General Certificate of Education
Advanced Subsidiary Examination
June 2012

Human Biology HBI3X/TN

Unit 3X AS Externally Marked Practical Assignment

Teachers' Notes Confidential

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

Estimated entries must be submitted to AQA in order for centres to receive hard copies of the materials to be used by candidates.

Open on receipt

Teachers' Notes

CONFIDENTIAL

These notes must be read in conjunction with *Instructions for the Administration of the EMPA* for GCE Human Biology published on the AQA website.

Trypsin: hero and villain

Task 1 - Showing the action of trypsin on milk

Trypsin is important in the digestion of proteins in the gut. In Task 1, this will be shown by the action of trypsin on milk protein. Casein is the milk protein digested by trypsin but candidates are not expected or required to know the name of this protein.

Materials

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

- 5 cm^3 of 5% trypsin solution
- 20 cm^3 of 1% milk powder solution labelled 'milk'
- water
- 250 cm^3 beaker
- sufficient warm water for water bath
- 4 test tubes
- test tube rack
- 2 graduated pipettes or syringes capable of measuring up to 1 cm^3 and in divisions of 0.1 cm^3
- thermometer capable of measuring up to 40 ^oC
- access to a clock or watch that shows division of a minute **only** in whole seconds
- marker pen

Managing the investigation

To show protein digestion, milk powder is used rather than fresh milk which does not always go clear as required. In Task 1 and Task 2, the milk powder solution should be labelled and referred to as 'milk'. 'Marvel' and 'Tesco dried milk' were successfully used in trials. Other brands also work but composition does vary and they should be trialled first. Trypsin is readily available from biological and chemical suppliers such as Philip Harris or Griffin Education.

The water bath temperature is approximate and not critical in Task 1. A thermostatically controlled water bath **should not** be used; candidates should each have their own beaker. It is sufficient to use hot water direct from the tap, cooling the water if necessary. This should allow the starting temperature to be within the range of 25-35 ^{o}C. There is no requirement to maintain the temperature during the task; it should not deviate much in the time required. It is intended, as per instructions, that only the milk is warmed and **not** the enzyme solution as well, but if candidates do so in error, they should **not** be corrected.

The clock that is provided **must not** allow readings smaller than whole seconds. A wall clock or a candidate's own watch could be used. In trials, it took between 50 and 80 seconds for the milk to go clear.

The task must be trialled before use.

Candidates **must not** be given information about an EMPA until one week before Task 1. One week before Task 1 candidates should be given the following information.

You will investigate the effect of the enzyme trypsin on the digestion of protein, the use of chromatography in the separation of amino acids and how trypsin can both benefit and damage the body.

There **must** be no further discussion and candidates **must not** be given any further resources to prepare for the assessment.

In Task 1 teachers must not give candidates the following information

- how to make up solutions
- how long to leave tubes in the water bath
- how to determine when a reaction is complete

Task 2 - Investigating the effect of different trypsin concentrations on the rate of digestion of milk protein

As in Task 1 candidates are not expected or required to know the name of the protein in milk. Furthermore, they are not required to have prior knowledge of the effect of enzyme concentration on an enzyme-controlled reaction.

Materials

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

- 20 cm^3 of 1% trypsin solution
- 150 cm^3 of 1% milk powder solution labelled 'milk'
- water
- large beaker capable of holding 10 test tubes
- sufficient warm water for water bath
- 10 test tubes
- test tube rack
- graduated pipettes or syringes capable of measuring up to 1 cm^3 and in divisions of 0.05 cm^3
- thermometer capable of measuring up to 40 ^oC
- timer (no restriction on type)
- marker pen

Managing the investigation

As for Task 1, fresh milk is not used. The investigation was successfully trialled using 'Marvel' and 'Tesco dried milk'. Other brands also work but composition does vary and they should be trialled first. Trypsin is readily available from biological and chemical suppliers.

An individual beaker for use as a water bath is preferred but, in Task 2, a thermostatically controlled water bath set at 35 ^oC could be used instead. A thermostatically controlled water bath could be shared by candidates but the centre should ensure that there is no communication between candidates as they do so. Alternatively, supplies of hot water should be available to allow candidates to prepare and maintain individual water baths at about 35^oC during the investigation. At lower temperatures the casein will break down more slowly. In trials, the lowest enzyme concentration took between 480 and 600 seconds to go clear.

