cell	rolling-circle mechanism in the
		donor cell

24 All of the following statements about viruses are true **except**:

- A The genome of HIV is more likely to mutate than the genome of bacteriophages
- **B** Before entering a host cell, specific proteins of viruses bind to receptors on specific host cells.
- **C** All viruses produce RNA as an intermediate molecule during the production of new viruses.
- **D** HIV and influenza viruses produce DNA as an intermediate molecule during the production of new viruses.

25 Arrange the following statements on signal transduction pathway for insulin in order.

- 1 Auto-crossphosphorylation
- 2 Increase in uptake of glucose through facilitated diffusion
- 3 Relay proteins bind to specific activated tyrosine residues
- 4 Activated relay proteins activate their respective transduction pathways
- 5 Insulin binds to receptor tyrosine kinase (RTK) at the receptor site
- 6 Vesicles containing glucose transporters move to and fuse with the plasma membrane
- 7 Changes in the 3D conformation activates the tyrosine kinase domain of receptor
- **A** 5, 1, 7, 3, 4, 6, 2
- **B** 5, 7, 1, 3, 4, 6, 2
- **C** 2, 5, 1, 7, 3, 4, 6
- **D** 2, 5, 1, 7, 4, 3, 6

26 The polymerase chain reaction is summarised in the flowchart below.



Which statement completes the flow chart?

- **A** Complementary strands of DNA are separated.
- **B** Free nucleotides join on the end of DNA strands.
- **C** Small sections of DNA are formed.
- **D** Strands of DNA bind to RNA primers.

27 A gene involved in the development of cancer was studied using the technique of gel electrophoresis.



Which of the following can possibly be concluded from the results of this study?

- **A** Mutation of gene resulting in a hyperactive protein
- **B** Additional DNA nucleotides are inserted within the mutant allele
- **C** Amplification of gene related to cancer
- **D** Over-expression of the gene related to cancer

28 Some of the features of different types of stem cells are listed.

- 1 They are able to develop into all cell types of the body to form a whole organism
- 2 They can develop into a wide range of different types of cell
- 3 They have active telomerase enzyme
- 4 They can only develop into a limited range of cell types

Which of the following will be shown by embryonic stem cells?

- **A** 1 and 2
- **B** 1 and 3
- **C** 2 and 3
- **D** 3 and 4
- **29** Most cases of cervical cancer is caused by infection with Human Papilloma Virus (HPV). Vaccines consisting of HPV antigens are available for prevention of cervical cancer.

Which of the following statements is true?

- A The HPV vaccine offers passive artificial immunity.
- **B** The HPV vaccine offers active natural immunity.
- **C** HPV vaccine stimulates the production of specific antibodies by T cells
- **D** HPV vaccine will be ineffective against the influenza virus due to different 3D conformation from influenza viral antigens
- **30 1-5** represents different components of the immune system. Arrows may represent processes such as activation of other cells and differentiation of cells. Which of the following could be a possible representation of **1-5**?



	1	2	3	4	5
Α	Antigen-	T cytotoxic cell	Antibodies	B cell	Memory B cell
	Presenting Cell				
В	Antigen-	B cell	T helper cell	T cytotoxic cell	Memory T cell
	Presenting Cell				
С	Antigen-	T helper cell	B cell	Plasma cell	Antibodies
	Presenting Cell				
D	Antigen-	T helper cell	B cell	Plasma cell	Memory B cell
	Presenting Cell				

Civics Group	Index Number	Name (use BLOCK LETTERS)		H2
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	ST. ANDREW'S JUNIOR COLLEGE 2017 JC2 BT2			
H2 BIOLOGY		9744/2		
Paper 2				
Tuesday	12 th September 2017	2 hours		
READ THESE INSTRU	CTIONS FIRST			
Write your name, civics group and index number on all the work you hand in. Write in dark blue or black pen on both sides of the paper. You may use a soft pencil for any diagram, graph or rough working. Do not use staples, paper clips, highlighters, glue or correction fluid.				
Section A (Structured Answer all questions. Write your answers in th	Questions) he spaces provided on the question paper.			
The number of marks is question or part question	given in brackets [] at the end of each n.	For Examiners' Use Section A		

Conceptual error (CE)	Lack of Keywords (K)	Misreading the question (Q)

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This document consists of **24** printed pages.

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QUESTION 1

Hormones, insulin and glucagon, are proteins that regulate the concentration of blood glucose level. Type 2 diabetes is characterized both by insulin resistance, a condition in which various tissues in the body no longer respond properly to insulin action, and by subsequent progressive decline in beta (β)-cell function to the point that the cells can no longer produce enough additional insulin to overcome the insulin resistance. Researchers are actively exploring use of stem cells as a potential source of deriving new β -cells to treat type 2 diabetes.



Fig. 1

The pancreas is located in the abdomen, adjacent to the duodenum (the first portion of the small intestine). A cross section of the pancreas shows the islet of Langerhans which is the functional unit of the endocrine pancreas. Encircled is the beta cell that synthesizes and secretes insulin. Beta cells are located adjacent to blood vessels and can easily respond to changes in blood glucose concentration by adjusting insulin production.

- (a) Cells that secrete proteins contain a lot of rough endoplasmic reticulum (rER) and a large Golgi body.
 - (i) Describe how the rER is involved in the production of insulin.

.....[1]

(ii) Describe how the Golgi body is involved in the secretion of insulin.

.....[2]

(b) Using type II diabetes as an example, explain how environment affects phenotype.

(c) With reference to Fig. 1, explain how the binding of insulin to receptors on muscle cells leads to a lowering of blood glucose concentration.

.....[2]

(d) Suggest how a change in the amino acid sequence of the receptor found in the plasma membrane of the muscle cell could make the cell resistant to insulin.

(e) Describe how phospholipids are arranged in a plasma membrane.

(f) Phospholipids are a type of lipid. Lipids, in general, are made up of glycerol and fatty acids monomers covalently bonded together. Name the covalent bond and describe the breakage of this bond.

Experiments have indicated that pancreatic stem cells (PSCs) can serve as sources of insulin secreting cells.

(g) State the source of PSCs and explain the PSCs' normal functions.

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(h) Suggest an advantage of using the patient's own PSCs to regenerate tissue or organs.

.....[1]

[Q1: 18 marks]

QUESTION 2

Stephen Lyon Crohn, also known as "The man who can't catch AIDS", was a man notable for a genetic mutation, which caused his inability to contract AIDS.

Crohn had the "delta-32" mutation on the CCR5 co-receptor, a protein on the surface of cells involved in the immune system.

CCR5 "delta-32" mutation involves a 32-base-pair deletion in the CCR5 co-receptor gene locus.

(a) Describe the role of CCR5 co-receptor protein in the entry of Human HIV into host cells.

(b) Describe briefly how the information on DNA is used to synthesize the pre-mRNA transcript for CCR5 co-receptor.

 Individuals can either gain full or partial resistance to HIV, depending on the number of mutated CCR5 alleles they possess at the CCR5 gene locus. Partial resistance to HIV occurs when the entry of the virus is severely hampered (but not completely prohibited).

Another Caucasian woman named Susan, is homozygous with the CCR5 "delta-32" mutation. Her husband has the same mutation on **one** CCR5 allele. The couple has a son and a daughter. Both son and daughter has 50% chance of having partial resistance and 50% chance of having full resistance.

(c) Explain if the inheritance of the CCR5 alleles is autosomal or X-linked.

.....[2]

(d) Suggest why some individuals have partial resistance to HIV.

.....[2]

The son has blood group O while his wife is heterozygous for blood group A.

(e) With the help of a genetic diagram, show all the possible outcomes for their offspring. [5]

QUESTION 3

Epidermal growth factor (EGF) is released by cells, and is picked up either by the cell itself or by neighbouring cells. It regulates the production of a number of proteins in target cells. Protein produced and its effect depends on the type of target cell.

Fig. 3 shows how EGF regulates 3 genes.



.....[1]

(b) The *c-Fos* gene can be a proto-oncogene.

Use the information in Fig. 3 to explain how a mutation of the c-Fos gene can result in the formation of a tumour.

(c) Gene B has been associated with a significant number of human cancers. Scientists used polymerase chain reaction (PCR) to make multiple copies of gene B extracted from a patient's cancer tissue sample.

The reaction mixture includes the sample of DNA to be copied plus the following ingredients:

- DNA primers
- buffer solution
- heat-stable DNA polymerase (Taq polymerase)
- deoxyribonucleoside triphosphates (deoxyATP, deoxyTTP, deoxyCTP and deoxyGTP)
- (i) Suggest why a buffer needs to be present in the reaction mixture.

.....[1]

(ii) The deoxyribonucleoside triphosphates that are added to the reaction mixture are the monomers used for making the new DNA strands.

Suggest **one further** reason for adding the deoxyribonucleoside triphosphates to the reaction mixture.

.....[1]

(iii) In the first stage of PCR, the mixture is heated to a temperature of around 90°C to denature the DNA. Suggest why high temperatures are needed to separate the two DNA strands.

(iv) At the end of several cycles of PCR, many copies of the DNA sample in the reaction mixture will have been made. The DNA samples are then separated out to produce a DNA banding pattern.

State the technique used to separate out the DNA samples **and** describe how this technique works.

[Q3: 12 marks]

QUESTION 4

Researchers have identified a gene that gives bacteria resistance to a type of antibiotics called polymyxins. Despite being discovered around 60 years ago, polymyxins maintained their effectiveness as antibiotics as they were seldom used due to concerns about their toxicity.

In recent years, rampant use of common antibiotics (e.g. penicillin) has led to the emergence of bacterial strains which are resistant to these antibiotics. This has become more and more of a global concern. Polymyxins are now a last line of defense against bacteria because of its previous lack of use.

(a) With reference to the reproductive cycle of bacteriophages, suggest how bacteriophage infections may lead to a spread of antibiotic resistance between bacterial populations.

[3]

Bacteria reproduce by the process of binary fission.

(b) Explain the significance of binary fission in bacteria.

The process of binary fission involves semi-conservative DNA replication.

(c) State two differences in the formation of the leading and lagging strands during DNA replication.

.....[2]

Bacteria rely on sugar sources e.g. lactose for survival.

(d) Describe the consequence of mutating the *lacl* gene of the bacterial lac operon, on usage of lactose.

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[Q4: 12 marks]

QUESTION 5

A student set up an experiment to investigate the effect of carbon dioxide on photosynthesis. First, he de-starched a small potted plant by putting it in the dark for two days. Then, he chose two leaves and inserted them into conical flasks, **A** and **B**, fitted with rubber stoppers. Lithium hydroxide was placed in Flask **B** to absorb all carbon dioxide present. The plant was then left under a table lamp for 15 minutes. Fig 5.1 shows the experimental setup.





He removed a sample of each leaf every 5 minutes (by punching out a leaf disc of approximately 0.2 cm in diameter, using a single-hole puncher) and return the leaves to their respective flasks immediately. Each leaf disc was then tested for the presence of ribulose bisphosphate (RuBP) and starch. Table 5.2 shows the results he obtained.

Flack	Concer	tration of	RuBP / µ	u molm ⁻²	Concentration of starch / μ molm ⁻²												
TIASK	0 min	5 min	10 min	15 min	0 min	5 min	10 min	15 min									
Α	0.0	2.2	3.0	3.1	0.0	2.1	3.4	6.5									
В	0.0	2.7	4.2	6.8	0.0	0.3	0.5	0.5									

(a) State two other variables which must be kept constant to maximize the validity of the results obtained for this experiment.

.....[1]

(b) With reference to Table 5.2, describe the relationship between the presence of carbon dioxide and concentration of starch.

(c) Explain the absence of RuBP in both leaves at the start of the experiment.

(d) The increase in RuBP concentration for the leaf in Flask A reached a plateau from 10 min to 15 min of exposure to light but continued to increase in the leaf in Flask B up to 15 min. Explain why.
The student watered the potted plant too excessively, causing the soil to become waterlogged. Fortunately, the roots of this plant could carry out anaerobic respiration under low oxygen conditions in the soil.

(e) Outline the process of anaerobic respiration in the roots under waterlogged conditions.

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[Q5: 12 marks]

QUESTION 6

The maggot fly, *Rhagoletis pomonella*, is native to the United States, and up until the discovery of the Americas by Europeans, fed solely on hawthorns. But when Europeans arrived on the Americas, they brought with them a new potential food source for the flies: apples.

At first, the flies are not attracted to apples. But over time, a minuscule change in the connections of two channels in the brain - one for detecting hawthorn odours and the other for apple odours - caused some flies to switch host plant (i.e. they jumped to apple trees). It was observed that the maggot flies strongly preferred to mate and lay their eggs on the type of tree they were born on.

When geneticists took a closer look in the late 20th century, they found that the two types - those that feed on apples and those that feed on hawthorns - have become genetically distinct groups.

(a) Identify the type of reproductive isolation shown in this case study.

.....[1]

(b) Discuss the processes that could have led to the genetic distinctions between the apple-eating flies and hawthorn-eating flies.

(c) Suggest how researchers can conclusively determine whether the apple-eating flies are a different species from the hawthorn-eating flies.

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A nuclear small subunit (18S) rRNA gene has been sequenced for various insect species and used to reconstruct their evolutionary relationship, which is shown in a phylogenetic tree in Fig. 6.1.



Fig. 6.1

(d) Comment on the evolutionary relationship between *Rhagoletis pomonella*, *Rhagoletis zoqui* and *Callophrys xami*.

.....[1]

(e) Discuss two advantages of using 18S rRNA gene over morphological comparisons for the reconstruction of the insect phylogeny.

[Q6: 12 marks]

QUESTION 7

Influenza is a prevalent illness all over the world. Due to the high rate of change in the structure of the virus, annual vaccinations are recommended.

To overcome this **problem** of the constant need for developing new vaccines against influenza viruses, scientists are attempting to produce vaccines relating to antibodies which recognize part of the virus that does not change—the stalk of haemagglutinin. This research may lead to the development of "universal" influenza vaccines which can remain effective over long periods of time (Journal of Virology, 2017).

Fig. 7.1 shows the structure of haemagglutinin.



Fig. 7.1

(a) With reference to the context above, explain why there is a "constant need for developing new vaccines against influenza viruses".

[3]

(b) With reference to the influenza virus and active immunity, define a vaccine and state how it provides protection against the virus.

 Vaccinations can control diseases by resulting in *herd immunity*, in which large percentage of population which has become immune to the virus through vaccinations, provides a measure of protection for individuals who are not immune.

(c) Explain why.

.....[1]

Tuberculosis (TB) is an infectious disease that generally affects the lungs. Most infections are latent and do not have symptoms. Latent infections can progress to active form of the disease which, if left untreated, kills about half of those infected.

The most common classic symptom of active TB is chronic cough with bloodcontaining sputum.

(d) Name the organism which causes tuberculosis (TB).

.....[1]

In the alveoli tissues, the bacteria that causes TB binds directly with mannose receptors on alveolar macrophages using a bacterial glycolipid, and is engulfed by alveolar macrophages.

(e) With reference to a cellular organelle in the macrophages, describe how macrophages attempt to process engulfed bacteria.

(f) Describe the cause of the chronic cough symptom of TB in its **active** stage.

.....[1]

[Q7: 10 marks]

QUESTION 8

Proteaceae are a family of flowering plants predominantly distributed in the tropical and sub-tropical regions of Australia and South Africa. Threatened species may be vulnerable to climate change, because of their narrow temperature tolerance, small population sizes and restricted distributions.

A study modelled climate-induced changes on the range size (geographical distribution of a species) of 282 Proteaceae species. Figures 8.1a and 8.1b show the time course of range changes for wind-dispersed (**A**) and ant-dispersed (**B**) Proteaceae species (under 'full migration' dispersal assumptions, which assumes no limitation to migration). Error bars represent standard error (for example, smaller standard errors indicates that the observations are closer to the actual value).



Fig. 8.1a



Fig. 8.1b

(a) Describe the differences in climate-induced changes in range size between the wind-dispersed and ant-dispersed Proteaceae species from Year 2000 to 2050.

(b) The Intergovernmental Panel for Climate Change (IPCC) predicted an increase of 2°C in global temperatures by 2050. Suggest reasons for the larger range size of ant-dispersed Proteaceae species compared to that of wind-dispersed species in 2050.

It was predicted that not only will the range size for ant-dispersed Proteaceae species remain high in 2050, it is likely that the plants would colonise new regions, particularly in the higher latitudes and altitudes.

(c) Explain why the Proteaceae species can potentially threaten the native species of the regions they expand into.

 Many indigenous cultures in tropical regions have used Proteaceae for medicinal preparations. For example, bioactive ingredients can be obtained from infusions of the roots, bark, leaves, or flowers of many Proteaceae species, which can used as topical applications for skin conditions or internally as tonics, aphrodisiacs, and medicines to treat headaches, cough, diarrhea, indigestion, stomach ulcers, and kidney disease.

(d) Discuss how climate change can affect the biodiversity of the Proteaceae species and its consequence to the production of biomedicines.

