MICROSCALE STANDARD CURVE - GLUCOSE CONCENTRATION

Supports delivery of:

- National 4 Biology: Unit 2, Key Area 6 – Biological actions in response to internal and external changes to maintain stable body conditions; research causes of diabetes.
- National 5 Biology: Unit 2, Key Area 2b – Blood glucose regulation.
- Higher Human Biology: Unit 2, Key Area 8c – Type 1 and type 2 diabetes.
- Advanced Higher Biology: Unit 1, Key Area 1b: Liquids and solutions – production of a standard curve to determine an unknown.

The Lancet reported, in 2020, that almost 270,000 people are now living with Type 2 diabetes in Scotland (population 5.4 million). The prevalance of the disease has increased by 40% in 10 years.

Country Focus

Diabetes in Scotland: a rising tide

The prevalence of type 2 diabetes in Scotland has increased by 40% in the past decade; almost 270 000 people are now living with type 2 diabetes among Scotland's population of 5·4 million.[1] Some of this increase is driven by the demographic shift in the population; the proportion of those aged 65 years and older increased from 16% to 19% over this period, partly reflecting past birth

SGLT2 inhibitors are now licensed for cardiovascular disease prevention and widespread use is advocated in many guidelines, there will be increasing pressure on the national drugs budget.

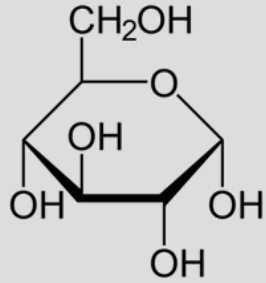
Health care in Scotland is provided within 14 NHS Boards and most Boards have a Managed Clinical Network coordinating multidisciplinary diabetes care for their area. Most type 2 diabetes is managed solely in primary care but

30% but whether this decline has been sustained more recently is unknown.[7]

Ample scope remains for further reduction in risk factors. Despite high rates of measurement of risk factors and use of drugs (eg, blood pressure was recorded in 88·6% and cholesterol in 84·2% in 2018), at least two thirds of those with type 2 diabetes have two or more cardiovascular disease risk factors other than diabetes

Management of the symptoms of diabetes requires careful monitoring of blood glucose concentrations. In this colour-based reaction, a standard curve will be produced using solutions of known glucose concentration; this will subsequently be used to estimate the glucose concentration in unknown samples. This practical activity can be linked to the testing of urine/blood for glucose and the diagnosis and management of diabetes mellitus.

Glucose is a monosaccharide reducing sugar. In this reaction, glucose donates electrons to permanganate (purple/pink in colour), which then becomes colourless. The time taken for the loss of colour is inversely correlated to increasing glucose concentration.



$$MnO_4^- + 8H^+ + 5e^- \rightarrow Mn^{2+} + 4H_2O$$

pink in solution — colourless in solution

A series of glucose solutions of known concentration are tested for the time taken for decolourisation of permanganate. These values are recorded and plotted against the glucose concentration. This "standard curve" is then used to determine the glucose concentration of unknown solutions. This practical activity can be linked to the testing of urine/blood for glucose and the diagnosis and management of diabetes mellitus.

HEALTH & SAFETY

Click here to access a model risk assessment for the materials involved in this activity. This may require adaptation for your own learners and setting.

Significant Hazards	**Who might be harmed? What action is needed?**
1 mol/L sulfuric acid At 1M, sulfuric acid may cause skin or respiratory irritation.	Technicians, Teachers or Learners could be harmed. For learners: Wear eye protection. The laboratory should be well ventilated. For technicians / teachers: See full risk assessment document above.
0.4 g/L potassium permanganate. Harmful if swallowed. Strong skin and eye irritant. Dust harmful to lungs. Hazardous to aquatic life.	Technicians could be harmed during preparation. Technicians should wear eye protection and rubber / plastic glves during preparation, as outlined in the full risk assessment. For teachers and learners, the concentration of potassium permanganate used in the experiment poses no significant hazards.

