Cambridge Assessment International Education

Cambridge International AS & A Level

CANDIDATE NAME

CENTRE NUMBER

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5462871735

BIOLOGY **9700/51**

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 **16** pages. Any blank pages are indicated.

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[Turn over

1 Antibiotic resistance in bacteria is a global problem that has caused scientists to research into antibacterial substances other than antibiotics. Honey has properties that make it a good antibacterial substance. For example, honey contains hydrogen peroxide, which is known to kill bacteria.

The most effective honey tested so far for antibacterial activity is Manuka honey. It contains less hydrogen peroxide than many other types of honey, but it does contain an antibacterial compound, methylglyoxal (MGO), which is not found in other types of honey.

A student decided to investigate the effect of two antibacterial substances on the bacterium *Bacillus subtilis*, which respires aerobically:

- MGO in Manuka honey
- an antibiotic solution used in cell cultures to prevent contamination.

The student wanted to find the lowest concentration of each antibacterial substance that would kill or inhibit the growth of *B. subtilis*.

(a) The student used a broth culture for the investigation. To make a broth culture, a small quantity of *B. subtilis* is added to a clear nutrient solution. A fresh (newly made) broth culture of *B. subtilis* is also clear.

Fig. 1.1 is a diagram of a fresh broth culture of *B. subtilis*.

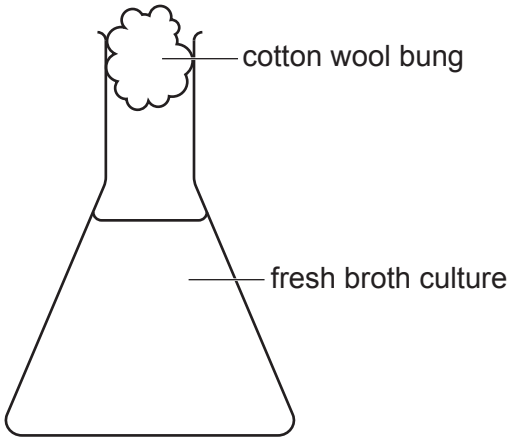


Fig. 1.1

(i) A sterile cotton wool bung was used in the top of the flask containing the fresh broth culture of *B. subtilis* to protect the culture from contamination.

Explain why it is better to use a sterile cotton wool bung in a flask containing broth culture of *B. subtilis*, rather than using a sterile rubber bung.

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..... [1]

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(ii) Before comparing the two antibacterial substances, the student carried out a trial experiment.

The student transferred a sample of fresh broth culture to a culture tube and incubated the tube at 25 °C for 24 hours.

Fig. 1.2 summarises the results of the trial experiment.

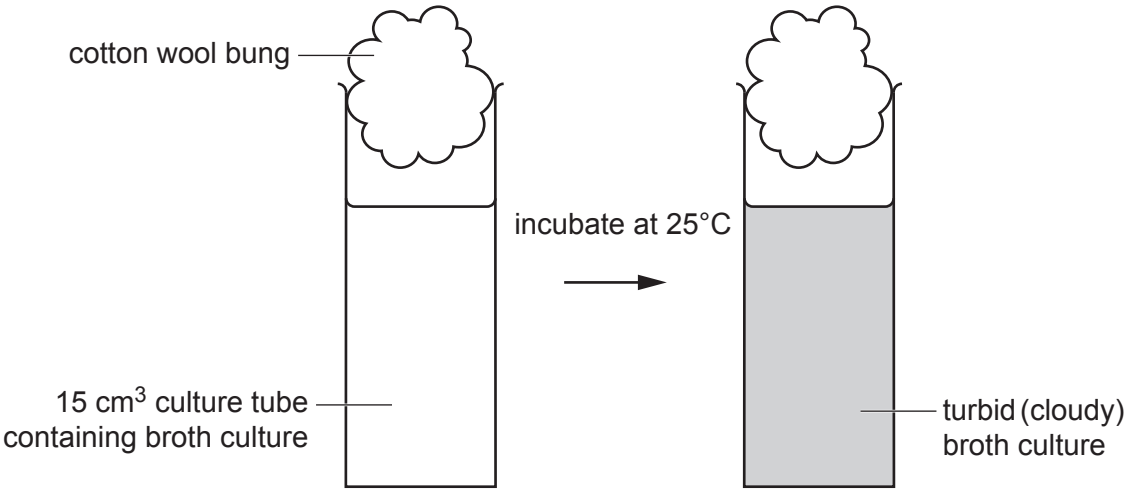


Fig. 1.2

The student decided that turbidity of the broth culture is a measure of bacterial population growth (bacterial growth).

