

CANDIDATE
NAME

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CENTRE
NUMBER

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BIOLOGY

9700/33

Advanced Practical Skills 1

February/March 2016

2 hours

Candidates answer on the Question Paper.

Additional Materials: As listed in the Confidential Instructions.

READ THESE INSTRUCTIONS FIRST

Write your Centre number, candidate number and name on all the work you hand in.

Write in dark blue or black pen.

You may use an HB pencil for any diagrams or graphs.

Do **not** use staples, paper clips, glue or correction fluid.

DO **NOT** WRITE IN ANY BARCODES.

Answer **all** questions.

Electronic calculators may be used.

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, fasten all your work securely together.

The number of marks is given in brackets [] at the end of each question or part question.

For Examiner's Use	
1	
2	
Total	

This document consists of **11** printed pages and **1** blank page.

Before you proceed, read carefully through the **whole** of Question 1 and Question 2.

Plan the use of the **two hours** to make sure that you finish all the work that you would like to do.

If you have enough time, think about how you can improve the accuracy of your results, for example by obtaining and recording one or more additional measurements.

You will **gain marks** for recording your results according to the instructions.

- 1 The concentration of protein in a person's urine may be measured to identify certain health problems.

You are required to:

- prepare different concentrations of the protein solution, **P**
- standardise and carry out the tests for protein concentration
- record the results of tests for the known concentrations of protein and an unknown sample, **U**
- estimate the concentration of protein in **U**.

The protein concentration can be measured by using potassium hydroxide solution and copper sulfate solution.

Fig. 1.1 shows the result of adding potassium hydroxide solution and copper sulfate solution to a sample containing protein.

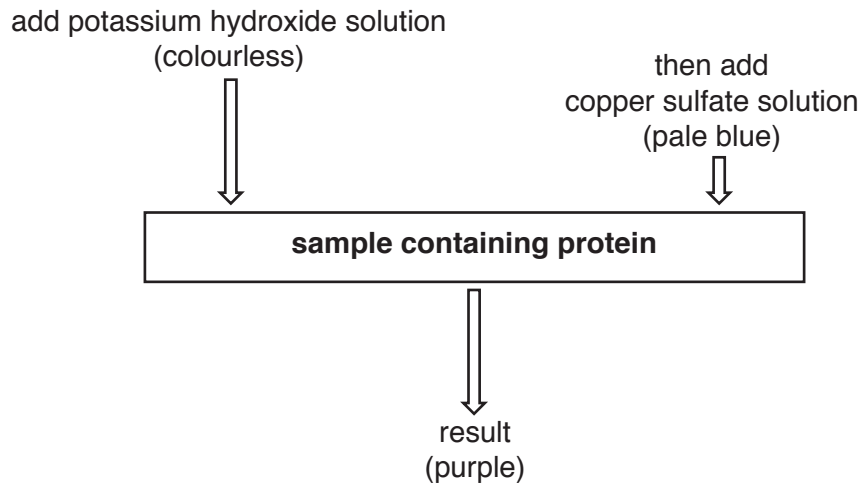


Fig. 1.1

Fig. 1.2 shows an example of a solution containing no protein (pale blue) and an example of a solution containing a high concentration of protein (dark purple), after testing.

