Cambridge Assessment International Education

Cambridge International AS & A Level

CANDIDATE NAME

CENTRE NUMBER

CANDIDATE NUMBER

6503887165

BIOLOGY **9700/52**

Paper 5 Planning, Analysis and Evaluation **October/November 2022**

1 hour 15 minutes

You must answer on the question paper.

No additional materials are needed.

INSTRUCTIONS

- Answer **all** questions.
- Use a black or dark blue pen. You may use an HB pencil for any diagrams or graphs.
- Write your name, centre number and candidate number in the boxes at the top of the page.
- Write your answer to each question in the space provided.
- Do **not** use an erasable pen or correction fluid.
- Do **not** write on any bar codes.
- You may use a calculator.
- You should show all your working and use appropriate units.

INFORMATION

- The total mark for this paper is 30.
- The number of marks for each question or part question is shown in brackets [ ].

This document has **12** pages. Any blank pages are indicated.

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1 Egg albumen (egg white) is a good source of protein. Some eggs contain up to 10.0% w/v (100 g dm^{-3}) protein.

A biuret assay can be used to determine the concentration of protein in egg albumen.

- Biuret reagent is a mixture of copper(II) sulfate solution and sodium hydroxide solution.
- In a biuret assay, biuret reagent is added to a solution.
- If protein is present, copper(II) ions (Cu^{2+}) in the biuret reagent bind with the nitrogen atoms that form peptide bonds in the protein.
- This binding causes the reagent to change from a clear, pale blue to a purple colour.
- Different concentrations of protein result in different intensities of purple colour.

A colorimeter can be used to measure differences in the intensity of the purple colour in samples tested.

Fig. 1.1 shows a colorimeter that measures the absorbance of light when it passes through different coloured solutions.

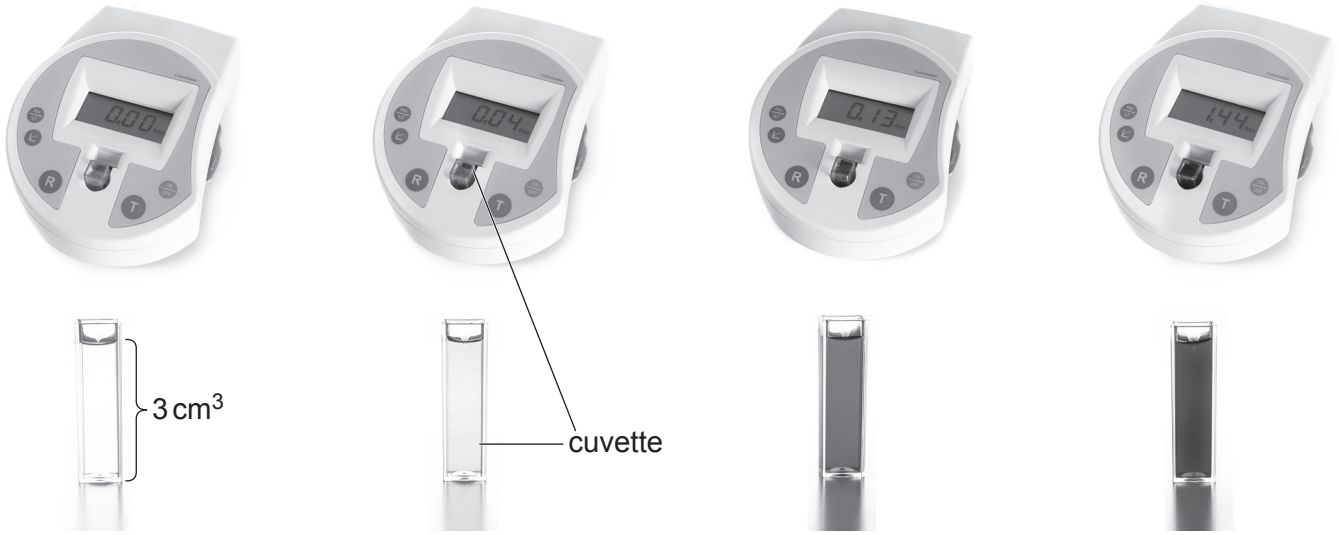


Fig. 1.1

(a) Outline how a colorimeter is set up so that a test cuvette can be placed in the slot to obtain a correct measurement of intensity of a coloured solution.

