ASI School ID:

AUSTRALIAN SCIENCE OLYMPIADS

NATIONAL QUALIFYING EXAM 2011

BIOLOGY – SECTION C

TO BE COMPLETED BY THE STUDENT USE CAPITAL LETTERS

Student Name: ……..…..…….

Home Address: ..……..……..

……..... **Post Code:** ……...

Telephone: (…….) …… **Mobile:** …….

E-Mail: …..……... **Date of Birth:** ……/……/..……

□ **Male** □ **Female** **Year 10** □ **Year 11** □ **Other:** …….

Name of School: ……..……..**State:** ……...

Students competing in the 2011 National Qualifying Examinations must be in Year 11 or an earlier year in 2011.

The Australian Olympiad teams in Biology, Chemistry and Physics will be selected from students participating in the Science Summer School. To be eligible for selection for the Summer School students will need to be an Australian citizen or permanent resident at the time offers are made.

To be eligible for selection in one of the Australian Science Olympiad teams, students must be eligible to hold an Australian passport by the time of team selection (March 2012)

Signature: …… Date: ……

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Examiners Use Only:

BIOLOGY

2011 National Qualifying Examination

Time Allowed:

Reading Time: 10 minutes

Examination Time: 120 minutes

INSTRUCTIONS

- *Attempt all questions in ALL sections of this paper.*
- Permitted materials: Non-programmable, Non-graphical calculator, pens, pencils, erasers and a ruler.
- Answer SECTIONS A and B on the MULTIPLE CHOICE ANSWER SHEET PROVIDED. Use a pencil.
- Answer SECTION (C) in the answer booklet provided. Write in pen and use pencil only for graphs.
- Ensure that your diagrams are clear and labelled.
- All numerical answers must have correct units.
- Marks will not be deducted for incorrect answers.
- **Do not write on this question paper. It will not be marked.**

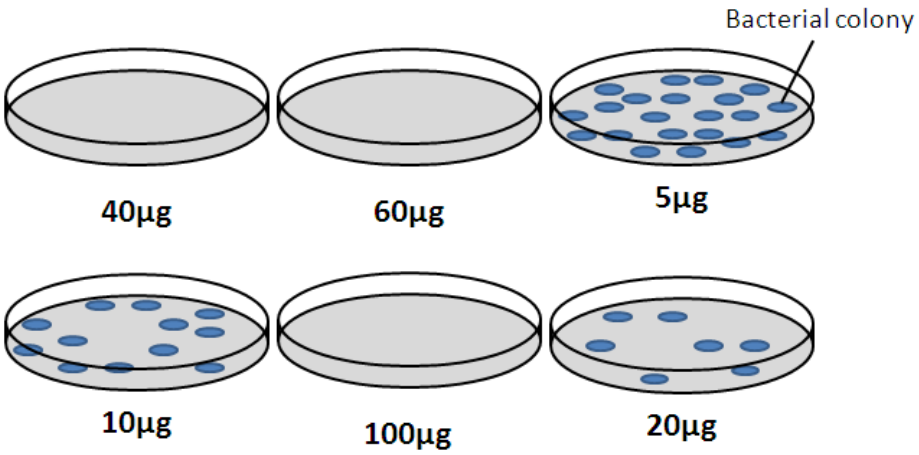
MARKS

SECTION A	45 multiple choice questions	45 marks
SECTION B	12 short answer questions	26 marks
SECTION C	6 written answer questions	40 marks
	Total marks for the paper	111 marks

SECTION C:

25. For a given antibiotic, the minimum inhibitory concentration (MIC) is defined as the minimum concentration known to inhibit visible bacterial growth. To determine the MIC of cefuroxime, an antibiotic, the following experiment was set up:

- 6 agar plates were set up each containing different amount of cefuxorime dissolved in 20mL of growth medium (the amounts were 5µg, 10 µg, 20 µg, 40 µg, 60 µg and 100 µg).
- The same number of bacteria were inoculated onto each plate.
- Plates were incubated at 37˚C overnight and examined the following day. The results are shown below.