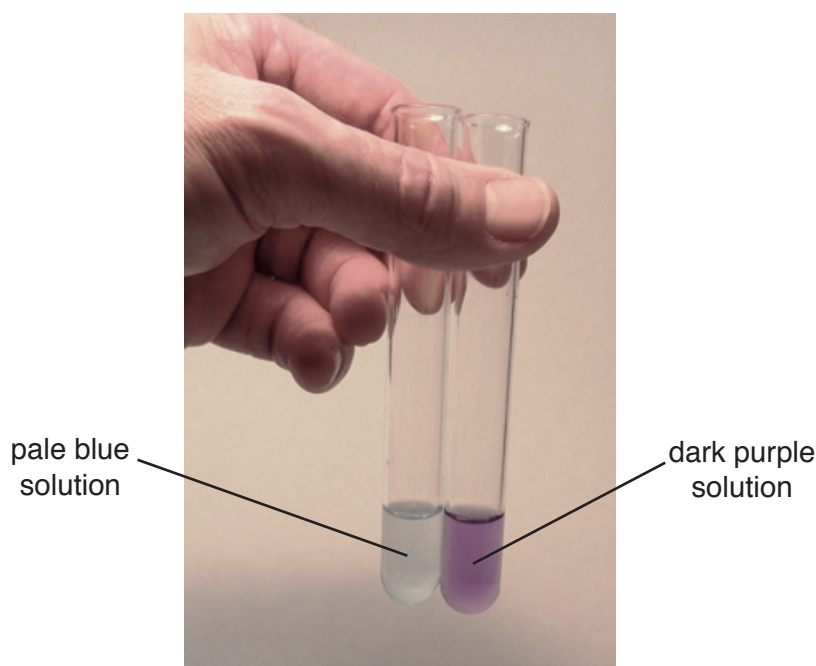


Fig. 1.2

You are provided with:

labelled	contents	hazard	volume/cm ³
P	1.0% protein solution	none	25
U	sample of unknown protein concentration	none	10
W	distilled water	none	25
K	potassium hydroxide solution	corrosive	15
C	copper sulfate solution	none	15

You are advised to wear suitable eye protection, especially when using the potassium hydroxide solution, **K**. If **K** comes into contact with your skin, wash off with cold water.

- (a) When carrying out a practical procedure, the hazards of using the solutions need to be considered. Then the level of risk needs to be assessed as low or medium or high.

State the hazard with the greatest level of risk when using the solutions, then state the **level** of risk of the procedure: low or medium or high.

hazard

level of risk

[1]

- (b) (i) Table 1.1 shows how to make up two of the concentrations of protein solution you will use.

Decide which concentrations of protein solution to prepare using simple dilution of the 1.0% protein solution, **P**.

Complete Table 1.1 to show how you will prepare the other concentrations.

Table 1.1

volume of 1.0% protein solution, P /cm ³	volume of distilled water, W /cm ³	percentage concentration of protein solution
5	0	1.0
0	5	0.0

[3]

Proceed as follows:

1. Prepare the concentrations of protein solution, as shown in Table 1.1, in the beakers provided.
2. Put 1 cm³ of the 1.0% protein solution into a test-tube.
3. Put 1 cm³ of **K** into the same test-tube. Shake gently to mix.
4. Using the syringe labelled **C**, put 1 cm³ of **C** into the same test-tube. Shake gently to mix.

You are required to standardise this test (step 2 to step 4) to be able to use the results to estimate the concentration of protein in **U**.

- (ii) State which variable you will need to standardise when testing the other protein solutions and **U**.

.....[1]

Read step 5 to step 8 before proceeding.

5. Carry out the standardised test for each of the protein solutions prepared in step 1.
6. Record your observations of the **colour** of each solution in (b)(iii).

- 7. In a test-tube rack, put the test-tubes in order of palest blue to darkest purple.
- 8. Record in **(b)(iii)** each colour as a **number** using the scale shown in Fig. 1.3.

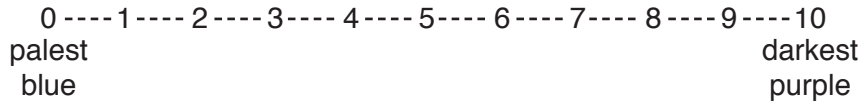


Fig. 1.3

- (iii)** Prepare the space below and record your observation for each test-tube as a colour and its number, using the scale in Fig. 1.3.

[5]

- 9. Carry out the standardised test with a sample of **U**.
- (iv)** Using the scale in Fig. 1.3 and the result for this test of **U**, state the number that represents the colour when compared with the results in **(b)(iii)**.

number for U

Use your results to estimate the concentration of protein in **U**.

estimated concentration of protein in U [1]

- (v)** Identify **one** significant source of error when estimating the concentration of protein in **U**.

.....
 [1]

- (c) Scientists may use another method to measure the protein concentration in urine samples. A sample of urine is put into a test-tube and an equal volume of reagent **X** is added.

When the mixture is shaken, a cloudy precipitate of protein forms.

- (i) State **two** of the variables which need to be standardised when using this method to compare different samples of urine.

Describe a method which can be used to standardise each of these variables.

variable 1

description

.....

.....

variable 2

description

.....

.....

[3]

A colorimeter can be used to measure the quantity of light absorbed (absorbance) by a solution.

