



General Certificate of Education  
Advanced Subsidiary Examination  
June 2010

**Biology**

**BIO3X/TN**

**Unit 3X AS Externally Marked Practical Assignment**

**Teachers' Notes**

**Confidential**

**To be given immediately to the teacher(s) responsible for GCE 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: GCE Biology* published on the AQA Website.

**The effect of pH on the rate of breakdown of lactose by the enzyme lactase****Materials****Task 1**

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

- 5 cm<sup>3</sup> of a 1% solution of lactase (1% of the enzyme product in water. This should be made up fresh on the day)
- 15 cm<sup>3</sup> fresh milk (not UHT)
- 15 cm<sup>3</sup> buffer pH 6.5
- 6 Clinistix® strips (glucose test strips)
- access to a Clinistix® colour chart labelled as described on page 3
- access to a water bath at 30 °C
- thermometer
- a stop watch or timer
- 2 boiling tubes
- rack for boiling tubes
- 2 syringes or pipettes to measure 2 cm<sup>3</sup> and 10 cm<sup>3</sup>

Note: the water baths could be provided as large containers of water at 30 °C; these should maintain the temperature sufficiently for the duration of the investigation.

**Task 2**

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

- 12 cm<sup>3</sup> of a 1% solution of lactase
- 60 cm<sup>3</sup> fresh milk (not UHT)
- 60 cm<sup>3</sup> of buffer solutions at pH values of 5.0, 6.0, 6.5, 7.0 and 8.0
- 30 Clinistix® strips (glucose test strips)
- access to a Clinistix® colour chart labelled as described
- access to a water bath at 30 °C
- thermometer
- a stop watch or timer
- 5 boiling tubes
- rack for boiling tubes
- 2 syringes or pipettes to measure 2 cm<sup>3</sup> and 10 cm<sup>3</sup>
- chinagraph pencil or labels or permanent marker pen

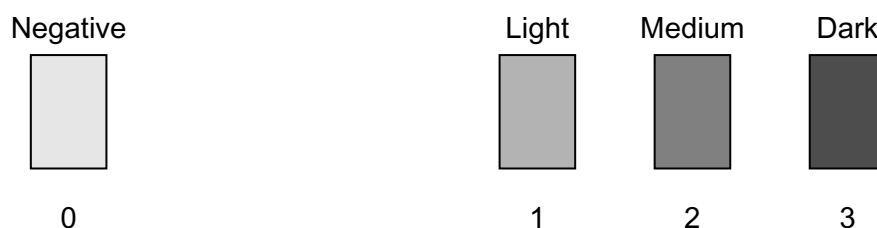
## Technical Information

The tasks must be trialled before use.

### Clinistix®

Only narrow strips of Clinistix® are needed and the commercially prepared strips can be cut into halves lengthways by the teacher or technician to reduce costs.

The Clinistix® colour chart should be labelled so that the colours are numbered as shown.



### The buffer

A citrate-phosphate buffer should be used. To prepare 100 cm<sup>3</sup> buffer use 0.2 mol dm<sup>-3</sup> disodium hydrogen phosphate (Na<sub>2</sub>HPO<sub>4</sub>) and 0.1 mol dm<sup>-3</sup> citric acid as follows.

pH	Volume of disodium hydrogen phosphate / cm <sup>3</sup>	Volume of citric acid / cm <sup>3</sup>
5.0	51.5	48.5
6.0	63.2	36.8
6.5	74.5	25.5
7.0	82.4	17.6
8.0	97.2	2.8

### The milk

This should be fresh whole milk, semi-skimmed or skimmed milk but **must not** be UHT milk.

### Lactase enzyme

Any lactase enzyme can be used e.g. Lactozym which is available from

<http://www.ncbe.reading.ac.uk/ncbe/MATERIALS/ENZYMES/lactozym.html>

Note: some enzyme preparations contain glucose, these should not be used.

These tasks were trialled by several teachers using the following materials. Other sources of materials may be used but the task must be trialled by the teacher or technician.

Clinistix® from Bayer

Semi-skimmed fresh milk

Lactozym enzyme

### **Managing Task 1**

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

- You will investigate a method of measuring the concentration of glucose.

There should be no further discussion of this topic.

