INTERNATIONAL BIOLOGY OLYMPIAD e. V. IBO

Write the numbers in Table 3.

Table 3 (4 marks) {one mark is given for each complete row of data; 0.33 of a mark is given for each correct value in a row. Marks are given for correct number, +/- 0.5 of a whole number}

	Sample F	Sample M	Sample D
Haematocrit (%)	42.3 %	32.8 %	25.5 %
MCV (fl)	79.2 fl	90.7 fl	83.7 fl
MCH (pg)	26.9 pg	28.7 pg	28.2 pg
MCHC (g/dl)	34.0 g/dl	31.7 g/dl	33.7 g/dl

Task 1c

For each patient, show your workings for each parameter, in Box 1a (for Father (F)), Box 1b (for Mother (M)) and Box 1c (for Daughter (D)).

Box 1: Workings for patient F (Father) (4 marks) {Marks are given for correct number, +/- 0.5 of a whole number; no marks are given for correct calculation but incorrect number}

Element	Show your working here
Haematocrit (%)	RBC fraction/Total plasma x 100 (58/137) x 100 = 42.3 %
MCV (FL)	Hct/RBC or 1l/RBC x Hct $0.424/5.35x10^{12} = 7.9x10^{-14} = 79.3x10^{-15}$ l (fl)
MCH (pg)	Hb/RBC 14.4 g/dl x 10 = 144 g/l $144/5.35x10^{12} = 2.69x10^{-11} = 26.9x10^{-12}$ g (pg)
MCHC (g/dl)	Hb/Hct 14.4/0.424 = 34.0 g/dl

Task 1d

Using the values you have obtained (included in Table 3), establish the classification in terms of size of blood cells and levels of haemoglobin of the blood of sample F, M and D.

You should answer from one of the following options for each patient (N.B. you can use any of the options more than once):

A. Macrocytic, normochromic
B. Microcytic, hypochromic
C. Normocytic, normochromic

Patient	Classification
Father (F)	C
Mother (M)	C
Daughter (D)	C

(3 marks: one mark for each correct answer) {Marks are given for correct letter only; one mark for each correct answer}

Box 2 (one mark) {Marks are given for a correct calculation (appreciating there is more than one way to derive the correct answer)}

Show an example of your workings for the calculation of volumes to be used of the highest PEP concentration.

Options/examples of method include:

To make 1.5 mM PEP from 10 mM PEP

10 mM/1.5 mM = 6.667 (a dilution factor)

1000 µl/6.667 = 150 µl of concentrated PEP in 1000 µl

OR

C1V1 = C2V2

10 mM * x = 1.5 mM * 1000

x = (1.5 * 1000)/10 = 150 µl in 1000 µl

Table 5, Reaction Volumes. You should write the substrate concentrations, PEP [S], you have decided to use in the first row at the top of each column, and the volumes of each solution in the respective boxes. (3 marks) {Marks are given for the correct numbers depending on the choice of PEP concentration; examples are shown in this marking crib. Marks are given for rows for A, B and C with each correct cell awarding 0.2 marks. The PEP concentrations used must be included in the top row but carry no marks, however failure to include this results in zero marks.}

	Concentration 1 e.g. 0.2 mM	Concentration 2 e.g. 0.4 mM	Concentration 3 e.g. 0.8 mM	Concentration 4 e.g. 1.0 mM	Concentration 5 e.g. 1.5 mM
Solution A (µl)	500	500	500	500	500
Solution B (µl)	430	410	370	350	300
Solution C (µl)	20	40	80	100	150
Plasma	50 µl	50 µl	50 µl	50 µl	50 µl
Final volume	1000 µl	1000 µl	1000 µl	1000 µl	1000 µl

Task 2c

Perform the experiment as described and record the absorbance readings using the tables provided (Tables 6.1, 6.2 and 6.3).

{Example of Table 6.1. Marks are awarded for a correct orientation of numbers, i.e. descending from approximately 1.2 to approximately 0.02 for higher concentrations and the inclusion of a correctly calculated rate. The column includes the calculation of initial change (rate) in absorbance per minute, which the students are asked to highlight (there are no marks for highlighting the numbers, but this will aid marking). 1 mark is awarded per column of data with a further 0.2 marks per calculated rate. 0.2 marks for each rate will be awarded in the rate is within +/- 10% of the known value}.