There is no restriction on the type of timer that is used in Task 2. Candidates could be provided with more test tubes if available or they should be advised to wash them out if they need to reuse them.

The task must be trialled before use.

In Task 2 teachers must not give candidates the following information

- how to determine when a reaction is complete
- how many repeats to include.

Centre Number						Candidate Number				
Surname										
Other Names										
Candidate Signature										

For Examiner's Use
Total Task 1

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Human Biology HBI3X/PM1

Unit 3X AS Externally Marked Practical Assignment

Task Sheet 1

To be completed before Task Sheet 2.

For submission by 15 May 2012

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

Trypsin: hero and villain

Introduction

Trypsin is an enzyme important in the digestion of proteins in the gut.

Task 1 - Showing the action of trypsin on milk

In Task 1, you will investigate the effect of trypsin on the protein in milk. Milk will go clear as the protein in the milk is digested.

Materials

You are provided with

- 5% trypsin solution
- milk
- water
- beaker and a supply of warm water
- test tubes
- test tube rack
- graduated pipettes or syringes
- thermometer
- timer
- 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 approximately 35 °C by half filling the beaker with warm water.
2. Label two test tubes **A** and **B**.
3. Put 5 cm^3 of milk into tube **A** and place the tube in the water bath.
4. Use the 5% trypsin solution and water to make 1 cm^3 of 1% trypsin in tube **B**.
5. Add the contents of tube **B** to tube **A** and immediately start the timer.
6. Measure, in seconds, how long it takes for the contents of tube **A** to go clear.
7. Repeat steps 2 to 6.

You will need to decide for yourself

- when the milk has gone clear.

Recording your results

Record your results in the table.

Trial number	Time taken for milk to go clear / seconds
1	
2	

Turn over for Question 1

Questions on Task 1

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

1 Complete the table with headings, units and volumes to show how you made 1 cm^3 of 1% trypsin solution (step 4).

2 You had to decide when the milk had gone clear (step 6). This is the end point for the reaction.
How did you try to ensure that your end point was the same each time?

3 You only repeated your experiment with trypsin once (step 7).
What is the advantage of carrying out several repeat experiments?

4 Apart from carrying out repeats, give **two** ways in which the method used in this investigation could be improved.

5 Were the data you collected qualitative or quantitative? Explain your answer.

6 Another student carried out the same investigation as you but used a timer that measured to 0.01 of a second. His results would **not** be any more accurate than yours.
Suggest why.

END OF TASK 1

Centre Number						Candidate Number				
Surname										
Other Names										
Candidate Signature										

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Total Task 2

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Human Biology HBI3X/PM2

Unit 3X AS Externally Marked Practical Assignment

Task Sheet 2

To be completed before the EMPA Written Test.

For submission by 15 May 2012

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

Trypsin: hero and villain

Introduction

Trypsin is an enzyme important in the digestion of proteins in the gut.

Task 2 - Investigating the effect of different trypsin concentrations on the rate of digestion of milk protein

In Task 2, you will investigate the effect of different concentrations of trypsin solution on the rate of digestion of the protein in milk.

Materials

You are provided with

- 1% trypsin solution
- milk
- water
- beaker and warm water
- 10 test tubes
- test tube rack
- graduated pipettes or syringes
- thermometer
- timer
- marker pen

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

Outline Method

To assist your planning, note that it could take up to 10 minutes for the contents of your tubes to go clear.

Read these instructions carefully before you start your investigation.

1. Set up a water bath at approximately 35 ^{o}C by half filling the beaker with warm water. Maintain the temperature by topping up with hot water, or replace the water if necessary.
2. Label five test tubes **A** to **E**.
3. Put 5 cm^3 of milk in each of tubes **A** to **E** and place all of these tubes in the water bath.

4. Label five test tubes **1** to **5**.
5. Use the 1% trypsin solution and water to make five different concentrations of trypsin solution in tubes **1** to **5** as shown in the table.