### **Managing Task 2**

One week before Task 2, teachers may give their candidates the following information.

- You will investigate the effect of pH on enzyme activity.

There should be no further discussion of this topic.

Centre Number						Candidate Number				
Surname										
Other Names										
Candidate Signature										

For Examiner's Use Total Task 1



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## Biology

## BIO3X/PM1

### Unit 3X AS 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.

## Task 1

### Introduction

Lactase is an enzyme. It breaks down lactose to glucose and galactose.

You are going to investigate the breakdown of lactose by recording the amount of glucose produced.

You will use glucose test strips to test for glucose. When a test strip is dipped into a solution of glucose, it changes colour. The colour change depends on the concentration of glucose in the solution.

The colour of the test strip can be compared to a colour chart to give a value for the concentration of glucose.

Glucose test strips do not give a positive reaction with any other substance.

In this task you are going to investigate how the breakdown of lactose can be followed using a glucose test strip.

### Materials

You are provided with

- solution of lactose
- fresh milk
- buffer solution
- glucose test strips
- access to a glucose test strip colour chart
- access to a water bath at 30 °C
- thermometer
- a stop watch
- boiling tubes
- rack for boiling tubes
- syringes or pipettes to measure 2 cm<sup>3</sup> and 10 cm<sup>3</sup>

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

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## Outline method

Read the following instructions carefully before you start your investigation.

1. Put 10 cm<sup>3</sup> of milk and 10 cm<sup>3</sup> of buffer into one boiling tube.
2. Put 2 cm<sup>3</sup> lactase into a second tube.
3. Put both tubes in a water bath and leave them for 3 minutes.
4. Add the lactase to the milk and buffer mixture.
5. Shake the mixture, return the tube to the water bath and start the stop watch.
6. Immediately dip a glucose test strip into the mixture and remove.
7. Wait **exactly 10 seconds** then compare the colour of the test strip with the colour chart.
8. Use the scale on the colour chart to record the concentration of glucose. It should be recorded as 0, 1, 2, or 3.
9. Repeat steps 6 to 8 every minute for 5 minutes with a new glucose test strip each time.
10. Record your results in the table.

Time / minutes	Amount of glucose present as colour scale value
0	
1	
2	
3	
4	
5	

**Questions on Task 1**

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

- 1 Suppose the temperature of the water you used decreased during your investigation. Explain how a decrease in temperature would affect the results in your table.
- 2 Some types of milk contain lactase. Give **one** reason why these types of milk would **not** be suitable in this task.
- 3 (a) You used glucose test strips to measure the concentration of glucose in this task. You should keep some aspects of using the test strips constant to obtain reliable data. Give **two** ways in which you should standardise your technique.
- 3 (b) You recorded the amount of glucose present as 0, 1, 2 or 3. Given a solution of glucose of concentration  $1 \text{ mol dm}^{-3}$  and distilled water, you could find the actual concentration of glucose which corresponded to amount 2 on your colour value scale. Describe how you would do this.
- 4 Lactose is a reducing sugar. Use this information to explain why it is **not** appropriate to use Benedict's reagent to measure the breakdown of lactose.  

(1 mark)
- 5 In the next part of the investigation you will measure the rate at which lactose is broken down under different conditions. Use the results in your table to suggest how you could measure the rate of breakdown of lactose.

**END OF TASK 1**



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Other Names										
Candidate Signature										

For Examiner's Use Total Task 2



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## Biology

## BIO3X/PM2

### Unit 3X AS 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.

**Task 2**

In this part of the investigation you will examine the effect of pH on the rate of breakdown of lactose by lactase at 30 °C.

**Materials**

You are provided with the following

- solution of lactase
- fresh milk
- buffer solutions at pH values of 5.0, 6.0, 6.5, 7.0 and 8.0
- glucose test strips
- access to a glucose test strip colour chart
- access to a water bath at 30°C
- thermometer
- a stop watch
- boiling tubes
- rack for boiling tubes
- syringes or pipettes to measure 2 cm<sup>3</sup> and 10 cm<sup>3</sup>
- marker pen or labels

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

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## Outline method

**Read these instructions carefully before you start your investigation.**

Glucose test strips change colour over a period of time. To get an accurate reading, the colour should be recorded **exactly 10 seconds** after the strip has been removed from the liquid.