Explain how this concept can be used in an investigation to measure the extent of bacterial growth.

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(b) The student decided to test the antibiotic solution before testing the Manuka honey.

In addition to normal laboratory apparatus and materials, the student was provided with:

- a fresh broth culture of *B. subtilis*
- a clear antibiotic stock solution
- nutrient solution to dilute the antibiotic stock solution
- 15 cm^3 flat-bottomed glass culture tubes with sterile cotton wool bungs
- a choice of graduated pipettes to measure volumes accurately: 0.2 cm^3, 2.0 cm^3, 10.0 cm^3, 25.0 cm^3.

The student:

- prepared dilutions of the antibiotic stock solution and added a volume of each to different culture tubes
- added a volume of fresh broth culture of *B. subtilis* to each culture tube
- incubated the culture tubes in an incubator
- allowed time for bacterial growth to occur and then checked each culture tube
- recorded and analysed the results
- decided on the lowest concentration of antibiotic solution that appeared to kill or inhibit the growth of *B. subtilis*.

Outline a control for this part of the investigation.

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(c) In the next part of the investigation, the student used a stock solution of Manuka honey. The student remembered that hydrogen peroxide could be present but could not think of a way to break down the hydrogen peroxide to remove it from the solution.

Describe how the student can improve the investigation by removing hydrogen peroxide from the Manuka honey solution **and** explain why this improvement makes the results more valid.

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(d) The student was provided with a stock solution of Manuka honey containing an MGO concentration of 600 μg cm^{-3}. This was a clear solution, labelled '100% honey'.

The same apparatus and materials were available.

Describe how the student could prepare a 10% solution of honey using the stock solution.

Construct a table to show how the dilution is made for the 10% solution and the other concentrations that the student could use.

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Space for table.

[2]

(e) State the independent variable **and** dependent variable for the part of the investigation involving Manuka honey solution.

independent variable .....

dependent variable .....
[2]

(f) Predict the results the student would expect when investigating the effect of Manuka honey on *B. subtilis*.

Explain the reasoning behind the prediction.

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(g) Describe how the student could determine the lowest concentration of Manuka honey solution that would kill or inhibit the growth of *B. subtilis*.

- Do **not** repeat any detail given in **(d)** of how to prepare the different concentrations of Manuka honey solution
- Do **not** give details of using aseptic technique (techniques to prevent contamination of the student, the environment or other people).

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

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(h) MRSA, methicillin-resistant *Staphylococcus aureus*, is an example of antibiotic resistance in bacteria. There is evidence that medical-grade Manuka honey is effective in treating wounds infected with MRSA. This honey has been sterilised by gamma irradiation and filtered to remove contaminants.

A study was carried out to see if another type of honey, Germania honey, is as effective as Manuka honey in killing bacteria removed from wounds of 50 people with MRSA.

Five different concentrations of each type of honey were compared. The concentrations were numbered 1 to 5, with 1 being the highest concentration and 5 the lowest concentration.

At the concentrations where there was no visible growth in a broth culture of *S. aureus*, the researchers transferred samples onto nutrient agar plates containing no antibacterial substance. Incubation of these plates confirmed that there was no bacterial growth.

The results were analysed using the chi-squared (χ^2) test.

Table 1.1 shows the results of the study and the statistical analysis using the χ^2 test.

Table 1.1

concentration of honey M=Manuka G=Germania	number of cultures with bacterial growth	number of cultures with no bacterial growth	χ^2 value	significant
M1	2	48	5.005	yes
G1	9	41		
M2	5	45	13.306	
G2	21	29		
M3	11	39	14.923	
G3	30	20		
M4	43	7	3.052	
G4	48	2		
M5	47	3	1.042	no
G5	49	1		

(i) State a null hypothesis for the investigation.

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..... [1]

(ii) Table 1.2 shows some critical values for χ^2 at different probabilities.

Table 1.2

degrees of freedom	probability						
	0.99	0.95	0.90	0.10	0.05	0.01	0.001
1	0.0002	0.0039	0.0158	2.706	3.841	6.635	10.827
2	0.0201	0.1026	0.2107	4.605	5.991	9.210	13.815

Use Table 1.2 to decide whether the χ^2 values for concentrations 2, 3 and 4 in Table 1.1 are significant or not significant. Write your decision in the final column of Table 1.1:

- write **yes** if the value is significant
- write **no** if the value is **not** significant

[1]

(iii) This study compared the effectiveness of the two honey varieties in treating wounds infected with MRSA.

