H

RAFFLES INSTITUTION
2023 Year 6 Preliminary Examination

Higher 2

CANDIDATE NAME												
CIVICS GROUP	2	3	S	0	3		INDEX NUMBER					

BIOLOGY

9744/04

Paper 4 Practical

14th August 2023
2 hours 30 minutes

Candidates answer on the Question Paper.
Additional Materials: As listed in the Confidential Instructions.

READ THESE INSTRUCTIONS FIRST

Write your index number, CT group and name on all the work you hand in.
Give details of the practical shift and laboratory, where appropriate, in the boxes provided.
Write in dark blue or black pen.
You may use a 2B pencil for any diagrams or graphs.
Do not use staples, paper clips, highlighters, glue or correction fluid.

Answer **all** questions in the spaces provided on the Question Paper.

The use of an approved scientific calculator is expected, where appropriate.
You may lose marks if you do not show your working or if you do not use appropriate units.

At the end of the examination, hand in the Question Paper containing your answers.
The number of marks is given in brackets [ ] at the end of each question or part of the question.

Shift
Laboratory

For Examiner's Use	
1	
2	
3	
Total	

This document consists of **19** printed pages and 1 blank page.

Raffles Institution
Internal Examination

Answer **all** questions.

1 The kidneys are the organs that remove waste products from the blood and produce urine.

Urine can be tested as part of a health check.

People who have kidney disease or a urinary tract infection may have unusually high concentrations of protein in their urine.

You will be testing a solution that represents a sample of urine from a patient with a possible kidney disease or a urinary tract infection and will be referred to as 'mock urine'.

You will determine the concentration of protein in this sample of mock urine.

You are provided with the materials shown in Table 1.1.

Table 1.1

labelled	contents	hazard	volume / cm^3
P	10.00% protein solution	none	20
W	distilled water	none	50
C	0.15% copper sulfate solution	harmful irritant	20
K	5.00% potassium hydroxide solution	harmful irritant	20
U	mock urine	none	10
X	unknown	none	10
U+X	mixture of **U** and **X**	none	10

You are required to wear suitable eye protection and gloves.

If any solution comes into contact with your skin, wash off immediately under cold water.

(a) You will need to carry out a serial dilution of the 10.00% protein solution, **P**, to reduce the concentration by half between each successive dilution.

You will need to prepare four concentrations of protein solution in addition to the 10.00% protein solution, **P**.

After the serial dilution is completed, you will need to have 5 cm^3 of each concentration available for use.

(i) In the space below, draw a table to show how you will prepare the serial dilution.

[3]

Carry out step **1** to step **9**.

1. Prepare the concentrations of protein solution, as decided in **(a)(i)**, in the plastic vials provided. Use a glass rod to mix the protein solutions.

2. Label five of the test-tubes with the concentrations you prepared in step **1**.

3. Put 1cm^3 of each concentration of protein solution into the appropriately labelled test-tubes.

4. Label another test-tube 0.00% and put 1cm^3 of distilled water, **W**, into this test-tube.

5. Put 1cm^3 of **K** into each of the labelled test-tubes. Shake gently to mix.

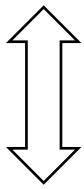
6. Put 1cm^3 of **C** into each of the labelled test-tubes. Shake gently to mix.

7. Leave the test-tubes aside. After 1 minute, shake gently to mix.

8. Observe the colour of the liquid in each test-tube. To see the colour more clearly, it may help to hold a piece of white paper behind the test tube. You may see the same colour in more than one test-tube.

9. Record your results in **(a)(ii)** using the symbols shown in Table 1.2.

Table 1.2

dark purple	+++++
	++++
	+++
	++
very light purple	+
blue	-

(ii) Record your results in an appropriate table. You may use the same symbols for more than one test-tube.

[2]

You are provided with a sample of mock urine, **U**. This represents a sample of urine from a patient being tested for possible kidney disease or urinary tract infection. You are also provided with solution **X**, taken from the human gastrointestinal tract.

10. Label three test-tubes, **U**, **X** and **U+X** respectively.

11. Put 1cm^3 of **U**, **X** and **U+X** into each of the test-tubes respectively.

12. Repeat steps **5** to step **8** for the contents of **U**, **X** and **U+X**.

(iii) Fig. 1.1 shows a scale of protein concentrations used in this investigation. The position for 0.00% and 10.00% are shown on the scale.

Complete the scale in Fig. 1.1 by showing the positions of the protein concentrations you prepared in step **1**.

