

Wednesday 18 June 2025 – Morning

A Level Biology B (Advancing Biology)

H422/03 Practical skills in biology

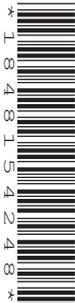
Time allowed: 1 hour 30 minutes

You must have:

- a ruler (cm/mm)

You can use:

- a scientific or graphical calculator



Please write clearly in black ink. **Do not write in the barcodes.**

Centre number

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Candidate number

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First name(s)

Last name

INSTRUCTIONS

- Use black ink. You can use an HB pencil, but only for graphs and diagrams.
- Write your answer to each question in the space provided. If you need extra space use the lined pages at the end of this booklet. The question numbers must be clearly shown.
- Answer **all** the questions.
- Where appropriate, your answer should be supported with working. Marks might be given for using a correct method, even if your answer is wrong.

INFORMATION

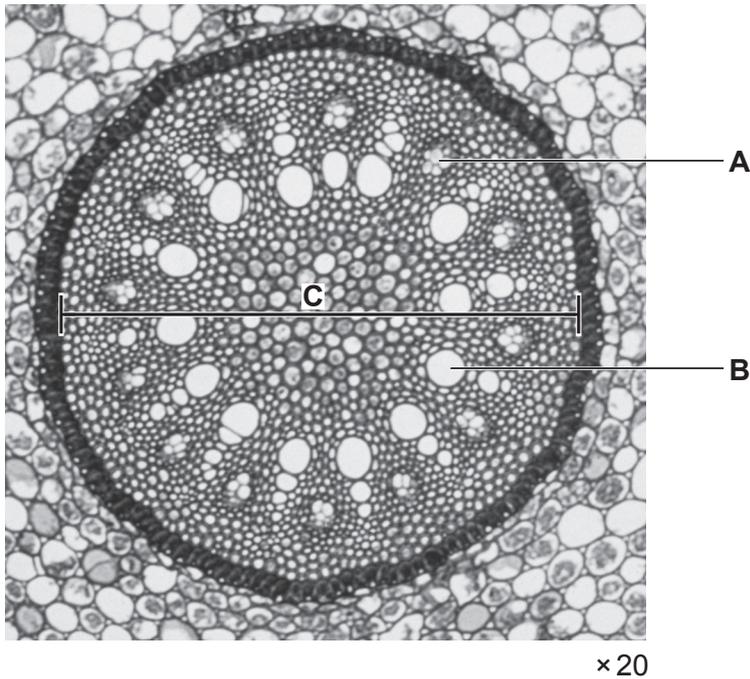
- The total mark for this paper is **60**.
- The marks for each question are shown in brackets [].
- Quality of extended response will be assessed in questions marked with an asterisk (*).
- This document has **20** pages.

ADVICE

- Read each question carefully before you start your answer.

1 Two functions of the roots of plants are to take up water from the soil and to store starch.

(a) The photomicrograph shows a transverse section of a root in a monocotyledonous plant.



(i) The cells labelled **A** and **B** are two different types of cell in the vascular tissue of the root.

Identify the types of cell labelled **A** and **B**.

A Sieve tube

B Xylem vessel

[2]

(ii) The diameter of one region of the root is represented by the line **C** in the image.

The magnification used to produce the image was $\times 20$.

Calculate the actual diameter of **C**.

Give your answer in mm. $\text{image diameter} \times 8 = 68 \text{ mm}$
 $\frac{68}{20} = 3.4 \text{ mm}$

Diameter = 3.4 mm [2]

(iii) One risk when dissecting plant tissues, such as roots, is the potential for injury when using sharp instruments.

State **one other** health and safety risk that should be considered before dissecting plant tissues.

