General Certificate of Education
Advanced Level Examination
June 2010

Human Biology HBI6X/TN

Unit 6X A2 Externally Marked Practical Assignment

Teachers' Notes Confidential

A copy should be given immediately to the teacher(s) responsible for GCE Human Biology

Open on receipt

Teachers' Notes

CONFIDENTIAL

These notes must be read in conjunction with *Instructions for the Administration of the Externally Marked Practical Assignment* published on the AQA Website.

The use of the enzyme urease by bacteria in the stomach

Candidates will investigate the effect of temperature on the activity of the enzyme urease. This enzyme allows some bacteria to survive the antibacterial action of acidic gastric juice. The method is based on the colour change of an indicator (phenol red) in the presence of alkaline substances such as the ammonia produced by the action of urease on urea. In each task, one drop of dilute hydrochloric acid will be added to the solutions under test to ensure that conditions are acidic to begin with.

Task 1

Materials

In addition to access to general laboratory equipment, each candidate needs

- 5 cm^3 phenol red solution
- 50 cm^3 5% urease solution
- 50 cm^3 1% urea solution
- 5 cm^3 hydrochloric acid, concentration 0.1 mol dm^{-3}
- distilled water
- a large beaker (400 cm^3 or 500 cm^3) to use as a water bath, or access to a thermostatic water bath (candidates could share a water bath but there should be no other cooperation)
- thermometer capable of measuring over the range 10 °C to 30 °C
- 2 small beakers
- 18 test tubes
- test-tube rack
- timer
- 3 graduated pipettes or syringes capable of measuring up to 5 cm^3
- 2 dropping pipettes
- marker pen

Managing the investigation

Candidates should be provided with a 5% urease solution from which they will produce a dilution series. They are required to find a concentration of urease that will produce an end-point in a manageable time scale. You will decide the concentration to use in Task 2, so it will not matter if candidates make the wrong concentrations.

This experiment was successfully trialled using powdered 'Urease Active Meal (crude urease)' obtained from Philip Harris. The temperature range given for this enzyme is 20 ºC to 70 ºC. The enzyme is also available from other suppliers such as Griffin Education or Timstar Laboratory Suppliers Ltd. For the purpose of the investigation, it will not matter if the urease that you obtain is not from a bacterial source, but it must be active at temperatures above 45 ºC. The urease solution will need to be filtered to produce a clear liquid. This can take up to about 20 minutes but will allow colour changes to be more readily visible. A stock solution of phenol red was used but a 0.1% concentration of phenol red was also found to be suitable for use. Urea is readily available from biological or chemical suppliers.

The task will need to be trialled before use.

One week before Task 1, teachers may give their candidates the following information.

You will investigate the effect of temperature on an enzyme-controlled reaction. This reaction is related to the bacteria that live in the human gut.

There should be no further discussion of this topic.

In this investigation, teachers must not give candidates the following information

- how to interpret colour changes
- how to produce a dilution series
- how long to leave the test tubes in the water bath before mixing.

Task 2

Materials

In addition to access to general laboratory equipment, each candidate needs

- 5 cm^3 phenol red solution
- 25 cm^3 urease solution (concentration determined by centre)
- 100 cm^3 5% urea solution
- 5 cm^3 hydrochloric acid, concentration 0.1 mol dm^{-3}
- 3 large beakers (400 cm^3 or 500 cm^3) to use as water baths, or access to thermostatic water baths (candidates could share a water bath but there should be no other cooperation)
- thermometer or thermometers capable of measuring over the range 20 ºC to 80 ºC
- 2 small beakers
- 15 test tubes
- test-tube rack
- timer
- 3 graduated pipettes or syringes capable of measuring up to 5 cm^3
- 2 dropping pipettes
- marker pen
- AQA Students' Statistics Sheet provided as part of Task Sheet 2

Managing the investigation

The results from Task 1 should be used to find an appropriate concentration of urease to use in Task 2. The teacher must decide on an appropriate concentration of urease to use in Task 2. All candidates should be provided with the same concentration of urease. It should be made clear to them that this concentration was determined by the results from Task 1.