Table 6.1 Sample F (6 marks)

Concentration	**1**	**2**	**3**	**4**	**5**
[S]	0.2	0.4	0.8	1.2	1.5
Absorbance at 0 s	1.036	0.990	0.959	1.009	1.000
Absorbance at 30 s	0.872	0.732	0.664	0.710	0.708
Absorbance at 60 s	0.712	0.475	0.358	0.402	0.398
Absorbance at 90 s	0.570	0.227	0.069	0.162	0.098
Rate (ΔA/Δt min)	0.313	0.520	0.609	0.616	0.610

Task 2d

Calculate the initial velocity (v_0) and write your answers in the tables below for each patient (Father (F), Mother (M) and Daughter (D)). Your values should be given to the nearest 3 decimal places (d.p.).

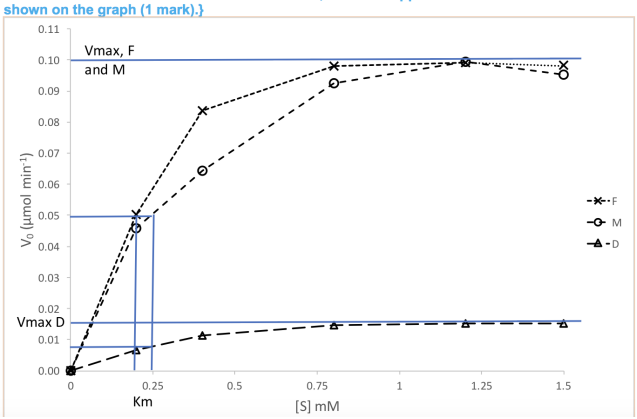
{Marks are awarded for the inclusion of correctly calculated numbers for V_0. 1 Mark is given for each F, M and D sample. Example data give for the Father (F)}

Table 7.1 Patient F (1 Mark)

[S]	V_0
0.2	0.050
0.4	0.084
0.8	0.098
1.2	0.099
1.5	0.098

Task 2e

Use your data to construct a Michaelis-Menten plot of v_0 over [S] for each patient (F, M and D) on the graph area below. You should draw one graph with three data plots; one each for Father (F), Mother (M) and Daughter (D). Use a cross (X) to mark data from the Father (F), a circle (O) to mark data from the Mother (M) and a triangle (Δ) to mark data from the Daughter (D).**{Example of plot. Two marks are awarded for each correctly labelled axis. Two marks are awarded for each data plot; two each for Father, Mother and Daughter. Markers will look for an approximation of the curve following the data points to make an approximation of the best fit (1 mark). In addition, the students are asked to indicate their estimations, where an approximation of V_{max} and K_M are shown on the graph (1 mark).}**



Task 2f

Using the three data plots, estimate V_{max} and K_M; write the estimates in the table below using the correct units and to the nearest 2 decimal places (d.p.), for each sample from the graph you have drawn.

(6 marks)

Patient	V_{max}	K_M
Father (F)	0.10 µmol min^{-1}	0.20 mM
Mother (M)	0.10 µmol min^{-1}	0.25 mM
Daughter (D)	0.02 µmol min^{-1}	0.25 mM

{(6 marks: one mark for each value) **{Calculations, derived from numbers generated from the plots are expected to be shown here. Marks are awarded for figures that are within a range of +/- 10% of values calculated by the staff team. It is expected that there will be some variation across the cohort.}**

Task 2g (3 marks: one mark for each completed table)

Determine [S]/v_0 for each substrate concentration and each sample and write your answers in the tables below for each patient (Father (F), Mother (M) and Daughter (D)). Your values should be given to to the nearest 1 decimal places (d.p.).