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(b) A student carried out a biuret assay to determine the concentration of protein in egg albumen from eggs produced by a chicken that had been fed on a new type of chicken feed. The eggs produced by the chicken before eating the new feed contained 6.0% w/v of protein in the egg albumen.

Egg albumen is diluted to obtain a concentration that is suitable for testing in the biuret assay. For example, egg albumen with 6.0% w/v protein is diluted by a factor of 10 to obtain a 0.6% w/v solution for a biuret assay.

To carry out the biuret assay on egg albumen of unknown protein concentration, a calibration curve needs to be produced. This involves using standard solutions of known concentrations of protein.

(i) The range of concentrations that can be measured using a colorimeter in a biuret assay is 0.1 – 1.0% w/v.

A stock solution of 1.0% w/v protein can be diluted using distilled water to prepare the standard solutions of protein.

Describe how the student could prepare a 0.1% solution of protein using the stock solution.

Construct a table to show how the dilution is made for the 0.1% solution and for other concentrations the student could use to produce a calibration curve for the biuret assay.

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Space for table.

[2]

(ii) Identify the independent variable **and** dependent variable for the preparation of the calibration curve.

independent variable .....

dependent variable .....

[1]

(iii) Complete Fig. 1.2 by labelling the axes and sketching the expected calibration curve.



Fig. 1.2

[2]

(iv) The biuret assay assumes that if the same intensity of colour in the biuret test is obtained with the test sample and a protein standard, then the protein concentration is the same, even though they are different proteins. This is summarised in Fig. 1.3.

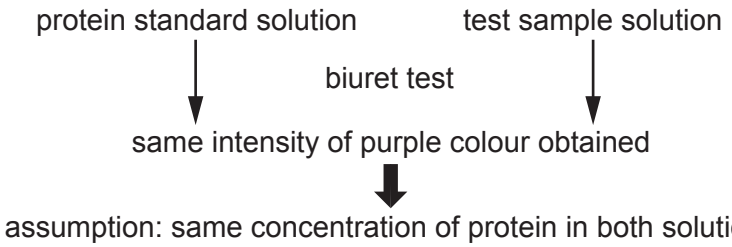


Fig. 1.3

Use the information on the biuret test given on page 2 to explain why different proteins of the same concentration will result in the same intensity of purple colour in the biuret test.

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(c) Describe how the student would:

- use the standard protein concentrations prepared in **(b)(i)** to obtain a calibration curve
- determine the concentration of protein in the egg albumen from eggs of chickens fed on the new chicken feed.

Do **not** repeat any detail given in **(a)** of how to use the colorimeter or in **(b)(i)** of how the standard protein solutions would be prepared.

Your method should be set out in a logical way and be detailed enough to let another person follow it.

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[Total: 15]

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2 A main feature of Huntington's disease (HD) is a loss of neurones from a region of the brain known as the striatum. A study of brain tissue taken from people with HD showed higher than normal (increased) urea concentrations in the anterior striatum and cerebellum regions of the brain.

Urea is a toxic waste product of amino acid and protein metabolism. It is produced from ammonia, which is also toxic, in an enzyme-controlled cycle known as the urea cycle.

Scientists carried out a study using transgenic (genetically modified) sheep, known as *OVT73*, to investigate the increased urea concentrations. *OVT73* have the mutant allele of the *Huntingtin* (*HTT*) gene. At the time of the study, *OVT73* were 5 years old and had no detectable loss of striatal neurones or disease symptoms.

Samples of tissue were taken from the same region of the anterior striatum in:
- six *OVT73* (three females and three males)
- a control group of non-transgenic sheep.

(a) Describe **two** features of an appropriate control group for this investigation.

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(b) A whole-genome RNA-sequencing experiment was carried out on the tissue samples using a commercially available diagnostic testing kit. This experiment produced gene expression profiles for comparison.

From the hundreds of genes that showed differential gene expression, 24 genes were described as genes of interest because they were involved in urea metabolism or in urea transport out of cells. None of these were genes coding for enzymes in the urea cycle.