In Task 2, the rate of colour change from yellow to pink-red should be found at three different temperatures, determined by the centre, but these temperatures should be within the range of 25 to 75 ºC. Literature reports indicate that the optimum temperature for urease is within a range of about 45 to 70 ºC. This variation does not matter for the context of the investigation. The three temperatures suggested are 30 ºC, 50 ºC and 70 ºC but the centre will need to decide for itself, through an appropriate trial, which temperatures to use. This makes allowance for any variability in samples of urease. However, this emphasises the need to use a sample of urease that will function at temperatures above 45 ºC.

Centres are again reminded that they should **not** purchase a urease sample that is intended to function over a narrow temperature range, and that for the purpose of the investigation, it will not matter if the source of the urease is not a bacterium. The urease solution should be filtered to produce a clear liquid. This will allow colour changes to be more readily visible.

For processing of data, where a candidate does not obtain sufficient results for all 3 temperatures, the teacher should provide supplementary data. This is provided *after* the candidate's attempt at the table and should be annotated as 'teacher's data'. In this investigation, five sets of data will be considered sufficient for a statistical analysis.

The task will need to be trialled before use.

In this investigation, teachers must not give candidates the following information

- how to interpret colour changes
- the number of repeats required
- what variables to monitor or control
- which statistical test would be appropriate to use.

Centre Number						Candidate Number				
Surname										
Other Names										
Candidate Signature										

For Examiner's Use
Total Task 1

General Certificate of Education
Advanced Level Examination
June 2010

Human Biology HBI6X/PM1

Unit 6X A2 Externally Marked Practical Assignment
Task Sheet 1

To be completed before Task Sheet 2

For submission by 15 May 2010

For this paper you must have:
- a ruler with millimetre measurements
- a calculator.

The use of the enzyme urease by bacteria in the stomach

Introduction

Some species of bacteria cause diseases of the stomach. Most bacteria are killed by acidic gastric juice. Gastric juice is produced by the stomach lining. Some species of bacteria survive the antibacterial action of gastric juice by secreting the enzyme urease. This enzyme catalyses a reaction that produces ammonia. The ammonia neutralises the acid in gastric juice.

Phenol red is a pH indicator that turns from yellow in acidic conditions to pink-red in alkaline conditions.

Task 1 – Finding a suitable concentration of urease to use

In this task, you will find the concentration of urease that produces a result in a suitable time so that you can use this in Task 2.

Materials

You are provided with

- phenol red solution
- 5% urease solution
- 1% urea solution
- hydrochloric acid
- distilled water
- a large beaker that you can use as a water bath, or access to a thermostatic water bath
- thermometer
- small beakers
- test tubes
- test-tube rack
- timer
- graduated pipettes or syringes
- dropping pipettes
- marker pen.

You may ask your teacher for any other apparatus you require.

Outline method

Read these instructions carefully before you start your investigation.

1. Set up a water bath at room temperature.
2. Label six test tubes A to F.
3. Put 1 cm^3 5% urease in tube A.
4. Use the 5% urease solution and distilled water to make urease solutions of concentrations 4%, 3%, 2% and 1%.
5. Set up tubes B to F.
   - Tube B should contain 1 cm^3 4% urease solution.
   - Tube C should contain 1 cm^3 3% urease solution.
   - Tube D should contain 1 cm^3 2% urease solution.
   - Tube E should contain 1 cm^3 1% urease solution.
   - Tube F should contain 1 cm^3 distilled water.
6. Place tubes A to F in the water bath.
7. Label another six test tubes 1 to 6.
8. Add 5 cm^3 urea solution to each tube.
9. Add 2 drops of phenol red and 1 drop of hydrochloric acid to each of tubes 1 to 6 and place all six tubes in the water bath.
10. After a suitable time, add the contents of tube 1 to tube A, quickly mix the contents and start the timer.
11. In the table below, record how long it takes for the phenol red to turn from yellow to pink-red.
12. Repeat steps 10 and 11 but add the contents of tube 2 to tube B, then tube 3 to tube C, then tube 4 to tube D, then tube 5 to tube E and finally tube 6 to tube F.
13. Tube F will act as a control experiment.

You will need to decide for yourself

- how to make the different concentrations of urease solution
- how long to leave the test tubes in the water bath before mixing
- when the colour change has occurred.

Recording your results

Record your results in the table.