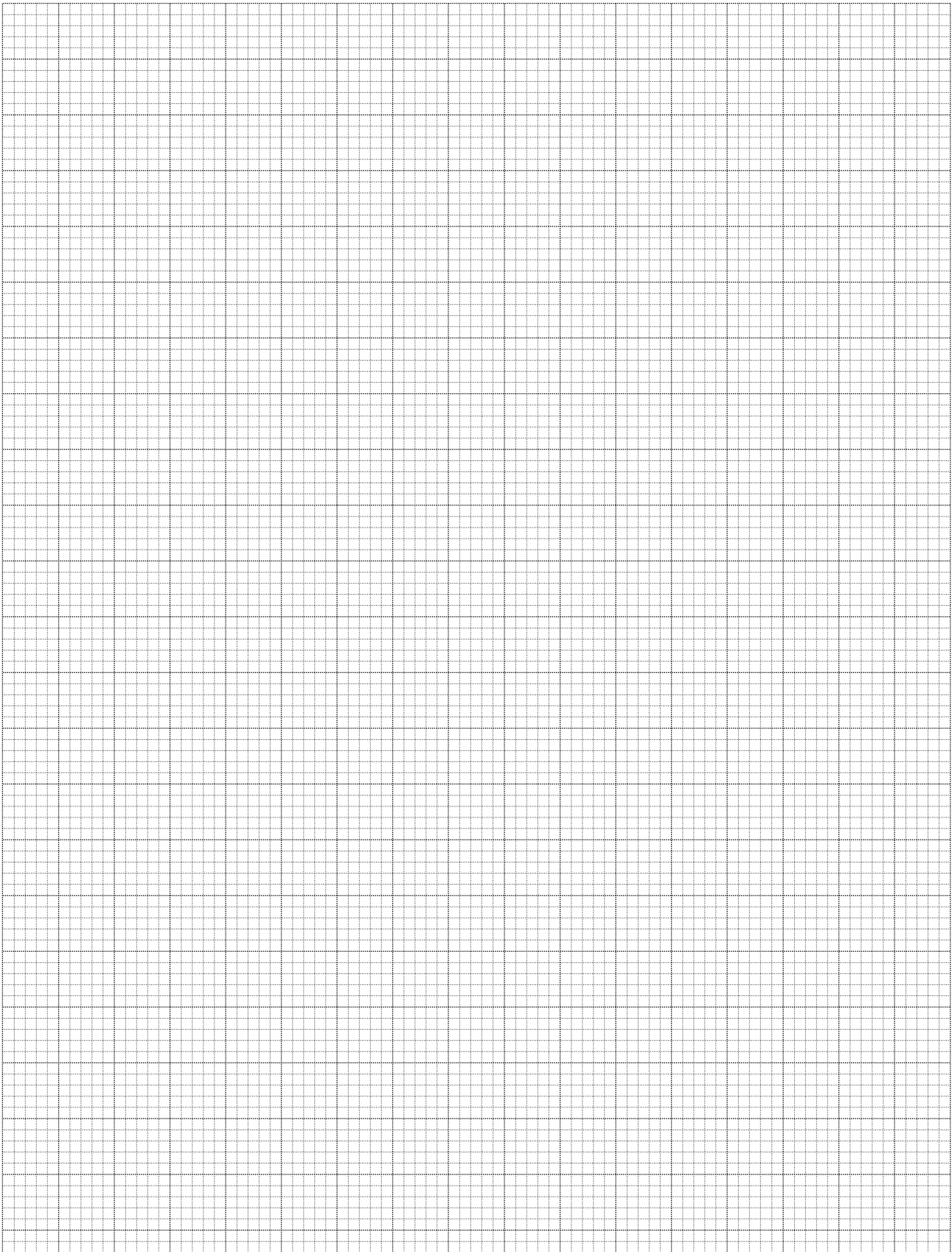
1. Put 10 cm<sup>3</sup> of milk and 10 cm<sup>3</sup> pH 7.0 buffer into one boiling tube.
2. Put 2 cm<sup>3</sup> lactase into a second tube.
3. Put both tubes in a water bath and leave them for 3 minutes.
4. Add the lactase to the milk and buffer mixture.
5. Shake the mixture and return the tube to the water bath.
6. Start the stop watch and immediately dip a glucose test strip into the mixture and remove.
7. Wait **exactly 10 seconds** then compare the colour of the glucose test strip with the colour chart.
8. Dip a new glucose test strip into the mixture every minute and record the time when glucose first appears.
9. Repeat steps 1 to 8 with buffers at pH 5.0, 6.0, 6.5 and 8.0 instead of buffer at pH 7.0.
10. If there is no colour change after 10 minutes, you should stop testing.