[1]

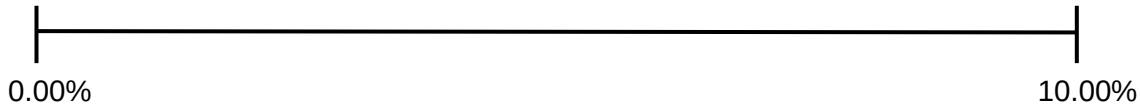


Fig. 1.1

(iv) Use your results in **(a)(ii)** and **(a)(iii)** to estimate the protein concentration of **U**, **X** and **U+X**. Show your estimates on Fig. 1.1 by drawing labeled arrows (↓) at the correct positions on the scale.

[2]

Table 1.3 shows the total mass of protein present in urine over 24 hours for people with different medical conditions.

Table 1.3

medical condition	total mass of protein in urine /mg24h^{-1}
no condition	<100
urinary tract infection	100–200
kidney tubular disease	200–500
glomerular disease	>500

The 10.00% protein solution you used in **(a)(i)** represents a urine sample collected over a period of 24 hours that contains 1000mg of protein.

(v) Using your result in **(a)(iv)**, estimate the concentration of protein in **U** over a period of 24 hours and state the possible medical condition of the patient indicated by **U**.

Show your working in the space provided.

medical condition ……..……..[2]

(vi) Solution **X** was extracted from the human gastrointestinal tract.

Suggest the identity of solution **X** and explain the basis of your suggestion.

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……[3]

(b) A colorimeter can be used to measure the intensity of the purple colour in the samples tested.

Fig. 1.2 shows a colorimeter that measures the absorbance of light when it passes through coloured solutions of different colour intensities.

Fig. 1.2

(i) Outline how a colorimeter is set up so as to obtain a correct measurement of the colour intensity of a coloured solution placed in the cuvette.

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……[2]

(ii) State the limitation of the experiment described in part **(a)**, that can overcome by the use of the colorimeter.

……

……[1]

A student prepared a series of protein solutions and performed a biuret test on these solutions to plot a calibration curve. The absorbance of these solutions as recorded by the student is shown below:

Table 1.4

1.0%	0.15
2.0%	0.36
3.0%	0.57
4.0%	0.78
5.0%	1.00

(iii) Plot a calibration curve using the data given, on the grid in Fig. 1.3.