..... Plant tissue could cause allergic
 reaction

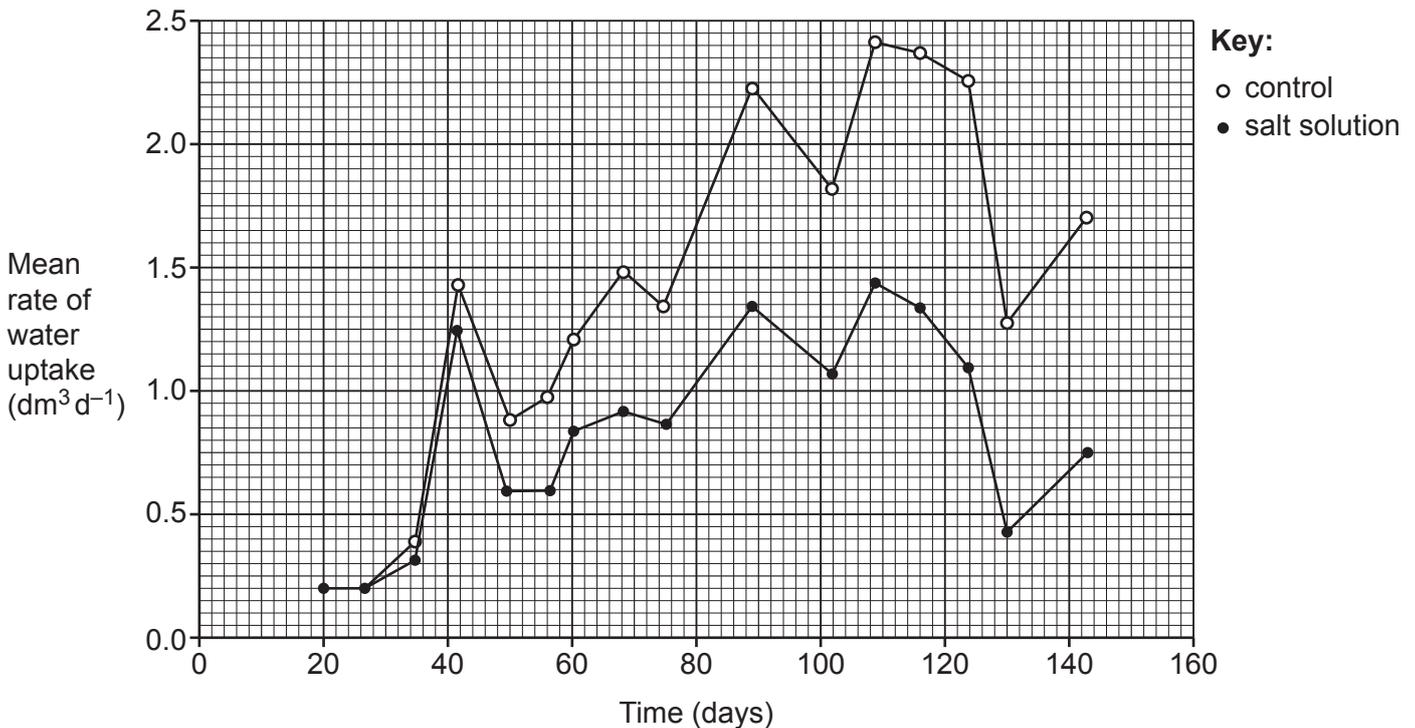
[1]

- (b) A scientist investigates how the rate of water uptake by roots is affected by the presence of salt (NaCl) in the surrounding soil.

This is the method they use:

- Grow 110 tomato plants in pots of soil in a greenhouse.
- Add 50 mmol dm^{-3} salt solution to the soil of 55 plants, every day.
- The other 55 plants are a control group.
- Calculate the rate of water uptake per day ($\text{dm}^3 \text{ d}^{-1}$) by the roots of each plant.

The results of the investigation are shown in the graph.



- (i) State how the group of control plants would be set up differently to the group of plants given salt solution **and** explain the purpose of this control group.

⇒ Set-up:-

add equal volume of distilled water.

⇒ Explanation:-

to allow comparison.

[2]

- (ii) Calculate the largest percentage difference in water uptake between the plants in the control group and the plants given salt solution.

Use your answer to complete the sentence.

$$\text{Day 124} \\ \frac{2.25 - 1.1}{1.1} \times 100 = 105\%$$

On day 124 the water uptake of the control plants was 105% greater than the water uptake of the plants given the salt solution. [2]

(iii) Suggest what conclusions can be made from the results shown in the graph.

- water was taken up even when salt was present.
- in both groups, rate of water uptake fluctuates during the experiment.
- Salt has no effect on rate of water uptake between 20 and 26.

[2]

(c) The roots of plants can store starch in high concentrations.

Outline how the starch concentration of a sample of root tissue could be estimated using colorimetry.

- Reagent used :- Iodine solution.
- prepare starch solution from tissue samples.
- calibrate colorimeter.
- Select red filter
- Measure absorbance
- Plot a calibration curve.
- estimate concentration of starch in the sample using calibration curve.