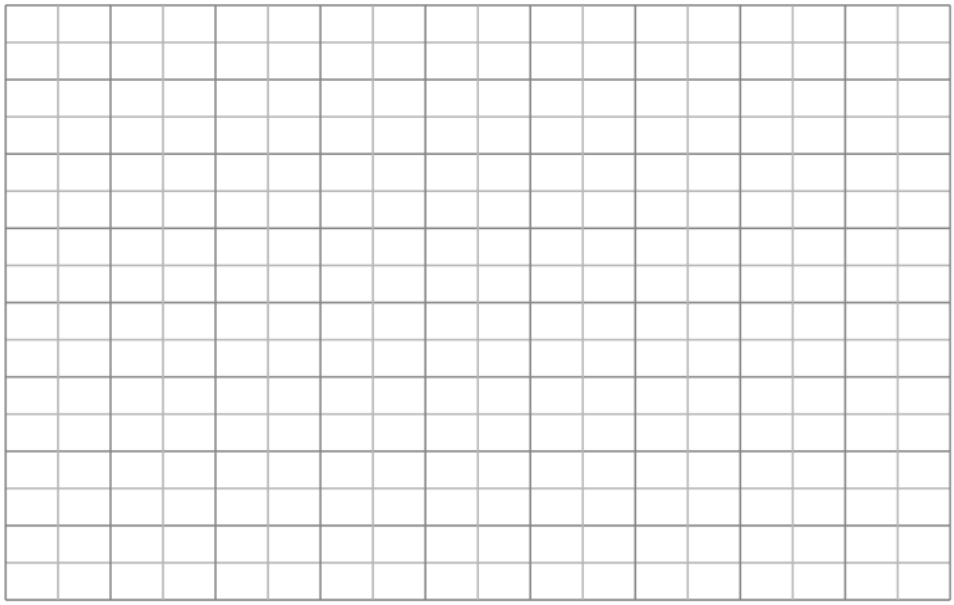
(i) Of the concentrations shown, what is the MIC for cefuxorime? **(1 mark)**

2µg/mL or equivalent

A mouse was given a single oral dose of 5mg of cefuroxime and blood was taken at various time intervals to determine the concentration of cefuroxime in the serum, as shown in the table below.

Time after administration (h)	0	0.5	1.5	3	4	5.5	6.5	8
Serum cefuroxime concentration (µg/mL)	0	3.5	6	7	3.4	1.7	0.8	0.4

(i) On the axes provided below, plot the above data giving appropriate labels for each axis and a title. **(5 marks)**



(ii) Provide an explanation for the shape of the graph. **(2 marks)**

Slight delay in increase as absorption occurs. Serum concentration peaks, then decreases as drug is moved out of compartment/broken down/excreted.

When deciding on antibiotic dosing it is important to take into account the time period for which the serum concentration of antibiotic is above the MIC (referred to as the t>MIC). This gives an indication of how effectively the dose will control bacterial infection.

(iii) Using the graph above, determine what the t>MIC is for the dose of cefuroxime administered to the mouse. **(1 mark)**

5 hours

(iv) If you were to perform this test for the MIC with a different bacterial species would you expect to find the same MIC? Why/why not? (**2 marks)**

No. Different bacteria have different susceptibilities to antibiotics and the levels needed to inhibit growth are different.

(v) Cefuroxime is most effective if doses are timed such that the serum concentration is greater than the MIC for over 50% of the time. Explain how dose regimes that do not achieve this could lead to antibiotic resistance. (**3 marks)**

(vi) Antibiotic concentrations that inhibit growth are referred to as bacteriostatic whereas those that kill the bacteria are termed bacteriocidal. Outline an experiment that could test whether the MIC determined is bacteriocidal or bacteriostatic, including a description of how you would interpret the results. (Hint: you could start with a swab taken from the plate with the MIC for cefuroxine). You may choose to use a flow chart or diagram to aid your explanation. (**3 marks)**

Swab taken from plate with MIC and inoculated on NA/NB and incubated. If growth occurs, it is bacteriostatic and if none bacteriocidal. Either a positive or negative or negative control should be mentioned.