Tube number	Volume of 1% trypsin used / cm^3	Volume of water used / cm^3	Concentration of trypsin solution / %
1	0.10	0.90	0.10
2	0.25	0.75	0.25
3	0.50	0.50	0.50
4	0.75	0.25	0.75
5	1.00	0.00	1.00

6. Place tubes **1** to **5** in the water bath and leave for a suitable length of time.
7. After this time, add the contents of tube **1** to tube **A** and start the timer immediately.
8. Record, in seconds, the time taken for the contents of the tube to go clear.
9. Repeat steps 7 and 8 but adding tube **2** to tube **B**, then tube **3** to tube **C**, then tube **4** to tube **D**, then tube **5** to tube **E**.

You will need to decide for yourself

- how long to leave the tubes in the water bath
- how many repeats to use for each concentration of trypsin
- when the contents of a tube have gone clear.

Turn over for Question 7

Presenting data

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

Processing data

8 Use your data to calculate the relative rate of reaction with each concentration of trypsin. To help the plotting of your graph, use the following formula:

$$\text{Relative rate of reaction of trypsin} = \frac{1000}{t}$$

Where t is the time taken for the contents of the tube to go clear and the relative rate of reaction will use the units of $\frac{1000}{t}$

9 Use the graph paper to plot an appropriate graph of your processed data.

END OF TASK 2

Centre Number						Candidate Number				
Surname						Other Names				

Notice to Candidate. The work you submit for assessment must be your own. If you copy from someone else or allow another candidate to copy from you, or if you cheat in any other way, you may be disqualified.

Candidate Declaration. I have read and understood the Notice to Candidate and can confirm that I have produced the attached work without assistance other than that which is acceptable under the scheme of assessment.

Candidate Signature		Date	

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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June 2012

Human Biology HBI3X

Unit 3X AS Externally Marked Practical Assignment Written Test

For submission by 15 May 2012

For this paper you must have:	**Time allowed**
• your Task Sheet 2, your results and your calculations • a ruler with millimetre measurements • a calculator.	• 1 hour 15 minutes
Instructions:	**Information**
• 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 not write outside the box around each page or on blank pages. • Do all rough work in this book. Cross through any work you do not want to be marked.	• 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.

Details of additional assistance (if any). Did the candidate receive any help or information in the production of this work? If you answer yes give the details below or on a separate page.

Yes ☐ No ☐

Teacher Declaration:
I confirm that the candidate has met the requirements of the practical skills verification (PSV) in accordance with the instructions and criteria in section 3.8 of the specification.

Practical Skills Verification	**Yes** ☐

Signature of teacher ..... Date .....

Section A

These questions relate to your investigation with trypsin.

Use your Task Sheet 2, your results, processed data and your graph to answer the questions.

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

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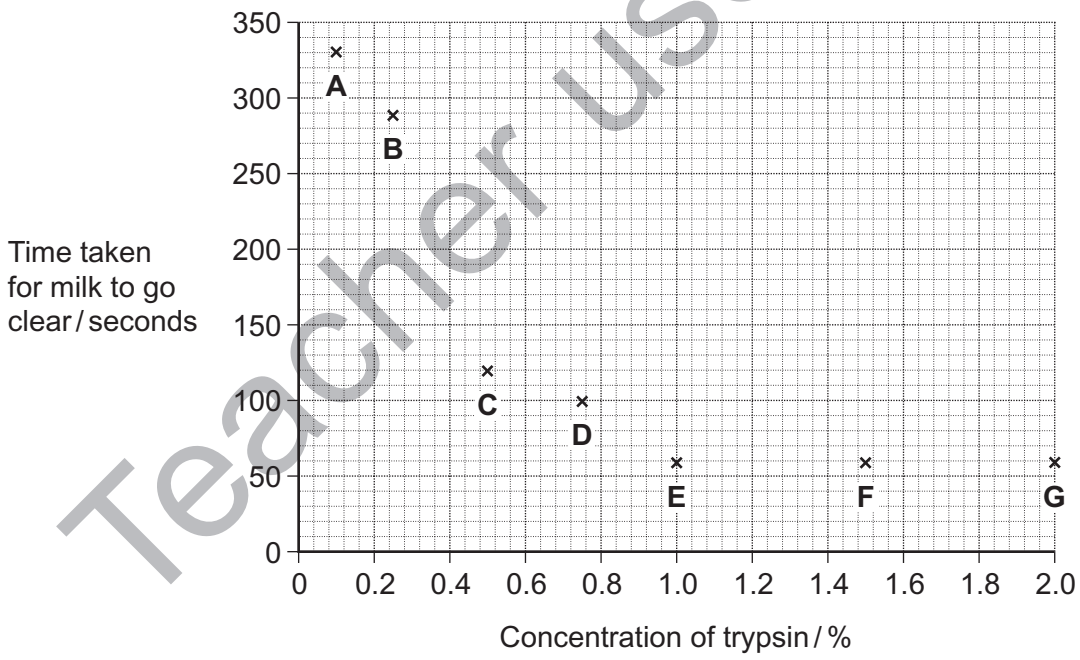
10 How did you decide what was a suitable length of time to leave your tubes in the water bath before mixing (step 6 and step 7)?