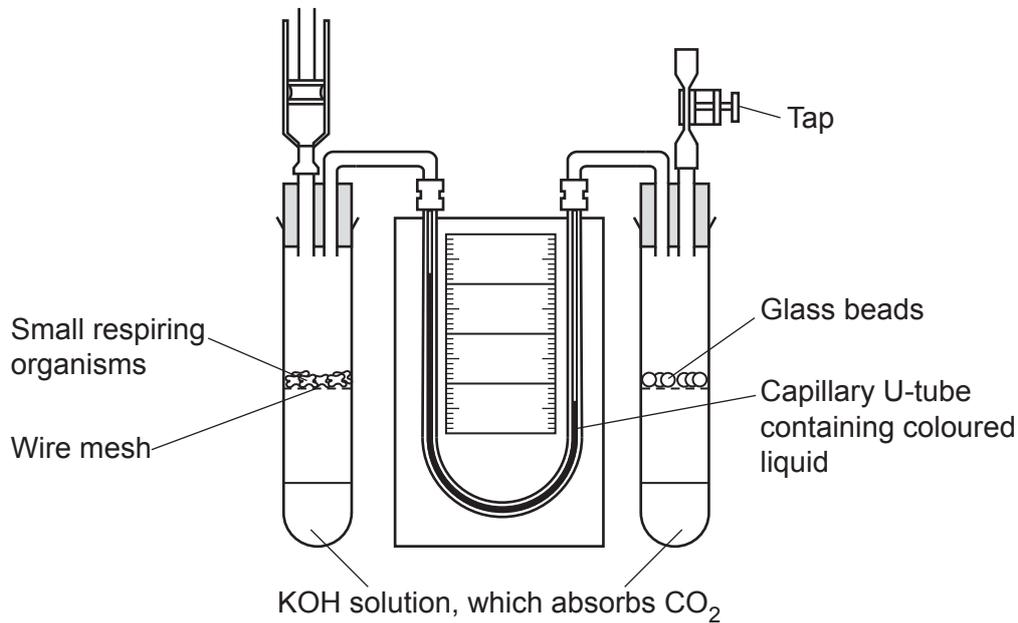
[4]

5
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- 2 Respirometers can be used to measure the respiratory quotient and the rate of respiration of organisms.

The diagram shows a respirometer that can be used for these measurements.



The oxygen (O₂) used by organisms for respiration can be estimated by measuring the distance moved by the coloured liquid in the capillary U-tube over a set time period. The volume of O₂ can then be calculated.

- (a) A student measures the volume of O₂ used and the volume of carbon dioxide (CO₂) produced per minute in four species of bean.

The results are shown in the table.

Species of bean	Volume of O ₂ used in one minute (mm ³)	Volume of CO ₂ produced in one minute (mm ³)
A	0.063	0.050
B	0.038	0.027
C	0.061	0.065
D	0.072	0.052

- (i) Describe how the values for the volume of CO_2 produced in one minute would be measured and calculated using a respirometer.

• Remove KOH solution from respirometer, replace with water.

• Record distance moved by colored liquid.

• Change caused by CO_2 production = distance moved by liquid with KOH present - distance moved without KOH.

• calculate volume using πr^2

[3]

- (ii) Calculate the RQ of **species A**, using the values in the table.

$$= 0.050 / 0.063$$

$$= 0.794$$

$$\text{RQ} = 0.794 \quad [1]$$

- (iii) The student thinks the results for one of the species are anomalous.

State which species has anomalous results **and** explain why the student thinks the results are anomalous.

• Species C because

RQ for C would be greater than 1.00

• Species C has an RQ of 1.07

[2]

(b)* Plan an investigation to test the effect of temperature on the **rate of respiration** in germinating beans and obtain valid results.

You do **not** need to include details of how to set up or use a respirometer in your plan.

⇒ Experimental plan

- at least four temperatures within a suitable range (up to 40°C)
- use of thermostatically controlled water bath
- Measure and calculate oxygen use over a set time period
- Calculate a rate of O₂ use per unit mass

⇒ Appropriate Statistical test

- Spearman's rank for correlation
- Unpaired t-test

⇒ Control variables.

- Use a same species.
- Same age of beans

[6]

Extra answer space if required.

- Same number of beans.
- Equilibrate for the same duration of time before each test.

⇒ Safety

- Allergic reaction from plant material
- cuts from broken glassware.