26. Marsupial moles (order *Notoryctemorphia*) inhabit the sandy desert regions of South Australia, Western Australia and the Northern territory. Much remains unknown about the lives of these unusual mammals, what is known includes the following:

- They tunnel through the sand, filling in the tunnel behind them and giving the appearance of "swimming" through the sand.
- It is believed that they also produce some permanent burrows.
- Marsupial moles spend short periods of time above the ground.
- They are mainly insectivorous.
- They have a horny shield on their nose and the neck is very rigid, due to fusion of the vertebrae.
- In contrast to other marsupials, the marsupium opens towards the posterior of the animal.

(i) Explain how one of the above features serves as an adaptation to subterranean life. (**2 marks)**

Marsupium opening – prevents the pouch from filling up with sand as the animal tunnels. Nose covering – prevents abrasion of the nose and face whilst tunneling.
Rigid neck – Provides support for the spine as the animal tunnels but resisting forces

The paws of various marsupials are shown below.

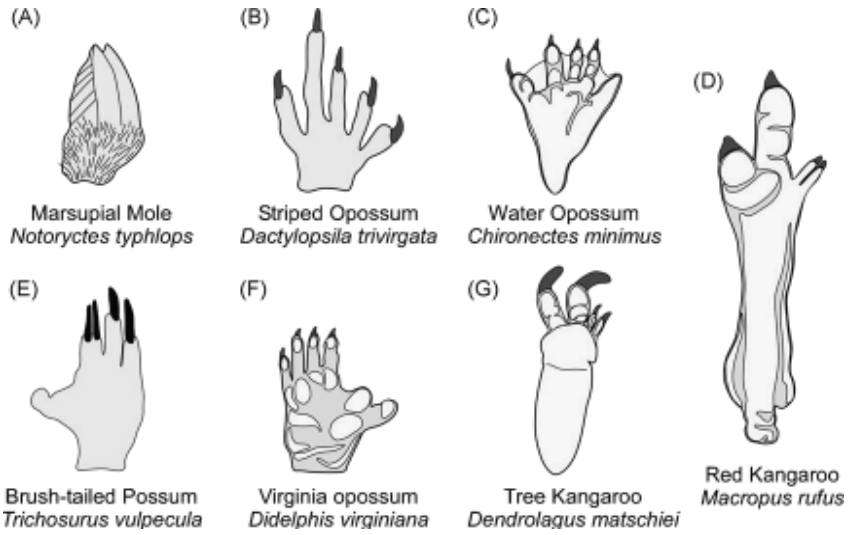


Figure 1 Taken from Karlen S.J. and Krubitzer L., The functional and anatomical organization of marsupial neocortex: evidence for parallel evolution across mammals. Prog Neurobiol (2007) 82(3):122-41.

(ii) By comparing the paw of the marsupial mole to another marsupial, explain how this paw is adapted to the marsupial mole's life. (**2 marks)**

Third and fourth claws are triangular shaped, forming a scoop, which is used to scoop sand when tunnelling through it.

Unusually, the eyes of the marsupial mole are completely covered by skin and lack a lens or pupil. The gene coding for one of the proteins involved in sight in mammals, interphotoreceptor retinoid binding protein (IRBP), was sequenced in several marsupials and the resulting sequences were aligned for comparison. A portion of the sequence from the coding strand is shown below, in which a mutation has occurred that has caused a stop codon to come into the reading frame.