[2]	

[Q8: 10 marks]

END OF PAPER

Civics Group	Index Number	Name (use BLOCK LETTERS)	H2
		ST. ANDREW'S JUNIOR COLLEGE 2017 JC2 PRELIMS	
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Paper 3	3		
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our name, civics group and index number on all the work you vvrite hand in.

Write in dark blue or black pen on both sides of the paper.

You may use a soft pencil for any diagram, graph or rough working. Do not use staples, paper clips, highlighters, glue or correction fluid.

Section A (Structured Questions)

Answer **all** questions.

Write your answers in the spaces provided on the question paper.

Section B (Essay Question)

Conceptual error

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Answer **one** essay question. Write your answers on the separate answer paper provided. All working for numerical answers must be shown.

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Section A

Answer **all** the questions in this section.

QUESTION 1

Leigh disease is an inherited neuro-metabolic disorder that affects the central nervous system. As the disease progresses, the muscular system is debilitated throughout the body, as the brain cannot control the contraction of muscles. Leigh disease can be caused by a deficiency of the pyruvate dehydrogenase complex (PDHC), most commonly due to a mutation in the X-linked gene, PDHA1, which codes for the PDHC α -subunit.

A married couple (both of whom are normal individuals) is concerned about their chances of having a child with Leigh disease because the woman's father had the disease.

(a) Draw a genetic diagram with appropriate symbols to determine the probability that they will have an affected child.

.....[6]

200 couples with the same genotypes as the above-mentioned couple were included in a genetic study of the Leigh disease. They had a total of 322 children with the following phenotypes:

85 normal girls

70 carrier girls

89 normal boys

- 78 boys with Leigh disease
- (b) A chi-squared test was carried out to test the significance of the difference between the observed (O) and the expected (E) results.

$$\chi^2 = \sum \frac{(O-E)^2}{E}$$

(i) Calculate the χ^2 value. Show your working.

.....[2]

 χ^2 value:

Fig. 1

Degrees of					probabilit	y			
Freedom	0.99	0.95	0.90	0.75	0.50	0.25	0.10	0.05	0.01
1	0.000	0.004	0.016	0.102	0.455	1.32	2.71	3.84	6.63
2	0.020	0.103	0.211	0.575	1.386	2.77	4.61	5.99	9.21
3	0.115	0.352	0.584	1.212	2.366	4.11	6.25	7.81	11.34
4	0.297	0.711	1.064	1.923	3.357	5.39	7.78	9.49	13.28

(ii) Fig. 1 shows the table of probabilities. State the probability that the observed results does not differ significantly from the expected results.

.....[1]

(iii) State what conclusions may be drawn from the probability found in (ii).

 Leigh disease can also be due to a mutation in the mtDNA (mitochondrial DNA) affecting the MT-ATP6 gene. The mutated allele results in the production of a non-functional subunit in the ATP synthase complex which allows protons to pass through the channel without generating the proton motive force.

(c) State whether a mitochondrion containing the mutated allele would have a higher, lower or equal oxygen consumption rate relative to a mitochondrion which has the normal allele.

.....[1]

(d) Explain your answer in (c).

The DNA sequence of the MT-ATP6 gene has remained relatively conserved over a long evolutionary period. It is present in a wide diversity of organisms, ranging from the simplest worms to the great apes. Differences in the nucleotide sequence of the gene in different species can be identified using multiple sequence alignments.

(e) Discuss how the comparison of MT-ATP6 DNA nucleotide sequences of different species demonstrates evolutionary change.

.....[2]

[Q1: 17 marks]

QUESTION 2

Celiac disease is an autoimmune disorder that is caused by an improper immune response to the protein gluten, found in wheat, rye, and barley, that damages the lining of the small intestine. There is no cure for celiac, and the only effective treatment is a gluten-free diet.

Recent studies indicated that harmless intestinal viruses, such as the reovirus, can cause the immune system to overreact to gluten, raising the possibility of such viruses contributing to the development of the disease.

Fig. 2.1 shows the general structure of a reovirus. Unlike Human Immuno-deficiency Viruses (HIV), reoviruses are not retroviruses.



Fig. 2.1

(a) With reference to Fig. 2.1, contrast the structure of the reovirus with that of HIV.

.....[2]

(b) Suggest how new double-stranded viral RNA genome is synthesized during the reproductive cycle of a reovirus.

.....[2]

Researchers also looked at patients with celiac disease and found that they had much higher levels of antibodies against reoviruses than those without the disease.

Those with higher levels of antibodies also had higher levels of the molecule IRF-1 (interferon regulatory factor 1), a regulator of gene transcription which plays a key role in the loss of gluten tolerance.

(c) Describe three ways in which the structure of antibodies contribute to its function.



Fig. 2.2 shows the structure of an immunoglobulin gene which codes for antibodies.



Fig. 2.2

(d) With reference to Fig. 2.2, explain how variability at the DNA level result in variability in the antigen-binding sites of antibodies.

(e) Antibodies are proteins. Draw a diagram of the monomer which makes up antibodies. There is no need to annotate the diagram.

.....[1]

Antibodies are secreted by plasma cells.

Fig. 2.3 shows a plasma cell and a B lymphocyte.



Fig. 2.3

(f) With reference to Fig. 2.3,

(i) State 2 ways in which the structure of plasma cell differs from the B lymphocyte.

(ii) Explain the reasons for the differences you described.

 A student researcher tried to reproduce the results of the study regarding elevated antibody levels against reoviruses. She studied the levels of antibodies in 3 human subjects infected with reoviruses and another 3 who are not infected.

Table 2.4 shows his results.

	Table 2.4	
		Antibody titre / unit ml ⁻¹
Ş	Subjects not infected with reovirus	30 ± 10
Ş	Subjects infected with reovirus	122 ± 25

(g) With reference to the results on subjects not infected with reoviruses, explain what is standard deviation and its implications.

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QUESTION 3

(a) A student investigated growth in the roots of broad bean, *Vicia faba*. The student cut sections of the root tip of this plant and viewed them with a light microscope.

Fig. 3.1 is a photomicrograph of one of the sections. The cell labelled ${\bf D}$ is in interphase.



Fig. 3.1

Complete the table below by:

- naming the stages of mitosis in the correct sequence following interphase
- identifying **one** example from the cells labelled **A** to **H** that is in each stage of mitosis that you have named.

.....[3]

stage of mitosis	label from Fig. 3.1

(b) In animal cells, centrioles are responsible for assembling microtubules to make the spindle at the beginning of mitosis.

Describe the role of the spindle during mitosis.

- (c) Scientists investigated three genes, P, Q and R, involved in controlling cell division. They studied the effect of mutations in these genes on the risk of developing lung cancer.
 - (i) Suggest the differences in the cell cycle of a cancer cell compared with that of a normal cell of the same type.

The scientists analysed genes, **P**, **Q** and **R** from healthy people and people with lung cancer.

- If a person had at least one copy of normal allele for a gene, they used the symbol N.
- If a person had two mutant alleles for a gene, they used the symbol M.

They used their data to calculate the risk of developing lung cancer for people with different N and M genotypes. A risk value of 1.00 indicates no increased risk. **Table 3.1** shows the scientists' results.

Table 3.	1
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Gene P	Gene Q	Gene R	Risk value of developing lung cancer / AU
N	N	N	1.00
М	N	N	1.30
N	N	M	1.78
N	М	N	1.45

N = at least one copy of the normal allele is present

M = two copies of the mutant allele are present

(ii) What do these data suggest about the relative importance of the mutant alleles of genes P, Q and R on **increasing** the risk of developing lung cancer?

Chemotherapy is the use of a drug to treat cancer. An experiment was set up to study a new drug, SA128, which kills dividing cells. A group of four men suffering from lung cancer was given the drug. The number of cancer cells per unit volume of blood was measured right before treatment and again after the 2-week treatment.

		Number of c	ancer cells	per unit vol	ume of bloo	d
Subject	TSH	OON	CCK	TTS	Average	Standard deviation
Before drug treatment	2000	1600	1800	1200		
After drug treatment	1000	500	800	300		

Table 3.2 : Effect of SA128 on cancer cells.

(iii) Calculate the **average** and complete Table 3.2.

(iv) Using the formula below, calculate the standard deviations of number of cancer cells per unit volume of blood before and after drug treatment, SA128. Complete Table 3.2. Express your answers to 3 significant figures.

.....[1]

standard deviation s =

$$=\sqrt{\frac{\sum(x-\overline{x})^2}{n-1}}$$

 $\overline{}$

[1]

<u>Legend</u> \sum is 'Sum of' x is observation \overline{x} is the mean n is the sample size (number of observations) (v) Using the formula below as well as the average and standard deviation values calculated in (c)(iii) and (c)(iv),



Calculate the t_{calculated} value.

-[1]
 - (vi) Using the critical t-values in **Table 3.3**, determine if there is a significant difference in the results using the new drug, SA128.

 	 	 	 	[2]

df	.10	.05
1	3.078	6.314
2	1.886	2.920
3	1.638	2.353
4	1.533	2.132
5	1.476	2.015
6	1.440	1.943
7	1.415	1.895
8	1.397	1.860
9	1.383	1.833
10	1.372	1.812
11	1.363	1.796
12	1.356	1.782
13	1.350	1.771
14	1.345	1.761
15	1.341	1.753
16	1.337	1.746
17	1.333	1.740
18	1.330	1.734
19	1.328	1.729
20	1.325	1.725
21	1.323	1.721
22	1.321	1.717
23	1.319	1.714
24	1.318	1.711
25	1.316	1.708
26	1.315	1.706
27	1.314	1.703
28	1.313	1.701
29	1.311	1.699
30	1.310	1.697
40	1.303	1.684
60	1.296	1.671
120	1.289	1.658
с	1,282	1.645

Table 3.3: t-test table of t critical values

Section B

Answer **one** question in this section.

Your answers should be illustrated by large, clearly labelled diagrams, where appropriate.

Your answers must be in continuous prose, where appropriate.

Your answers must be set out in sections (a), (b) etc., as indicated in the question.

- 4 (a) The effects of global warming on the spread of malaria beyond the tropics is a debatable issue. Discuss the arguments/evidences that support your stance on the matter, and provide a balanced account of counter-arguments.
 - (b) Discuss the effects of climate change (as a result of greenhouse gas emissions. [13]

[Q4 Total: 25]

5	(a)	Explain what is meant by primary, secondary, tertiary and quaternary	
		structure of haemoglobin.	[13]

(b) Using a named example, discuss how genetic variation may be preserved in natural population by heterozygote advantage. [12]

[Q5 Total: 25]

END

	ST. ANDREW'S JUNIOR COLLEGE 2017 JC2 PRELIMS	E	
H2 BIOLOGY		9	744/4
Paper 4: Practica	I Exam		
Friday	25 th August 2017	2 hours 30 mi	nutes
READ THESE INSTRUCTION Write your name, civics group hand in. Write in dark blue or black pe You may use a soft pencil for Do not use staples, paper clip Answer all questions. Write your answers in the spa	NS FIRST o and index number on all the work you n on both sides of the paper. any diagram, graph or rough working. os, highlighters, glue or correction fluid. aces provided on the question paper.	Shift Lab	
		For Examin	ier's Use
INFORMATION TO CANDID	ATES	Section A	\ge
The number of marks is giver	n in brackets [] at the end of each	1	/ 21
question or part question.		2	/19
		3	/15
1			

Name (use BLOCK LETTERS)

Civics Group

Index Number

[Turn over

/55

Total

H2

IMPORTANT:

Candidates with access to microscope at the start of the paper are given the **first 1h 15 min** to use it. Please answer **QUESTION 2(b)** within this time frame.

Candidates with no access to microscope at the start of the paper will be given access **1h 15min after the start of the paper**. You may proceed with **QUESTION 1** first.

QUESTION 1

Investigation into the effect of enzyme concentration on the hydrolysis of starch

The enzyme amylase catalyses the hydrolysis of starch.

You are required to investigate the effect of the concentration of amylase on the time taken to completely hydrolyse starch.

lodine solution turns from yellowish brown to blue-black when starch is present. The time taken for the complete hydrolysis of starch can be found by removing a sample of an amylase and starch mixture at regular time intervals, and adding it to a drop of iodine solution. The starch has been completely hydrolysed when the iodine solution remains yellowish brown after adding the sample. This is the end-point. In judging the end-point, specks of blue-black in an otherwise yellowish brown solution can be ignored.

You are provided with:

- 40.0 cm³ of 2.0% amylase solution, labelled **E**, which is an irritant,
- 100.0 cm³ of distilled water, labelled W,
- 40.0 cm³ of starch solution, labelled **S**,
- lodine solution, labelled **iodine**, which is a stain.

Read steps 1 to 9 before starting.

Proceed as follows:

You are required to prepare different concentrations of the amylase solution and set up a control.

1 Carry out **simple** dilutions of the amylase solution, **E**, to obtain a range of concentrations in which the concentration of the amylase is reduced by 0.4% between each successive dilution.

Prepare 10.0 cm³ for each concentration of amylase solution, **using the plastic** containers provided.

Concentration of amylase solution / %	volume of E / cm^3	volume of W / cm ³
2.0		

Table 1.1

2 Prepare a suitable control for this investigation.

Describe the control that you have prepared. Explain the rationale of the control.

.....[2]

You are required to investigate the effect of different amylase concentrations on the time taken to completely hydrolyse starch. You will test samples from mixtures of a starch solution and different concentrations of amylase solution at a chosen time interval until each end-point is reached, up to a maximum of **180 seconds**.

3 Decide on a suitable time interval at which samples from each mixture of amylase solution and starch solution will be tested for complete hydrolysis of starch.

State the chosen time interval with a reason for this choice.

Time interval

Reason	
	[4]
	 [1]

4 The enzyme's optimum temperature is 35°C. Using the hot and tap water provided, set up a water-bath in the beaker labelled **water-bath**, so that you can maintain this temperature throughout the investigation.

5 Put uniform-sized drops of iodine solution on the white tile, labelled with the times that a sample from each mixture of amylase solution and starch solution will be removed and tested, as shown in **Fig. 1.1**.



Fig. 1.1

6 Put 3.0 cm³ of **S** into a test-tube and 2.0 cm³ of 2.0% amylase solution into a separate test-tube. Put the test-tubes into the water-bath for at least one minute in order to equilibrate to 35° C.

7 The reaction will start as soon as **S** and the amylase solution are mixed.

Add the starch solution, **S**, to the 2.0% amylase solution, and start timing immediately. Using a Pasteur pipette, remove a sample of the mixture at the first chosen time and add **one drop** to the first drop of iodine solution on the white tile. Continue removing and testing samples at the chosen time interval until the end-point is reached, up to a maximum of **180 seconds**. *Make sure that the mixture or enzyme and starch are maintained at* 35°C throughout the experiment.

8 Repeat steps **5** – **7** to collect the result for each of the other concentrations of amylase solution and the control that you have prepared. Record **'more than 180'** for any mixtures that have not reached the end-point by 180 seconds.

9 Use the space below to record your results.

10 Explain how the concentration of amylase affects the rate of hydrolysis of starch.

11 Temperature was one variable which was controlled in this investigation.

Identify one variable that affects enzyme reactions, which was not controlled in this investigation.

.....[1]

12 Suggest how you would control this variable.

.....[1]

13 For a biotechnological process involving an enzyme to work most efficiently, the enzyme must work at its maximum rate, R.

An enzyme can be used to catalyse the conversion of ethanol (substrate) to acetaldehyde (product).

The effect of the concentration of ethanol (A) on the maximum rate of the production of acetaldehyde (R), is shown in **Table 1.2**.

Concentration of ethanol (A) / mol dm ⁻³	Maximum rate (R) / min ⁻¹
0.00800	0.0700
0.0150	0.110
0.0500	0.170
0.100	0.220
0.300	0.270

Table 1.2

A linear graph can be drawn by plotting 1/R against 1/A.

This can be used to find R (maximum rate of production) for any particular ethanol concentration in this range.

Complete **Table 1.3** for the values of A and R in the last row of Table 1.2, by calculating 1/A and 1/R to the appropriate number of decimal places. [1]

Table	1.3
-------	-----

1/A / mol ⁻¹ dm ³	1/R / min
125.0	14.3
66.7	9.1
20.0	5.9
10.0	4.6

15 Find the maximum rate of production (R) which would be achieved if the ethanol concentration (A) was 0.1 mol dm^{-3} .

Show clearly how you obtained R.

R =min⁻¹ [2]

[TOTAL : 21]

[4]

14 Using the data from **Table 1.3**, draw a graph on the grid provided.

QUESTION 2

Fig 2.1 is a photomicrograph of a stained transverse section through part of a leaf from a different type of plant.

You are not expected to be familiar with this specimen.



Fig. 2.1

(a) Draw a large **plan diagram** of Fig. 2.1 in the space provided below. Please refer to the coloured photo micrograph provided on the student's bench. [2]

REMINDER:

Candidates with access to microscope at the start of the paper are given the **first 1h 15 min** to use it. Please answer **QUESTION 2(b)** within this time frame.