9
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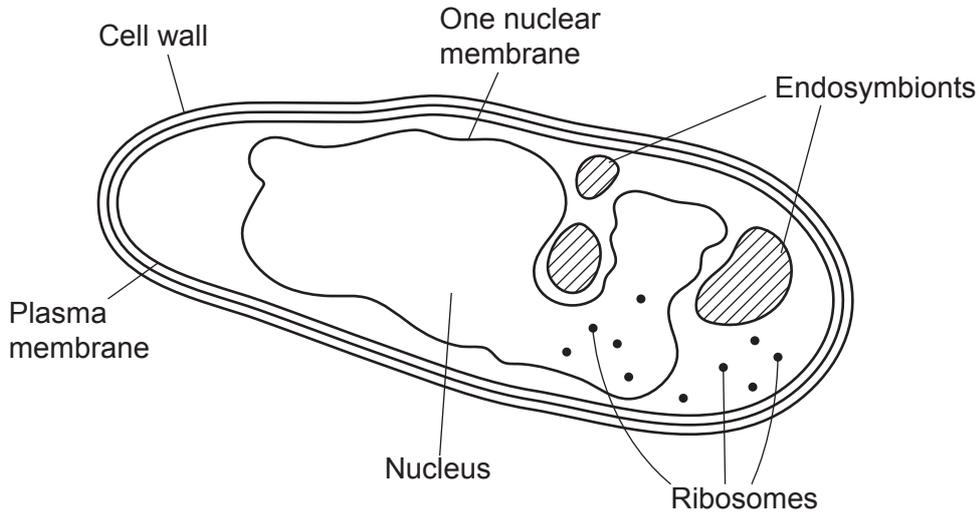
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3 *Parakaryon myojinensis* is a species of single-celled organism that was discovered in 2012.

P. myojinensis is unique among species that have been discovered because it has features of both eukaryotes and prokaryotes. It also has features that are neither eukaryotic nor prokaryotic.

A student uses a sharp pencil to draw a *P. myojinensis* cell from an electron micrograph.

A representation of the student's drawing of *P. myojinensis* is shown in the image.



Endosymbionts are organisms that can also be found in some eukaryotic and prokaryotic cells.

(a) State **two** ways in which the student could improve their drawing.

1 Do not use Shading

2 Addition of scale bar.

[2]

- (b) The student concludes that 'although it does not seem to have mitochondria, *P. myojinensis* is more similar to a eukaryote than a prokaryote'.

Discuss the evidence from the image that supports **and** does **not** support this conclusion.

⇒ Support because nucleus present

⇒ Does not support because;

- eukaryotes do not have ribosomes

- eukaryotes have a double membrane.

- eukaryotes have other membrane-bound organelles present

[3]

- (c) Suggest what the absence of mitochondria indicates about the process of respiration in *P. myojinensis*.

it may rely on anaerobic respiration

[1]

- (d) The student is interested in whether the cell wall of *P. myojinensis* is made from peptidoglycan or a different substance.

State how to test for the presence of peptidoglycan in the cell wall of *P. myojinensis*.

- Gram Staining

- Use of crystal violet stain and potassium iodide solution

- Purple color if peptidoglycan is present

[2]

(e)* *Rhizobium* species are other examples of single-celled organisms.

Describe how to culture *Rhizobium*, in vitro.

Your answer should include steps that would reduce the risk of contamination of the culture.

- Disinfect workspace
- Sterilise scissors to obtain *Rhizobium*.
- Select a root nodule from leguminous plant
- wash the nodule
- crush the nodule
- Add distilled water
- Working aseptically e.g use of bunsen flame to generate convection current away from workspace

⇒ prepare agar mixture that includes

- A respiratory substrate
- A nitrogen source
- A source of essential ions
- A pH buffer
- Boil the mixture, then cool and pour in Sterile dish. [6]

Extra answer space if required.

- allow agar to set
 - Add 1cm^3 of *rhizobium* suspension with sterile pipette and spread suspension with sterile loop
 - Seal and place petri dish in incubator.
- ⇒ Risk assessment
- cut from scalpels.
 - Skin irritation
 - incubate at $25-30^\circ\text{C}$ to prevent pathogenic from being cultured.

4 Mutations in some genes are associated with an increased risk of developing cancer.

(a) The table lists three genes associated with cancer: *Myc*, *p53* and *Ras*.

Complete the table by putting a tick (✓) in the appropriate box or boxes on each line to show whether a gene has the feature.