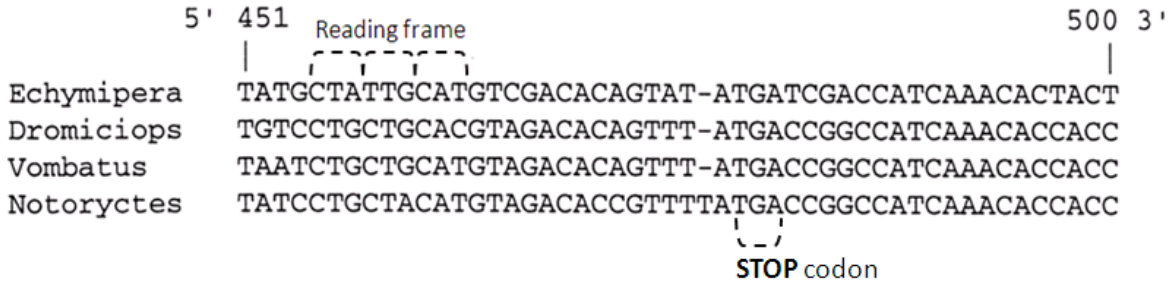


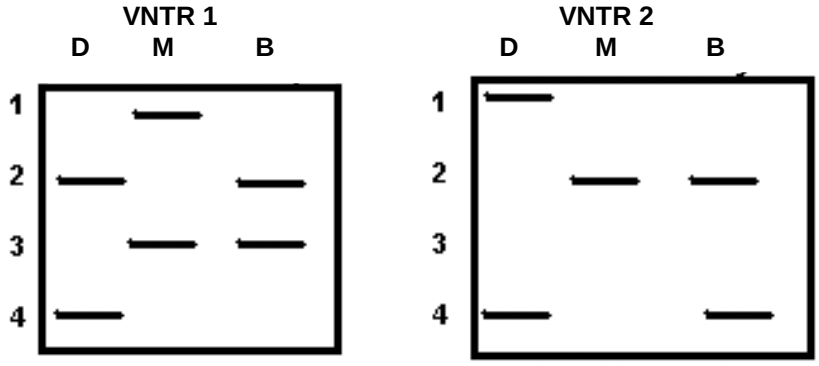
Figure 2 Springer et al., The interphotoreceptor retinoid binding protein gene in therian mammals: implications for higher level relationships and evidence for loss of function in the marsupial mole. Proc Natl Acad Sci (1997) 94(25) 13754-9

(iii) What would be the effect of this mutation on the function of IRBP? (**2 marks)**

(vii) Explain, with reference to selective pressures, the presence of this mutation in the genome of the marsupial mole. (**3 marks)**

No need for functioning IRBP because of lack of eyes. Therefore no selective pressure on gene to retain function. Thus mutations are able to accumulate.

27. A celebrity has been named in a paternity suit. The defendant (labeled D in the autoradiogram), the mother (labeled M), and the baby (labeled B) have each been typed for two loci VNTR1 and VNTR2, as shown in the autoradiograms below. Each of these VNTR loci has four alleles. For VNTR1, the frequencies of the alleles 1, 2, 3, and 4 in the general population are 0.2, 0.4, 0.3, and 0.1, respectively. For VNTR2, the frequencies of alleles 1, 2, 3, and 4 are 0.1, 0.1, 0.2, and 0.6, respectively.



Do the autoradiograms indicate that D could be the father of the baby B? Yes or No?

Eshan's gel question.

28. There are many reagents that scientists can use to label specific cellular components with fluorescent markers or fluorochromes. On excitation with a laser these fluorescent markers emit light of a specific wavelengths that can then be detected. Different types of fluorochromes emit light at different wavelengths which can be detected separately, hence cells can be labelled with many different fluorochromes specific for different cellular components simultaneously.

The table below shows the binding properties and emission wavelengths of three commonly used fluorochromes.

Fluorochrome	**Binds**	**Emission wavelength**
DAPI	DNA	460nm
Mitotracker red®	Mitochondria	600nm
phalloidin-FITC	Actin (cytoskeleton)	520nm

One technique that uses fluorescent labelling is fluorescence microscopy. In this technique, thin sections of tissues (ideally one cell thick) are generated by slicing the tissue to be examined and placing them on glass slides. These sections are then incubated with a cocktail of fluorescent

markers for the components under examination. During this incubation the fluorescent markers will bind their target cellular components. Excess fluorescent markers are then washed away and the slide can be viewed using a fluorescence microscope.