## Presenting data

- 6** Record the results of your investigation in an appropriate table in the space below.

You will be awarded up to 1 mark for the quality of your practical work.

- 7 Use the graph paper to plot an appropriate graph of the data you collected in Task 2.



**END OF TASK 2**

Centre Number						Candidate Number				
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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	



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## Biology

## BIO3X

### Unit 3X AS 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 graph
- 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.

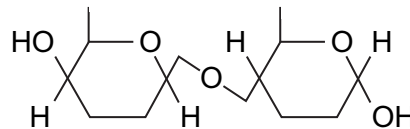
### Section A

These questions are about your investigation into the effect of pH on the breakdown of lactose by lactase.

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

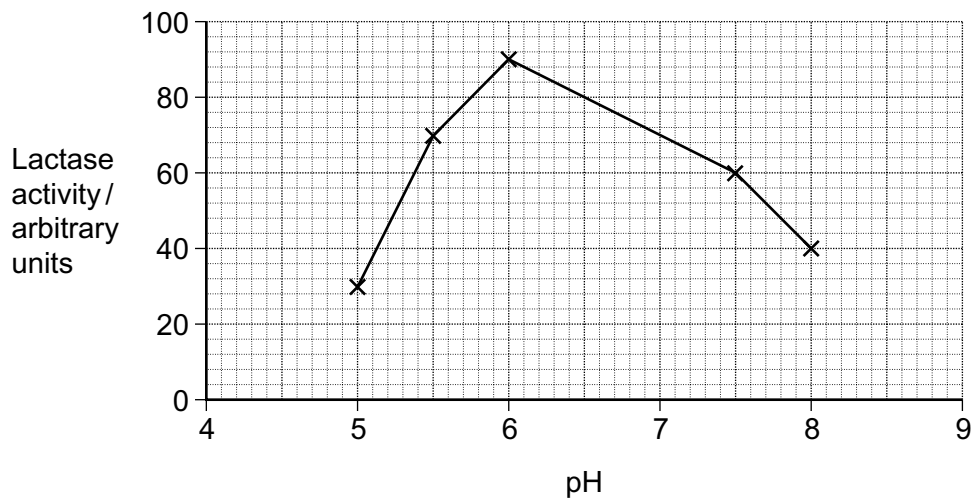
Answer **all** questions in the spaces provided.

- 8 (a)** You were told to leave the tubes in the water bath for 3 minutes before adding the enzyme to the milk and buffer (Step 3). Explain why.
- 8 (b)** Explain how you would know if 3 minutes was long enough to leave the tubes before mixing.
- 9** The diagram shows the structure of a molecule of lactose.



Draw a diagram to show the monosaccharides formed when lactose is hydrolysed.

- 10** A student carried out an investigation into the effect of pH on the activity of lactase. The graph shows the results.



- 10 (a)** Explain the shape of the curve.
- 10 (b)** This student concluded that the optimum pH for the action of lactase was pH 6.0. Was this a valid conclusion? Explain your answer.
- 10 (c)** Glucose test strips contain immobilised enzymes. Immobilised enzymes are not usually affected by the changes in pH in the range used in this investigation.

Describe how you would investigate whether the enzymes on the glucose test strips were affected by the pH of the buffers used in your investigation.

## Resource Sheet

### Resource A

Doctors compared two tests for lactase deficiency.

Doctors investigated three groups of people. The people in all three groups were not allowed to eat or drink for 8 hours before the test. They each then drank a solution containing 50 g of lactose made with a radioactive form of carbon called  $^{14}\text{C}$ .