Tube	Percentage concentration of urease	Time taken for phenol red to turn pink-red / seconds
A	5	
B	4	
C	3	
D	2	
E	1	
F	0	

Questions on Task 1

Answer **all** questions in the spaces provided.

1 Complete the headings on the table and the empty boxes to show how you made the different concentrations of urease solution.

Percentage concentration of urease solution	**Volume of water /.....**	**Volume of 5% urease solution /.....**
4		
3		
2		
1		

2 Urease catalyses the reaction

urea + water → ammonia + carbon dioxide

What name is given to the type of reaction that urease catalyses?

3 (a) You left tube 1 and tube A for a time in the water bath before mixing their contents. Explain why.

3 (b) You were told to place the test tubes in a water bath at room temperature. Do you think it was necessary to use a water bath?
Explain your answer.

3 (c) Describe how you monitored the temperature of the water bath.

4 What was the purpose of using tube F as a control?

5 Use your data to suggest the urease concentration that you will use in Task 2.
Explain why you chose this concentration.

6 pH is a factor that affects enzyme activity. Do you think a buffer solution should have been added to the contents of the tubes before mixing?
Explain your answer.

END OF TASK 1

Centre Number						Candidate Number				
Surname										
Other Names										
Candidate Signature										

For Examiner's Use
Total Task 2

General Certificate of Education
Advanced Level Examination
June 2010

Human Biology HBI6X/PM2

Unit 6X A2 Externally Marked Practical Assignment
Task Sheet 2

To be completed before the EMPA Written Test

For submission by 15 May 2010

For this paper you must have:
- a ruler with millimetre measurements
- a calculator.

The use of the enzyme urease by bacteria in the stomach

Introduction

Some species of bacteria cause diseases of the stomach. Most bacteria are killed by acidic gastric juice in the stomach. Some species of bacteria survive the antibacterial action of gastric juice by secreting the enzyme urease. This enzyme catalyses a reaction that produces ammonia. The ammonia neutralises the acid in gastric juice.

Phenol red is a pH indicator that turns from yellow in acidic conditions to pink-red in alkaline conditions. The optimum temperature for bacterial urease activity is not the same as for human enzymes.

For Task 2, you will investigate the effect of temperature on the activity of urease. You will mix a solution of urease with a solution of urea, in the presence of phenol red. You will do this at a particular temperature and measure how long it takes for the phenol red to turn from yellow to pink-red. You will repeat the experiment at two other temperatures. Your teacher will tell you the concentration of the urease solution and the three temperatures to use.

Task 2 – Finding the optimum temperature for bacterial urease

Materials

You are provided with

- phenol red solution
- 25 cm^3 urease solution
- 100 cm^3 5% urea solution
- hydrochloric acid
- large beakers that you can use as water baths, or access to thermostatic water baths
- thermometer
- small beakers
- test tubes
- test-tube rack
- timer
- graduated pipettes or syringes
- dropping pipettes
- marker pen
- AQA Students' Statistics Sheet included at the back of this Task Sheet

You may ask your teacher for any other apparatus you require.

Method

Read these instructions carefully before you start your investigation

1. Set up a water bath at the temperature you are investigating.
2. Put 5 cm^3 of the urea solution, 2 drops of phenol red and 1 drop of hydrochloric acid into a test tube.
3. Put 1 cm^3 of urease solution into a second tube.
4. Put both tubes into a water bath at the first temperature you are investigating.
5. Leave them for 5 minutes, then mix the contents of the tubes and start the timer.
6. Record the time for the phenol red solution to go from yellow to pink-red.
7. Repeat steps 1 to 6 for the two other temperatures.

You will need to decide for yourself

- how many repeats to use at each temperature. You will need enough data to be able to carry out a statistical test on your results
- whether there are other variables to control that might influence the data to be collected
- how to ensure the reliability of the end-point of each experiment
- what statistical test to use when analysing your results.

