General Certificate of Education
Advanced Level Examination
June 2011

Human Biology HBI6T/P11/task

Unit 6T A2 Investigative Skills Assignment
Task Sheet

The effect of temperature on the rate of respiration of microorganisms such as those found in probiotic foods

Introduction

Microorganisms are used in probiotic foods. These microorganisms might colonise the intestine, and could reduce the number of harmful microorganisms which live there.

Yeast is a eukaryotic microorganism used in some foods. The rate of respiration of yeast can be assessed by using a dye called methylene blue. This blue dye can be added to a yeast culture. Respiration by the yeast removes oxygen from the culture liquid and causes the dye to become colourless.

$$\text{Blue-coloured methylene blue} \xrightarrow[\text{removes oxygen}]{\text{Respiration}} \text{Colourless methylene blue}$$

Materials

You are provided with

- yeast culture 200 g dm^{-3}
- glucose solution 10 g dm^{-3}
- methylene blue indicator
- boiling tubes
- boiling tube rack or large beaker to stand boiling tubes in
- bungs suitable for boiling tubes
- permanent marker pen or chinagraph pencil
- three waterbaths, set at about 25 °C, 40 °C and as close as possible to 100 °C (or the means to set these up using beaker, Bunsen burner, heat-resistant mat, gauze, tripod, matches or spills)
- thermometers
- test tubes
- test tube rack
- syringe, pipette or dropper
- glass rod
- distilled water
- timer

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

A copy of the AQA Students' Statistics Sheet is provided at the back of this Task Sheet. This may be used at any Stage during the investigation and examination.

Outline Method

Read these instructions carefully before you start your investigation.

1. Label three boiling tubes **X**, **Y** and **Z**.
2. To each tube add 10 cm^3 glucose solution.
3. To tubes **X** and **Y** add 1 cm^3 methylene blue solution. To tube **Z** add 1 cm^3 distilled water.
4. Stand all three tubes in a waterbath at about 25 °C.
5. Label three test tubes **1**, **2** and **3**.
6. To test tubes **1**, **2** and **3** add 1 cm^3 yeast culture, being sure to stir the stock culture before you measure out your samples. Stand tubes **1** and **3** in the waterbath at 25 °C.
7. Stand test tube **2** in a waterbath at (close to) 100 °C for 2 minutes.
8. Allow tube **2** to cool, then stand it in the waterbath at about 25 °C.
9. Leave the tubes in the waterbath at 25 °C for a suitable length of time.
10. Pour the contents of test tube **1** into boiling tube **X**, then pour test tube **2** into boiling tube **Y**, and test tube **3** into boiling tube **Z**. Place bungs in all the boiling tubes. Turn these tubes upside down gently twice and then remove the bungs.
11. Place the boiling tubes back in the waterbath at about 25 °C. Start your timer.
12. Inspect the colour of the liquid in the tubes every minute. Do not disturb the tubes other than by lifting them gently to check the colour. There may be a ring of blue colour at the top of the liquid but you should ignore this. Record how long it takes for the liquid in the tubes **X** and **Y** to reach the same colour as the liquid in tube **Z**.
13. Repeat the steps needed for tube **X** as many times as you think are necessary.
14. Repeat steps 1–13 using a waterbath at 40 ºC.

You will need to decide for yourself

- how long to leave the tubes in the waterbath before adding the yeast culture
- how many repeats to carry out in step 13
- which statistical test to carry out.

ISA HBI6T/P11 Candidate Results Sheet: Stage 1

The effect of temperature on the rate of respiration of microorganisms such as those found in probiotic foods

Centre Number | | | | | | Candidate Number | | | | |

Candidate Name .....

Record your data in a table in the space below.

Hand in this sheet at the end of each practical session. *(3 marks)*

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Turn over ▶

ISA HBI6T/P11 Candidate Results Sheet: Stage 2

The effect of temperature on the rate of respiration of microorganisms such as those found in probiotic foods

Centre Number | | | | | | Candidate Number | | | | |

Candidate Name .....