(b) You are required to measure the diameter of the field of view using the clear plastic ruler.

Proceed as follows:

- 1. Put the clear plastic ruler on the stage of the microscope and view the scale lines using low power (x100).
- 2. Measure the diameter of the field of view and record this in (b)(i).
- (i) Diameter of the field of viewmm [1]

Fig. 2.2 is the same photomicrograph as in Fig. 2.1 showing the field of view at the same magnification as the field of view you have just measured.



Fig. 2.2

(ii) Using appropriate measurements, calculate the fraction of the diameter of the field of view occupied by the leaf in **Fig. 2.2** along the line **X–Z**.

fraction of diameter of field of view[1]

(iii) Using your answers to (b)(i) and (b)(ii) calculate the depth of the midrib, as shown by line Y–Z. Give your answer to the nearest μm. You may lose marks if you do not show your working.

.....μm [2]

(iv) A student used a clear plastic ruler to measure the field of view of a microscope. The student replaced the ruler with a slide of a leaf and **estimated** the diameter of the midrib. Using these results the student calculated the actual diameter of the midrib.

State how this student could have modified their method to obtain a more accurate result. State the apparatus the student would use and describe the method.

apparatus	
mathad	
	 [3]
	r - 1

(c) One technique used for studying antigen-antibody reactions is immunodiffusion.

Wells are cut into an agar support medium to contain antigens and antibodies. Antibodies and antigens diffuse out of the wells into the agar. If an antigen meets a complementary antibody a reaction occurs causing a band of precipitate to appear.

Fig. 2.3 shows the results of an immunodiffusion test with known antigens P and Q and the antibodies to these antigens.



Fig. 2.3

In an investigation, the serum from two test organisms was tested for the presence of antibodies to specific antigens. Both organisms had been previously exposed to both antigens. The serum was placed in wells at the edge of the petri dish and the antigens in a central well.

Fig. 2.4 shows the test set-up.



Fig. 2.4

(i) Suggest **one** variable that must be controlled in this procedure.

.....[1]

(ii) State the independent variable in this investigation.

.....[1]

(iii) Both test organisms had antibodies against antigen **X**, but only organism **2** had antibodies against antigen **Y**.

On Fig. 2.4 draw lines to represent where precipitation might have occurred for both organisms. [2]

(iv) Suggest one disadvantage of immunodiffusion for detecting antigens.

.....[1]

A **naturally occurring** mutant of *Plasmodium* sp. has been tested for use as a 'whole organism' vaccination against malaria. The mutant organism develops normally in mosquito vectors and infects the salivary glands in the same way as non-mutant wild type *Plasmodium* sp. In mice, the mutant infects liver cells but does not multiply and cannot enter red blood cells.

Trials using mice were carried out and the effectiveness of the mutant organism as a vaccine tested by injecting non-mutant wild type *Plasmodium* sp. into vaccinated and non-vaccinated mice.

Table 2.1 shows the results of investigations in mice using the mutant *Plasmodium* sp.

	number of mutan	percentage of mice not		
test group	first inoculation	first booster inoculation	second booster inoculation	infected by wild type <i>Plasmodium</i> sp.
1	0	0	0	0
2	50 000	25000	25000	100
3	10 000	10000	10 000	100
4	10 000	10000	0	70

Table 2.1

(v) Suggest the purpose of including each of the following test groups.

oup 1	
oups 2 and 3	
oup 4	
	[3]

(vi) Using the information in the question, outline a procedure that might be used to obtain mutant *Plasmodium* sp. to use in the vaccination trials.

[TOTAL : 19]
QUESTION 3: PLANNING QUESTION

Effect of citrate on rate of respiration

Enzymes catalysing essentially irreversible reactions are potential sites of control in cellular respiration. One of these enzymes is phosphofructokinase, which can be regulated by the reversible binding of citrate to its allosteric site. (Citrate is produced as an intermediate compound during Krebs cycle.)

Using this information and your own knowledge, design an experiment to determine the effect of citrate concentration on the rate of cellular respiration.

You must use:

- 10 mM citrate,
- purified homogenate of enzymes found in the cytosol,
- 5% glucose solution,
- pH buffer,
- distilled water,
- Benedict's solution,
- apparatus shown in **Fig. 3.1**, can be used to separate proteins from ions and disaccharides,



Fig. 3.1

- syringes,
- white card,

- stopwatch,
- thermometer,
- bunsen burner with tripod, gauze and bench mat,
- thermostatically controlled water bath,
- normal laboratory glassware e.g. test-tubes, beakers, measuring cylinders, graduated pipettes, glass rods, etc.,

Your plan should:

- have a clear and helpful structure such that the method you use is able to be repeated by anyone reading it,
- be illustrated by relevant diagrams, if necessary,
- identify the independent and dependent variables,
- describe the method with the scientific reasoning used to decide the method so that the results are as accurate and reliable as possible,
- show how you will record your results and the proposed layout of results tables and graphs,
- use the correct technical and scientific terms,
- include reference to safety measures to minimize any risks associated with the proposed experiment.

[Total: 15]

.....

.....

.....

.....

SAJC H2 PRELIM 2017 PAPER 1 ANSWER SCHEME

1	В	11	D	21	D
2	Α	12	D	22	Α
3	В	13	С	23	Α
4	В	14	Α	24	D
5	В	15	Α	25	В
6	D	16	D	26	Α
7	Α	17	С	27	Α
8	D	18	Α	28	С
9	В	19	Α	29	D
10	В	20	В	30	D

Civics Group	Index Number	Name (use BLOC	K LETTERS)		H2	I
		ST. ANDREW 2017	I'S JUNIOR COLL JC2 PRELIMS	EGE		
H2 BIOLO	DGY				9744/2	
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Hormones, insulin and glucagon, are proteins that regulate the concentration of blood glucose level. Type 2 diabetes is characterized both by insulin resistance, a condition in which various tissues in the body no longer respond properly to insulin action, and by subsequent progressive decline in beta (β)-cell function to the point that the cells can no longer produce enough additional insulin to overcome the insulin resistance. Researchers are actively exploring use of stem cells as a potential source of deriving new β -cells to treat type 2 diabetes.



Fig.	1
------	---

The pancreas is located in the abdomen, adjacent to the duodenum (the first portion of the small intestine). A cross section of the pancreas shows the islet of Langerhans which is the functional unit of the endocrine pancreas. Encircled is the beta cell that synthesizes and secretes insulin. Beta cells are located adjacent to blood vessels and can easily respond to changes in blood glucose concentration by adjusting insulin production.

(a) Cells that secrete proteins contain a lot of rough endoplasmic reticulum (rER) and a large Golgi body.

- (i) Describe how the rER is involved in the production of insulin.
-[1] 1 (RER has) bound <u>ribosomes</u> for protein synthesis [REJECT: make amino acid] [ACCEPT: amino acids joined together / polypeptide]
 - 2 Chemical modification / post-translational modification of polypeptide

Note:

Point 2 is accepted in view that students have not learnt about the processing of insulin in detail. Chemical modification of insulin e.g cleavage of pro-insulin to insulin is done in the Golgi body

(ii) Describe how the Golgi body is involved in the secretion of insulin.

-[2]
 - 1 (Golgi body) further chemically modifies (insulin);
 - 2 packages (insulin) into secretory vesicles which move towards the cell surface membrane (and fuse with it, to release insulin out of the cell);
- (b) Using type II diabetes as an example, explain how environment affects phenotype.

.....[3]

- 1 people with functional pancreas/with no type I diabetes have **functional genes** which code for insulin release;
 - (insulin is secreted when blood glucose level increases);
- 2 **overeating** of sugary foods for a long period of time causes repeated stimulation of the pancreas;

which responds by secreting high levels of insulin;

- 3 **repeated exposure** of target cells to large amounts of insulin **desensitizes** the cells' responsiveness to insulin;
- 4 result in the target cells **failing to take in glucose**; (blood glucose stays high) resulting in type II diabetes;

(c) With reference to Fig. 1, explain how the binding of insulin to receptors on muscle cells leads to a lowering of blood glucose concentration.

.....[2]

- 1 Insulin binding results in the entry of glucose into the muscle cell by facilitated diffusion / via carrier (protein)/channel (protein)/glut4 protein/glucose transporter (down a concentration gradient)
- 2 Glucose used to form glycogen in the muscle cell (in Fig).
- (d) Suggest how a change in the amino acid sequence of the receptor found in the plasma membrane of the muscle cell could make the cell resistant to insulin.

.....[2] [Max 1]

- 1 Different amino acid sequence lead to different interactions between <u>R groups</u> of amino acids,
- 2 leading to different tertiary structure / three-dimensional structure (of receptor) ;

[Compulsory]

- 3 (so insulin) does not fit / bind / is not complementary ;
 [REJECT: any reference to 'active site', 'enzyme-substrate complex' or insulin not fitting/binding to an enzyme]
- (e) Describe how phospholipids are arranged in a plasma membrane.
-[3]
- 1 (phospholipid molecules arranged as a) bilayer ; [ACCEPT : double layer]
- 2 Polar <u>phosphate</u> head / charged phosphate group (of phospholipid molecules) faces outwards and interacts with aqueous medium of the external environment and the cytoplasm ;
- 3 **Non-polar** hydrocarbon chains of fatty acids in phospholipid molecules form the **interior** of the plasma membrane / cell membrane / cell surface membrane ;
- (f) Phospholipids are a type of lipid. Lipids, in general, are made up of glycerol and fatty acids monomers covalently bonded together. Name the covalent bond and describe the breakage of this bond.

.....[2]

- 1 ester bond ; [Reject: ester]
- 2 Addition of 1 water molecule across each ester bond (via hydrolysis reaction) ;
- 3 Products of hydrolysis are the **hydroxyl group (-OH) in the glycerol molecule** and the **carboxyl group (-COOH) of a fatty acid** ;

Experiments have indicated that pancreatic stem cells (PSCs) can serve as sources of insulin secreting cells.

- (g) State the source of PSCs and explain the PSCs' normal functions.
-[2]
- 1 Pancreas;
- 2 Give rise to pancreatic cells, to growth, **repair and maintenance** of pancreatic tissues.
- (h) Suggest an advantage of using the patient's own PSCs to regenerate tissue or organs.

.....[1]

1 No immune response (to own tissue) / tissue will not be rejected

[Reject: "cells will not be rejected" as context is on tissue regeneration]

[Q1: 18 marks]

Stephen Lyon Crohn, also known as "The man who can't catch AIDS", was a man notable for a genetic mutation, which caused his inability to contract AIDS.

Crohn had the "delta-32" mutation on the CCR5 co-receptor, a protein on the surface of cells involved in the immune system.

CCR5 "delta-32" mutation involves a 32-base-pair deletion in the CCR5 co-receptor gene locus.

- (a) Describe the role of CCR5 co-receptor protein in the entry of Human HIV into host cells.
- 1. Viral <u>Gp120</u> recognises and binds to CCR5 co-receptor (in addition to CD4 receptor on T cell)
- 2. thus, triggering an allosteric change in viral Gp41 which then pierces through the host plasma membrane

/ causing fusion between viral envelope and host plasma membrane

- (b) Describe briefly how the information on DNA is used to synthesize the pre-mRNA transcript for CCR5 co-receptor.
-[3]
- 1 <u>Ref. RNA polymerase</u> II and General <u>transcription factors</u> / Transcription initiation complex bind to <u>promoter</u> sequence / <u>TATA</u> box; and **unzips** DNA helix;
- 2 adds complementary ribonucleotides to the 3' end of growing RNA chain ;
- 3 ref. termination of transcription; cleaving occurs 10 to 35 nucleotides downstream of <u>polyadenylation signal</u> (of pre-mRNA);

Individuals can either gain full or partial resistance to HIV, depending on the number of mutated CCR5 alleles they possess at the CCR5 gene locus. Partial resistance to HIV occurs when the entry of the virus is severely hampered (but not completely prohibited).

Another Caucasian woman named Susan, is homozygous with the CCR5 "delta-32" mutation. Her husband has the same mutation on **one** CCR5 allele. The couple has a son and a daughter. Both son and daughter has 50% chance of having partial resistance and 50% chance of having full resistance.

(c) Explain if the inheritance of the CCR5 alleles is autosomal or X-linked.

-[2] 1. Autosomal:
- 2. If autosomal, the mother / Susan will pass down the mutant allele to both son and daughter, the father will pass down either mutant or normal allele to son and daughter, children will either be fully resistant (homozygous) or partially resistant (heterozygous)

/ If X-linked, the mother / Susan will pass down the mutant allele to both son and daughter, the father will pass down mutant allele to only the daughter (as son inherits the Y chromosome), both son and daughter would be fully resistant

Explanation:

Key: allele A: normal allele; allele a: delta-32 mutant allele

If autosomal,

Mother has 2 delta 32 resistance alleles (aa)

Father has 1 susceptible allele and 1 resistance allele (Aa)

Son or daughter will either be aa (fully resistant) or Aa (50%)

If X linked,

Mother has delta 32 resistance allele (X^a X^a)

Father has 1 resistance allele and a Y chromosome (X^a Y)

Daughter: X^a X^a

Son: X^aY

(d) Suggest why some individuals have partial resistance to HIV.

.....[2]

- 1. Their genotype is heterozygous / they have one mutant allele and one normal allele;
- 2. Expression of **both** the normal allele and the mutant allele/the normal allele and mutant allele are co-dominant to each other; leads to **both functional and non-functional** CCR5 co-receptor.

The son with partial resistance to HIV, marries a woman with no resistance.

The son has blood group O while his wife is heterozygous for blood group A.

(e) With the help of a genetic diagram, show all the possible outcomes for their offspring.

.....[5]

Key: "A": normal CCR5 allele

"a": mutant CCR5 allele

Parents phenotype: parental genotype ;; Wife No resistance AA I^A I^o

Al٥



al

gametes;;

Punnett Sq showing offspring genotype

Gametes	AI ^A	Alo
Al°	AAI ^A Iº No resistance, Blood gp A	AAIºIº No resistance Blood gp O
alº	Aal ^A l ^o Partial resistance, Blood gp A	Aalºlº Partial resistance, Blood gp O

AIA

F1 phenotypic ratio:

1 no resistance, blood gp A: 1 no resistance, blood gp O: 1 partial resistance, blood gp A: 1 partial resistance, blood gp O

Mark scheme:

- 1 Parental genotypes
- 2 Parental gametes; circled
- 3 Punnett square showing all offspring genotypes
- 4 corresponds phenotypes to genotypes / legend for Punnett square
- 5 Offspring phenotypic ratio

[Q2: 14 marks]

Question 3

Epidermal growth factor (EGF) is released by cells, and is picked up either by the cell itself or by neighboring cells. It regulates the production of a number of proteins in target cells. Protein produced and its effect depends on the type of target cell.

Fig. 3 shows how EGF regulates 3 genes.





(a) Name the two transcription factors in Fig. 3.

.....[1] 1 <u>Phosphorylated</u> ERK ; AND c-Fos (protein)

(b) The *c-Fos* gene can be a proto-oncogene.

Use the information in Fig. 3 to explain how a mutation of the *c*-*F*os gene can result in the formation of a tumour.

.....[3]

- 1 gain of function mutation of proto-oncogene to form oncogene
- [Compulsory point] that code for **abnormal** c-Fos protein [Reject: overexpression] which is constitutively active / degradation-resistant;
- 3 lead to increased expression of gene B / form more gene B product, thus, over-stimulation of the cell cycle / cell keeps dividing [only award mark if point 2 is correct]
- (c) Gene B has been associated with a significant number of human cancers. Scientists used polymerase chain reaction (PCR) to make multiple copies of gene B extracted from a patient's cancer tissue sample.

The reaction mixture includes the sample of DNA to be copied plus the following ingredients:

- DNA primers
- buffer solution
- heat-stable DNA polymerase (Taq polymerase)
- deoxyribonucleoside triphosphates (deoxyATP, deoxyTTP, deoxyCTP and deoxyGTP)
- (i) Suggest why a buffer needs to be present in the reaction mixture.

.....[1]

1 to control the pH

/ to stop the polymerase denaturing

- / to optimise pH for polymerase activity
- (ii) The deoxyribonucleoside triphosphates that are added to the reaction mixture are the monomers used for making the new DNA strands.

Suggest **one further** reason for adding the deoxyribonucleoside triphosphates to the reaction mixture.

[1]

1 Ideas that it is a source of energy / AW;

(hydrolysis of the dATP to dAMP and PP release energy which is used in the catalysis of phosphodiester bonds in the polynucleotide chain)

- (iii) In the first stage of PCR, the mixture is heated to a temperature of around 90°C to denature the DNA. Suggest why high temperatures are needed to separate the two DNA strands.
-[2]
 - 1 Idea of many hydrogen bonds between **complementary** strands together ;
 - 2 Hydrogen bonds break because of increased kinetic energy / vibrations ;
 - (iv) At the end of several cycles of PCR, many copies of the DNA sample in the reaction mixture will have been made. The DNA samples are then separated out to produce a DNA banding pattern.