MATERIALS & METHOD

AIM

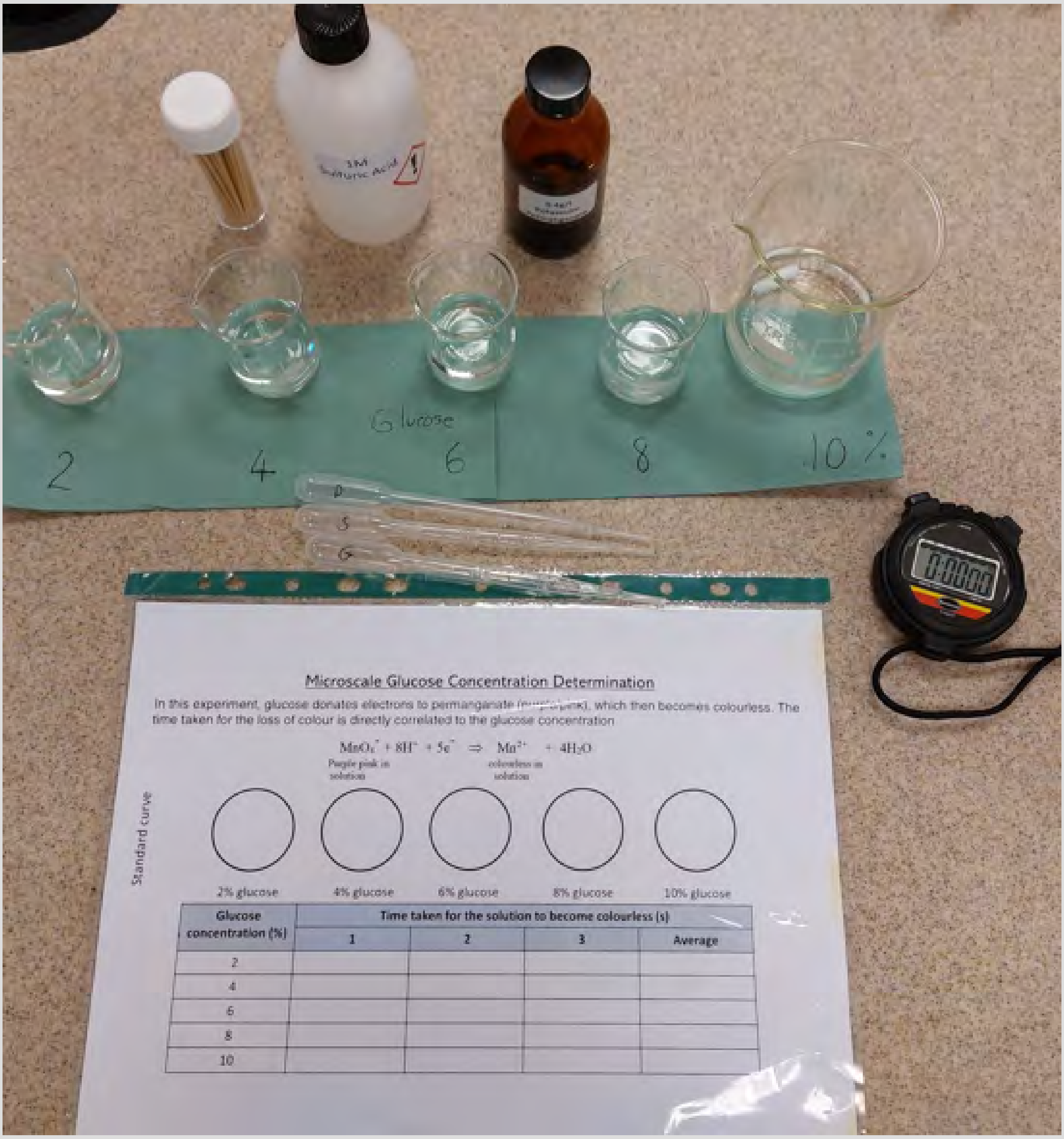
To determine the concentration of glucose in unknown samples.