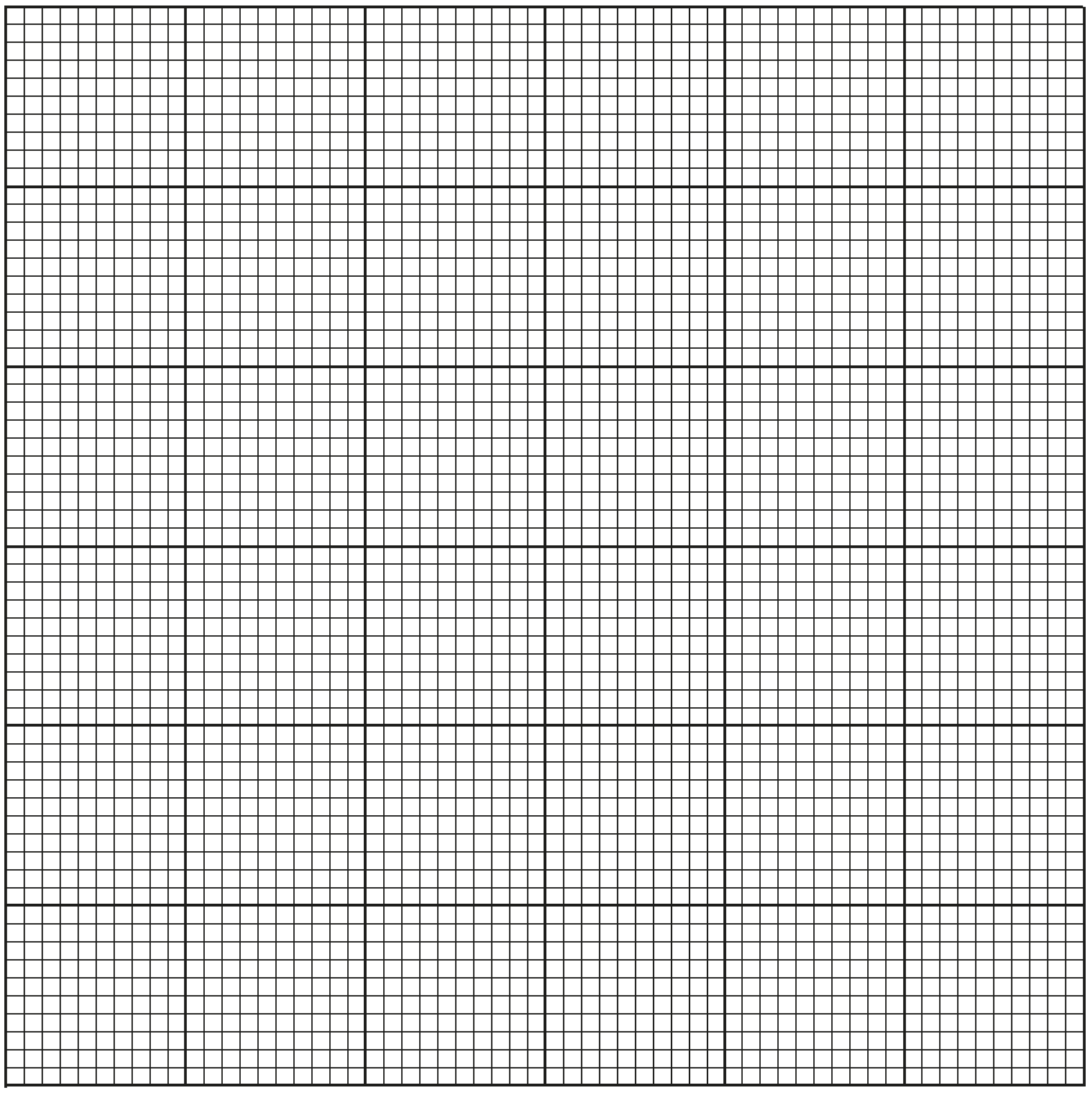

Fig. 1.3 [4]

The colorimeter has a limitation in that it is unable to determine the absorbance value of solutions above 1.0. In such situations, the sample must be diluted first before its absorbance value is determined. The concentration can then be determined using the standard curve and multiplied by the dilution factor.

A separate experiment was carried out involving sample **R**, taken from patient **R** who has severe glomerular disease (protein concentration is between 6 and 8%). The student had to modify the experimental procedure involving the colorimeter, to enable precise determination of the protein concentration in the urine taken from patient **R**.

(iv) Describe how the student could modify the previous experiment to determine the concentration of protein in sample **R**.

*Do **not** repeat any detail of how the standard protein solutions are prepared, the biuret test and how the colorimeter would be used.*

Your method should be set out in a logical way and be detailed enough to let another person follow it.

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……[5]

[Total: 25]

2 **W** is a fruit from the genus *Actinidia* and native to China. You are provided with half a specimen of a variety of **W**, **W1** and another variety **W2**.

You will need to wear gloves and goggles for this part of the experiment.

You are not expected to be familiar with this specimen.

(a) Compare the morphology of **W1** and **W2** in the space below.

[3]

(b) (i) Cut a 5mm thick transverse slice of specimen **W1** on the cutting board. The slice should contain a full complement of seeds and should not be taken from the extreme ends of the fruit.

Blot dry the cut surface with a paper towel. Cover one cut surface of the slice with bromocresol green, **G**. Leave it to stain for one minute. **G** stains proteins blue.

In the space provided, draw a large, detailed diagram of the cut surface of the slice as shown by the shaded area in Fig. 2.1.

You are required to use a sharp pencil for drawings.

Your drawing should show details of the arrangement of different regions and their correct shapes and proportions. Indicate where the highest concentration of protein is present.

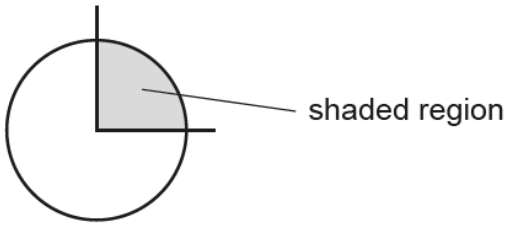

Fig. 2.1

[4]

(ii) You are required to estimate the total number of seeds that were present in the fruit from which **W1** was taken.

- Cut the 5mm slice of **W1** into equal quarters.
- Place one of these quarters on the cutting board and, using the forceps, squeeze out all of its seeds.

Count the number of seeds present: ……

Repeat this for another quarter.

Count the number of seeds present: …… [1]

Fig. 2.2 shows, in longitudinal view of the fruit, the area where the seeds are found and the position from which **W1** was taken.