State the technique used to separate out the DNA samples **and** describe how this technique works.

.....[4]

- 1 <u>Gel</u> electrophoresis ;
- 2 (Load 10 µl of sample into the wells in agarose gel ;

Gel electrophoresis conducted at 100V till tracking dye move to ³/₄ length of gel) DNA is **negatively-charged** (due to negatively-charged sugar-phosphate backbone) move towards the **positively-charged** electrode

- 3 through an agarose matrix which acts as a molecular sieve ;
- **4** DNA fragments separated by size ; where shorter DNA fragments move faster [Reject: further] than longer ones;

[Q3: 12 marks]

Researchers have identified a gene that gives bacteria resistance to a type of antibiotics called polymyxins. Despite being discovered around 60 years ago, polymyxins maintained their effectiveness as antibiotics as they were seldom used due to concerns about their toxicity.

In recent years, rampant use of common antibiotics (e.g. penicillin) has led to the emergence of bacterial strains which are resistant to these antibiotics. This has become more and more of a global concern. Polymyxins are now a last line of defense against bacteria because of its previous lack of use.

- (a) With reference to the reproductive cycle of bacteriophages, suggest how bacteriophage infections may lead to a spread of antibiotic resistance between bacterial populations.
-[3]
- 1 During generalised / specialised <u>transduction</u>, **host/bacteria DNA** can be incorporated into the phage capsid **randomly (for generalised transduction)/occasionally/by mistake** during viral assembly;
- 2 The resulting transducing phages infect other bacteria and newly infected cell acquires the donor bacterial DNA
- 3 Genetic recombination occurs and **expression** of antibiotic resistance genes result in phenotype of antibiotic resistance

Bacteria reproduce by the process of binary fission.

(b) Explain the significance of binary fission in bacteria	
	[2]

- 1 Ref. asexual reproduction for unicellular organism
- 2 Ensuring that offspring are <u>genetically identical</u> to the parent / Desirable alleles/traits are passed down

3 Rapid increase in cell numbers (under favourable conditions) [Any 2] The process of binary fission involves semi-conservative DNA replication.

- (c) State two differences in the formation of the leading and lagging strands during DNA replication.
-[2]
- 1 Presence of DNA ligase in lagging strand to ligate Okazaki fragments;
- 2 Presence of Okazaki fragments in lagging strand but none in leading strand;
- 3 Presence of more than 1 (RNA) primer/primase in lagging strand; (**REJECT**: "no primer needed in leading strand". This is incorrect!)
- 4 Strands are synthesized in opposite directions;
- 5 <u>Leading</u> strand is synthesized <u>continuously</u> vs <u>lagging</u> strand is synthesized <u>discontinuously</u> in the form of okazaki fragments
- [Any 2]

Bacteria rely on sugar sources e.g. lactose for survival.

- (d) Describe the consequence of mutating the *lacl* gene of the bacterial lac operon, on usage of lactose.
-[5]
- 1 lac repressor has a change in <u>3D conformation</u> at the **DNA-binding domain** / allosteric site so that allolactose / inducer binds tightly
- 2 lac repressor is inactive and is no longer able to bind to the operator (lacO);
- 3 RNA polymerase can constitutively access and transcribe the <u>structural</u> genes / lacZ, lacY and lacA;
- 4 β-galactosidase, lac permease and lactose transacetylase / (inducible) enzymes to utilize lactose are **constitutively** synthesised ;
- 5 Bacteria can utilize lactose even in the absence of lactose;

OR

- 1 lac repressor has a change in <u>3D conformation</u> at the **DNA-binding domain** / **allosteric site** so that allolactose / inducer cannot bind
- 2 lac repressor (super repressor) binds tightly/continuously to the operator (lacO);
- 3 RNA polymerase cannot access and transcribe the <u>structural</u> genes / lacZ , lacY and lacA;
- 4 β-galactosidase, lac permease and lactose transacetylase / (inducible) enzymes to utilize lactose are **not** synthesised ;
- 5 Bacteria cannot utilize lactose even in the presence of lactose;

[Q4: 12 marks]

A student set up an experiment to investigate the effect of carbon dioxide on photosynthesis. First, he de-starched a small potted plant by putting it in the dark for two days. Then, he chose two leaves and inserted them into conical flasks, **A** and **B**, fitted with rubber stoppers. Lithium hydroxide was placed in Flask **B** to absorb all carbon dioxide present. The plant was then left under a table lamp for 15 minutes. Fig 5.1 shows the experimental setup.





He removed a sample of each leaf every 5 minutes (by punching out a leaf disc of approximately 0.2 cm in diameter, using a single-hole puncher) and return the leaves to their respective flasks immediately. Each leaf disc was then tested for the presence of ribulose bisphosphate (RuBP) and starch. Table 5.2 shows the results he obtained.

Flask	Concer	ntration of	RuBP / µ	ւ molm ⁻²	Concentration of starch / µ molm ⁻²			
	0 min	5 min	10 min	15 min	0 min	5 min	10 min	15 min
Α	0.0	2.2	3.0	3.1	0.0	2.1	3.4	6.5
В	0.0	2.7	4.2	6.8	0.0	0.3	0.5	0.5

Table 5.2

(a) State two other variables which must be kept constant to maximize the validity of the results obtained for this experiment.

.....[1]

- 1 Size of the leaves chosen (in flask A and flask B)
- 2 Distance from light source/light intensity
- 3 Temperature
- 4 Size of flask (affecting the volume of gas)

[Reject: leaves must be from the same plant as diagram already show only one pot of plants; pH of soil as fluctuations in pH will affect both leaves equally and hence, not affect the results]

- (b) With reference to Table 5.2, describe the relationship between the presence of carbon dioxide and concentration of starch.
-[2]
 - 1 In the presence of carbon dioxide (i.e. Flask A), starch concentration increased from 0.0 to 6.5 μ molm⁻² from 0 15 min.
 - 2 while in the absence of carbon dioxide (i.e. Flask B), starch concentration increased much <u>less</u>, from <u>0.0 to 0.5 μ molm⁻² from <u>0 15 min</u>.</u>

OR

- 1 The presence of carbon dioxide leads to a higher concentration of starch
- 2 E.g. at 15min, starch concentration in the presence of carbon dioxide (i.e. Flask A) is at <u>6.5 μ molm⁻²</u> while in the absence of carbon dioxide (i.e. Flask B), starch concentration is at <u>0.5 μ molm⁻²</u>

(c) Explain the absence of RuBP in both leaves at the start of the experiment.

-[2]
 - 1 In the dark (during de-starching), the leaves carried out carbon fixation to convert **RuBP to PGA**.
 - 2 However, in the dark, no <u>ATP</u> and <u>NADPH</u> were produced,
 - 3 hence, chloroplasts are unable to convert **PGA to PGAL** and to regenerate **RuBP from PGAL**.
- (d) The increase in RuBP concentration for the leaf in Flask A reached a plateau from 10 min to 15 min of exposure to light but continued to increase in the leaf in Flask B up to 15 min. Explain why.

.....[3]

- 1 (In Flask A, concentration of RuBP reaches a plateau because) the RuBP that is being used up (in carbon fixation) equals the RuBP that is regenerated (from PGAL in the Calvin cycle).
- 2 (In Flask B, exposure to light causes) the production of <u>ATP</u> and <u>NADPH</u> in the light-dependent reactions, which will convert existing PGA to PGAL (don't double penalize from part (c)), and **regenerate RuBP from PGAL**.
- 3 However, in the absence of carbon dioxide, carbon fixation does not occur, and RuBP accumulates in the leaf.

The student watered the potted plant too excessively, causing the soil to become waterlogged. Fortunately, the roots of this plant could carry out anaerobic respiration under low oxygen conditions in the soil.

- (e) Outline the process of anaerobic respiration in the roots under waterlogged conditions.
-[4]
 - 1 Glucose is converted to pyruvate via <u>glycolysis</u> in the <u>cytosol</u>.
 - 2 Pyruvate is then decarboxylated to acetaldehyde/ethanal with the release of <u>CO₂</u>.
 - 3 This reaction is catalysed by <u>pyruvate decarboxylase</u>.
 - 4 NADH then reduces acetaldehyde/ethanal to ethanol,
 - 5 catalysed by <u>alcohol dehydrogenase</u>.
 - 6 NAD+ is **regenerated** for use in glycolysis to generate some **ATP** continuously.

[Q5: 12 marks]

The maggot fly, *Rhagoletis pomonella*, is native to the United States, and up until the discovery of the Americas by Europeans, fed solely on hawthorns. But when Europeans arrived on the Americas, they brought with them a new potential food source for the flies: apples.

At first, the flies are not attracted to apples. But over time, a minuscule change in the connections of two channels in the brain - one for detecting hawthorn odours and the other for apple odours - caused some flies to switch host plant (i.e. they jumped to apple trees). It was observed that the maggot flies strongly preferred to mate and lay their eggs on the type of tree they were born on.

When geneticists took a closer look in the late 20th century, they found that the two types - those that feed on apples and those that feed on hawthorns - have become genetically distinct groups.

(a) Identify the type of reproductive isolation shown in this case study.

- 1 Behavioural isolation [Reject: Sympatric isolation; Geographical isolation]
- (b) Discuss the processes that could have led to the genetic distinctions between the apple-eating flies and hawthorn-eating flies.

(Behavioural isolation) reduced the gene flow between flies which jumped to apple trees and flies which remained on hawthorn trees.

- 2 Processes of <u>natural selection</u> and <u>genetic drift</u> occurred.
- 3 Different tree habitats exert different selection pressures
- 4 Individuals which are better adapted to their respective environments survive better, reproduce more and pass on their advantageous alleles to their offspring.
- 5 Different **mutations/genetic differences** accumulate in the different subpopulations of the flies. [Reject: speciation occurred]
- (c) Suggest how researchers can conclusively determine whether the apple-eating flies are a different species from the hawthorn-eating flies.

.....[1]

1 Observe for <u>mating</u> between apple-eating flies and hawthorn-eating flies, to produce <u>viable and fertile</u> offspring.

A nuclear small subunit (18S) rRNA gene has been sequenced for various insect species and used to reconstruct their evolutionary relationship, which is shown in a phylogenetic tree in Fig. 6.1.



- (d) Comment on the evolutionary relationship between *Rhagoletis pomonella*, *Rhagoletis zoqui* and *Callophrys xami*.
- Rhagoletis pomonella and Rhagoletis zoqui are more closely related to each other than either one is to Callophrys xami.

/ Rhagoletis pomonella and Rhagoletis zoqui share a more recent common ancestor with each other than with Callophrys xami.

(e) Discuss two advantages of using 18S rRNA gene over morphological comparisons for the reconstruction of the insect phylogeny.

.....[4]

Quantifiable and open to statistical analysis

- 1 Molecular data such as nucleotide and amino acid sequences are **quantifiable**, in abundance and **open to statistical analysis**. [Reject: the rRNA gene is in abundance]
- 2 Large quantities of data are required for statistical analysis; however there is **little morphological data available**.

Unambiguous and objective

- 3 Molecular data can be easily described in an **unambiguous/objective [reject: accurate]** manner.
- 4 Morphological data may be **subjective** and may differ depending on the way in which it was classified In addition some characteristics may be analogous.

Not affected by convergent evolution

- 5 Molecular data provides a clear model of evolution by comparing the nucleotide and/or amino acid sequence as the **rate of molecular change in genes and proteins is regular** like a molecular clock.
- 6 Morphological evidence could be due to <u>convergent evolution</u> as similar morphology may not have been inherited from common ancestor /Rate of morphological change is not regular due to convergent or divergent evolution (hence cannot be used to reconstruct evolutionary relationships accurately)

Based strictly on heritable material

- 7 Molecular data is based strictly on **heritable** material.
- 8 Morphological data is based on anatomical characters which may be influenced by **environmental** factors as well as variation due to genotype of the organism.

Greater number of characters can be compared

- 9 DNA information provides **abundance of data** for analysis and it allows easy homology assessment.
- 10 Morphological **traits are few** and it is often difficult to assess homology for less complex structures.

[Any 2 pairs; no marks awarded if no attempt is made to compare between both molecular and morphological methods]

Influenza is a prevalent illness all over the world. Due to the high rate of change in the structure of the virus, annual vaccinations are recommended.

To overcome this **problem** of the constant need for developing new vaccines against influenza viruses, scientists are attempting to produce vaccines relating to antibodies which recognize part of the virus that does not change—the stalk of haemagglutinin. This research may lead to the development of "universal" influenza vaccines which can remain effective over long periods of time (Journal of Virology, 2017).

Fig. 7.1 shows the structure of haemagglutinin.



Fig. 7.1

(a) With reference to the context above, explain why there is a "constant need for developing new vaccines against influenza viruses".

.....[3]

- 1. <u>Antigenic drift</u> where there are spontaneous <u>mutations</u> in RNA genome coding for antigens <u>haemagglutinin (and neuraminidase)</u>,
- 2. due to lack of proof-reading in RNA-dependent RNA polymerase,
- 3. change <u>3D conformation/tertiary structure</u> of haemagglutinin (and neuraminidase),
- 4. cannot be detected by **existing** memory B cells / antibodies present in the immune system, (thus same strain can infect the same person who is vaccinated previously)

OR

- 1. <u>Antigenic shift</u> where **two (or more)** different strains of the influenza virus infects the **same host cell**
- 2. There is reassortment of the viral RNA segments,
- 3. (giving rise to a new combinations of RNA segments in new viral particles, hence) **new combinations** of surface antigens haemagglutinin and neuraminidase arises; a new virus strain results,
- 4. cannot be detected by **existing** memory B cells / antibodies present in the immune system.

- (b) With reference to the influenza virus and active immunity, define a vaccine and state how it provides protection against the virus.
-[2]
- 1. A vaccine contains antigen consisting of dead/attenuated <u>influenza</u> virus/ parts of the influenza virus.
- which stimulates artificial active immunity by production of antibodies by plasma cells/production of memory cells after introduction/injection into body; [Reject: vaccines produce antibodies]

Vaccinations can control diseases by resulting in *herd immunity*, in which large percentage of population which has become immune to the virus through vaccinations, provides a measure of protection for individuals who are not immune.

(c) Explain why.

[1]
1. (When a large number of individuals are immune,) chains of infection are likely to be disrupted, which stops or slows the spread of disease.

/ The more people who are immune, the smaller the probability that those who are not immune will come into contact with an infectious individual.

Tuberculosis (TB) is an infectious disease that generally affects the lungs. Most infections are latent and do not have symptoms. Latent infections can progress to active form of the disease which, if left untreated, kills about half of those infected.

The most common classic symptom of active TB is chronic cough with blood-containing sputum.

(d) Name the organism which causes tuberculosis (TB).

.....[1]

1 Mycobacterium tuberculosis

In the alveoli tissues, the bacteria that causes TB binds directly with mannose receptors on alveolar macrophages using a bacterial glycolipid, and is engulfed by alveolar macrophages.

(e) With reference to a cellular organelle in the macrophages, describe how macrophages attempt to process engulfed bacteria.

.....[2]

- 1. <u>Lysosomes</u> contain **hydrolytic enzymes**, (e.g. proteases), which hydrolyzes bacteria
- 2. after lysosomes fused with phagosomes/endocytic vesicle containing bacteria

- (f) Describe the cause of the chronic cough symptom of TB in its active stage.
- I. Bacteria destroy alveoli/causes cavities in the lungs; this leads to less surface area for diffusion of gases.

OR

1. Damaged areas (may become infected with other bacteria) form pockets of pus; this **increases diffusion distance** between alveolar sac and alveolar capillaries

[Q7: 10 marks]

Proteaceae are a family of flowering plants predominantly distributed in the tropical and sub-tropical regions of Australia and South Africa. Threatened species may be vulnerable to climate change, because of their narrow temperature tolerance, small population sizes and restricted distributions.

A study modelled climate-induced changes on the range size (geographical distribution of a species) of 282 Proteaceae species. Figures 8.1a and 8.1b show the time course of range changes for wind-dispersed (**A**) and ant-dispersed (**B**) Proteaceae species (under 'full migration' dispersal assumptions, which assumes no limitation to migration). Error bars represent standard error (for example, smaller standard errors indicates that the observations are closer to the actual value).







- (a) Describe the differences in climate-induced changes in range size between the wind-dispersed and ant-dispersed Proteaceae species from Year 2000 to 2050.
- [2]
 1 The range size for wind-dispersed Proteaceae species decreases from 2350 square hectares (accept 2300 2400) in year 2000 to 1000 square hectares in year 2050 while
 - 2 the range size for ant-dispersed species **decreased** from 1600 to 1300 square hectares from year 2000 to 2020 but **increased back** to 1600 square hectares in year 2050.
- (b) The Intergovernmental Panel for Climate Change (IPCC) predicted an increase of 2°C in global temperatures by 2050. Suggest reasons for the larger range size of ant-dispersed Proteaceae species compared to that of wind-dispersed species in 2050.