7 Record the results of your investigation in an appropriate table in the space below.

8 Compare any **two** temperatures of your choice from the results you have collected.

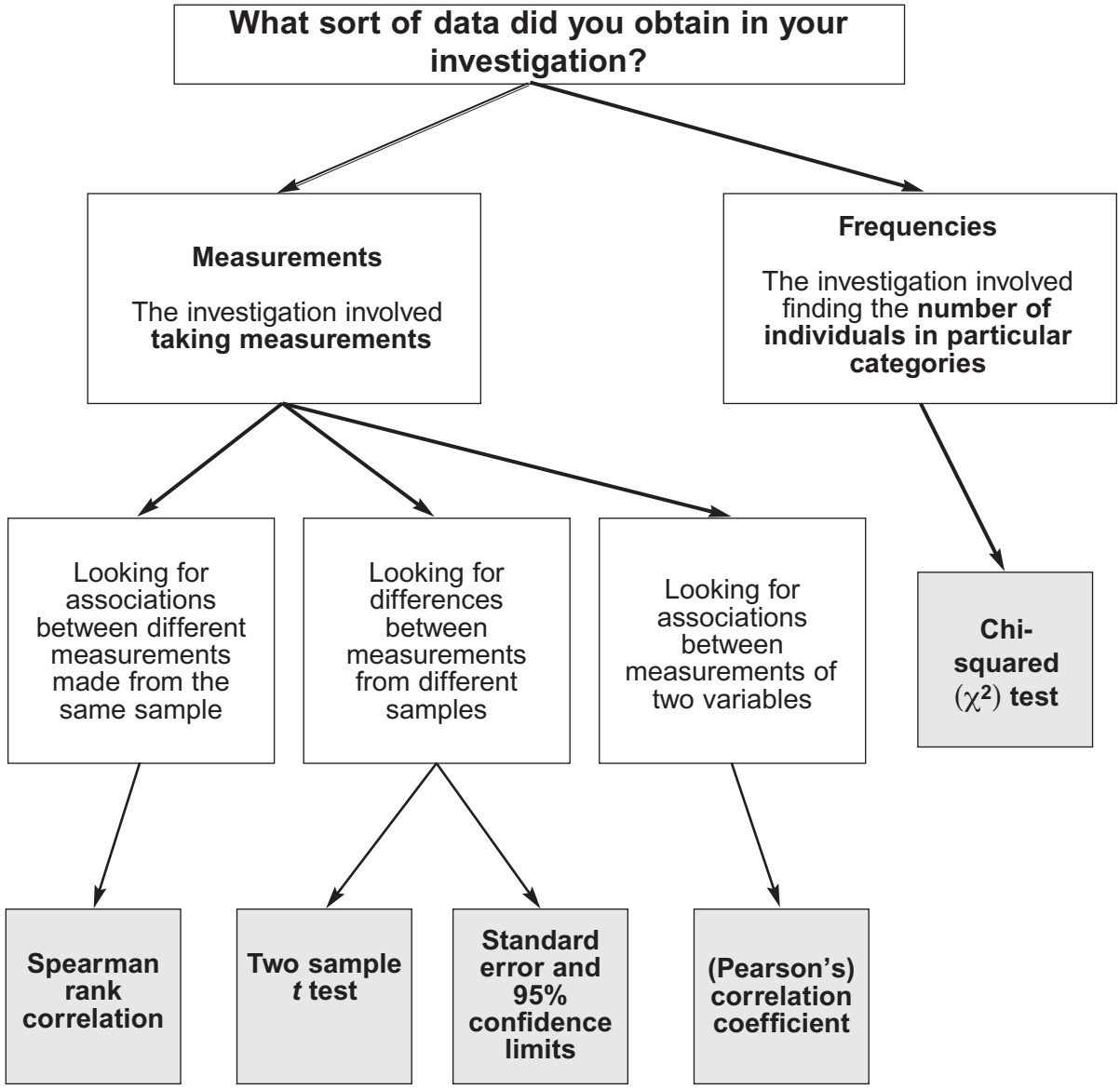
Use the space below to analyse your data with a suitable statistical test. You may use a calculator and the Students' Statistics Sheet that has been provided to perform this test.

You should

- state your null hypothesis
- give your choice of statistical test
- give reasons for your choice of statistical test
- carry out the test and calculate the test statistic
- interpret the test statistic in relation to the null hypothesis being tested.

END OF TASK 2

Students' Statistics Sheet



For use in the A2 ISA and EMPA assessment

Statistical tests and tables of critical values

Tables of critical values

A table of critical values is provided with each statistical test. If your calculated test statistic is less than, or equal to, the critical value, then the result of your statistical test is significant. This means that your null hypothesis should be rejected.