A section of mammalian liver is taken and stained with the three fluorescent labels in the table above. It is then viewed using a fluorescence microscope. It is noted that most of the liver cells contain a large organelle that fluoresces at 460nm, whilst emissions at 600nm and 520nm were observed throughout the remaining cytoplasm.

i. What is the large organelle fluorescing at 460nm? Explain your reasoning with reference to the fluorescent markers used.

It is the nucleus (1 mark). DAPI fluoresces at 420nm and binds DNA, most cellular DNA is in the nucleus (1 mark).

ii. Mitotracker red® labels mitochondria. Why can't individual organelles be visualised?

Mitochondria are too small to be resolved under a light microscope. (1 mark)

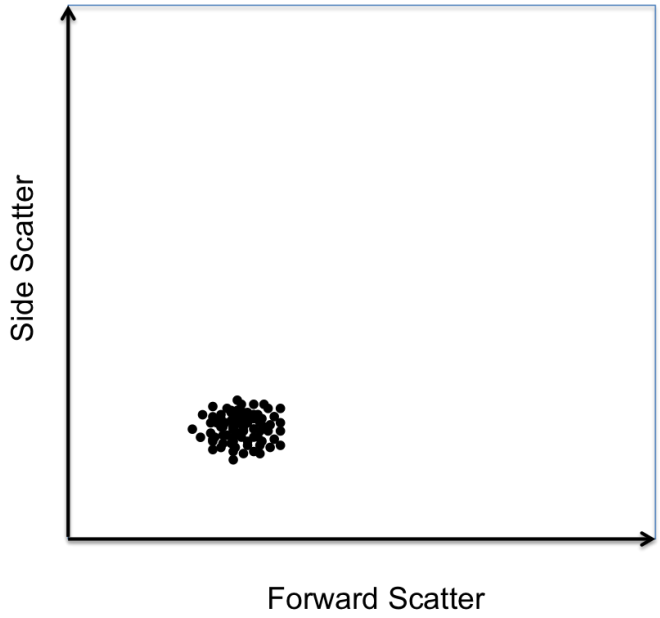
iii. There appear to be circular vessels within the liver section which contain large numbers of very small cells which show staining for phalloidin-FITC but neither DAPI nor Mitrotracker red®. What sort of cells are these, what is their function and why might they lack mitochondria?

Red blood cells (1 mark). RBCs are responsible for oxygen transport (1 mark). Mitochondria are responsible for aerobic respiration; the lack of mitochondria prevents the RBC from using up the oxygen they are transporting (1 mark).

Fluorescence assisted cell sorting (FACS) is another technique which can utilise fluorochromes and is used by scientists to look at the characteristics of individual cells in suspension. For example, it is commonly used to look at the properties of different types of blood cells. It works by taking a single cell suspension (a solution of cells where the cells are not bound to one another) and passes the cells one at a time past a laser beam. The FACS machine can then detect light scattered forward by the cell (forward scatter), light scattered sideways by the cell (side scatter), and, if the cell has been labelled with a fluorescent marker, light emitted from the fluorescent marker on excitation by the laser beam. It will then record these values for each individual cell.

Forward scatter correlates with cell volume, i.e. larger cells have greater forward scatter, whilst side scatter correlates with cell complexity or granularity, i.e. more granular cells have greater side scatter.

In the figure below the forward vs. side scatter properties of lymphocytes (a type of white blood cell) have been represented as a dot plot. Each dot represents a single cell and its position along each axis shows the forward and side scatter values as detected by the FACS machine. A group of cells that show similar properties for the parameters shown are often referred to as a population.



iv. Neutrophils, another type of white blood cell, are both larger than lymphocytes and more granular. On the dot plot above draw a circle using a **solid** line indicating where you would expect a population of neutrophils to be positioned.