.....[4]

[Ant-dispersed species] 1 Increase in temperature increases rate of metabolism / development of insects such as ants

Explanation for students' understanding:

Higher rate of metabolism causes ants to be more physically active and travel further distances in a day;

Higher rate of development of insects leads to eggs hatching and developing into adults faster, thus, increasing population size faster. The larger the ant colony size, the larger the foraging range

2 This increases the **distance/area** over which the **ants can disperse the seeds** of the ant-dispersed Proteaceae species.

OR

- 1 Increase in temperature may cause some ant colonies to migrate to higher latitudes/cooler regions
- 2 This increases the **area** over which the **ants can disperse the seeds** of the ant-dispersed Proteaceae species.

[Reject: This increases the range size of ant-dispersed species]

AND

[Wind-dispersed species]

- 3 The increase in temperature may result in **changes in wind patterns** (e.g. less wind / wind is less strong),
- 4 This restricts the **area** over which the wind can disperse the seeds of winddispersed species.

OR

- 3 Plants also adapt to increased temperatures by reducing the number of stomata per leaf (to reduce amount of water lost via evapo-transpiration).
- 4 The rate of photosynthesis decreases, and the populations of wind-dispersed species may reduce in number and hence range size.

It was predicted that not only will the range size for ant-dispersed Proteaceae species remain high in 2050, it is likely that the plants would colonise new regions, particularly in the higher latitudes and altitudes.

(c) Explain why the Proteaceae species can potentially threaten the native species of the regions they expand into.

.....[2]

- 1 Proteaceae species may become invasive species which **compete** more successfully with the native species for resources such as nutrients, sunlight and space (name at least one).
- 2 The **absence of natural predators** / herbivores which can feed on the new Proteaceae species will allow the plants to grow unchecked / more vigorously than native species (which have natural predators that control their population size).
- 3 [only award mark if point 1 or 2 is listed] This can cause the extinction of native species in these regions / the **survival** of native species is affected

Many indigenous cultures in tropical regions have used Proteaceae for medicinal preparations. For example, bioactive ingredients can be obtained from infusions of the roots, bark, leaves, or flowers of many Proteaceae species, which can used as topical applications for skin conditions or internally as tonics, aphrodisiacs, and medicines to treat headaches, cough, diarrhea, indigestion, stomach ulcers, and kidney disease.

(d) Discuss how climate change can affect the biodiversity of the Proteaceae species and its consequence to the production of biomedicines.

.....[2]

- 1 Tropical species are often sensitive to hot weather as many species have evolved to become **thermal specialists** *I* have a narrow temperature tolerance.
- 2 Hence they are very **vulnerable** / cannot adapt **to climate warming** [Reject: climate change as it is lifted from question stem]
- 3 With the <u>loss of biodiversity</u> due to climate changes, production of biomedicines decreases / potential uses of Proteaceae species for biomedical usage may be **lost forever**.

[Q8: 10 marks]

Civics Group	Index Number	Name (us	SE BLOCK LETTERS)		H2
		ST. AI	NDREW'S JUNIC 2017 JC2 PRE	R COLLEGE LIMS		
H2 BIOL	OGY				9	9744/3
Paper	3 (Mark Sc	heme)				
Monday		18 ^t	^h September 201 [°]	7	2	hours
Additiona	l Materials: An Co	iswer Pap over Shee	per et for Section B			
READ THES	E INSTRUCTIO	ONS FIRS	т			
Write your na hand in. Write in dark You may use Do not use st	me, civics grou blue or black pe a soft pencil fo aples, paper cli	p and ind en on botl r any diag ps, highliq	ex number on all h sides of the pap gram, graph or rou ghters, glue or co	the work you er. ugh working. rrection fluid.		
Section A (S Answer all qu Write your an	tructured Que stions. swers in the sp	stions) aces prov	vided on the ques	tion paper.		
Section B (E	ssay Question)			For Exami Use	ners'
Answer one a Write your an	essay question. swers on the se	eparate a	nswer paper prov	ided.	Section A	
All working fo	r numerical ans	swers mus	st be shown.		1	/17
					2	/18
					3	/15
Conceptual (CE)	error Lack of Keywor	ds (K)	Misreading the question (Q)			
			consists of 4.4		Total	/50
	I NIS (locument	i consists of 14 pr	inted pages.	гт	urn over

Section A

Answer **all** the questions in this section.

QUESTION 1

Leigh disease is an inherited neuro-metabolic disorder that affects the central nervous system. As the disease progresses, the muscular system is debilitated throughout the body, as the brain cannot control the contraction of muscles. Leigh disease can be caused by a deficiency of the pyruvate dehydrogenase complex (PDHC), most commonly due to a mutation in the X-linked gene, PDHA1, which codes for the PDHC α -subunit.

A married couple (both of whom are normal individuals) is concerned about their chances of having a child with Leigh disease because the woman's father had the disease.

(a) Draw a genetic diagram with appropriate symbols to determine the probability that they will have an affected child.

					[6]
Parental phenotype: Parental genotype:	Normal X ^D Y	male ⁄	X X	Norr	nal female X ^D X ^d
Gametes:	XD	Y		XD) (Xd)
F1 genotypes: F1 phenotypes: F1 phenotypic ratio:	X ^D X ^D Normal girl 1	X ^D X Carrier : 1	d girl	X ^D Y Normal boy : 1	X ^d Y Leigh disease boy : 1

Accept: 2 Normal girl

Probability of having an affected child = 1/4

Mark scheme:

- 1 Parental genotypes (showing X-linked symbols)
- 2 Gametes (circled)
- 3 F₁ genotypes
- 4 F1 phenotypes corresponding to genotypes
- 5 F1 Phenotypic ratio
- 6 Probability = 1/4 [Award mark if genotype is wrongly written]

[No marks for Points **1-5** if genotype is written wrongly e.g. "X" represents normal allele; "x" represents diseased allele; "X" represents allele for disease; "X^o" represents allele for no disease]

200 couples with the same genotypes as the above-mentioned couple were included in a genetic study of the Leigh disease. They had a total of $\frac{326}{322}$ children with the following phenotypes:

85 normal girls70 carrier girls89 normal boys78 boys with Leigh disease

(b) A chi-squared test was carried out to test the significance of the difference between the observed (O) and the expected (E) results.

$$\chi^2 = \sum \frac{(0-E)^2}{E}$$

(i) Calculate the χ^2 value. Show your working.

$$\chi^2 = \frac{3.5^2}{80.5} + \frac{11.5^2}{80.5} + \frac{7.5^2}{80.5} + \frac{3.5^2}{80.5}$$

= 2.596 (3 d.p.) (Follow the chi sq table) / 2.60 (2 d.p.)

OR

$$\chi^2 = \frac{(85 - 80.5)^2}{80.5} + \frac{(70 - 80.5)^2}{80.5} + \frac{(89 - 80.5)^2}{80.5} + \frac{(78 - 80.5)^2}{80.5}$$

- 1 Correct working
- 2 Answer given to 3 d.p. or 2 d.p.

Fig. 1

Degrees of		probability									
Freedom	0.99	0.95	0.90	0.75	0.50	0.25	0.10	0.05	0.01		
1	0.000	0.004	0.016	0.102	0.455	1.32	2.71	3.84	6.63		
2	0.020	0.103	0.211	0.575	1.386	2.77	4.61	5.99	9.21		
3	0.115	0.352	0.584	1.212	2.366	4.11	6.25	7.81	11.34		
4	0.297	0.711	1.064	1.923	3.357	5.39	7.78	9.49	13.28		

(ii) Fig. 1 shows the table of probabilities. State the probability that the observed results does not differ significantly from the expected results.

.....[1] 1 0.50 > p > 0.25

3

(iii) State what conclusions may be drawn from the probability found in (ii).

[3] [No error carried forward if (ii) is wrong]

- 1 Since 0.50 > p > 0.25 is greater than 0.05,
- 2 there is no significant difference between the observed and the expected values [Reject: ratio] at the 5% level. Any observed differences is due to chance.
- 3 The predicted ratio of 1 : 1 : 1 : 1 is correct / the inheritance is sex-linked [Reject: there is mendelian inheritance]

[Reject: there is independent assortment of the 2 genes \rightarrow this is a monohybrid cross and independent assortment is not relevant to this case]
Leigh disease can also be due to a mutation in the mtDNA (mitochondrial DNA) affecting the MT-ATP6 gene. The mutated allele results in the production of a non-functional subunit in the ATP synthase complex which allows protons to pass through the channel without generating the proton motive force.

- (c) State whether a mitochondrion containing the mutated allele would have a higher, lower or equal oxygen consumption rate relative to a mitochondrion which has the normal allele.
-[1] 1 Equal / Higher

(d) Explain your answer in (c).

.....[2]

[Explanation for Equal rate]

- 1. Oxygen acts as a <u>final electron acceptor</u> [Reject: final electron carrier] (in oxidative phosphorylation).
- Since the flow of electron through the ETC is not affected by the mutation / the donation of electrons from NADH and FADH₂ to the ETC is not affected, (the rate of oxygen consumption in the mutated mitochondrion will remain the same.)

OR

[Explanation for Higher rate]

- 1. Ref. reduction in ATP production in oxidative phosphorylation,
- 2. Therefore, rate of oxidative phosphorylation increases to **regenerate NAD⁺ for use in glycolysis** for alternative ATP production

The DNA sequence of the MT-ATP6 gene has remained relatively conserved over a long evolutionary period. It is present in a wide diversity of organisms, ranging from the simplest worms to the great apes. Differences in the nucleotide sequence of the gene in different species can be identified using multiple sequence alignments.

(e) Discuss how the comparison of MT-ATP6 DNA nucleotide sequences of different species demonstrates evolutionary change.

1 The **similarities** in the nucleotide sequences shows molecular <u>homology</u>

- / which suggests inheritance from a <u>common ancestor</u>.
- 2 As the descendants of same species evolve independently, more and more differences (mutations) are **accumulated** in their DNA.
- 3 Resulting in divergent evolution and descent with modification.

Examiner's comment:

Students who stated that "the more similar the sequences are, the more closely related the organisms are to each other" have misinterpreted the question to mean "explain how molecular homology demonstrates evolutionary relationship between different species"

QUESTION 2

Celiac disease is an autoimmune disorder that is caused by an improper immune response to the protein gluten, found in wheat, rye, and barley, that damages the lining of the small intestine. There is no cure for celiac, and the only effective treatment is a gluten-free diet.

Recent studies indicated that harmless intestinal viruses, such as the reovirus, can cause the immune system to overreact to gluten, raising the possibility of such viruses contributing to the development of the disease.

Fig. 2.1 shows the general structure of a reovirus. Unlike Human Immuno-deficiency Viruses (HIV), reoviruses are not retroviruses.



Fig. 2.1

(a) With reference to Fig. 2.1, contrast the structure of the reovirus with that of HIV.

-[2]
 - 1 Reovirus has no viral envelope but HIV has a viral envelope
 - 2 Reovirus has double-stranded RNA genome but influenza virus has singlestranded RNA genome
 - 3 Reovirus has 2 capsids while HIV has 1 capsid
 - 4 Segmented genome vs HIV non-segmented genome
 - 5 Absence of viral enzymes in Reovirus but presence of viral enzymes (list at least 2: integrase, protease, reverse transcriptase) in HIV
 - [Any 2]
- (b) Suggest how new double-stranded viral RNA genome is synthesized during the reproductive cycle of a reovirus.
-[2]
- 1 Viral RNA **unzips** to form single stranded RNA
- 2 **Viral** RNA-dependent **RNA polymerase** is used to make new RNA strands from RNA template by **complementary base pairing**

Researchers also looked at patients with celiac disease and found that they had much higher levels of antibodies against reoviruses than those without the disease.

Those with higher levels of antibodies also had higher levels of the molecule IRF-1 (interferon regulatory factor 1), a regulator of gene transcription which plays a key role in the loss of gluten tolerance.

(c) Describe three ways in which the structure of antibodies contribute to its function.

- 1 Constant region; determines the class of antibody (e.g. IgG, IgA, IgM etc) / allows binding to phagocyte (e.g. macrophage/neutrophil)
- Variable regions (of each light chain and heavy chain); forms an antigen binding site that provides a lock-and-key fit for **specific binding** to a particular epitope of an antigen.
- 3 **Disulfide bridges** between chains; **links** the heavy and light chains together
- 4 Hinge region; provides flexibility to change orientation/movement to bind antigen

Examiner's comment:

Students are reminded to write both structure and function.

[Reject: different combinations of heavy and light chains allow binding to different antigens on pathogens \rightarrow this addresses why a huge variety of pathogens can be recognised]



Fig. 2.2 shows the structure of an immunoglobulin gene which codes for antibodies.

Fig. 2.2

- (d) With reference to Fig. 2.2, explain how variability at the DNA level result in variability in the antigen-binding sites of antibodies.
-[3]
- 1 Somatic recombination (at the DNA level) of both light and heavy chain genes occurred
- 2 only **one V segment** and **one J segment** are arranged together, along with the C segment for the **light chain gene**
- 3 only one D segment and one J segment are first arranged together, followed by one V segment being arranged next to the pre-arranged DJ region, along with the C segments for the heavy chain gene

Reject: class switching as it does not affect the variable regions

- (e) Antibodies are proteins. Draw a diagram of the monomer which makes up antibodies. There is no need to annotate the diagram.
- [1]
 1 Correct structure of amino acid drawn showing the amino group, carboxyl group, alpha carbon, R group side chain and hydrogen atom



[Accept: zwitterion diagram] H[Reject: repeating n units of monomers linked together (-HN – C – COO-)n] Antibodies are secreted by plasma cells. R

Fig. 2.3 shows a plasma cell and a B lymphocyte.



Fig. 2.3

(f) With reference to Fig. 2.3,

(i) State 2 ways in which the structure of plasma cell differs from the B lymphocyte.

-[2] 1. plasma cell has Golgi apparatus but B lymphocyte does not; / plasma cell has Golgi vesicles / secretory, vesicles but B lymphocyte does not:
 - 2. plasma cell has **more** (rough) endoplasmic reticulum;
 - 3. plasma cell has more ribosomes;
 - 4. plasma cell has more mitochondria;
 - 5. plasma cell is larger at $7\mu m$ vs $4\mu m$ / contains **more** cytoplasm; [Any 2]

(ii) Explain the reasons for the differences you described.

.....[2]

check answer against (i)

1. [plasma cell has Golgi apparatus but B lymphocyte does not] Enables packaging / chemical modification/ adding carbohydrate / making glycoprotein to form antibody

/ [plasma cell has Golgi vesicles / secretory, vesicles but B lymphocyte does not] Enables for transport of antibody to plasma membrane for secretion from cell

- 2. [plasma cell has more (rough) endoplasmic reticulum] Enables fast / large production of protein / antibodies
- 3. [plasma cell has more ribosomes] Enables fast / large production of antibodies which are **proteins**
- 4. [plasma cell has more mitochondria] Provides more ATP which releases energy upon hydrolysis for production of protein / antibody
- 5. [plasma cell is larger / contains more cytoplasm] Allows more space for organelles e.g mitochondria, rER, Golgi apparatus etc

[Any 2 corresponding to (i) responses]

A student researcher tried to reproduce the results of the study regarding elevated antibody levels against reoviruses. She studied the levels of antibodies in 3 human subjects infected with reoviruses and another 3 who are not infected.

Table 2.4 shows his results.

l able 2.4		
	Antibody titre / unit ml ⁻¹	
Subjects not infected with reovirus	30 ± 10	
Subjects infected with reovirus	122 ± 25	

- - - - -

- (g) With reference to the results on subjects not infected with reoviruses, explain what is standard deviation and its implications.
-[3]
- 1 Standard deviation is the **deviation from the mean** antibody titre
- 2 and determines the **range** of antibody titre observed in subjects not infected with reovirus
- 3 [Evidence]The antibody titre ranges from 20 (30 10) to 40 (30 + 10) unit ml⁻¹ in subjects not infected with reovirus / the antibody titre deviates 10 unit ml⁻¹ from the mean of 30 unit ml⁻¹;
- 4 Standard deviation is an indication of the reproducibility of the data collected. Since standard deviation for subjects not infected is high, results are not very reproducible / the data don't change too much when the experiment is **repeated**

[Q2: 23 18 marks]

QUESTION 3

(a) A student investigated growth in the roots of broad bean, *Vicia faba*. The student cut sections of the root tip of this plant and viewed them with a light microscope.

Fig. 3.1 is a photomicrograph of one of the sections. The cell labelled \mathbf{D} is in interphase.



Fig. 3.1

Complete the table below by:

- naming the stages of mitosis in the correct sequence following interphase
- identifying one example from the cells labelled A to H that is in each stage of mitosis that you have named.