Spearman rank correlation test

Use this test when
- you wish to find out if there is a significant association between two sets of measurements from the same sample
- and you have between 5 and 30 pairs of measurements.

Record the data as values of X and Y.

Convert these values to rank orders, 1 for largest, 2 for second largest, etc.

Now calculate the value of the Spearman rank correlation, r_s, from the equation

$$r_s = 1 - \left[\frac{6 \times \Sigma D^2}{N^3 - N}\right]$$

Where N is the number of pairs of items in the sample.
D is the difference between each pair (X-Y) of measurements.

A table showing the critical values of r_s for different numbers of paired values.

Number of pairs of measurements	Critical value
5	1.00
6	0.89
7	0.79
8	0.74
9	0.68
10	0.65
12	0.59
14	0.54
16	0.51
18	0.48

Correlation coefficient (Pearson's correlation coefficient)

Use this test when
- you wish to find out if there is a significant association between two sets of measurements measured on interval or ratio scales
- the data are normally distributed.

Record the data as values of variables X and Y.

Now calculate the value of the (Pearson) correlation coefficient, r, from the equation

$$r = \frac{\Sigma XY - [(\Sigma X)(\Sigma Y)]/n}{\sqrt{\{\Sigma X^2 - [(\Sigma X)^2/n]\}\ \{\Sigma Y^2 - [(\Sigma Y)^2/n]\}}}$$

Where n is the number of values of X and Y.

A table showing the critical values of *r* for different degrees of freedom.

Degrees of freedom	Critical value	Degrees of freedom	Critical value
1	1.00	12	0.53
2	0.95	14	0.50
3	0.88	16	0.47
4	0.81	18	0.44
5	0.75	20	0.52
6	0.71	22	0.40
7	0.67	24	0.39
8	0.63	26	0.37
9	0.60	28	0.36
10	0.58	30	0.35

For most cases, the number of degrees of freedom = $n - 2$

The *t* test

Use this test when
- you wish to find out if there is a significant difference between two means
- the data are normally distributed
- the sample size is less than 25.

t can be calculated from the formula

$$t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{(s_1^2/n_1) + (s_2^2/n_2)}}$$

Where $\bar{x}_1$ = mean of first sample
$\bar{x}_2$ = mean of second sample
s_1 = standard deviation of first sample
s_2 = standard deviation of second sample
n_1 = number of measurements in first sample
n_2 = number of measurements in second sample

A table showing the critical values of *t* for different degrees of freedom.

Degrees of freedom	Critical value	Degrees of freedom	Critical value
4	2.78		
5	2.57	15	2.13
6	2.48	16	2.12
7	2.37	18	2.10
8	2.31	20	2.09
9	2.26	22	2.07
10	2.23	24	2.06
11	2.20	26	2.06
12	2.18	28	2.05
13	2.16	30	2.04
14	2.15	40	2.02

The number of degrees of freedom = $(n_1 + n_2) - 2$

Standard error and 95% confidence limits

Use this when
- you wish to find out if the difference between two means is significant
- the data are normally distributed
- the sizes of the samples are at least 30. For assessment purposes, five samples are acceptable providing that this is acknowledged either at a convenient place in the statistical analysis or in the conclusions.

Standard error
Calculate the standard error of the mean, SE, for each sample from the following formula:

$$SE = \frac{SD}{\sqrt{n}}$$

Where SD = the standard deviation
n = sample size

95% confidence limits
In a normal distribution, 95% of data points fall within ± 2 standard deviations of the mean.

Usually, you are dealing with a sample of a larger population. In this case, the 95% confidence limits for the sample mean are calculated using the following formula

95% confidence limits = $\bar{x} \pm 2 \times \frac{\mathrm{SD}}{\sqrt{n}}$ **OR** $\bar{x} \pm 2 \times \mathrm{SE}$

The chi-squared test

Use this test when
- the measurements relate to the number of individuals in particular categories
- the observed number can be compared with an expected number which is calculated from a theory, as in the case of genetics experiments.

The chi-squared (χ^2) test is based on calculating the value of χ^2 from the equation

$$\chi^2 = \Sigma \frac{(O - E)^2}{E}$$

Where O represents the observed results
E represents the results we expect.