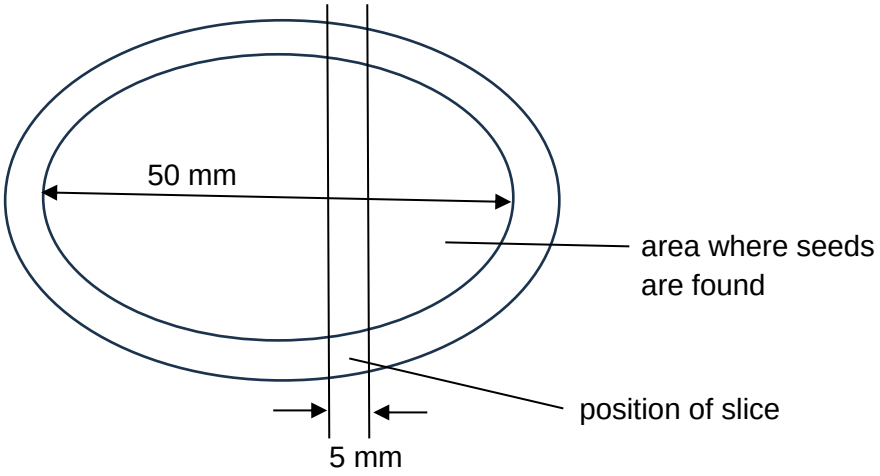

Fig. 2.2 (Not to scale)

(iii) Plan how you would estimate the number of seeds in the whole fruit, taking into consideration and explaining any allowance that you may think is necessary.

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……[4]

(iv) Using the method you described in **(b)(iii)** and data collected from **(b)(ii)**, estimate the number of seeds found in **W1**. Show your working clearly.

[2]

(c) A student noted that **W2** tasted sweeter than **W1.** He carried out a *t*-test to investigate the differences by measuring the concentration of sucrose in a sample of 30 fruits for each variety of **W**.

	W1	**W2**
Mean sucrose concentration/arbitrary units	15 ± 3	23 ± 4

The formula for *t*-test is:

$$t = \frac{\left|\bar{x}_1 - \bar{x}_2\right|}{\sqrt{\left(\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}\right)}} \qquad v = n_1 + n_2 - 2$$

Key to symbols

s = standard deviation
$\bar{x}$ = mean
n = sample size (number of observations)
v = degrees of freedom

(i) Complete the calculation to find the value of *t* for the concentration of sucrose in the fruits.

$$t = \frac{\quad}{\sqrt{\frac{4^2}{30} + \quad}}$$

$$= \frac{\quad}{0.9}$$

t = …… [2]

(ii) State the null hypothesis for **(c)(i)**.

……..……...

……[1]

Table 2.1 shows the critical values at $p < 0.05$ for the *t*-test and Table 2.2 shows the p values at different significance levels.

Table 2.1

degrees of freedom	18	20	21	22	23	24	25	26	27	28	29	30	40	60	∞
critical value	2.10	2.09	2.08	2.07	2.06	2.06	2.06	2.06	2.05	2.05	2.04	2.04	2.02	2.00	1.96

Table 2.2

Degrees of freedom	Significance level					
	20% (0.20)	10% (0.10)	5% (0.05)	2% (0.02)	1% (0.01)	0.1% (0.001)
1	3.078	6.314	12.706	31.821	63.657	636.619
2	1.886	2.920	4.303	6.965	9.925	31.598
3	1.638	2.353	3.182	4.541	5.841	12.941
4	1.533	2.132	2.776	3.747	4.604	8.610
5	1.476	2.015	2.571	3.365	4.032	6.859
6	1.440	1.943	2.447	3.143	3.707	5.959
7	1.415	1.895	2.365	2.998	3.499	5.405
8	1.397	1.860	2.306	2.896	3.355	5.041
9	1.383	1.833	2.262	2.821	3.250	4.781
10	1.372	1.812	2.228	2.764	3.169	4.587
11	1.363	1.796	2.201	2.718	3.106	4.437
12	1.356	1.782	2.179	2.681	3.055	4.318
13	1.350	1.771	2.160	2.650	3.012	4.221
14	1.345	1.761	2.145	2.624	2.977	4.140
15	1.341	1.753	2.131	2.602	2.947	4.073
16	1.337	1.746	2.120	2.583	2.921	4.015
17	1.333	1.740	2.110	2.567	2.898	3.965
18	1.330	1.734	2.101	2.552	2.878	3.922
19	1.328	1.729	2.093	2.539	2.861	3.883
20	1.325	1.725	2.086	2.528	2.845	3.850
21	1.323	1.721	2.080	2.518	2.831	3.819
22	1.321	1.717	2.074	2.508	2.819	3.792
23	1.319	1.714	2.069	2.500	2.807	3.767
24	1.318	1.711	2.064	2.492	2.797	3.745
25	1.316	1.708	2.060	2.485	2.787	3.725
26	1.315	1.706	2.056	2.479	2.779	3.707
27	1.314	1.703	2.052	2.473	2.771	3.690
28	1.313	1.701	2.048	2.467	2.763	3.674
29	1.311	1.699	2.043	2.462	2.756	3.659
30	1.310	1.697	2.042	2.457	2.750	3.646
40	1.303	1.684	2.021	2.423	2.704	3.551
60	1.296	1.671	2.000	2.390	2.660	3.460
120	1.289	1.658	1.980	2.158	2.617	3.373
∝	1.282	1.645	1.960	2.326	2.576	3.291