The 24 genes of interest were identified from RNA sequencing using mRNA transcripts.

Explain why it was more appropriate to use RNA sequencing rather than DNA sequencing for the identification of these genes of interest.

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[Turn over

(c) A second sample of tissue was taken from the same region of the anterior striatum of each of the same individuals. A different commercial diagnostic testing kit was used to obtain a comparison of gene expression for the 24 genes of interest.

Analysis of the results identified ten genes with differential gene expression at a statistically significant level of $p \leq 0.05$.

Table 2.1 shows the results for these ten genes and how much greater the value for *OVT73* is, calculated as a ratio.

Mean count is a standardised value based on the quantity of mRNA transcripts obtained from the diagnostic testing.

Table 2.1

SE = standard error

gene name	***OVT73* / mean count ± SE**	**control / mean count ± SE**	**ratio (*OVT73* count ÷ control count)**
SLC14A1	1061.5 ± 149.9	483.6 ± 82.1	2.20
OXTR	110.3 ± 14.6	50.5 ± 13.5	2.18
SMOC2	113.4 ± 14.8	62.2 ± 13.3	1.82
SLC5A7	458.5 ± 37.9	300.4 ± 29.7	1.53
ETV5	2651.7 ± 252.6	1780.4 ± 192.6	1.49
RHCG	118.6 ± 12.3	80.7 ± 11.4	1.47
SIAH3	230.5 ± 9.3	167.2 ± 10.6	1.38
CBS	546.0 ± 24.7	456.0 ± 18.5	1.20
ITGB4	60.2 ± 8.1	94.2 ± 8.3	0.64
CPAMD8	22.0 ± 4.0	42.4 ± 8.1	0.52

(i) State which statistical test would have been used to establish the statistical significance between the control and *OVT73* data sets.

Explain why the test chosen was suitable.

statistical test .....

explanation .....

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(ii) State the null hypothesis for the investigation.

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(iii) With reference to Table 2.1, explain the advantage of calculating the ratios.

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(d) The results of the two RNA-sequencing experiments indicate that there is a difference between *OVT73* and the control group in the expression of each of the ten genes shown in Table 2.1.

State **one** feature of the study that contributes to the validity of these results and **one** feature that would improve the validity of these results.

feature that contributes to validity .....

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feature to improve validity .....

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[2]

(e) Differential expression of the ten genes shown in Table 2.1 was compared for tissue samples taken from a **different** region of the anterior striatum.

Only gene *SLC14A1* showed differential gene expression at a significant level. This gene also showed statistically significant differential gene expression in the cerebellum and motor cortex regions of the brain.

The scientists then measured the concentrations of urea in tissue samples taken from these three regions of the brain.

The results are shown in Table 2.2.

Table 2.2

	mean urea concentration ± SE / nmol dm^{-3} urea mg^{-1} of protein		**ratio**	***p*-value**
tissue	***OVT73***	**control**		
anterior striatum	236.7 ± 35.2	154.2 ± 8.6	1.54	0.02
cerebellum	81.4 ± 17.5	46.6 ± 19.8	1.75	0.22
motor cortex	69.9 ± 10.3	52.7 ± 12.5		0.31

(i) Complete Table 2.2 by calculating the ratio for the motor cortex. [1]

(ii) With reference to Table 2.2, explain how the standard error (SE) values for mean urea concentration are good indicators of the estimates of statistical significance.

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(iii) *SLC14A1* codes for a transport protein that functions in the facilitated diffusion of urea out of cells.

Explain the relationship between the results shown in Table 2.2 and the increased gene expression shown by *SLC14A1*.

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(f) The results of the study show a relationship between the increased urea concentrations and Huntington's disease, but also highlight the need for further investigation.

Some possible causes of increased urea concentrations in cells are:

1. a changed expression in the genes coding for the enzymes of the urea cycle
2. an increase in protein breakdown related to cell death
3. an increase in protein breakdown to provide more energy to a cell that has an increased rate of metabolism.

Explain why the results of this study indicate that possible causes 1 and 2 are less likely to be involved in the **early** stages of HD than possible cause 3.

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[Total: 15]

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