State the conclusions that can be made from the results and statistical analysis of this study.

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[Total: 20]

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2 Holstein cattle have been selectively bred for their high milk production.

The seed of the cotton plant, *Gossypium* spp. is a good source of fibre, protein and carbohydrate. To improve milk production, whole cottonseed or cottonseed meal can be added to livestock feed. Cottonseed meal is cottonseed that has been processed by grinding.

Fig. 2.1**A** shows whole cotton seed and Fig. 2.1**B** shows cottonseed meal.



A



B

Fig. 2.1

Free gossypol is a toxin found in cotton seeds. Some of this free gossypol binds to protein to form bound gossypol. This occurs during cottonseed meal production and in the rumen (forestomach) of the cow during microorganism fermentation.

Free gossypol is easily absorbed. Bound gossypol is absorbed less easily and is not toxic.

An investigation was carried out on the effect of different dry-matter diets on lactating (milk-producing) Holstein cows:

- 30 healthy cows were fed on the same cottonseed-free diet for 14 days.
- The cows were then divided equally into five groups, **A** to **E**, and fed on one of five experimental diets for 42 days.
- The quantity of milk produced each day was recorded.
- The cows had no visible signs of illness during the 42 days.

For each diet, Table 2.1 shows:

- the cottonseed content and total gossypol (free and bound) content
- the mean daily dry matter taken in during feeding (intake)
- the mean daily lactation performance (milk yield).

Table 2.1

SE = standard error

group	description of dry matter diet	cottonseed content / percentage of dry matter		total gossypol in diet / mg kg^{-1}	dry matter intake $kg\ d^{-1}$ SE = 1.4	milk yield / $kg\ d^{-1}$ SE = 1.3
		whole cottonseed	cottonseed meal			
A	cottonseed replaced by soybean meal	0	0	0	24.8	27.6
B	whole cottonseed	15.0	0	1039.7	23.6	29.7
C	cottonseed meal	0	7.0	900.1	23.2	27.9
D	mixed 1 (whole cottonseed and cottonseed meal)	7.5	3.5	959.7	22.6	28.7
E	mixed 2 (whole cottonseed and cottonseed meal)	15.0	7.0	1922.0	24.0	32.6

The differences in the dry matter intake for groups **A** to **E** were **not** statistically significant.

(a) Explain why the cows were all fed on the same cottonseed-free diet for 14 days.

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..... [1]

(b) Using the information provided and the results shown in Table 2.1, a student made some statements about the effect of feeding different diets to lactating Holstein cows.

(i) The student stated that if the investigation is repeated, the groups of cows will produce milk in this order:

(highest quantity) **E** → **B** → **D** → **C** → **A** (lowest quantity).

With reference to Table 2.1, explain why the statement made by the student is **not** supported by the data.

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(ii) Scientists are developing cotton plants that are genetically modified to produce seeds lacking gossypol. Table 2.1 suggests that there may be a link between total gossypol in the diet and milk yield.

The student stated that repeating the investigation with no gossypol in diets **B** to **E** would result in a lower milk yield for each group.

Explain whether or not Table 2.1 provides enough evidence to support the statement made by the student.

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..... [2]

(c) Higher concentrations of gossypol absorbed into the circulation can cause an illness in cows known as gossypol toxicity. Low concentrations of gossypol can be detoxified in the liver.

(i) During the investigation, the gossypol intake for each cow was measured and analysed.

At the end of the investigation (day 42), the blood plasma gossypol concentration of each cow was also measured.

Fig. 2.2 shows the free gossypol intake for each cow, plotted against the concentration of gossypol in plasma on day 42.