.....[3]

Mark scheme:

- 1 one mark for the stages of the cell cycle in the correct sequence ie. Prophase, metaphase, anaphase and telophase under column heading "stage of mitosis";
- 2 one mark for each two correct matching of each stage with a cell ; (mark for 1st answer given)

stage of mitosis	label from Fig. 3.1
prophase	A / H ;
metaphase	G ;
anaphase	C / E / F ;
telophase	B ;

(b) In animal cells, centrioles are responsible for assembling microtubules to make the spindle at the beginning of mitosis.

Describe the role of the spindle during mitosis.

- (kinetochore) microtubules attached to <u>kinetochore</u> on <u>centromere</u> of duplicated chromosomes (during prophase);
- 2 arranging / aligning / orienting / AW, duplicated chromosomes at the equator / metaphase plate ; [**REJECT**: centre of the cell]
- 3 centromere divides ; microtubules shorten; sister chromatids/chromosomes pulled to opposite poles [Reject: opposite ends] (of cell);
- **4** Ref. Equivalent and complete collection of chromosomes at both poles of the cell;

OVP:

- 5 Overlapping/polar microtubules (slide past each other) elongate the cell;
- (c) Scientists investigated three genes, P, Q and R, involved in controlling cell division. They studied the effect of mutations in these genes on the risk of developing lung cancer.
 - (i) Suggest the differences in the cell cycle of a cancer cell compared with that of a normal cell of the same type.
 - 1 Cell cycle shorter / interphase shorter / division more frequent ; [Reject: cell cycle is irregular / inappropriate]
 - 2 (cell cycle) **checkpoints** not controlled / unregulated ; / uncontrolled cell division / AW ;
 - **3** Cell cannot be stimulated to undergo apoptosis (even when errors occur during the cell cycle);

The scientists analysed genes, P, Q and R from healthy people and people with lung cancer.

- If a person had at least one copy of normal allele for a gene, they used the symbol N.
- If a person had two mutant alleles for a gene, they used the symbol M.

They used their data to calculate the risk of developing lung cancer for people with different N and M genotypes. A risk value of 1.00 indicates no increased risk. **Table 3.1** shows the scientists' results.

Gene P	Gene Q	Gene R	Risk value of developing lung cancer / AU
N	N	N	1.00
М	N	N	1.30
N	N	М	1.78
N	М	Ν	1.45

Table 3.1

N = at least one copy of the normal allele is present

M = two copies of the mutant allele are present

(ii) What do these data suggest about the relative importance of the mutant alleles of genes P, Q and R on increasing the risk of developing lung cancer?

.....[2]

- 1 [general trend] order of mutant alleles that increases the risk of developing lung cancer from highest to lowest : R > Q > P ;
- 2 [quote data to support ans] Being homozygous for the mutation in R produces highest risk of <u>1.78</u> A.U.; in Q produces next highest risk of <u>1.45</u> A.U.; in P produces least risk of <u>1.30</u> A.U.

Chemotherapy is the use of a drug to treat cancer. An experiment was set up to study a new drug, SA128, which kills dividing cells. A group of four men suffering from lung cancer was given the drug. The number of cancer cells per unit volume of blood was measured right before treatment and again after the 2-week treatment.

	Number of cancer cells per unit volume of blood					
Subject	TSH	OON	CCK	TTS	Average	Standard deviation
Before drug treatment	2000	1600	1800	1200		
After drug treatment	1000	500	800	300		

Table 3.2 : Effect of SA128 on cancer cells.

(iii) Calculate the average and complete Table 3.2.

[1]

	Number of cells per unit volume of blood					
Subject	TSH	OON	ССК	TTS	Average	Standard deviation
Before drug treatment	2000	1600	1800	1200	<mark>1650</mark>	<mark>342</mark>
After drug treatment	1000	500	800	300	<mark>650</mark>	<mark>311</mark>

(iv) Using the formula below, calculate the standard deviations of number of cancer cells per unit volume of blood before and after drug treatment, SA128. Complete Table 3.2. Express your answers to 3 significant figures.

.....[1]

standard deviation s

$$x = \sqrt{\frac{\sum (x - \overline{x})^2}{n - 1}}$$

<u>Legend</u> \sum is 'Sum of' x is observation \overline{x} is the mean n is the sample size (number of observations) (v) Using the formula below as well as the average and standard deviation values calculated in (c)(iii) and (c)(iv),



Calculate the t_{calculated} value.

.....[1] 1. One mark for correct calculation of t_{calculated} value (step 2) – 4.327 (3 dp)

Examiner's comments:

No error carried forward if values calculated in (iii) and (iv) are wrong unless wrong dp.

(vi) Using the critical t-values in **Table 3.3**, determine if there is a significant difference in the results using the new drug, SA128.

.....[2]

- 1 One mark for correct calculation of **DoF** (Ans: <u>6</u>) and identification of t_{critical} value of <u>1.943</u> (step 3, 4)
- 2 One mark for correct comparison of t_{calculated} and t_{critical} and making a correct conclusion that there is significant difference in the results for before and after treatment with drug, SA128. (step 5)

t_{calculated} of 4.327 is <u>higher than</u> t_{critical} (1.943). There is a <u>significant difference</u> between the results before and after treatment with drug, SA128.

Examiner's comments:

No error carried forward if DF or T critical is wrong

Working for Reference:

Step 1: Null hypothesis:

There is <u>no significant difference</u> between the results before and after treatment using the new drug, SA128.

Step 2: Calculation of experimental t-value $x_1 - x_2 = 1650 - 650 = 1000$ $(s_1)^2 = (342)^2 = 116964$ $(s_1)^2 / n = 116964 / 4 = 29241$ $(s_2)^2 = (311)^2 = 96721$ $(s_2)^2 / n = 96721 / 4 = 24180$ $t = 1000 / \sqrt{29241 + 24180}$ t_{calculated} = **4.327 (3 dp according to T table)**

Step 3: Calculation of Degrees of Freedom Degrees of Freedom is sum of sample sizes for control and test minus 2 = 8 - 2 = 6.

Step 4: Stating of t_{critical} value (from Table of significance, looking at p=0.05) t_{critical} value for 6 DoF and p value 0.05 is **1.943**.

Step 5: Comparison between t_{calculated} and t_{critica}l t_{calculated} of 4.327 is <u>higher than</u> t_{critical} (1.943). Null hypothesis is not accepted.

Conclusion:

There is a <u>significant difference</u> between the results before and after treatment with drug, SA128.

df	.10	.05
1	3.078	6.314
2	1.886	2.920
3	1.638	2.353
4	1.533	2.132
5	1.476	2.015
6	1.440	1.943
7	1.415	1.895
8	1.397	1.860
9	1.383	1.833
10	1.372	1.812
11	1.363	1.796
12	1.356	1.782
13	1.350	1.771
14	1.345	1.761
15	1.341	1.753
16	1.337	1.746
17	1.333	1.740
18	1.330	1.734
19	1.328	1.729
20	1.325	1.725
21	1.323	1.721
22	1.321	1.717
23	1.319	1.714
24	1.318	1.711
25	1.316	1.708
26	1.315	1.706
27	1.314	1.703
28	1.313	1.701
29	1.311	1.699
30	1.310	1.697
40	1.303	1.684
60	1.296	1,671
120	1.289	1.658
с	1.282	1.645

Table 3.3: t-test table of t critical values

[Q3 Total: 15]

Section B

Answer **one** question in this section.

Your answers should be illustrated by large, clearly labelled diagrams, where appropriate.

Your answers must be in continuous prose, where appropriate.

Your answers must be set out in sections (a), (b) etc., as indicated in the question.

- 4 (a) The effects of global warming on the spread of malaria beyond the tropics is a debatable issue. Discuss the arguments/evidences that support your stance on the matter, and provide a balanced account of counter-arguments.
 - (b) Discuss the effects of climate change (as a result of greenhouse gas emissions. [13]

[Q4 Total: 25]

- **5** (a) Explain what is meant by primary, secondary, tertiary and quaternary structure of haemoglobin. [13]
 - (b) Using a named example, discuss how genetic variation may be preserved in natural population by heterozygote advantage. [12]

[Total: 25]

4(a) The effects of global warming on the spread of malaria beyond the tropics is a contentious issue. Discuss the arguments/evidences that support your stance on the matter, and provide a balanced account of counter-arguments. [12]

Arguments and evidences **supporting** the theory that global warming causes spread of malaria beyond the tropics

- 1 Malaria can only occur in climates where mosquitoes are present to transmit the disease.
- 2 Global warming leads to temperatures in the sub-tropical regions becoming more optimal for both mosquitoes and malaria parasite.
- 3 For the optimal development of Anopheles mosquitoes, the temperatures should be **around 20 to 30°C**.
- 4 Ref. higher temperature leads to higher rate of **development** / metabolic rate / survival of **both mosquitoes**
- 5 Ref. **parasite** plasmodium development peaks at 26°C (thus, the closer the subtropical temperatures is to 26°C, the higher the rate of plasmodium development)
- 6 In addition, global warming can lead to higher humidity (in the sub-tropics), more stagnant pools of water available for mosquitoes to lay eggs
- 7 [Observation on sub-tropical regions] There was an apparent association between an **increased incidence in malaria and increased minimum temperatures** during December - January of the preceding year.
- 8 [Observation on sub-tropical regions] A rise in minimum temperature of 1°C, from 15.5 to 16.5°C could account for 24 additional cases per 1000 of population.
- 9 [concluding remark] The above effects of temperature and climate on malaria have led many to believe that global warming will result in the spread of this disease, to higher altitudes and higher latitudes (sub-tropical regions) where it is cooler.
- 10 AVP

[Max 8 marks]

Arguments and evidences **against** the theory that global warming causes spread of malaria beyond the tropics

- 1 (There is common assumption that a faster development of plasmodium parasite leads to higher rate of transmission. But a latest study shows that temperature has a more complex effect.) As temperature rises, **parasites** do develop faster, but fewer of them become infectious.
- 2 In sub-tropical regions, an increase of temperature to above 24°C led to higher parasite development but a decrease in malaria risk.
- 3 This is because the **parasite may not be able to cope** with the **higher temperatures** or
- 4 Mosquito immune systems may work better at warmer temperatures.
- 5 Many other arguments and evidence have not taken into consideration other more significant factors in the recent upsurge of malaria, such as the **resistance to**

drugs, poor vector control, changes in land uses and human population growth and migration.

- 6 Ref. elaboration based on any of the other significant factors
- 7 AVP:
- a. [Poor vector control] Ref. less use of DDT insecticides → more proliferation of mosquitoes
- b. [Changes in land use] Ref. extension of villages of towns, deforestations, building of dams, human populations have come closer to the wilderness and hence are in closer proximity to mosquito breeding grounds
- c. [Population growth] An increase in population will result in increase in malaria, if there is no simultaneous improvement in healthcare facilities and living standards
- d. [Migration] Migration and human travel can cause the spread of malaria from one area to another (where the people have low immunity to the disease)

QwC: at least 3 points in each set of arguments

4(b) Discuss the effects of climate change (as a result of greenhouse gas emissions).

- 1 Emission of greenhouse gases result in global warming.
- 2 Which causes the melting of ice sheets and sea ice.

[Any 2]

<u>Rise in sea level</u>

- 3 Melting of ice sheets results in extra water entering the ocean, leading to **increase in sea level.**
- 4 Melting of sea ice can lead to a positive feedback loop which **further** accelerates the melting of polar ice caps.
- 5 As oceans become warmer, the water also expands, increasing the volume of the ocean water and leading to further rise in sea level.
- 6 Low-lying islands / coastal regions may become submerged.

[Any 1 pair]

Stress on fresh-water supplies

- 7 Many island nations will have their supplies of **drinking water reduced** because sea water will invade their freshwater aquifers.
- 8 **Melting of** fresh water (i.e. **ice sheets**) into the sea also **turns the already scarce fresh water into salt water**, decreasing the freshwater availability.
- 9 Global warming also results in **more evaporation**, which leads to increased amount of water in the atmosphere, and hence **heavier rainfall**.
- 10 Which leads to more rapid movement of water from the atmosphere back to the oceans, reducing our ability to store and use it.
- 11 At higher temperature, **more precipitation** will occur **as rain** rather than snow.
- 12 **Reservoirs fill quickly** to maximum capacity. **Any excess rain water is lost** as runoff which cannot be stored / Less water will penetrate the ground surface, causing reduced soil moisture and reduced groundwater replenishment.

[Any 2]

Heat-waves and heavy rains

- **13** A warmer climate creates an atmosphere that can **collect**, **retain**, **and drop more water**, **changing weather patterns**.
- 14 This causes wet areas to become wetter and dry areas become drier. /This can lead to **heat waves** and **heavy rain**.
- 15 Heatwaves associated with low humidity may result in wildfire.
- 16 Heavy rain increases the amount of runoff into rivers and lakes, washing sediment, nutrients, pollutants, trash, animal waste, and other materials into water supplies, making them unusable, unsafe, or in need of water treatment.

[Any 2]

Death of coral reefs

- 17 Heat stress can cause **coral bleaching** / coral polyps expel the zooxanthellae.
- 18 This is because at higher temperatures, **zooxanthellae photosynthesis is disrupted** / zooxanthellae produces more toxic compounds

[13]

- 19 If temperatures remain above the bleaching threshold for prolonged periods of time, **corals will eventually die** from starvation and disease.
- 20 **Ocean acidification** affects hard corals as they cannot absorb the calcium carbonate to maintain their skeletons (and the stony skeletons that support corals will dissolve).

[Any 2]

Migration of fishes and insects

- 21 Rising ocean temperatures can directly affect the metabolism, life cycle, and behaviour of marine species.
- 22 Many **fish species**, especially their young and larvae, have highly specific temperature ranges and will **move to cooler waters to survive**.
- 23 Warmer temperatures may **disrupt the migratory behaviour and timing of several fish species** (for example, by impeding their ability to orient themselves for effective navigation.)
- 24 **Insect distribution and migration** are also greatly influenced by increased temperatures as they are cold-blooded and will **move to habitats with temperatures that are optimal for their growth and reproduction**.
- 25 These insect migrations have **ramifications for many ecosystems**, e.g. insects play important ecological functions such as pollination, vectors of diseases and parasites, killing of other pest insects or are crop pests themselves (name one examples).

[Any 2]

Release of greenhouse gases in frozen organic matter

- 26 Global warming leads to accelerated melting of permafrost.
- 27 As permafrost thaws, the **frozen organic matter starts to decay** and is digested by microbes. The digestion **releases carbon dioxide and methane**.

QwC: Obtain at least 1 mark each from 3 different categories of effects of climate change.

5(a) Explain what is meant by primary, secondary, tertiary and quaternary structure of haemoglobin. [13]

Primary structure

- 1 Refers to the type, <u>number</u> and <u>sequence</u> of amino acids in a linear polypeptide chain
- 2 making up each haemoglobin polypeptide (individual α and β subunits)
- 3 ref (each α -chain is) <u>141</u> amino acids long and (each β -chain is) <u>146</u> amino acids long
- 4 <u>Peptide bond</u> involved in joining all amino acid monomers together

Secondary structure

- 5 Refers to the folding of the polypeptide into **regular structures**
- 6 α -helices / coiling of polypeptide chain into a regular helical conformation.
- 7 <u>Hydrogen bonds</u> between peptide bonds found within the **same** polypeptide chain, **between C=O group** on the peptide bond of one amino acid the **NH** group on peptide bond of another amino acid

Tertiary structure

- 8 the folding of the polypeptide chain into its unique **3-dimensional shape**; / ref. **globular** shape of haemoglobin
- 9 Amino acids far away in primary structure are brought close together (by R group interaction);
- 10 Non-polar/hydrophobic (side chains of) amino acids are buried in the interior; Polar and charged/hydrophilic (side chains of) amino acids are on the surface;
- 11 Bonds involved include hydrophobic interactions, hydrogen bonds and ionic bonds **between R groups of amino acids** in each polypeptide chain (name at least 2)

Quaternary structure

- 12 Refers to the arrangement of the polypeptide subunits within a protein that is made up of more than one polypeptide chain
 - / spatial arrangement of more than one polypeptide chain
- 13 ref. to the **association** of 2α and 2β subunits to form functional haemoglobin molecule
- 14 Bonds involved include hydrophobic interactions, hydrogen bonds and ionic bonds between R groups of amino acids **in the four subunits / between the 4 polypeptide chains** (name at least 2)

Teachers' comments:

It is important to state the definitions of each level of folding and tailor your points to the haemoglobin case study. Note that **disulfide bonds are not present** in haemoglobin.

QwC: Obtain at least 1 mark each from 4 different levels of protein structure

5(b) Using a named example, discuss how genetic variation may be preserved in natural population by heterozygote advantage. [12]

Hb^sHb^s individuals

- 1 Individual homozygous for Hb^S / genotype Hb^SHb^S suffer from <u>sickle cell</u> <u>anaemia</u>
- 2 the normal haemoglobin (haemoglobin A) in red blood cells is replaced entirely by abnormal haemoglobin (haemoglobin S).
- 3 Sickled red blood cells tend to stick together and obstruct blood flow in capillaries, depriving multiple organs of oxygen, resulting in organ damage / Sickled red blood cells rupture easily, resulting in anaemia and fatigue. May result in early death.
- 4 Therefore, there is a higher potential for the removal of the Hb^s allele from the gene pool.