SKILL FOCUS

Producing a standard curve that can be used to determine the concentration of glucose in an "unknown" sample.

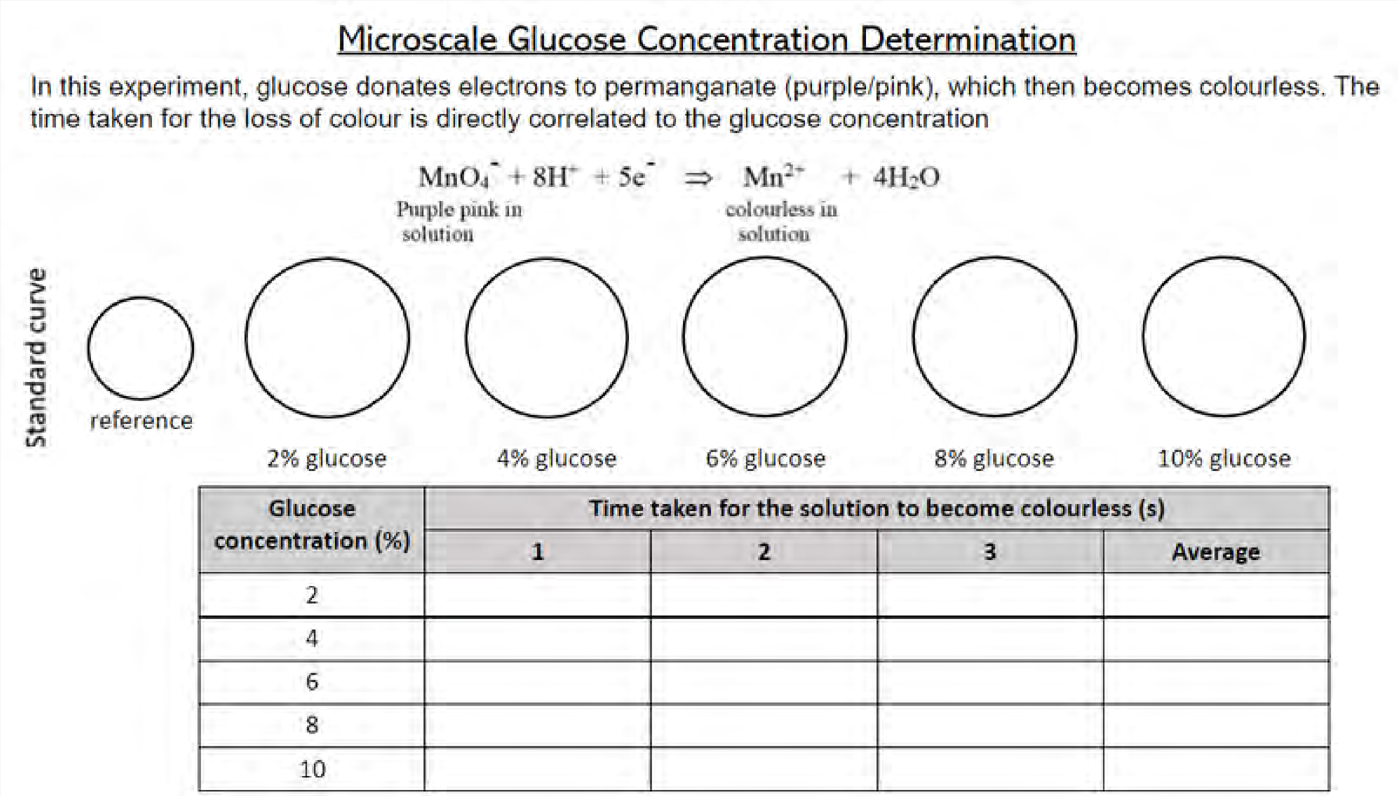
MATERIALS REQUIRED (PER PAIR)

- 3 x $1cm^3$ plastic pipettes
- beaker of water to rinse pipettes
- $20cm^3$ standard glucose solutions (2%, 4%, 6%, 8% and 10% glucose)
- $10cm^3$ $1mol\ dm^3$ sulfuric acid
- $10cm^3$ $0.4g\ dm^3$ potassium permanganate
- stopwatch
- cocktail sticks
- activity board
- $20cm^3$ test glucose solutions ("unknowns")



STEP 1 - THE STANDARD CURVE

Add a few drops of water to the "reference" circle on the activity board. This will allow a quick and easy comparison of a colourless solution (i.e. the end-point of the assay).



