Cambridge Assessment International Education

Cambridge International AS & A Level

CANDIDATE NAME

CENTRE NUMBER

CANDIDATE NUMBER

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.

DC (DE) 320747

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*.

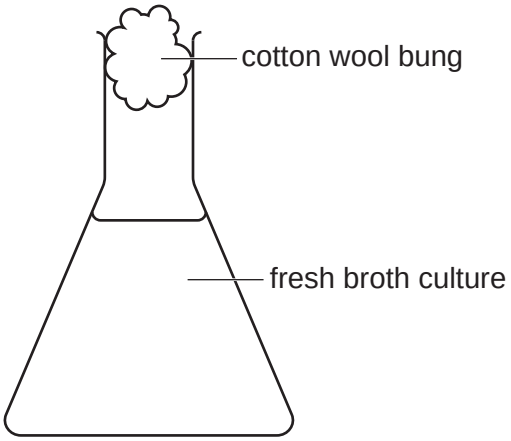

Fig. 1.1

(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.

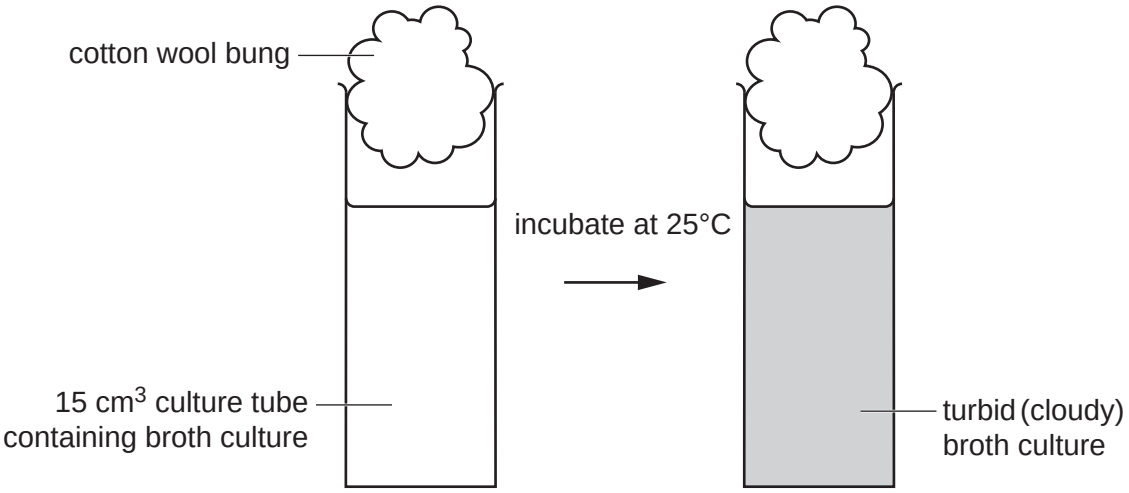

Fig. 1.2

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.

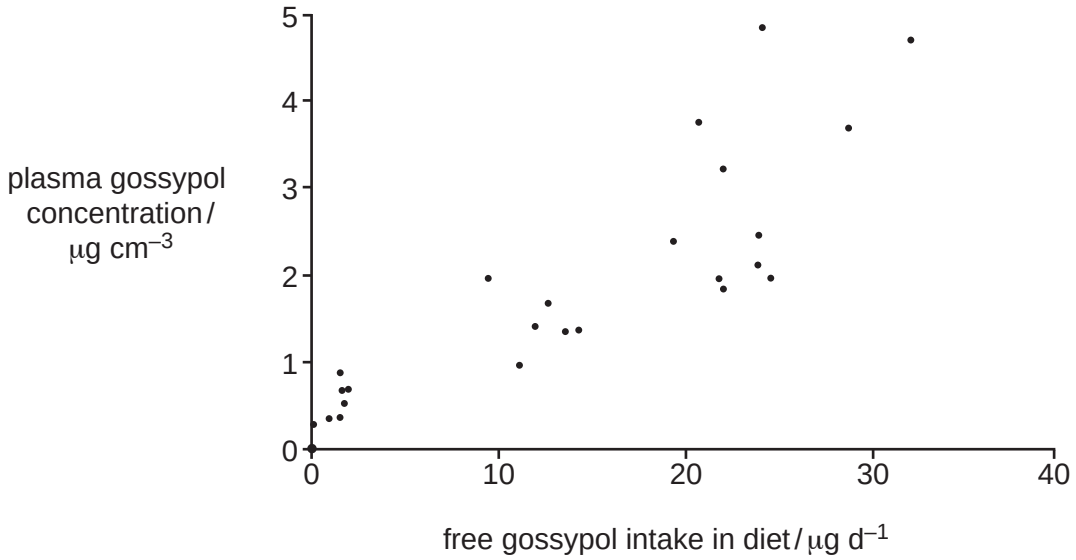

Fig. 2.2

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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