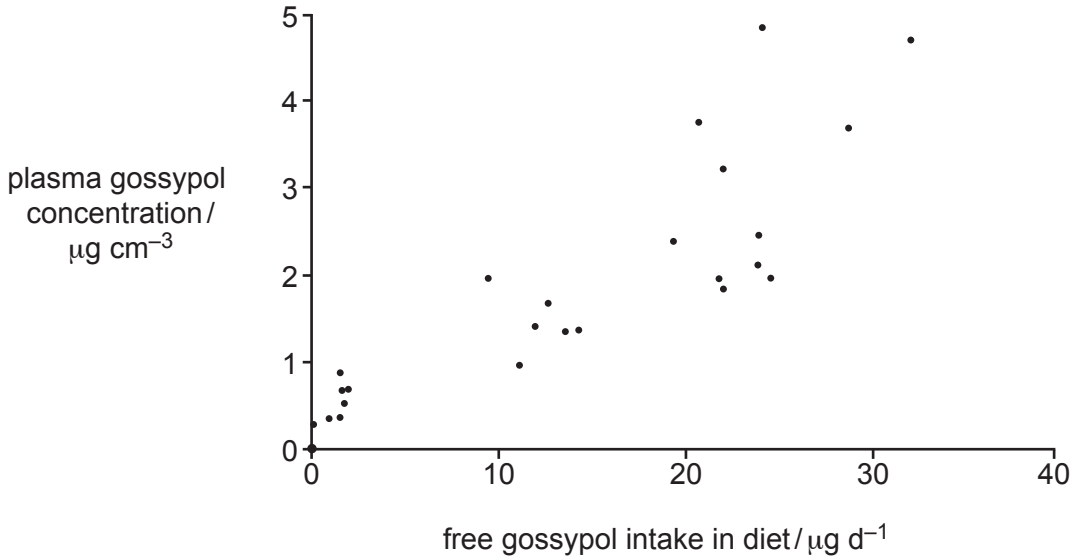


Fig. 2.2

With reference to Fig. 2.2, explain whether there is a relationship between free gossypol intake and plasma gossypol concentration on day 42.

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(ii) Gossypol produced by cotton plants can be found in two forms, the (–) isomer and the (+) isomer. The (–) isomer has higher biological activity within cells and is much more toxic than the (+) isomer. Both forms can bind protein to become bound gossypol.

Table 2.2 summarises the mean daily gossypol intakes for the five different experimental groups of lactating Holstein cows.

Table 2.2

group	description of dry matter diet	total gossypol intake / g d^{-1}	free gossypol intake / g d^{-1}	bound gossypol intake / g d^{-1}	(–) isomer intake / g d^{-1}
A	cottonseed replaced by soybean meal	0.0	0.0	0.0	0.0
B	whole cottonseed	23.5	22.4	1.1	9.4
C	cottonseed meal	21.0	1.5	19.5	8.4
D	mixed 1 (whole cottonseed and cottonseed meal)	21.9	12.1	9.8	8.8
E	mixed 2 (whole cottonseed and cottonseed meal)	45.7	25.2	20.5	18.3

The investigators knew that different diets presented different levels of risk of causing gossypol toxicity in cows. For example, the diet for group **A** cows did not present any risk of toxicity.

With reference to Table 2.2, discuss the extent to which the different experimental diets for groups **B**, **C**, **D** and **E** present a risk of causing gossypol toxicity.

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[Total: 10]

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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.
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- Do **not** write on any bar codes.
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- 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 [ ].

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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.