Microscale Glucose Concentration Determination

In this experiment, glucose donates electrons to permanganate (purple/pink), which then becomes colourless. The time taken for the loss of colour is directly correlated to the glucose concentration

$$MnO_4^- + 8H^+ + 5e^- \Rightarrow Mn^{2+} + 4H_2O$$

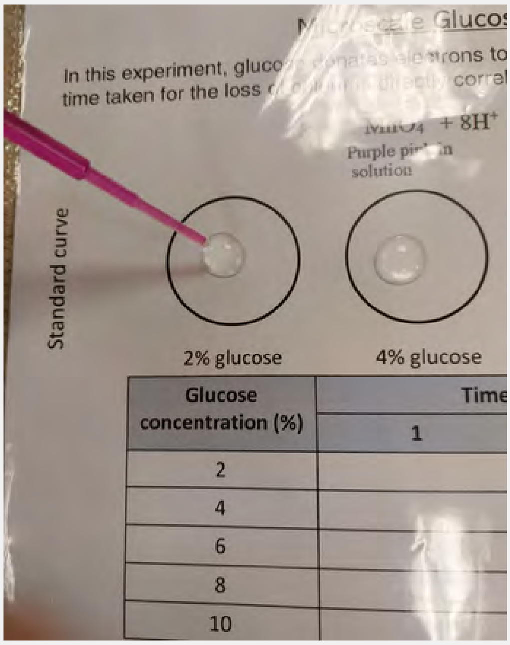
Purple pink in solution — colourless in solution

Glucose concentration (%)	Time taken for the solution to become colourless (s)			
	1	2	3	Average
2				
4				
6				
8				
10				

STEP 2

To the "Standard Curve" activity board, add:

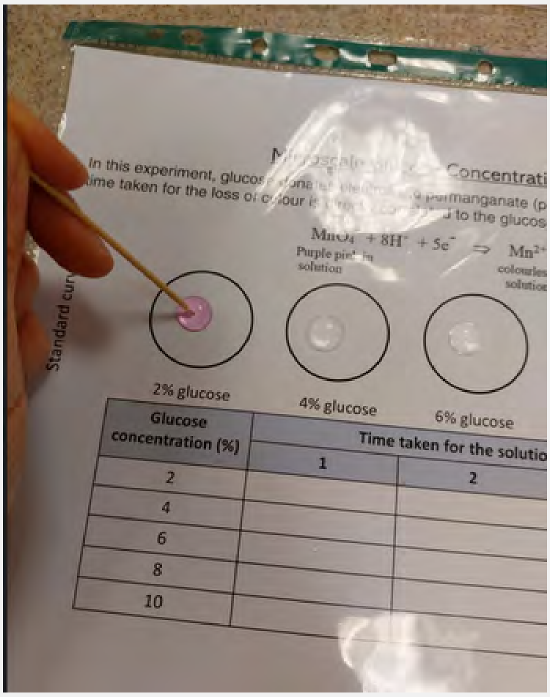
- **6 drops of glucose solutio**n (the appropriate concentration to each reaction circle).
- **3 drops sulfuric acid** to each reaction circle.