After adding reagent **X**, a scientist used a colorimeter to obtain the absorbance of:

- six solutions of known protein concentration
- a urine sample (of unknown protein concentration).

A calibration graph was drawn by plotting the absorbance against the known concentrations of protein in the six solutions. The graph can be used to estimate the concentration of protein in the urine sample.

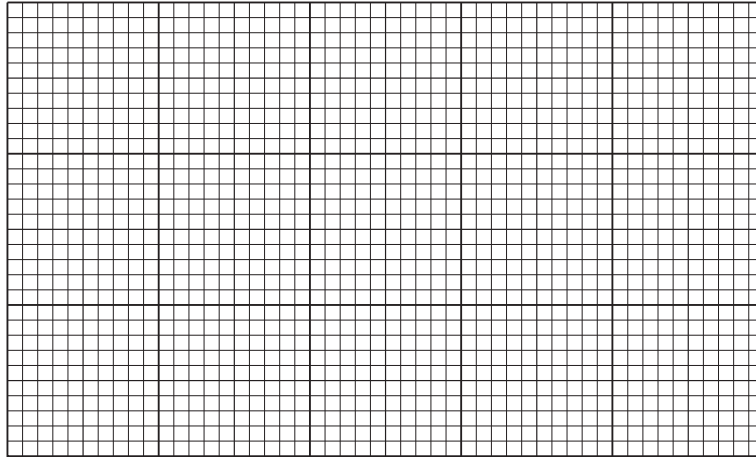
Table 1.2 shows the results for the absorbance of solutions of known protein concentration.

Table 1.2

protein concentration / $\mu\text{g cm}^{-3}$	absorbance
0	0.00
100	0.06
200	0.36
450	0.28
760	0.47
900	0.52

You are required to use a sharp pencil for graphs.

(ii) Plot a graph of the data shown in Table 1.2.



[4]

(iii) Draw a circle on the graph to show the anomalous result.

[1]

(iv) Use your graph to estimate the concentration of protein in a urine sample with an absorbance of 0.49.

Show on your graph how you estimated the concentration of protein.

concentration of protein

[2]

[Total: 22]

2 You are provided with two samples of onion that have been kept in two different solutions, **S1** or **S2**.

You are required to:

- make slides of the inner epidermis of onions that have been kept in **S1** and **S2**
- observe and draw **two** cells from each epidermis.

Proceed as follows:

1. Label one **dry and clean** microscope slide '**S1**' and put the slide on a paper towel.
2. Put a few drops of **S1** onto the slide.
3. Remove a piece of the onion from **S1** and, using forceps or fingers, peel off the inner epidermis, as shown in Fig. 2.1.

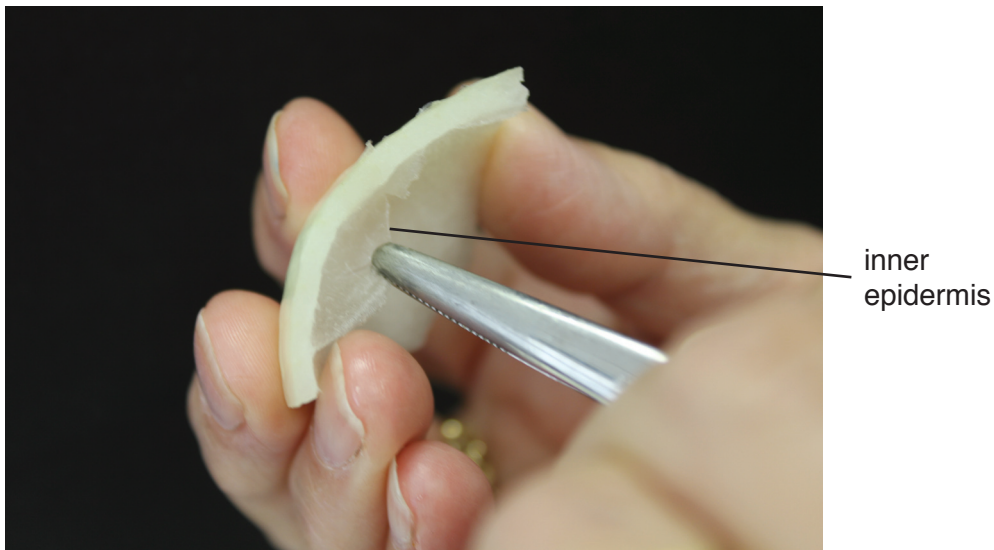


Fig. 2.1

4. Cut one piece of the inner epidermis that will fit under a coverslip. Put the remaining inner epidermis back into **S1**.
5. Place the inner epidermis on the slide as shown in Fig. 2.2. If the epidermis is folded, you may need to add more drops of **S1** so that it floats and uncurls.