Hb^AHb^A individuals

- 5 <u>Hb^AHb^A</u> individuals are at greater risk of dying of malaria compared to heterozygotes.
- 6 Therefore, there is a higher potential for the removal of the Hb^A allele from gene pool.

Hb^AHb^S individuals

- 7 Individual who are heterozygous / genotype Hb^AHb^S suffer from sickle cell trait
- 8 produce both normal and abnormal haemoglobin
- 9 <u>Hb^AHb^s</u> individuals are a <u>selective advantage</u> and are able to survive and reproduce in malaria-infected areas.]
- 10 (This condition is known as heterozygote advantage as) it preserves the Hb^s allele in the population
- 11 A mosquito transmits *Plasmodium*, the malaria parasite to humans. The malaria <u>parasite</u> spends part of its life cycle in red blood cells.
- 12 When malaria parasite invade the bloodstream, the red blood cell that contains haemoglobin S is sickled-shaped and is **quickly destroyed by the body**, **trapping the parasites within the sickle cell and stopping the infection**.
- 13 The slowdown in blood flow in sufferers of sickle cell trait also hampered the parasite's ability to travel and rapidly infect new cells.

QwC: Mentioning of how Hb^AHb^A and Hb^SHb^S are at selective disadvantage and how heterozygotes are at selective advantage / Points touching on individuals of each of the 3 genotypes

THE END

	Index Number	Name (use BLOCK LETTERS)		H2
		ST. ANDREW'S JUNIOR COLLEGE 2017 JC2 PRELIMS		
H2 BIOLC	DGY			9744/4
Paper 4	4: Practica	II Exam		
Friday		25th August 2017	2 hours 30 r	minutes
READ THESE	INSTRUCTIO	NS FIRST		
Write your nar hand in. Write in dark b You may use a Do not use sta Answer all que Write your ans	ne, civics group blue or black pe a soft pencil for aples, paper clip estions. swers in the spa	o and index number on all the work you en on both sides of the paper. any diagram, graph or rough working. os, highlighters, glue or correction fluid. aces provided on the question paper.	Shift Lab	
			For Exam	niner's Use
	N TO CANDID	ATES	Section A	
The number o question or pa	f marks is giver art question.	n in brackets [] at the end of each	1	/ 21
	·		2	/19
				,10
			Total	/55

This document consists of **xx** printed pages.

[Turn over

IMPORTANT:

Candidates with access to microscope at the start of the paper are given the **first 1h 15 min** to use it. Please answer **QUESTION 2(b)** within this time frame.

Candidates with no access to microscope at the start of the paper will be given access **1h 15min after the start of the paper**. You may proceed with **QUESTION 1** first.

QUESTION 1

Investigation into the effect of enzyme concentration on the hydrolysis of starch

The enzyme amylase catalyses the hydrolysis of starch.

You are required to investigate the effect of the concentration of amylase on the time taken to completely hydrolyse starch.

lodine solution turns from yellowish brown to blue-black when starch is present. The time taken for the complete hydrolysis of starch can be found by removing a sample of an amylase and starch mixture at regular time intervals, and adding it to a drop of iodine solution. The starch has been completely hydrolysed when the iodine solution remains yellowish brown after adding the sample. This is the end-point. In judging the end-point, specks of blue-black in an otherwise yellowish brown solution can be ignored.

You are provided with:

- 40.0 cm³ of 2.0% amylase solution, labelled **E**, which is an irritant,
- 100.0 cm³ of distilled water, labelled W,
- 40.0 cm³ of starch solution, labelled **S**,
- lodine solution, labelled **iodine**, which is a stain.

Read steps 1 to 9 before starting.

Proceed as follows:

You are required to prepare different concentrations of the amylase solution and set up a control.

1 Carry out **simple** dilutions of the amylase solution, **E**, to obtain a range of concentrations in which the concentration of the amylase is reduced by 0.4% between each successive dilution.

Prepare 10.0 cm³ for each concentration of amylase solution, **using the plastic** containers provided.

Complete Table 1.1 to show how you will prepare the different concentrations of amylase solution.

.....[2]

Concentration of amylase solution / %	volume of E / cm ³	volume of W / cm ³
2.0	10.0	0.0
1.6	8.0	2.0
1.2	6.0	4.0
0.8	4.0	6.0
0.4	2.0	8.0

Table 1.1

a) Correct selection of [amylase]

 b) Correct volumes of enzyme to make each concentration and correct volumes of water to make an appropriate total volume to 1dp e.g. 10.0 cm³ (must be the same volume for each concentration)

[Deduct one mark if no lines drawn within the table]

[Deduct one mark if volume precision is incorrect]

2 Prepare a suitable control for this investigation.

Describe the control that you have prepared. Explain the rationale of the control.

.....

.....[2]

a) [Setup] Replace enzyme with equal volume / 2.0 cm³ of distilled water/W

b) [Rationale] To prove that the disappearance of blue-black coloration observed is due to the action of amylase enzyme on starch

You are required to investigate the effect of different amylase concentrations on the time taken to completely hydrolyse starch. You will test samples from mixtures of a starch solution and different concentrations of amylase solution at a chosen time interval until each end-point is reached, up to a maximum of **180 seconds**.

3 Decide on a suitable time interval at which samples from each mixture of amylase solution and starch solution will be tested for complete hydrolysis of starch.

State the chosen time interval with a reason for this choice.

Time interval

a) Select time interval of 30s or less

+ Reason: Ref. short time interval will give more precise results [Reject: more accurate results]
/ Ref. a not too large time interval such that accuracy is not compromised
/ to ensure that at least 6 readings are obtained during the 180s
[Reject 1min due to a lack of readings]

4 The enzyme's optimum temperature is 35°C. Using the hot and tap water provided, set up a water-bath in the beaker labelled **water-bath**, so that you can maintain this temperature throughout the investigation.

5 Put uniform-sized drops of iodine solution on the white tile, labelled with the times that a sample from each mixture of amylase solution and starch solution will be removed and tested, as shown in Fig. 1.1.



Fig. 1.1

6 Put 3.0 cm³ of **S** into a test-tube and 2.0 cm³ of 2.0% amylase solution into a separate test-tube. Put the test-tubes into the water-bath for at least one minute in order to equilibrate to 35° C

7 The reaction will start as soon as **S** and the amylase solution are mixed.

Add the starch solution, **S**, to the 2.0% amylase solution, and start timing immediately. Using a Pasteur pipette, remove a sample of the mixture at the first chosen time and add **one drop** to the first drop of iodine solution on the white tile. Continue removing and testing samples at the chosen time interval until the end-point is reached, up to a maximum of **180 seconds**. *Make sure that the mixture or enzyme and starch are maintained at* 35°C throughout the experiment.

8 Repeat steps **5–7** to collect the result for each of the other concentrations of amylase solution and the control that you have prepared. Record **'more than 180'** for any mixtures that have not reached the end-point by 180 seconds.

9 Use the space below to record your results.

......[4]

Table showing time taken for different amylase concentration to completely hydrolyse starch / end-point

Concentration of amylase	Time taken for end-point / s			
2.0	40	1		
1.6	60	1	ſ	
1.2	100			Results are based or
0.8	120	1		20s time interval
0.4	160	1	Į	
0.0	More than 180			

a) Table format: Independent variable in leftmost vertical column;

b) Suitable column / row headings with correct units; [Reject: concentration of E as E means 2%; Reject: time **interval** when end point is reached]

c) Records results for 5 concentrations of amylase and control; to whole number
d) Trend of shortest time for highest concentration of amylase to longest time for lowest concentration of amylase, and control as "more than 180";

[Penalise 1m for writing units beside every reading e.g. 40s, 60s etc]

[Penalise 1m for absence of boundary and/or grid lines; do not double penalise if Q1 is already penalised]

10 Explain how the concentration of amylase affects the rate of hydrolysis of starch.

.....[3]

- a) As the concentration of amylase increases, the rate of hydrolysis of starch increases;
- b) Increase in <u>frequency of effective collisions between enzyme and substrate</u> molecules;
- c) Increase in concentration of <u>enzyme-substrate complexes</u> formed <u>per unit time</u>; (Increase in concentration of products formed per unit time)

d) Ref. Increase in amylase concentration; increase in number of active sites

11 Temperature was one variable which was controlled in this investigation.

Identify one variable that affects enzyme reactions, which was not controlled in this investigation.

.....[1]

a) pH

AVP:

12 Suggest how you would control this variable.

.....[1]

a) Add the same volume of <u>pH buffer</u> to each test [Reject: buffer as it is too vague; isotonic buffer; PBS buffer as it is isotonic buffer]

14 For a biotechnological process involving an enzyme to work most efficiently, the enzyme must work at its maximum rate, R.

An enzyme can be used to catalyse the conversion of ethanol (substrate) to acetaldehyde (product).

The effect of the concentration of ethanol (A) on the maximum rate of the production of acetaldehyde (R), is shown in Table 1.2.

Concentration of ethanol (A) / mol dm ⁻³	Maximum rate (R) / min ⁻¹
0.00800	0.0700
0.0150	0.110
0.0500	0.170
0.100	0.220
0.300	0.270

Table 1.2

A linear graph can be drawn by plotting 1/R against 1/A.

This can be used to find R (maximum rate of production) for any particular ethanol concentration in this range.

Complete Table 1.3 for the values of A and R in the last row of Table 1.2, by calculating 1/A and 1/R to the appropriate number of decimal places.

.....[1]

Table 1.3		
1/A	1/R	
/ mol ⁻¹ dm ³	/ min	
125.0	14.3	
66.7	9.1	
20.0	5.9	
10.0	4.6	
3.3	3.7	

a) Correct calculation of 1/A and 1/R, **and** rounding to 1 dp (follow the pattern in the prior rows)

15 Using the data from Table 1.3, draw a graph on the grid provided.

a) use of sensible scale [Reject: odd scales e.g. 3:10] that allow points to occupy at least half the grid in both x and y directions

b) all points plotted accurately to within half a small square on the grid

c) axes correctly labelled with correct units, ascending scale and equidistant intervals d) correct straight line of best fit [Reject: extrapolation of points]; cut through 1 point and ensured that there are equal number of points on either side of the best fit line



16 Find the maximum rate of production (R) which would be achieved if the ethanol concentration (A) was 0.1 mol dm^{-3} .

Show clearly how you obtained R.

R=min⁻¹ [2]

- a) Calculation of $1/A = 1/0.1 = 10.0 \text{ mol } \text{dm}^{-3}$
- b) Correct reading from graph (precision to half a small square); correct conversion from 1/R to R and answer expressed to 3 sig fig; allow ECF from incorrect calculation of 1/A

[TOTAL : 21]

QUESTION 2

Fig 2.1 is a photomicrograph of a stained transverse section through part of a leaf from a different type of plant.

You are not expected to be familiar with this specimen.



Fig. 2.1

(a) Draw a large plan diagram of Fig. 2.1 in the space provided below. Please refer to the coloured photo micrograph provided on the student's bench. [2]

Mark scheme:

- 1 at least 4 lines at leaf blade + size at least 60 mm thickness at mid-rib region + no shading ;
- 2 no cells drawn + at least 5 layers within mid-rib region

REMINDER:

Candidates with access to microscope at the start of the paper are given the **first 1h 15 min** to use it. Please answer **QUESTION 2(b)** within this time frame.

(b) You are required to measure the diameter of the field of view using the clear plastic ruler.

Proceed as follows:

- 1. Put the clear plastic ruler on the stage of the microscope and view the scale lines using low power (x100).
- 2. Measure the diameter of the field of view and record this in (b)(i).

a) Accept: 1.6 – 1.8 mm

Fig. 2.2 is the same photomicrograph as in Fig. 2.1 showing the field of view at the same magnification as the field of view you have just measured.



Fig. 2.2

(ii) Using appropriate measurements, calculate the fraction of the diameter of the field of view occupied by the leaf in Fig. 2.2 along the line **X–Z**.

 YZ = 4.4 - 4.5 cm XZ = 6.8 - 6.9 cm[Reject: answers in decimal places] Fraction of diameter = 4.4 / 6.8 = 11 / 17 or 4.4 / 6.9 = 44 / 69 or 4.5 / 6.8 = 45 / 68 or 4.5 / 6.9 = 15 / 23

 (iii) Using your answers to (b)(i) and (b)(ii) calculate the depth of the midrib, as shown by line Y–Z. Give your answer to the nearest µm. You may lose marks if you do not show your working.

.....μm [2]

a) shows answer to (b)(i) multiplied by answer to (b)(ii)b) decision to multiply by 1000 (to convert to μm)

(iv) A student used a clear plastic ruler to measure the field of view of a microscope. The student replaced the ruler with a slide of a leaf and **estimated** the diameter of the midrib. Using these results the student calculated the actual diameter of the midrib.

State how this student could have modified their method to obtain a more accurate result. State the apparatus the student would use and describe the method.

apparatus	
method	
	[3]

Apparatus :

1 stage micrometer and eyepiece graticule ;

Method:

2 <u>Calibration of eyepiece graticule:</u>

Place stage micrometer on the stage of the microscope and position stage scale such that it is **superimposed** on or aligned next to the smaller eyepiece scale under low power objective lens (x10).

Count the number of eyepiece unit to 1 stage unit.

Calculate the length of each eyepiece unit by dividing the length of 1 stage unit (e.g. 0.1mm) by the number of eyepiece units (e.g. 10).

/ Given that 1 unit of the stage scale measures 0.1 mm, the length of 1 eyepiece unit (division) can be measured by dividing the length of 1 stage unit by the number of eyepiece units that can be fitted on to it. E.g. 0.1 mm divide by 10 eyepiece unit = 0.01 mm = 10 μ m

3 Measurement of midrib using eyepiece graticule by **counting the number of eyepiece units** which is then **multiplied** by the **length of each eyepiece unit** (e.g.10 μm) (c) One technique used for studying antigen-antibody reactions is immunodiffusion.

Wells are cut into an agar support medium to contain antigens and antibodies. Antibodies and antigens diffuse out of the wells into the agar. If an antigen meets a complementary antibody a reaction occurs causing a band of precipitate to appear.

Fig. 2.3 shows the results of an immunodiffusion test with known antigens P and Q and the antibodies to these antigens.



Fig. 2.3

In an investigation, the serum from two test organisms was tested for the presence of antibodies to specific antigens. Both organisms had been previously exposed to both antigens. The serum was placed in wells at the edge of the petri dish and the antigens in a central well.

Fig. 2.4 shows the test set-up.



Fig. 2.4

(i) Suggest **one** variable that must be controlled in this procedure.

.....[1]

1 ref. to any one: thickness of agar / volume of agar / depth of wells consistency / concentration of, agar volume / diameter of wells distance of antigen wells from test organism wells / distance between wells temperature [do not allow: pH] volume of serum / antigen volume [REJECT: Concentration of serum/antibodies]

(ii) State the independent variable in this investigation.

.....[1]

- 1 the type of the serum / antibody / antibodies (from the test organism); [REJECT : antigen ; amount ; concentration ; types of test organisms – this is indirect independent variable]
- (iii) Both test organisms had antibodies against antigen X, but only organism 2 had antibodies against antigen Y.

On Fig. 2.4 draw lines to represent where precipitation might have occurred for both organisms. [2]



2 One line between 1 and antiger...,

[Additional guidance – Allow: 2 marks if lines do not intersect If the lines are reversed / spread outside dish max. 1

Do not allow: if the lines are inverted / lines cross wells]

(iv) Suggest one disadvantage of immunodiffusion for detecting antigens.

- 1.[1]
 - 1 ref. to all antibodies not forming precipitates / AW;
 - 2 ref. to sensitivity / AW ; (the idea that test will not detect low concentrations)
 - 3 ref. to more qualitative / difficult to quantify ;
 - 4 ref. to, slow rate / inability to diffuse of some antibodies ; (Allow of 'slow' is in the context of getting results of tests)
 - **5** ref. to problem of identifying individual antigen [because an antibody e.g Ig M may potentially bind to several types of antigens] / AW ;

[REJECT :

Antigens mutate ;

Relating to experiment design issue - Difficult to tell which band of precipitate belongs to which set of reaction between antigen and antibodies. Because when using immunodiffusion for detecting antigen, one antibody is placed central well at a time / AW ;]

A **naturally occurring** mutant of *Plasmodium* sp. has been tested for use as a 'whole organism' vaccination against malaria. The mutant organism develops normally in mosquito vectors and infects the salivary glands in the same way as non-mutant wild type *Plasmodium* sp. In mice, the mutant infects liver cells but does not multiply and cannot enter red blood cells.

Trials using mice were carried out and the effectiveness of the mutant organism as a vaccine tested by injecting non-mutant wild type *Plasmodium* sp. into vaccinated and non-vaccinated mice.