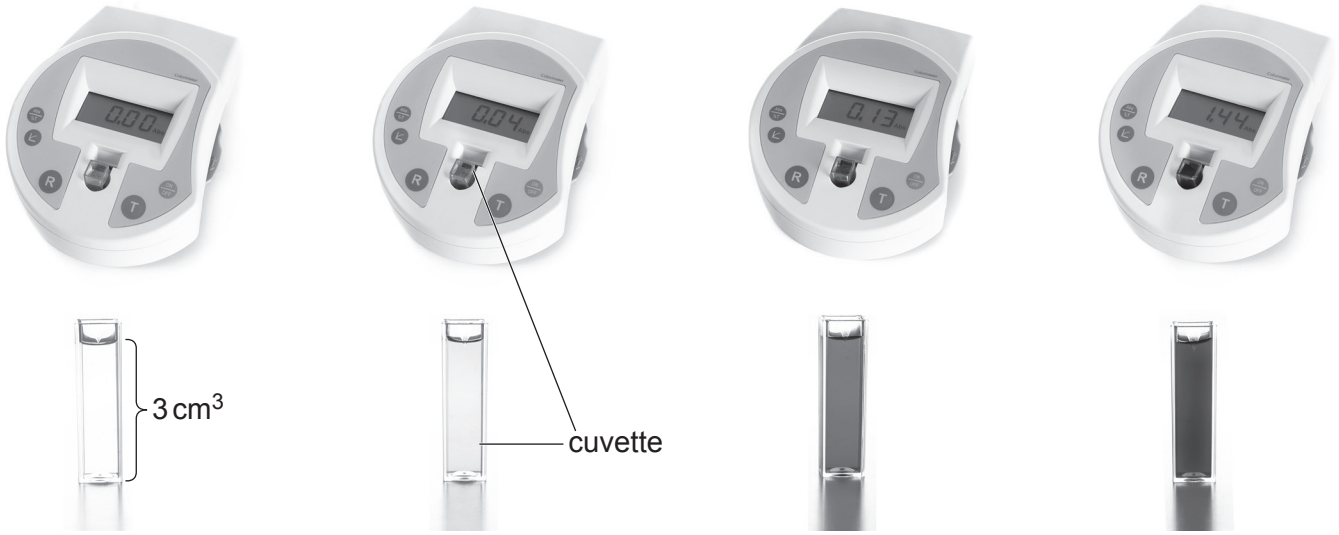


Fig. 1.1

(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.

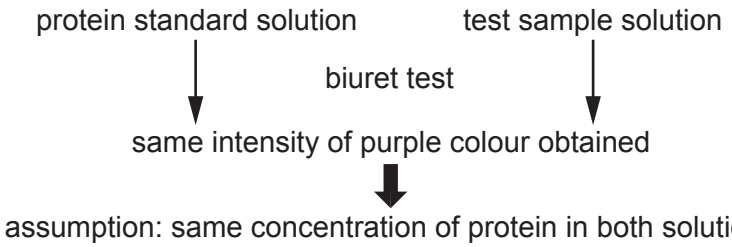


Fig. 1.3

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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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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[2]

(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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7875730187

BIOLOGY **9700/53**

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

1 hour 15 minutes

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No additional materials are needed.

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- 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 **16** pages. Any blank pages are indicated.

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1 Antibiotic resistance in bacteria is a global problem that has caused scientists to research into antibacterial substances other than antibiotics. Honey has properties that make it a good antibacterial substance. For example, honey contains hydrogen peroxide, which is known to kill bacteria.

The most effective honey tested so far for antibacterial activity is Manuka honey. It contains less hydrogen peroxide than many other types of honey, but it does contain an antibacterial compound, methylglyoxal (MGO), which is not found in other types of honey.

A student decided to investigate the effect of two antibacterial substances on the bacterium *Bacillus subtilis*, which respires aerobically:

- MGO in Manuka honey
- an antibiotic solution used in cell cultures to prevent contamination.

The student wanted to find the lowest concentration of each antibacterial substance that would kill or inhibit the growth of *B. subtilis*.

(a) The student used a broth culture for the investigation. To make a broth culture, a small quantity of *B. subtilis* is added to a clear nutrient solution. A fresh (newly made) broth culture of *B. subtilis* is also clear.

Fig. 1.1 is a diagram of a fresh broth culture of *B. subtilis*.

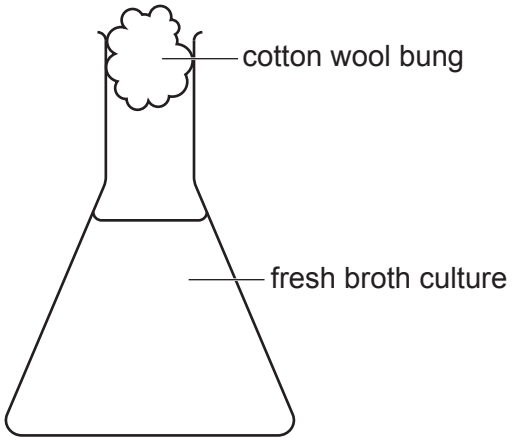


Fig. 1.1

(i) A sterile cotton wool bung was used in the top of the flask containing the fresh broth culture of *B. subtilis* to protect the culture from contamination.

Explain why it is better to use a sterile cotton wool bung in a flask containing broth culture of *B. subtilis*, rather than using a sterile rubber bung.

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(ii) Before comparing the two antibacterial substances, the student carried out a trial experiment.

The student transferred a sample of fresh broth culture to a culture tube and incubated the tube at 25 °C for 24 hours.

Fig. 1.2 summarises the results of the trial experiment.