*Note: It is important to prevent the epidermis from drying out, so add more drops of **S1** if needed.*

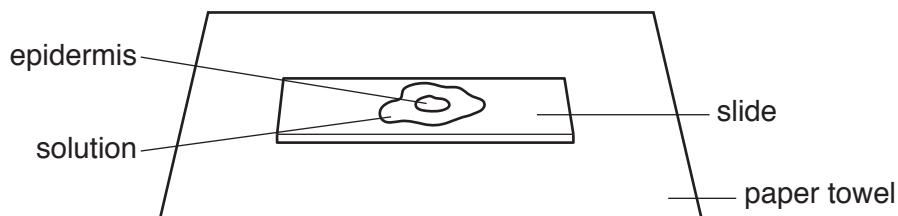


Fig. 2.2

6. Cover the epidermis with a coverslip and use a paper towel to remove any excess solution that is outside the coverslip.
7. Repeat step 1 to step 6 using the piece of onion in **S2** and mount the piece of inner epidermis in **S2**, on a slide labelled '**S2**'.

- (a) Use the microscope to select an area of cells on each of the slides **S1** and **S2** that shows the effect of keeping the epidermis in the solution.

Adjust the amount of light entering the microscope to observe cells clearly.

You are required to use a sharp pencil for drawings.

- (i) Draw **two** adjacent (touching) cells from each slide.

Use **one** ruled label line and label to identify the cell wall of **one** of the cells.

two cells from S1

two cells from S2

[5]

- (ii) Using your observations of the cells drawn in (a)(i), state which of the solutions, **S1** or **S2**, had a more negative water potential than the onion cells.

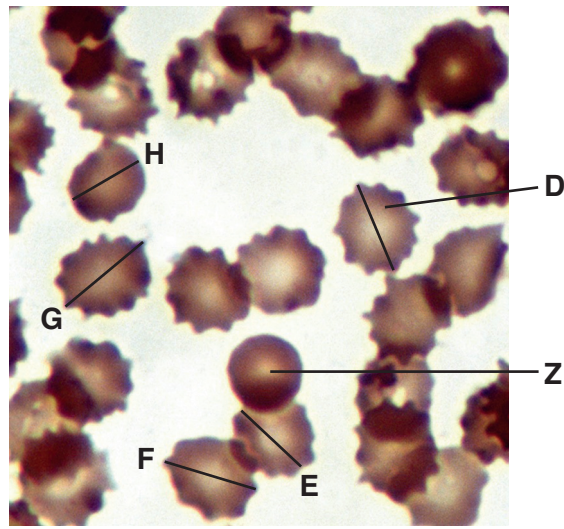
solution

Explain the reason for your answer.

.....
.....
.....
.....
.....

[3]

Fig. 2.3 is a photomicrograph of human red blood cells that have been kept in a solution. This has caused most of the cells to change shape (become crenated).



magnification $\times 1430$

Fig. 2.3

(b) (i) Draw the cells labelled **D** and **Z**.

cell D

cell Z

[3]

(ii) Annotate the drawings in (b)(i) to describe **one** observable difference between cell **D** and cell **Z**. [1]

(iii) Suggest **one** reason for the observable difference between cell **D** and cell **Z**.

.....
.....[1]

(c) (i) Use the magnification to find the actual diameter, in μm , of the cells labelled **D**, **E**, **F**, **G** and **H** in Fig. 2.3. For each cell, measure the diameter at the position shown by the line.

You may lose marks if you do not show your working.

D μm , **E**..... μm , **F**..... μm , **G**..... μm , **H**..... μm [3]

(ii) Using the actual diameters calculated in (c)(i), calculate the **mean** actual diameter of a crenated red blood cell.

You may lose marks if you do not show your working or if you do not use appropriate units.

mean actual diameter[2]

[Total: 18]

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