A table showing the critical values of χ^2 for different degrees of freedom.

Degrees of freedom	Critical value
1	3.84
2	5.99
3	7.82
4	9.49
5	11.07
6	12.59
7	14.07
8	15.51
9	16.92
10	18.31

The number of degrees of freedom = number of categories – 1

Centre Number						Candidate Number				
Surname										
Other Names										
Candidate Signature										

For Examiner's Use Total EMPA mark	
Examiner's Initials	
Section	Mark
Task 1	
Task 2	
Section A	
Section B	
TOTAL EMPA MARK	

General Certificate of Education
Advanced Level Examination
June 2010

Human Biology HBI6X

Unit 6X A2 Externally Marked Practical Assignment

Written Test

For submission by 15 May 2010

For this paper you must have:
- Task Sheet 2, your results and your statistical calculation
- a ruler with millimetre measurements
- a calculator.

Time allowed
- 1 hour 15 minutes

Instructions
- Use black ink or black ball-point pen.
- Fill in the boxes at the top of this page.
- Answer **all** questions.
- You must answer the questions in the spaces provided.
- Do all rough work in this book. Cross through any work you do not want to be marked.

Information
- The marks for questions are shown in brackets.
- The maximum mark for this paper is 30.
- You will be marked on your ability to:
  – use good English
  – organise information clearly
  – use scientific terminology accurately.

Section A

These questions relate to your investigation into the effect of temperature on the activity of urease.

Use your Task Sheet 2, your results and your statistical calculation to answer them.

Answer **all** questions in the spaces provided.

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9 How did you decide that the end-point had been reached in all your tubes?

10 A student combined the data from several people in her class. She did not think that the combined data would be reliable. Give **two** reasons why the combined data would **not** be reliable.

11 Which two temperatures did you use for your statistical analysis?
Explain why you chose these temperatures.

12 Another student carried out the same investigation.

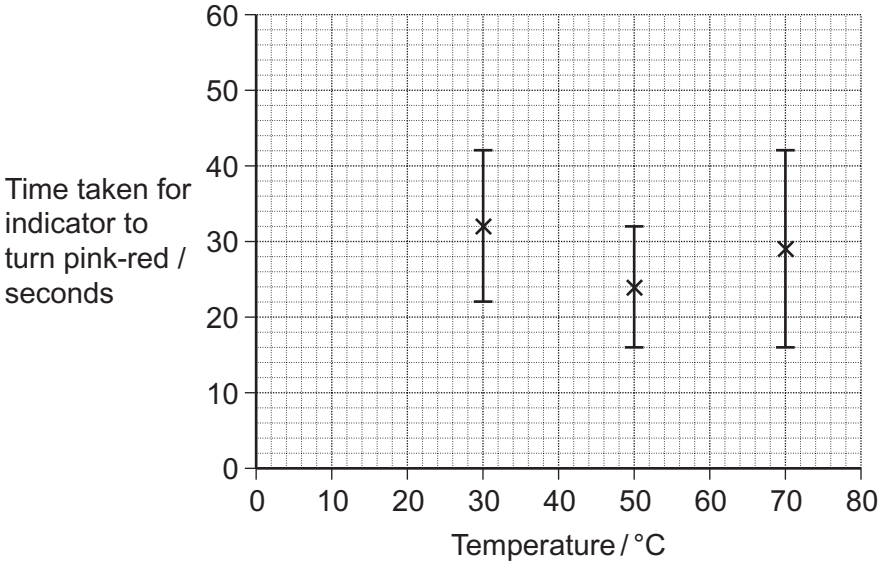
12 (a) The student performed a statistical test on the results he obtained for 30 ºC and 50 ºC. He obtained a value of $p = 0.0286$.

How should he interpret this value? Explain your answer.

12 (b) The student plotted his results on the graph shown in **Figure 1**. The bars show the standard deviations. His conclusion was that it was not possible to determine the optimum temperature for urease from the results he had obtained.

Do you agree with this conclusion or not? Explain your answer.

Figure 1



13 Gastric juice has antibacterial properties. Give **two** reasons why bacteria do **not** survive in the acidic gastric juice in the stomach.

14 A research worker investigated the pH at which no bacteria survived. Give **two** factors that he should have kept constant.