- Group **A** were the control group
- Group **B** were lactase deficient
- Group **C** had irritable bowel syndrome (IBS)

Both lactase deficiency and irritable bowel syndrome have similar symptoms.

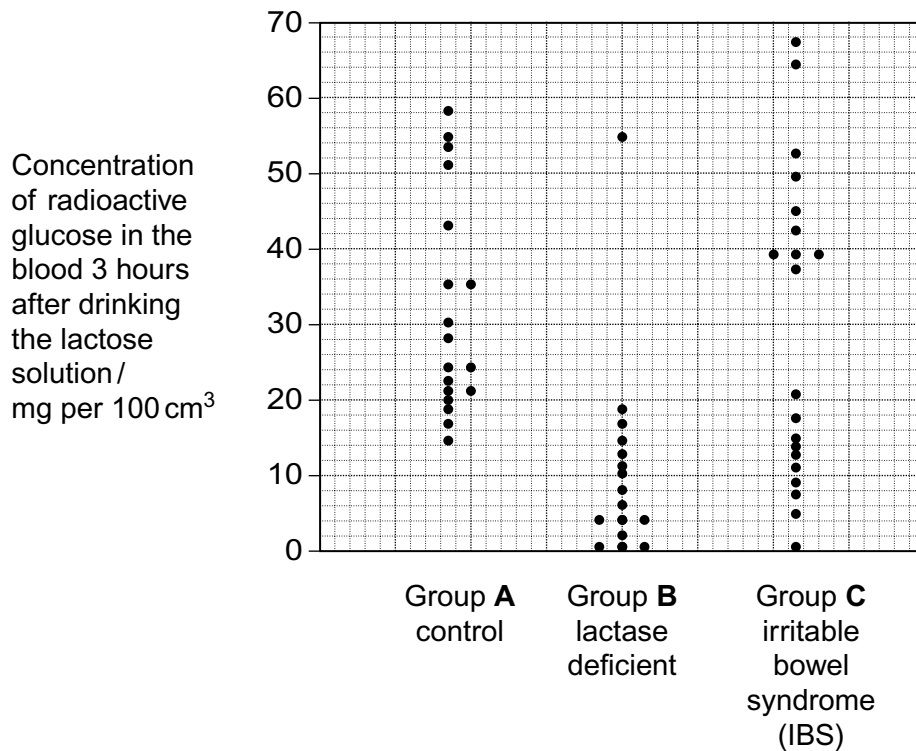
The doctors carried out two measurements on the people in each group.

#### Test 1 – The lactose tolerance test

The doctors measured the concentration of radioactive glucose in the blood of each person.

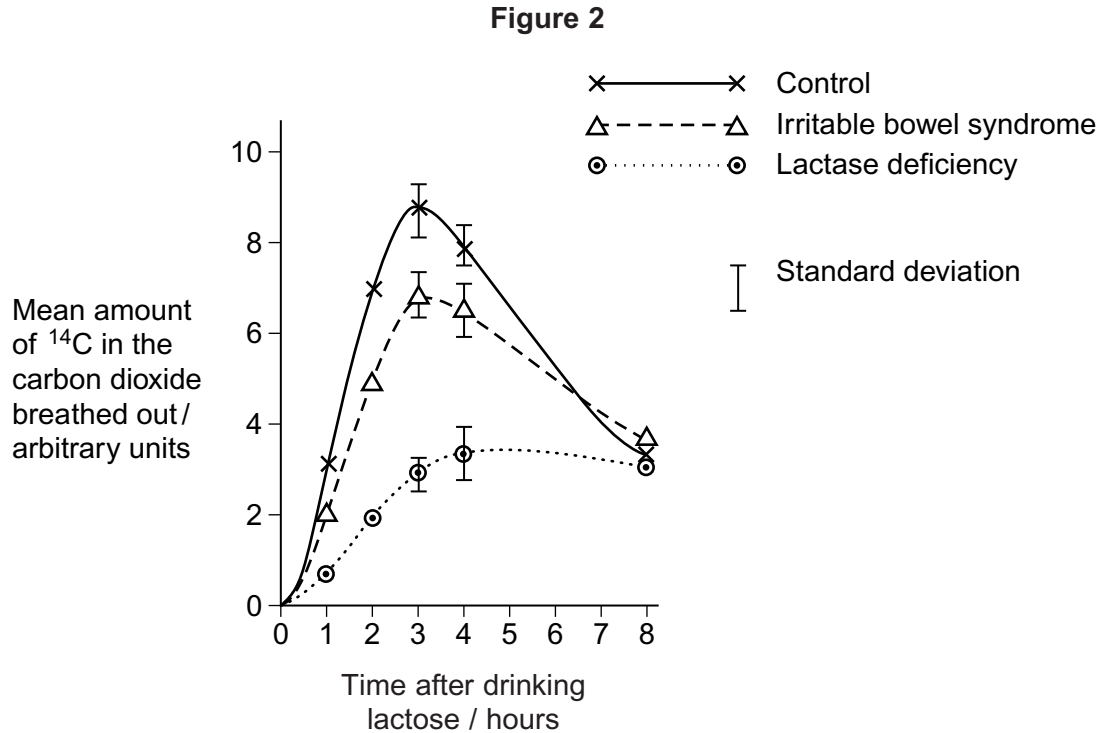
**Figure 1** shows the results. Each point shows the result for one person 3 hours after drinking the lactose solution.