Another student carried out the same investigation as you but also included experiments with a 1.5% trypsin solution and a 2.0% trypsin solution. She also set up a control experiment.

11 (a) Describe what the contents of the tube used in the control experiment should be.

11 (b) What relative rate of reaction would you expect her to obtain for the control experiment?

12 Before processing her data, she plotted her results on the following graph.



12 (a) Describe her results between **A** and **C**.

12 (b) Use your knowledge of enzymes to suggest an explanation of her results

12 (c) She described her graph as a scatter diagram. She drew a line of best fit and concluded that it showed a positive correlation. Explain why both her description and her conclusion were **not** correct.

13 Biuret reagent is used as an indicator to show the presence of protein in a solution. This indicator turns from blue to purple when added to a solution containing protein. When milk protein is hydrolysed by trypsin, amino acids are released. Amino acids do not change the colour of biuret reagent.

13 (a) What is meant by *hydrolysed*?

13 (b) At the end of the investigation, the student tested each tube with biuret reagent. She found that the indicator turned purple in each tube although the milk protein had been fully hydrolysed.

Explain why.

Resource Sheet

Introduction

Trypsin is an enzyme important in the digestion of proteins in the gut and is also found in other parts of the body.

Resource A

If trypsin is present in the lungs, it can damage proteins in lung tissue. Alpha-1-antitrypsin inhibits the action of trypsin by binding to the enzyme. Alpha-1-antitrypsin is also a protein. Alpha-1-antitrypsin is produced in the lungs when trypsin is present. Scientists investigated whether there was a correlation between different types of lung disease and the concentration of alpha-1-antitrypsin in lung tissue. They recruited four groups of people. The four groups investigated were:

- 49 healthy people
- 19 people with emphysema
- 31 people with bronchiectasis
- 34 people with asthma.

Their results are shown in **Figure 1**.

Figure 1

Group	Concentration of alpha-1-antitrypsin in lung tissue / arbitrary units			
	Range	Mean	Median	Standard deviation
Healthy people	5.4 to 10.5	7.4	7.5	±1.3
People with emphysema	6.3 to 10.6	9.0	9.2	±1.5
People with bronchiectasis	6.4 to 10.7	9.2	9.3	±1.5
People with asthma	5.7 to 16.9	8.1	7.9	±2.2

Resource B

Paper chromatography can be used to separate amino acids from a mixture in a solution.
An outline of the method is as follows.

- Add the mixture to chromatography paper
- Run in a suitable solvent
- Use a stain to show where spots of each amino acid are on the paper
- Identify the amino acids

Section B

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

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

Use the information provided in **Resource A** to answer **Questions 14** to **18**.

14 The binding of alpha-1-antitrypsin stops trypsin from working. Suggest how.

15 What is meant by 'healthy people' in this investigation?

16 Explain the meaning of the following terms used in **Figure 1**.

16 (a) Range

16 (b) Median

16 (c) Standard deviation

17 What do these results show about the concentration of alpha-1-antitrypsin in people with emphysema and bronchiectasis?

18 A biology student, reading the results of the investigation, concluded that people with lung disease have higher concentrations of alpha-1-antitrypsin in their lungs. Evaluate this conclusion.

Use the information provided in **Resource B** to answer **Question 19**.

19 Researchers investigated which amino acids were present in alpha-1-antitrypsin. They hydrolysed a sample of alpha-1-antitrypsin and obtained a mixture of amino acids.

Describe how you would adapt the method in **Resource B** to identify the amino acids present in this mixture. Credit will be given for the use of appropriate apparatus and techniques.

END OF QUESTIONS