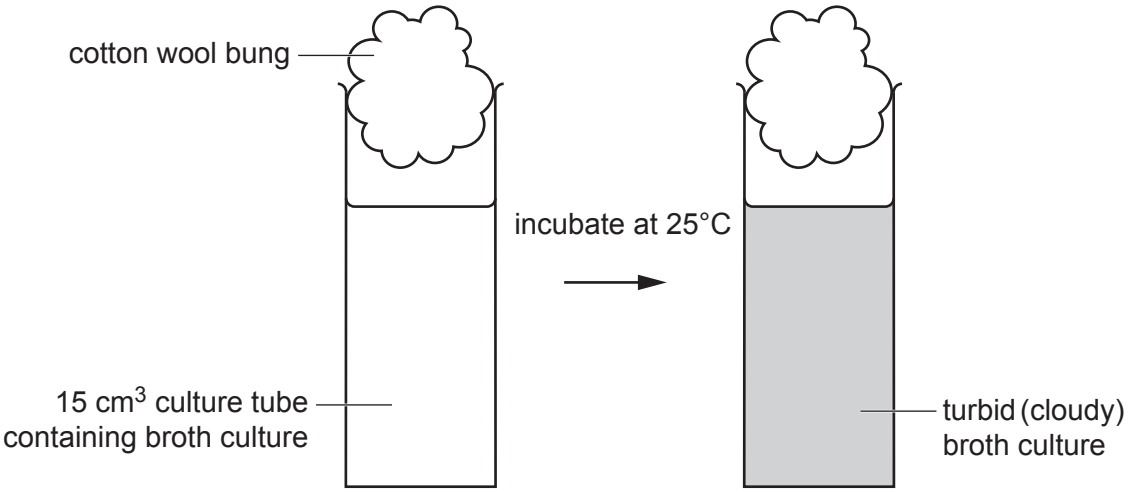


Fig. 1.2

The student decided that turbidity of the broth culture is a measure of bacterial population growth (bacterial growth).

Explain how this concept can be used in an investigation to measure the extent of bacterial growth.

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(b) The student decided to test the antibiotic solution before testing the Manuka honey.

In addition to normal laboratory apparatus and materials, the student was provided with:

- a fresh broth culture of *B. subtilis*
- a clear antibiotic stock solution
- nutrient solution to dilute the antibiotic stock solution
- 15 cm^3 flat-bottomed glass culture tubes with sterile cotton wool bungs
- a choice of graduated pipettes to measure volumes accurately: 0.2 cm^3, 2.0 cm^3, 10.0 cm^3, 25.0 cm^3.

The student:

- prepared dilutions of the antibiotic stock solution and added a volume of each to different culture tubes
- added a volume of fresh broth culture of *B. subtilis* to each culture tube
- incubated the culture tubes in an incubator
- allowed time for bacterial growth to occur and then checked each culture tube
- recorded and analysed the results
- decided on the lowest concentration of antibiotic solution that appeared to kill or inhibit the growth of *B. subtilis*.

Outline a control for this part of the investigation.

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(c) In the next part of the investigation, the student used a stock solution of Manuka honey. The student remembered that hydrogen peroxide could be present but could not think of a way to break down the hydrogen peroxide to remove it from the solution.

Describe how the student can improve the investigation by removing hydrogen peroxide from the Manuka honey solution **and** explain why this improvement makes the results more valid.

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(d) The student was provided with a stock solution of Manuka honey containing an MGO concentration of 600 μg cm^{-3}. This was a clear solution, labelled '100% honey'.

The same apparatus and materials were available.

Describe how the student could prepare a 10% solution of honey using the stock solution.

Construct a table to show how the dilution is made for the 10% solution and the other concentrations that the student could use.

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Space for table.

[2]

(e) State the independent variable **and** dependent variable for the part of the investigation involving Manuka honey solution.

independent variable .....

dependent variable .....
[2]

(f) Predict the results the student would expect when investigating the effect of Manuka honey on *B. subtilis*.

Explain the reasoning behind the prediction.

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(g) Describe how the student could determine the lowest concentration of Manuka honey solution that would kill or inhibit the growth of *B. subtilis*.

- Do **not** repeat any detail given in **(d)** of how to prepare the different concentrations of Manuka honey solution
- Do **not** give details of using aseptic technique (techniques to prevent contamination of the student, the environment or other people).

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

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(h) MRSA, methicillin-resistant *Staphylococcus aureus*, is an example of antibiotic resistance in bacteria. There is evidence that medical-grade Manuka honey is effective in treating wounds infected with MRSA. This honey has been sterilised by gamma irradiation and filtered to remove contaminants.