**Figure 1**



## Test 2 – The carbon dioxide breath test

In this test the doctors measured the amount of  $^{14}\text{C}$  in the carbon dioxide breathed out. The doctors took measurements at intervals for 8 hours after each volunteer had drunk the lactose solution. **Figure 2** shows the mean results for each group.



## Resource B

Biologists divided new-born rats randomly into four groups.

They fed the rats in each group on a standard diet which only differed in the carbohydrate content. When these rats were adult, the biologists measured the activity of lactase in the digestive system of the rats. **Figure 3** shows the mean results for each group.

**Figure 3**

Diet	Mean lactase activity / $\mu\text{mol}$ of lactose digested per hour ( $\pm$ standard deviation)
Low sucrose	57.9 ( $\pm$ 14.5)
High sucrose	184.2 ( $\pm$ 30.8)
Low starch	86.9 ( $\pm$ 13.3)
High starch	221.4 ( $\pm$ 25.4)



**Section B**

You should use the information on the Resource Sheet to answer these questions.

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

Use **Resource A** to answer Question **11**.

- 11** The people who took part in these tests were not allowed to eat or drink for 8 hours before the test. Explain why.

Use **Figure 1** to answer Questions **12** and **13**.

- 12 (a)** Give the range of results for the control group (group **A**)

- 12 (b)** Each person in the control group was given 50 g of lactose containing the same amount of radioactive carbon. All the products of lactose digestion were absorbed into their blood. The concentration of glucose was measured in mg per 100 cm<sup>3</sup> of blood.

Explain why the variation in the results may be due to differences in body mass.

- 13** In **Test 1** the doctors obtained different results for the three groups.

Would **Test 1** be useful to identify people who were lactase deficient? Use the data from all three groups to explain your answer.

Use **Figure 2** to answer Questions **14** to **17**.

- 14** Describe the common trend shown by **all** the curves in **Figure 2**.

- 15** Explain why the doctors stopped measuring the amounts of <sup>14</sup>C in the carbon dioxide breathed out after 8 hours.

- 16** Carbon dioxide in the breath contained the radioactive form of carbon,  $^{14}\text{C}$ . Explain how  $^{14}\text{C}$  in carbon dioxide came from  $^{14}\text{C}$  in glucose in the blood.
- 17** The doctors concluded that measuring the amount of  $^{14}\text{C}$  in the carbon dioxide in the breath after 3 hours was a better way of diagnosing lactase deficiency than the lactose tolerance test. Do you agree with the doctors' conclusion? Give the reasons for your answer.

You should use **Resource B** to answer Questions **18** and **19**.

- 18** Give **one** piece of evidence from **Figure 3** that indicates lactase activity is affected by diet.
- 19** Some students suggested from these data that increasing starch in the diet was the most effective way to increase lactase activity in lactase deficient people. Is this conclusion valid? Explain your answer.

**END OF QUESTIONS**

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**DO NOT WRITE ON THIS PAGE  
ANSWER IN THE SPACES PROVIDED**

**There are no questions printed on this page**

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ANSWER IN THE SPACES PROVIDED**