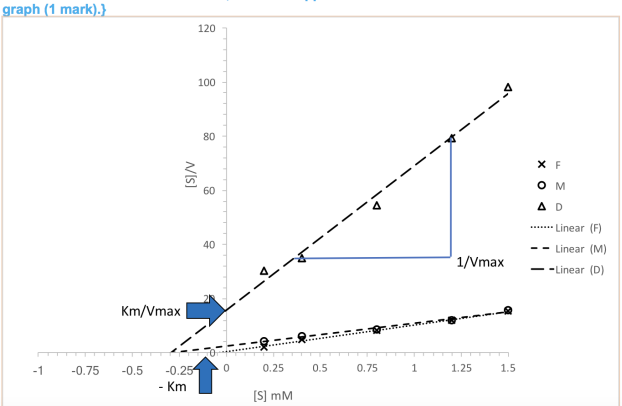
{Marks are given for each correct table, one mark for each table ((3 total) example for Patient F shown. Marks are not awarded for completing the [S] column as these figures (where correct) are awarded in previous questions.}

Patient F

[S]	S/V_0
0.2	2.0
0.4	4.8
0.8	8.2
1.2	12.1
1.5	15.3

Task 2h

Plot [S]/v_0 over [S] for each patient (F, M and D) on the graph area below. You should draw one graph with three data plots; one each for Father (F), Mother (M) and Daughter (D). Add a line of best fit for each data plot. Use a cross (X) to mark data from the Father (F), a circle (O) to mark data from the Mother (M) and a triangle (Δ) to mark data from the Daughter (D). **{Example of plot. Two marks are awarded for each correctly labelled axis. Two marks are awarded for each data plot; two each for Father, Mother and Daughter. Markers will look for an approximation of the curve following the data points to make an approximation of the best fit (1 mark). In addition, the students are asked to indicate their estimations, where an approximation of V_{max} and K_M are shown on the graph (1 mark).}**



Task 2i

Calculate V_{max} and K_M for each patient and include in the table below using the correct units to two decimal places (d.p.).

(6 marks)

Patient	V_{max}	K_M
F	**0.10 µmol min^{-1}**	**0.04 mM**
M	**0.12 µmol min^{-1}**	**0.35 mM**
D	**0.02 µmol min^{-1}**	**0.34 mM**

(6 marks: one mark for each value) **{Calculations, derived from numbers generated from the plots are expected to be shown here. Marks are awarded for figures that are within a range of +/- 10% of values calculated by the staff team. It is expected that there will be some variation across the cohort.}**

Task 3a

Complete the table below, indicating which size protein bands are visible for each patient (F, M or D), as either 'present' or 'not present'. Use the letter P to mark present, and NP to mark not present.

Task 3b

Indicate on the table whether any of the three patients (F, M or D) contain a PK tetramer by marking as either 'present' or 'not present'. Use the letter P to mark present, and NP to mark not present.

(5 marks) {1 mark for each correct complete row, the correct answers are shown in the table.}

		Patient F	Patient M	Patient D
	Molecular Weight	Present (P) or Not Present (NP)		
	232 kDa	P	P	NP
	58 kDa	P	P	NP
	40 kDa	NP	NP	P
	18 kDa	NP	NP	P
	PK tetramer	P	P	NP

Task 3d (1 mark for the correct answer; you cannot gain a mark for Task 3e unless you have answered Task 3d correctly).

Wild type alleles are designated **A**; mutant alleles are designated lower case **a** followed by a subscript.

Indicate, by drawing a circle around your choice of either A, B or C, father III-4's genetic PK gene arrangement in terms of:

A. Simple Heterozygote (Aa_1, Aa_2, Aa_3)
B. Compound Heterozygote (a_1a_2; a_1a_3; a_2a_3)
C. Homozygous (AA

Task 3e (1 mark for the correct answer; you can only receive a mark if you have answered Task 3d correctly).

Indicate, by drawing a circle around your choice of either A or B, brother IV-5's genetic PK gene arrangement in terms of:

A. Simple Heterozygote
B. Compound Heterozygote

{The correct answer for Task 3d is A; the correct answer for Task 3e is B. No marks are given for either Task 3d or 3e if the answer of Task 3d is incorrect; One mark is given for the correct answer to Task 3d, even if Task 3e is incorrect.}