(iii) State the conclusion that could be gathered from the *t*-test.

……...

……...

……...

……...

……...

……[3]

[Total: 20]

3 The study of blood and blood disorders is known as haematology in clinical medicine. Blood film preparation is a technique that is commonly used on blood samples to diagnose abnormal changes to the patient's blood system.

The blood film is prepared by placing a drop of blood on one end of a glass slide and another glass slide is used to spread the blood as a thin film on the slide.

Fig. 3.1 below shows how a blood film is prepared.

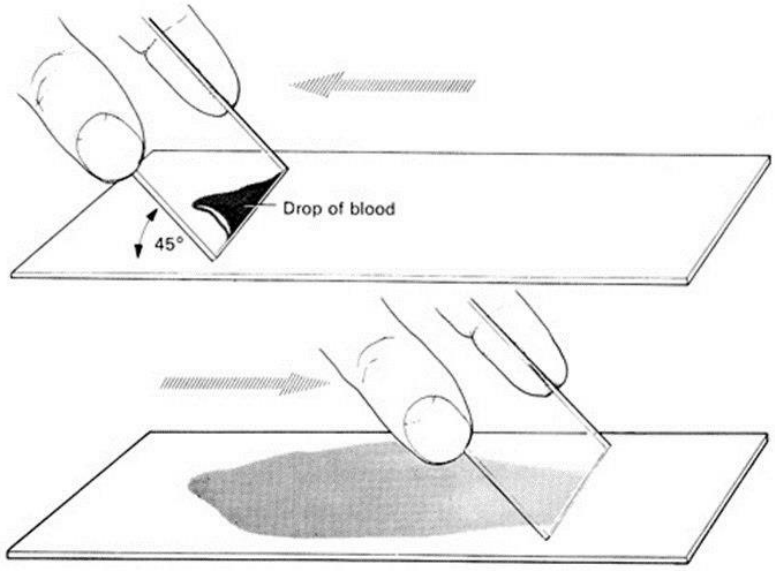

Fig. 3.1

The blood film is stained with mixture of methylene blue and eosin.

The appearance of the various cellular components of blood under the microscope is described below :

Table 3.1

type of cell	appearance
lymphocytes	Spherical cells that have a large darkly stained round nucleus that takes up much of the cell volume. It has little or no cytoplasm. The diameter of the nucleus is similar to the diameter of a red blood cell.
neutrophils	Spherical cells that have a darkly stained nucleus that is lobed (2 to 5 lobes), with thin threads connecting the lobes. The cytoplasm is stained light red.

(a) You are given slides **S1** and **S2** in a petri dish

(i) Using the slide **S2**, determine the diameter of the field of view at high power magnification.

[1]

The gap between the cover slip and slide is 100 µM.

Fig 3.2

(ii) Calculate the volume of blood under the field of view at high power magnification.

[2]

(b) **(i)** Place slide **S2** under higher power magnification.

Use slide **S2** to determine the dimensions of each eye piece graticule unit.

Show your working.

[1]

(ii) Place slide **S1** under high power magnification.

Locate a lymphocyte and use the markings on the eye piece to measure the diameter of the:

a) nucleus (N); and

diameter of N ……..……..[1]

b) cell (C).

diameter of C ……[1]

(iii) Calculate the N:C ratio of the lymphocyte, and express your answer in whole numbers.

ratio ……..[1]

(c) Place slide **S1** under high power magnification.

In the space below, make a labeled drawing of a neutrophil.

[3]

[Total: 10]

End of Paper

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