Table 2.1 shows the results of investigations in mice using the mutant *Plasmodium* sp.

	number of mutant Plasmodium cells given to the mice			percentage of mice not
test group	first inoculation	first booster inoculation	second booste inoculation	infected by wild type <i>Plasmodium</i> sp.
1	0	0	0	0
2	50 000	25000	25000	100
3	10 000	10000	10 000	100
4	10 000	10000	0	70

Table 2.1

(v) Suggest the purpose of including each of the following test groups.

group 1 groups 2 and 3 group 4

16

- 1 *group 1*: ref. to the idea of a control ; to show that percentage change of mice not infected is due to inoculation ;
- 2 group 2 & 3: ref. to idea of finding how many organisms, give immunity / needed; (e.g. how many are needed to make the vaccine work)
 [REJECT: to determine or justify the minimum or maximum number of organisms needed]
- **3** group 4: ref. to idea of finding the number of, innoculations / boosters, needed ; e.g. to see if another booster is needed

(vi) Using the information in the question, outline a procedure that might be used to obtain mutant *Plasmodium* sp. to use in the vaccination trials.

.....[2]

Salivary gland:

ref. to (information that mutant) <u>Plasmodium</u> breeds / develops / AW in mosquitoes ;

- 1 ref. to breeding mosquitoes / culturing in salivary gland tissue / AW ;
- 2 ref. to extracting (<u>Plasmodium</u>) from salivary glands / culture of cells from salivary glands;

Liver:

ref. to (information that mutant) <u>Plasmodium</u> develops / AW in mice's liver cells ;

- 1 ref. to breeding infected mice / culturing (Plasmodium) in liver tissue / AW ;
- 2 ref. to extracting (<u>Plasmodium</u>) from mice's infected

[TOTAL : 19]
QUESTION 3: PLANNING QUESTION

Effect of citrate on rate of respiration

Enzymes catalysing essentially irreversible reactions are potential sites of control in cellular respiration. One of these enzymes is phosphofructokinase, which can be regulated by the reversible binding of citrate to its allosteric site. (Citrate is produced as an intermediate compound during Krebs cycle.)

Using this information and your own knowledge, design an experiment to determine the effect of citrate concentration on the rate of cellular respiration.

You must use:

- 10 mM citrate,
- purified homogenate of enzymes found in the cytosol,
- 5% glucose solution,
- pH buffer,
- distilled water,
- benedict's solution,
- apparatus shown in Fig. 3.1, can be used to separate proteins from ions and disaccharides.



Fig. 3.1

- syringes,
- white card,
- stopwatch,
- thermometer,
- bunsen burner with tripod, gauze and bench mat,
- thermostatically controlled water bath,
- normal laboratory glassware e.g. test-tubes, beakers, measuring cylinders, graduated pipettes, glass rods, etc.,

Your plan should:

- have a clear and helpful structure such that the method you use is able to be repeated by anyone reading it,
- be illustrated by relevant diagrams, if necessary,
- identify the independent and dependent variables,
- describe the method with the scientific reasoning used to decide the method so that the results are as accurate and reliable as possible,
- show how you will record your results and the proposed layout of results tables and graphs,
- use the correct technical and scientific terms,
- include reference to safety measures to minimize any risks associated with the proposed experiment.

MARK SCHEME

INTRODUCTION

A: BACKGROUND KNOWLEDGE / RATIONALE

[1m for 2 out of 3 points]

1. PFK is an enzyme involved in <u>glycolysis</u> where <u>glucose is converted into pyruvate</u>.

- 2. The <u>rate of glucose consumption decreases</u> as the concentration of citrate increases, since citrate acts as an <u>inhibitor</u> to phosphofructokinase.
- 3. Therefore the rate of respiration decreases.

[1m for 2 out of 3 points]

- 4. Presence of glucose can be detected using the Benedict's test.
- 5. The colour of the mixture / precipitate reflects the amount / quantity of glucose present.
- 6. As the concentration of glucose increases, the colour of the mixture / precipitate <u>changes</u> from <u>blue</u> to <u>green</u> to <u>yellow</u> to <u>brick-red</u> (any two colour)

[Rationale] - 1m for #2,3

- [Award marks for this under dependent variable section] In this experiment, the effect of citrate on the rate of cellular respiration is determined by the <u>rate of glucose</u> <u>consumption / amount of glucose consumed per unit time</u> after <u>a fixed period of</u> <u>time</u>.
- 2. The concentration of glucose **remaining** can be estimated by comparing the results of the experiment against the results of the Benedict's test performed on <u>a range of glucose solutions of known concentration</u> / glucose standard solutions.
- 3. Concentration of glucose **consumed** can be calculated by subtracting the concentration of glucose remaining from the original 5%.

[Hypothesis] – 1m

1. As the <u>citrate concentration increases</u>, the rate of cellular respiration decreases as measured by the rate of glucose consumption.

B: VARIABLES AND CONTROLLED VARIABLES

[State the independent and dependent variables] – 1m for #1,2

- The independent variable is <u>citrate concentration</u> / mM ; 0 mM, 1.5 mM, 3.0 mM, 4.5 mM, 6.0 mM, 7.5 mM, 9.0 mM. [At least 5 readings, regular intervals; maximum 10 mM]
- 2. The dependent variable is **rate** of respiration, calculated by <u>concentration (in percentage) of glucose consumed **per unit time**</u>

[Other variables to keep constant: Can be written in detail in Procedure Section] – 1 m for every 2 points; total 2m

1. Volume of glucose

- Use a <u>syringe</u> to add the <u>same volume</u> (state volume here or in procedure) of fresh glucose solution from the same stock (stirred well before use) to keep the initial concentration of glucose constant.
- 2. <u>Temperature</u>
 - The optimum temperature, **e.g. 37°C**, is kept constant by using a <u>thermostatically</u> <u>controlled water bath</u>.
- 3. <u>pH</u>
 - Keep pH constant with a pH buffer of same volume (2cm3)
- 4. Duration of reactions
 - A <u>digital stopwatch</u> is used to ensure duration of reactions is kept constant **e.g. 3min.**

[Control] -1m

1. A control is set up with **2.0 cm³** of 0 mM citrate / **2.0 cm³** distilled water instead of citrate to show any change in colour of precipitate obtained from Benedict's test is due to presence of citrate.

C: DETAILED PROCEDURE - total 7m

1m

Part 1: Preparation of the glucose standards

- 1. Label 10 boiling tubes **0.5%**, **1.0%**, **1.5%**, **2.0%**, **2.5%**, **3.0%**, **3.5%**, **4.0%**, **4.5%** and **5.0%**. (minimum 5 tubes)
- Prepare <u>20.0 cm³</u> of various concentrations of glucose solutions as shown in the table below. <u>10.0 cm³ syringes</u> are used to add the liquids and glucose solution is placed in their respective boiling tubes.

Concentration of glucose	Volume of 5% glucose	Volume of distilled water /
solution to be prepared /%	solution / cm ³	cm ³
0.5	2.0	18.0
1.0	4.0	16.0
1.5	6.0	14.0
2.0	8.0	12.0
2.5	10.0	10.0
3.0	12.0	8.0
3.5	14.0	6.0
4.0	16.0	4.0
4.5	18.0	2.0

- 3. Label 10 test-tubes 0.5%, 1.0%, 1.5%, 2.0%, 2.5%, 3.0%, 3.5%, 4.0%, 4.5% and 5.0%.
- 4. Using a <u>5.0 cm³ syringe</u>, add <u>2.0 cm³ of each concentration of glucose solution</u> into their respective test-tubes.
- 5. Using a <u>5.0 cm³ syringe</u>, add <u>2.0 cm³ of Benedict's solution</u>. <u>Shake gently to mix the</u> <u>contents of the tube</u>.
- 6. Place the test-tubes in the boiling water for two minutes. Start the stopwatch.
- 7. After two minutes, stop the stopwatch. Remove the tubes from the boiling water and place them in a rack.
- 8. <u>Shake gently to mix the contents of the tube</u> and observe the contents of the testtubes immediately after mixing. Record the observations in a table, <u>noting any</u> <u>differences in terms of colour and cloudiness</u>.
- 9. <u>Set aside these tubes for comparison in Part 3.</u>

Part 2: Preparation of citrate solution

- 1. Label 7 boiling tubes <u>0 mM, 1.5 mM, 3.0 mM, 4.5 mM, 6.0 mM, 7.5 mM, 9.0 mM</u>. **[At least 5 readings, regular intervals; maximum 10 mM]**
- Prepare <u>10.0 cm³</u> of various concentrations of citrate solution as shown in the table below. 10.0 cm³ <u>syringes</u> are used to dispense the liquids and citrate solution into their respective boiling tube.

Concentration of citrate	Volume of 10 mM citrate	Volume of distilled water
/mM	/cm ³	/cm ³
1.5	1.5	8.5
3.0	3.0	7.0
4.5	4.5	5.5
6.0	6.0	4.0
7.5	7.5	2.5
9.0	9.0	1.0

Part 3:

- 3. Label another 7 boiling-tubes <u>0 mM, 1.5 mM, 3.0 mM, 4.5 mM, 6.0 mM, 7.5 mM, 9.0 mM</u>.
- 4. Using two clean 5.0 cm³ syringes, add <u>5.0 cm³ of **5% glucose solution**</u> [Note: concentration of glucose provided] and <u>2.0 cm³ of pH buffer (e.g. 6.2)</u> into a boiling-tube labeled 1.5 mM.
- 5. Place this <u>boiling tube and the purified homogenate of **enzymes** into a <u>thermostatically controlled water bath</u> at <u>37°C for 10 minutes to **equilibrate**</u>. Start the <u>stopwatch</u>.</u>
- 6. Also **equilibrate** the **citrate** solutions in a <u>thermostatically controlled water bath</u> at <u>37°C for at least 10 minutes</u>.
- After 10 minutes, stop the stopwatch. Using a <u>2.0 cm³ syringe</u>, add <u>2.0 cm³ of 1.5 mM citrate solution</u> and using a <u>5.0 cm³ syringe</u>, add <u>5.0 cm³ of enzyme homogenate</u> into the boiling-tube. [Note: enzyme homogenate should be the last to be added.] Shake gently to mix the contents of the tube.
- 8. Place the test tube into <u>a thermostatically controlled water bath of 37°C for 3 minutes</u>. Start the <u>stopwatch</u>.
- 9. After 3 minutes, <u>quench/stop the enzymatic reaction by placing the test tube in the boiling water bath for 2 minutes</u>.
- 10. After 2 minutes, stop the stopwatch and <u>pour the reaction mixture through the filtration</u> <u>set-up</u> / membrane filter.
- 11. Perform <u>Benedict's test</u> on the <u>filtrate</u> as it was done for the glucose standards (<u>steps</u> <u>4-8</u>, <u>Part 1</u>).

1m

1m

- 12. Place the tubes containing the filtrate and the glucose standards <u>against a white card.</u> <u>Compare with the glucose standards</u> (from Part I, Step 9) to estimate the concentration of glucose present. <u>Shake the tube gently to mix the contents</u> before comparison.
- 13. <u>Record</u> the <u>concentration of glucose remaining /% in a table</u>.
- 14. Calculate the concentration of glucose consumed (5% concentration of glucose remaining) / %
- 15. Repeat steps 4 to 14 using the other citrate concentrations as prepared in Part 2.

[Replicates and Repeats] - 1m

- 16. To ensure <u>reliability of results</u>, repeat steps 4 to 15 to obtain <u>a total of three readings</u> (triplicates) at each citrate concentration, and calculate the average.
- 17. To ensure <u>reproducibility of data</u>, <u>repeat the entire experiment</u> twice using <u>freshly</u> <u>prepared reagents and solutions</u> and <u>glucose standards</u>.

D: DATA MANIPULATION AND EXPECTED RESULTS

1. [Draw Table of results] -1m

Table showing the amount of glucose /% at different concentrations of citrate / mM

Concentration of citrate /mM	Concentra remaining	ation 1/ %	of (glucose		
	Reading 1	Reading 2	Reading 3	Ave	Concentration of glucose consumed / %	Rate of respiration / % min ⁻¹
0.0						
1.5						
3.0						
4.5						
6.0						
7.5						
9.0						

Concentration of glucose consumed = 5% - Concentration of glucose remaining

Rate of respiration = Concentration of glucose consumed / 3 min

2. [Draw a graph to show expected trends and/or results] -1m

Graph of rate of glucose consumed /% min ⁻¹ against concentration of citrate /mM



- SAFETY PRECAUTIONS -1m for 2 sets
 1. Wear safety goggles and gloves when handling chemical reagents which may be irritants (e.g. Benedict's solution, citrate solution) to the eyes and/or skin. Immediately flush the eye / wash with water when the chemical reagent comes into
 - contact with the eyes and/or skin. If irritation persists, call for medical help.
- 2. Handle glassware with care as broken glassware is sharp and can cause cuts.
- 3. AVP



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S/N	Apparatus/Reagents/Chemicals	Quantity per student
1	In a beaker, labelled S , 1.0% starch solution	at least 40 cm ³
2	In a beaker, labelled E , 5% amylase solution	at least 40 cm ³
3	In a beaker, labelled W, distilled water	at least 100 cm ³
4	In a dark bottle, labelled iodine , iodine (in potassium iodide) solution- used for testing for the presence of starch	at least 20 cm ³
5	Test-tube rack, suitable to hold 7 test-tubes	1
6	Test-tubes	7
7	Beakers or containers to hold up to 50 cm ³ / plastic containers	6
8	Beaker to contain about 400 cm ³ of water, labelled water-bath	1
9	Access to a source of cold (tap) water to prepare a water-bath at about 35 °C	
10	Access to a source of hot water at 50 °C or more to prepare a water-bath at about 35 °C	
11	250 cm ³ beakers	2
12	Thermometer, –10 to 110 °C	1
13	Paper towels	8
14	10 cm ³ syringes	2
15	5 cm ³ syringes	2
16	Pasteur pipette, 1 cm ³ or 3 cm ³	2
17	White tiles (10 cm × 10 cm)	1
18	Glass rod	1
19	Stopwatch	1
20	Glass marker pen	1
21	Safety glasses/goggles	1 pair
22	Coloured photo micrograph of Fig. 2.1	1
23	Light microscope	1 per pair of candidates
24	Plastic ruler	1

PREPARATION LIST FOR QUESTIONS 1 AND 2

INSTRUCTIONS FOR PREPARING APPARATUS FOR QUESTION 2

These instructions give details of the apparatus required by each candidate for each experiment in this paper. A summary of the questions that will be presented to the candidates is included, where appropriate, to allow the biology teacher to test the apparatus appropriately. **No access to the Question Paper is permitted in advance of the examination.**

Candidates must be provided with a microscope with:

- Eyepiece lens, ×10 (equal to 16 mm or 23")
- Low-power objective lens, ×10 (equal to 16 mm or 23")
- High-power objective lens, ×40 (equal to 4 mm or 16")
- Eyepiece graticule fitted within the eyepiece and visible in focus at the same time as the specimen.

To avoid confusion, only the lenses specified above should be fitted in the microscopes to be used in the examination. Any lenses which are **not** ×10 or ×40 should be removed or replaced. Each candidate must have sole, uninterrupted, use of the microscope for at least one hour.

Each candidate will require:

- (i) Clear plastic ruler, marked in mm
- (ii) Microscope (as described above)

For each candidate:

- the microscope **must** be set up on low power
- the slide must **not** be left on the stage of the microscope.

APPARATUS LIST FOR QUESTIONS 1 and 2

S/N	Apparatus/Reagents/Chemicals	Quantity per student
1	In a beaker, labelled S , 1.0% starch solution	at least 40 cm ³
2	In a beaker, labelled E, 5% amylase solution	at least 40 cm ³
3	In a beaker, labelled W, distilled water	at least 100 cm ³
4	In a dark bottle, labelled iodine , iodine (in potassium iodide) solution- used for testing for the presence of starch	at least 20 cm ³
5	Test-tube rack, suitable to hold 7 test-tubes	1
6	Test-tubes	7
7	Beakers or containers to hold up to 50 cm ³ / plastic containers	6
8	Beaker to contain about 400 cm ³ of water, labelled water-bath	1
9	Access to a source of cold (tap) water to prepare a water-bath at about 35 °C	
10	Access to a source of hot water at 50 °C or more to prepare a water-bath at about 35 °C	
11	250 cm ³ beakers	2
12	Thermometer, –10 to 110 °C	1
13	Paper towels	8
14	10 cm ³ syringes	2
15	5 cm ³ syringes	2
16	Pasteur pipette, 1 cm ³ or 3 cm ³	2
17	White tiles (10 cm × 10 cm)	1
18	Glass rod	1
19	Stopwatch	1
20	Glass marker pen	1
21	Safety glasses/goggles	1 pair
22	Coloured photo micrograph of Fig. 2.1	1
23	Light microscope	1 per pair of candidates
24	Plastic ruler	1