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

You should

- state your null hypothesis *(1 mark)*

- give your choice of statistical test *(1 mark)*

- give reasons for your choice of statistical test *(1 mark)*

- carry out the test and calculate the test statistic *(1 mark)*

- interpret the test statistic in relation to the null hypothesis being tested.
  Use the words *probability* and *chance* in your answer. *(2 marks)*

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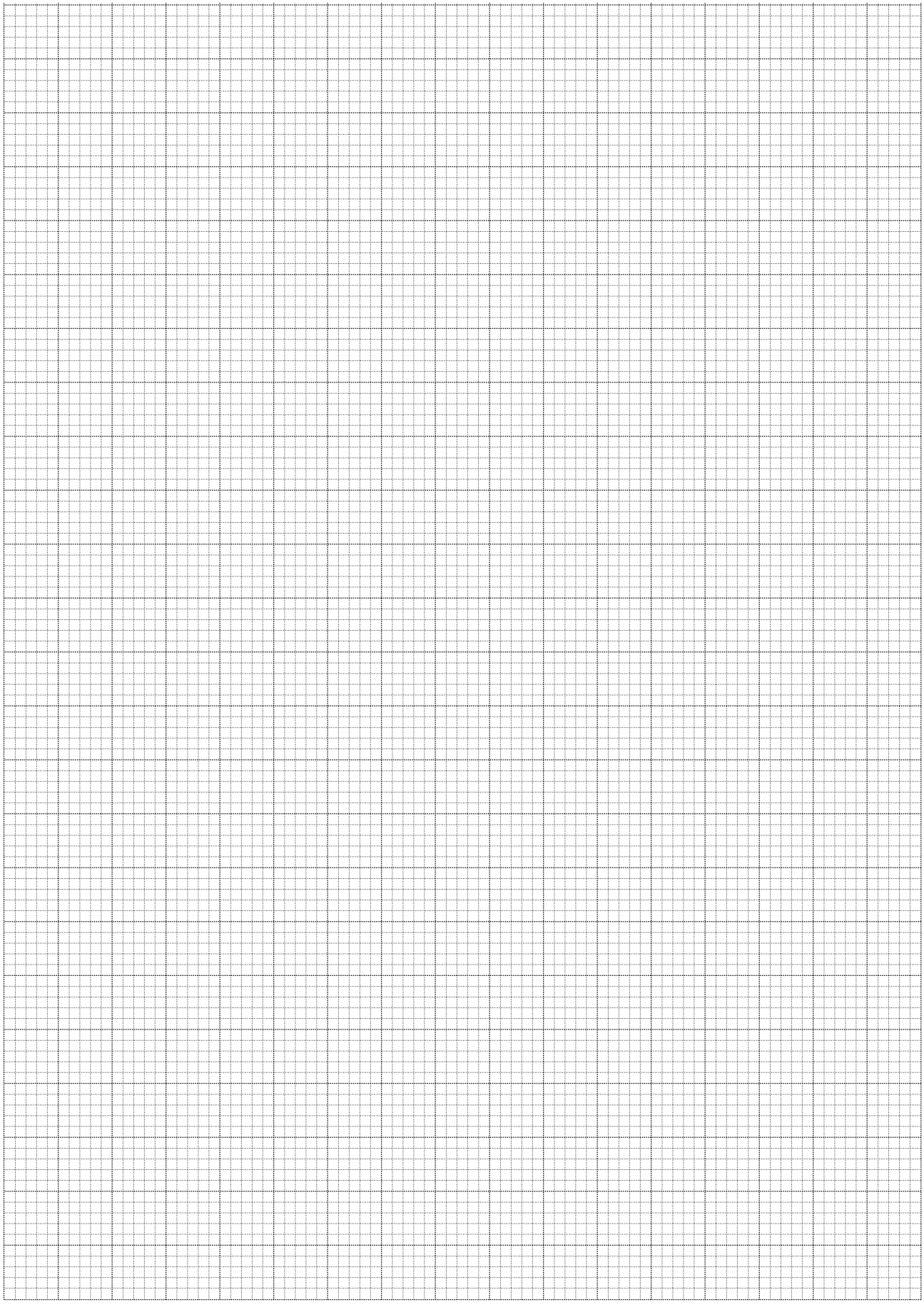
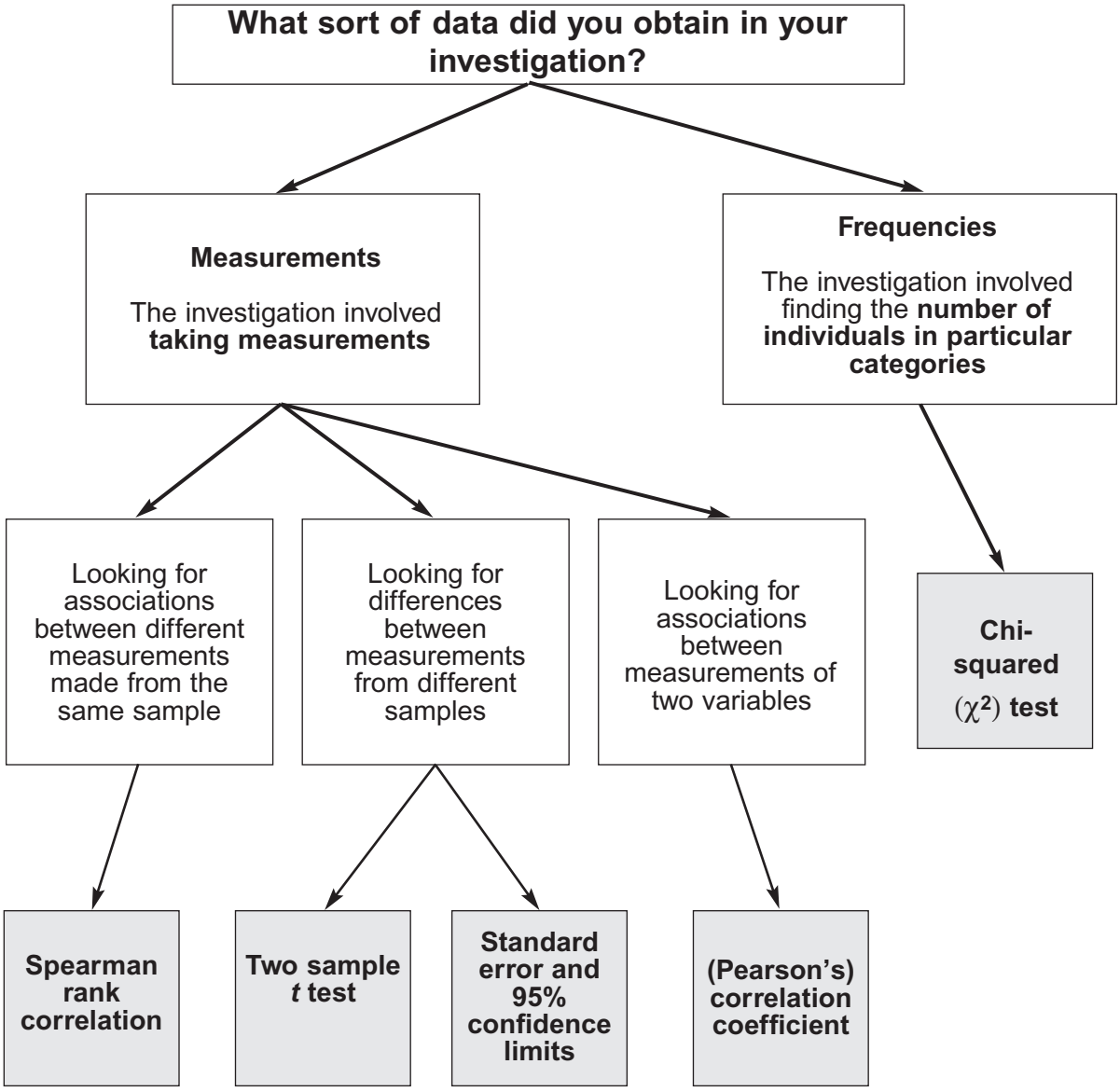
This graph paper is provided for use if you need it.

Hand in this sheet at the end of the practical session.



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 greater 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
- 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

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

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	0.42
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 datapoints 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 is 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-square (χ^2) test is based on calculating the value of χ^2 from the equation

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

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