A study was carried out to see if another type of honey, Germania honey, is as effective as Manuka honey in killing bacteria removed from wounds of 50 people with MRSA.

Five different concentrations of each type of honey were compared. The concentrations were numbered 1 to 5, with 1 being the highest concentration and 5 the lowest concentration.

At the concentrations where there was no visible growth in a broth culture of *S. aureus*, the researchers transferred samples onto nutrient agar plates containing no antibacterial substance. Incubation of these plates confirmed that there was no bacterial growth.

The results were analysed using the chi-squared (χ^2) test.

Table 1.1 shows the results of the study and the statistical analysis using the χ^2 test.

Table 1.1

concentration of honey M=Manuka G=Germania	number of cultures with bacterial growth	number of cultures with no bacterial growth	χ^2 value	significant
M1	2	48	5.005	yes
G1	9	41		
M2	5	45	13.306	
G2	21	29		
M3	11	39	14.923	
G3	30	20		
M4	43	7	3.052	
G4	48	2		
M5	47	3	1.042	no
G5	49	1		

(i) State a null hypothesis for the investigation.

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(ii) Table 1.2 shows some critical values for χ^2 at different probabilities.

Table 1.2

degrees of freedom	probability						
	0.99	0.95	0.90	0.10	0.05	0.01	0.001
1	0.0002	0.0039	0.0158	2.706	3.841	6.635	10.827
2	0.0201	0.1026	0.2107	4.605	5.991	9.210	13.815

Use Table 1.2 to decide whether the χ^2 values for concentrations 2, 3 and 4 in Table 1.1 are significant or not significant. Write your decision in the final column of Table 1.1:

- write **yes** if the value is significant
- write **no** if the value is **not** significant

[1]

(iii) This study compared the effectiveness of the two honey varieties in treating wounds infected with MRSA.

State the conclusions that can be made from the results and statistical analysis of this study.

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[Total: 20]

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2 Holstein cattle have been selectively bred for their high milk production.

The seed of the cotton plant, *Gossypium* spp. is a good source of fibre, protein and carbohydrate. To improve milk production, whole cottonseed or cottonseed meal can be added to livestock feed. Cottonseed meal is cottonseed that has been processed by grinding.

Fig. 2.1**A** shows whole cotton seed and Fig. 2.1**B** shows cottonseed meal.



A



B

Fig. 2.1

Free gossypol is a toxin found in cotton seeds. Some of this free gossypol binds to protein to form bound gossypol. This occurs during cottonseed meal production and in the rumen (forestomach) of the cow during microorganism fermentation.

Free gossypol is easily absorbed. Bound gossypol is absorbed less easily and is not toxic.

An investigation was carried out on the effect of different dry-matter diets on lactating (milk-producing) Holstein cows:

- 30 healthy cows were fed on the same cottonseed-free diet for 14 days.
- The cows were then divided equally into five groups, **A** to **E**, and fed on one of five experimental diets for 42 days.
- The quantity of milk produced each day was recorded.
- The cows had no visible signs of illness during the 42 days.

For each diet, Table 2.1 shows:

- the cottonseed content and total gossypol (free and bound) content
- the mean daily dry matter taken in during feeding (intake)
- the mean daily lactation performance (milk yield).

Table 2.1

SE = standard error

group	description of dry matter diet	cottonseed content / percentage of dry matter		total gossypol in diet / mg kg^{-1}	dry matter intake $kg\ d^{-1}$ SE = 1.4	milk yield / $kg\ d^{-1}$ SE = 1.3
		whole cottonseed	cottonseed meal			
A	cottonseed replaced by soybean meal	0	0	0	24.8	27.6
B	whole cottonseed	15.0	0	1039.7	23.6	29.7
C	cottonseed meal	0	7.0	900.1	23.2	27.9
D	mixed 1 (whole cottonseed and cottonseed meal)	7.5	3.5	959.7	22.6	28.7
E	mixed 2 (whole cottonseed and cottonseed meal)	15.0	7.0	1922.0	24.0	32.6

The differences in the dry matter intake for groups **A** to **E** were **not** statistically significant.

(a) Explain why the cows were all fed on the same cottonseed-free diet for 14 days.

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(b) Using the information provided and the results shown in Table 2.1, a student made some statements about the effect of feeding different diets to lactating Holstein cows.

(i) The student stated that if the investigation is repeated, the groups of cows will produce milk in this order:

(highest quantity) **E** → **B** → **D** → **C** → **A** (lowest quantity).