Resource Sheet

Introduction

The human gut normally supports a community of bacteria. These gut bacteria do not produce urease. Sometimes, pathogenic species of bacteria, such as *Helicobacter pylori*, invade the gut. These bacteria do produce urease.

Resource A

H. pylori bacteria attack the lining of the stomach, removing some of the mucus that protects the lining from digestive enzymes and acid. As a result, a stomach ulcer can form.

The urease test you used in Task 1 and Task 2 is a diagnostic test that has been developed to identify the presence of pathogenic bacteria in the gut. A colour change is due to ammonia produced by the action of the enzyme urease. The test can be performed on a sample of faeces with a positive result indicating that urease-producing bacteria are present.

Table 1 shows the results of urease tests conducted with samples of two species of bacteria.

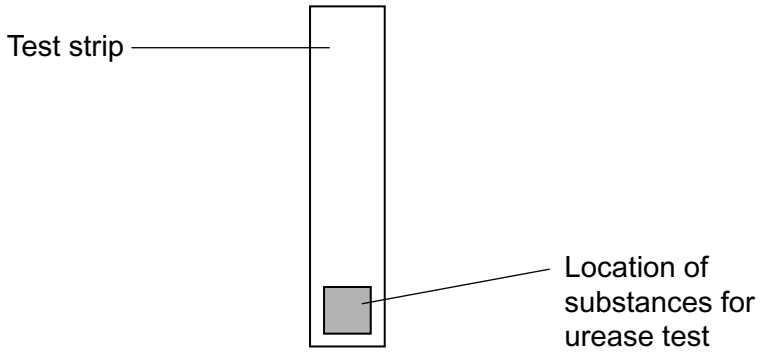
Table 1

Bacterium	**Result with phenol red**
Proteus mirabilis	Positive; colour change from original yellow to pink-red; reaction time slower than with *H. pylori*
Escherichia coli	Negative; ....

Resource B

Urease test strips can be used as a rapid test to indicate the presence of *H. pylori* or *P. mirabilis*. The urease test strip uses the same chemical reactions as the urease test. **Figure 2** shows a urease test strip.

Figure 2



Resource C

The urea breath test is a more recently developed test for the presence of *H. pylori*. A patient swallows a sample of urea made using a radioactive isotope of carbon, ^{14}C. Urease-producing bacteria break down the urea and produce ammonia and carbon dioxide. The patient's breath is tested for one of these products.

Resource D

H. pylori is present in 54% of the population in developed countries. Doctors investigated how often this bacterium is present in patients with different stomach illnesses. **Table 2** shows the results of this investigation.

Appropriate treatment for any of the different stomach illnesses cannot begin until the involvement of *H. pylori* has been confirmed or ruled out.

Table 2

Stomach illness	Percentage of patients infected by *H. pylori*
Gastric ulcer	100
Gastric cancer	94
Gastric lymphoma	92
Gastritis	92

Section B

Use the information in the **Resource Sheet** to answer the questions.

Answer **all** questions in the spaces provided.

Use **Resource A** to answer Question **15**.

15 (a) How would a urease test with *Escherichia coli* be determined as 'negative'?

15 (b) What information does this negative result give about *E. coli*?

15 (c) Would it be appropriate to use the urease test to detect *Proteus mirabilis*? Explain your answer.

Use **Resource B** to answer Question **16**.

16 Urease test strips can indicate the presence of *Helicobacter pylori.* Identify the substances on the end of the urease test strip.

Use **Resources C** and **D** to answer Questions **17** and **18**.

17 (a) A patient with a gastric ulcer was tested with a urea breath test.
Explain how this test showed that he had *H. pylori* in his gut.

17 (b) In order to plan a successful programme of treatment for conditions in **Table 2**, a doctor will need to know if a patient was *H. pylori* positive.
Explain why.

18 (a) Doctors studied 282 cases of gastric cancer.
How many of these cases were definitely **not** due to the presence of *H. pylori*?

18 (b) Is it valid to conclude that the number of patients affected by gastric lymphoma is the same as the number affected by gastritis?
Explain your answer.

Use **all Resources** to answer Question **19**.

19 Which test would be best for a national screening programme for *H. pylori*?
Explain your answer.

END OF QUESTIONS

There are no questions printed on this page

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