Feature	<i>Myc</i>	<i>p53</i>	<i>Ras</i>
A mutation that causes a loss of function that can lead to cancer.		✓	
It is a proto-oncogene.	✓		✓

[1]

(b) The polymerase chain reaction (PCR) can be used to detect alleles that are associated with cancer.

(i) Complete the table to show the temperatures used in the three stages of a PCR cycle and the processes that occur at each temperature.

Temperature (°C)	Process
97	Separation of DNA strands
55	Annealing of primers
75	DNA polymerase attaches nucleotides to the new strands

[2]

(ii) In theory, the number of DNA molecules can increase exponentially during PCR.

Calculate the theoretical maximum number of DNA molecules that could be present after 15 cycles of PCR, assuming that the process started with one DNA molecule.

Give your answer as a \log_{10} value.

$$2^{15} = 32768$$

$$= (10)^{4.515}$$

Number of DNA molecules = $(10)^{4.515}$ [2]

- (iii) Suggest **one** reason why the theoretical maximum number of DNA molecules might **not** be achieved.

Long numbers that expected
primers might not bond to template
DNA strand [1]

- (iv) A version of PCR known as quantitative PCR (qPCR) is used to detect alleles that are associated with an increased risk of cancer.

qPCR involves:

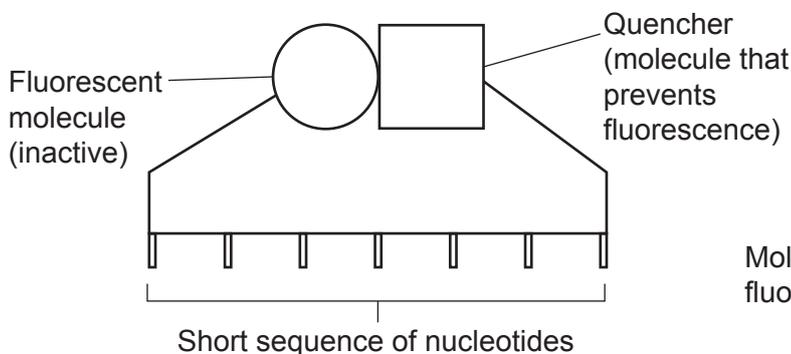
- extracting mRNA (transcribed from the target allele) from a cell
- converting the mRNA to complementary DNA (cDNA)
- conducting PCR on the cDNA sample using *Taq* DNA polymerase, allele-specific primers and fluorescent probes.

State the name of the enzyme that converts mRNA to cDNA.

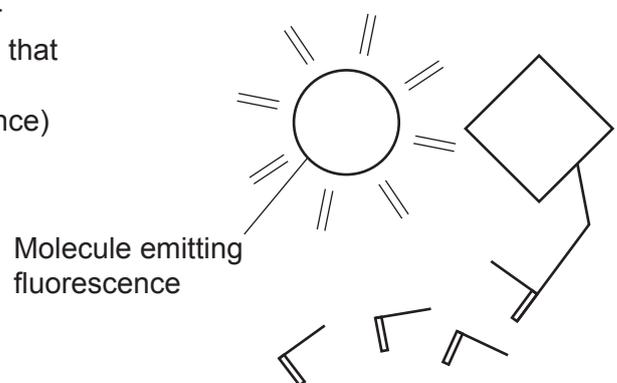
Reverse transcriptase [1]

- (v) The diagram shows a fluorescent probe used in qPCR.

Before the probe has bound to cDNA



After the action of *Taq* DNA polymerase



Suggest how fluorescent probes can be used in qPCR to estimate the concentration of cDNA derived from a cancer-associated allele.

- probe nucleotide sequence should be complementary to known sequence in the target allele.
 - fluorescence proportional to DNA concentration.
- [2]

(c) Electrophoresis is another technique that can be used to detect cancer-associated alleles.

CYP17 is a gene that has been linked to some cancers.