With reference to Table 2.1, explain why the statement made by the student is **not** supported by the data.

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(ii) Scientists are developing cotton plants that are genetically modified to produce seeds lacking gossypol. Table 2.1 suggests that there may be a link between total gossypol in the diet and milk yield.

The student stated that repeating the investigation with no gossypol in diets **B** to **E** would result in a lower milk yield for each group.

Explain whether or not Table 2.1 provides enough evidence to support the statement made by the student.

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(c) Higher concentrations of gossypol absorbed into the circulation can cause an illness in cows known as gossypol toxicity. Low concentrations of gossypol can be detoxified in the liver.

(i) During the investigation, the gossypol intake for each cow was measured and analysed.

At the end of the investigation (day 42), the blood plasma gossypol concentration of each cow was also measured.

Fig. 2.2 shows the free gossypol intake for each cow, plotted against the concentration of gossypol in plasma on day 42.

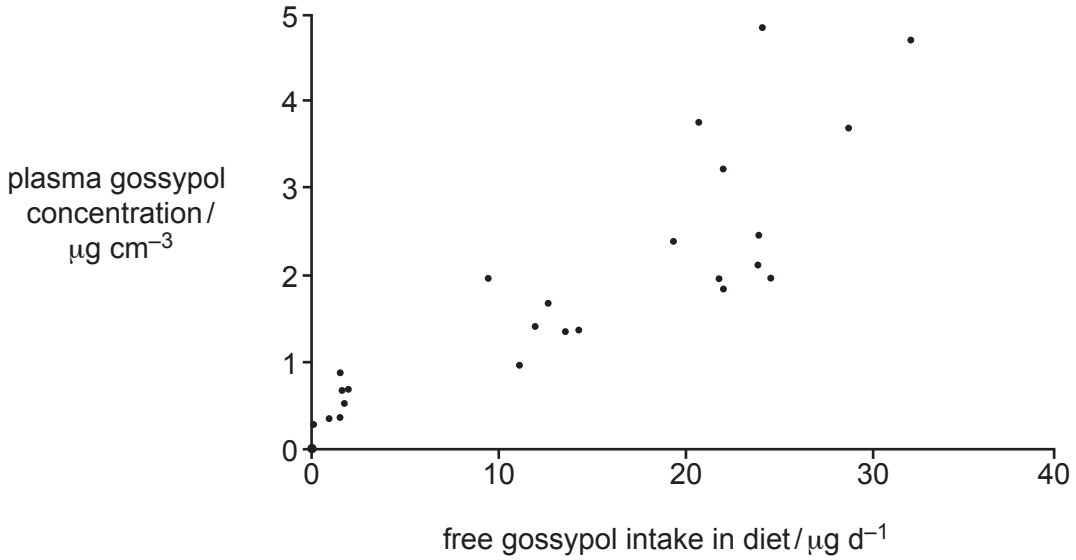


Fig. 2.2

With reference to Fig. 2.2, explain whether there is a relationship between free gossypol intake and plasma gossypol concentration on day 42.

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(ii) Gossypol produced by cotton plants can be found in two forms, the (–) isomer and the (+) isomer. The (–) isomer has higher biological activity within cells and is much more toxic than the (+) isomer. Both forms can bind protein to become bound gossypol.

Table 2.2 summarises the mean daily gossypol intakes for the five different experimental groups of lactating Holstein cows.

Table 2.2

group	description of dry matter diet	total gossypol intake / $g\,d^{-1}$	free gossypol intake / $g\,d^{-1}$	bound gossypol intake / $g\,d^{-1}$	(–) isomer intake / $g\,d^{-1}$
A	cottonseed replaced by soybean meal	0.0	0.0	0.0	0.0
B	whole cottonseed	23.5	22.4	1.1	9.4
C	cottonseed meal	21.0	1.5	19.5	8.4
D	mixed 1 (whole cottonseed and cottonseed meal)	21.9	12.1	9.8	8.8
E	mixed 2 (whole cottonseed and cottonseed meal)	45.7	25.2	20.5	18.3

The investigators knew that different diets presented different levels of risk of causing gossypol toxicity in cows. For example, the diet for group **A** cows did not present any risk of toxicity.

With reference to Table 2.2, discuss the extent to which the different experimental diets for groups **B**, **C**, **D** and **E** present a risk of causing gossypol toxicity.

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[Total: 10]

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