1 mark for a circle positioned with higher FSC and SSC than the lymphocyte population.

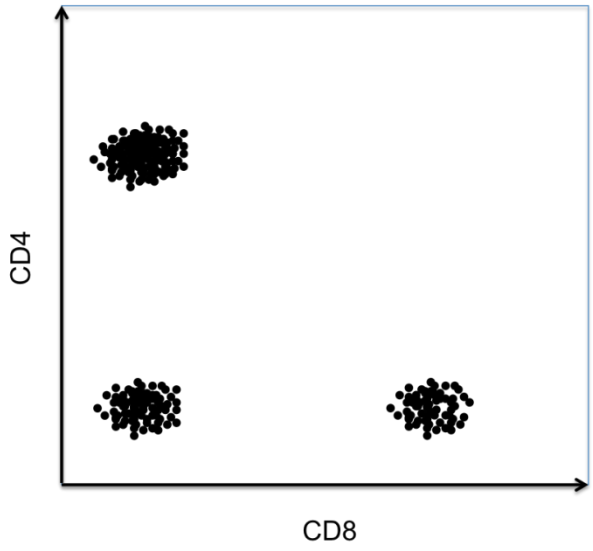
v. On the above dot plot draw a circle using a **dotted** line indicating where you would expect a population red blood cells to be positioned with regards to forward and side scatter parameters. Explain your reasoning in the space below.

Red blood cells are smaller than lymphocytes and therefore will have lower forward scatter (1/2 mark). They are less complex as they have limited organelles and will therefore have lower side scatter than lymphocytes (1/2 mark). 1 mark for correct positioning of RBC population on graph – bottom left – lower in both FSC and SSC than lymphocytes.

The table below summarises the sub-populations of lymphocytes, listing membrane proteins that can be used to identify them.

Lymphocyte sub-type	**Defining membrane proteins**
Cytotoxic T lymphocytes	CD3, CD8
T helper lymphocytes	CD3, CD4
B lymphocytes	CD19
Natural killer cells	CD16, CD56

vi. Lymphocytes were purified from the blood of a healthy individual and stained with fluorescent markers for CD8 and CD4. These cells were then analysed via FACS. When these cells were analysed with regards to CD8 and CD4 fluorescence the plot below was generated, showing 3 different populations of lymphocytes. Given that the population in the bottom left of the plot is considered negative for CD4 and CD8 staining, mark on the graph in which populations you would expect to find the four different sub-types of lymphocytes listed in the table above.



½ mark per correct placement of each subtype – CTLs – bottom right, T helpers – top left and both NKT and B cells in bottom left.

Karlen S.J. and Krubitzer L., The functional and anatomical organization of marsupial neocortex: evidence for parallel evolution across mammals. *Prog Neurobiol*. 82(3),122-41.

Springer et al., The interphotoreceptor retinoid binding protein gene in therian mammals: implications for higher level relationships and evidence for loss of function in the marsupial mole. *Proc Natl Acad Sc*. 94(25),13754-9

Wolfe-Simon, F., Switzer Blum, J., Kulp, T.R., Gordon, G.W., Hoeft, S.E., Pett-Ridge, J.F. Stolz, J.F., Webb, S.M., Weber, P.K., Davies, P.C.W., Anbar, A. D. & Oremland, R. S., (2010). A Bacterium that can grow by using Arsenic instead of Phosphorus. *Science*. in press.

For a brief overview of this story, see also NASA Science News, at:
http://science.nasa.gov/science-news/science-at-nasa/

Figure of the Miller-Urey experiment, freely availably at Wikipedia:
http://upload.wikimedia.org/wikipedia/commons/thumb/5/54/Miller-Urey_experiment-en.svg/500px-Miller-Urey_experiment-en.svg.png

Integrity of Competition

If there is evidence of collusion or other academic dishonesty, students will be disqualified. Markers' decisions are final