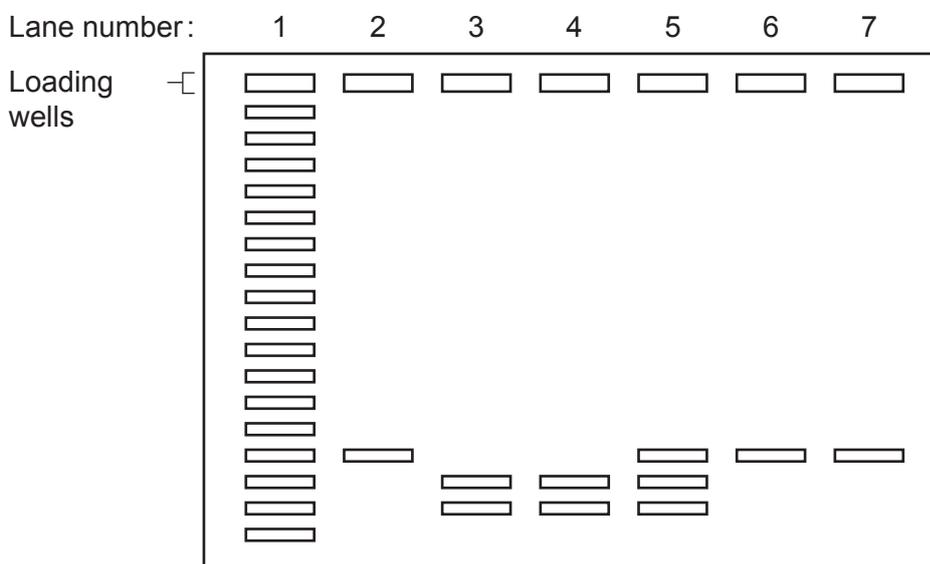
- The normal *CYP17* allele does not increase the risk of cancer.
- A mutant *CYP17* allele increases the risk of some cancers.

Restriction enzymes are used to produce fragments of the *CYP17* alleles for analysis in electrophoresis.

Restriction enzymes:

- produce one fragment when the normal *CYP17* allele is present
- produce two smaller fragments when the mutant *CYP17* allele is present.

The diagram shows the results of electrophoresis of DNA samples from 6 people. Lanes 2 to 7 in the diagram show only DNA fragments from *CYP17* alleles.



(i) Samples containing DNA fragments are placed in the loading wells of the electrophoresis box before beginning electrophoresis.

State **one other** substance that should be added to the box before beginning electrophoresis **and** explain the purpose of this substance.

Substance : Buffer

Purpose : to maintain a constant pH.

[2]

(ii) State **and** explain the purpose of the DNA fragments in lane 1 in the diagram.

Marker, DNA fragments in solution
 To compare the distance travelled
 by sample fragments

..... [2]

(iii) State the lane numbers that indicate:

- a genotype that is homozygous for the normal *CYP17* allele
- a genotype that is homozygous for the mutant *CYP17* allele.
- a heterozygous genotype for the *CYP17* gene

Homozygous normal 2, 6, 7

Homozygous mutant 3, 4

Heterozygous 5

..... [2]

- 5 Scientists analysed the effect of alcohol consumption by parents on the risk of a child being born with microcephaly.

Microcephaly is defined as a head circumference more than two standard deviations below the mean at a particular age.

The scientists obtained information on the number of units of alcohol consumed per week by both parents:

- during the three months before conception
- during the first three months of the mother's pregnancy.

The scientists compared the risk of the newborn baby having microcephaly when parents consumed:

- no alcohol
- more than 5 units of alcohol per week.

They performed statistical tests to test the hypothesis that there was a greater risk of microcephaly when parents consumed more than five units of alcohol per week.

The p values from the statistical tests are shown in the table.

	p value	
	Mother consuming >5 units per week	Father consuming >5 units per week
Before pregnancy	0.078	0.048
In the first three months of pregnancy	0.508	0.246

- (a) Describe the conclusions that can be made from the p values in the table.

⇒ Before pregnancy: Higher risk when fathers consume >5 units per week.

- no risk when mother consume >5 units per week.

⇒ In the first 3 months of pregnancy:- no effect of alcohol consumption on the risk. [2]

(b) Suggest what additional information is needed to increase confidence in the conclusions.

- Larger sample size
- ⇒ confounding variables that should have been controlled
- Smoking
 - Drug use
 - BMI
 - age of Parents.
- [2]

END OF QUESTION PAPER

EXTRA ANSWER SPACE

If you need extra space use these lined pages. You must write the question numbers clearly in the margin.

The page contains a large area for writing, defined by a vertical solid line on the left and horizontal dotted lines extending across the page. This area is intended for providing extra answer space for questions.

A large area of the page is reserved for writing, featuring a vertical solid line on the left side and horizontal dotted lines extending across the page.

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