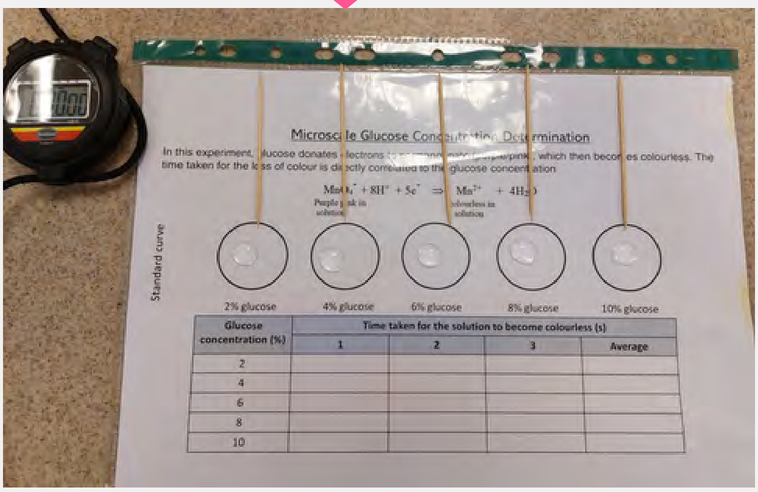


STEP 3

To initiate the reaction, add 1 drop of potassium permanganate to each reaction circle and mix using a cocktail stick.

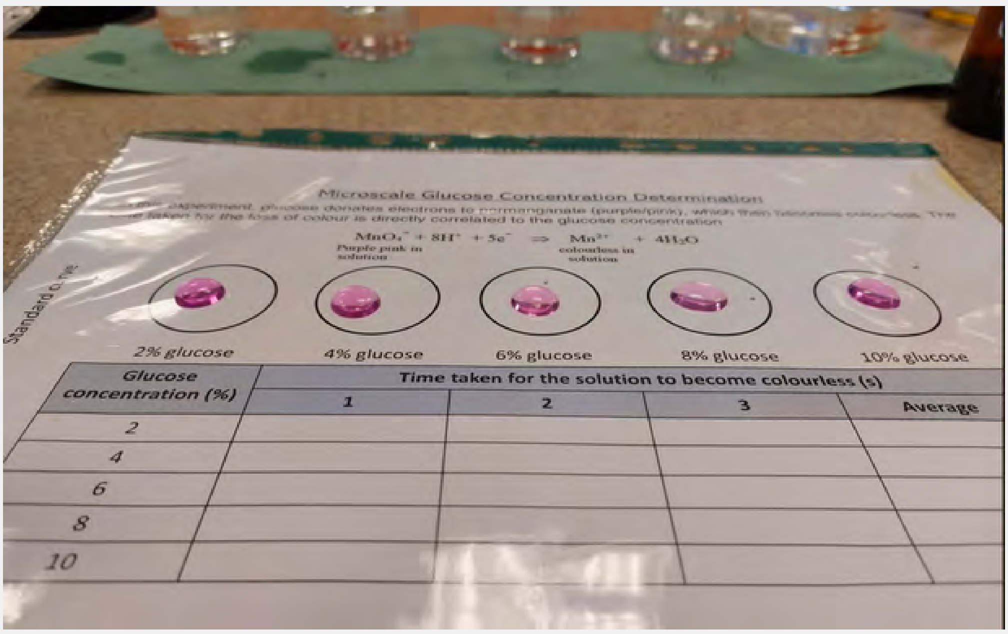
Use a new cocktail stick for each mix to prevent cross-contamination).





STEP 4

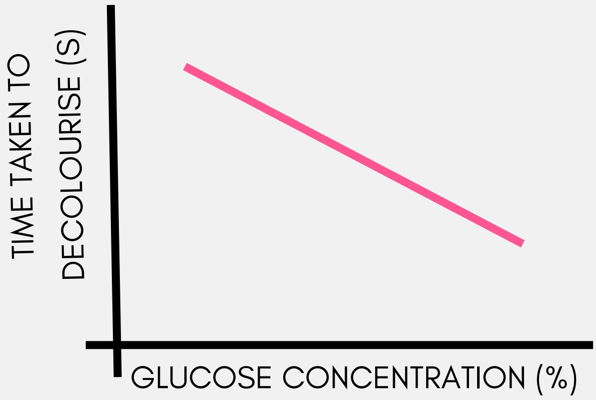
Record the time required for the pink colour of permanganate to disappear.



STEP 5

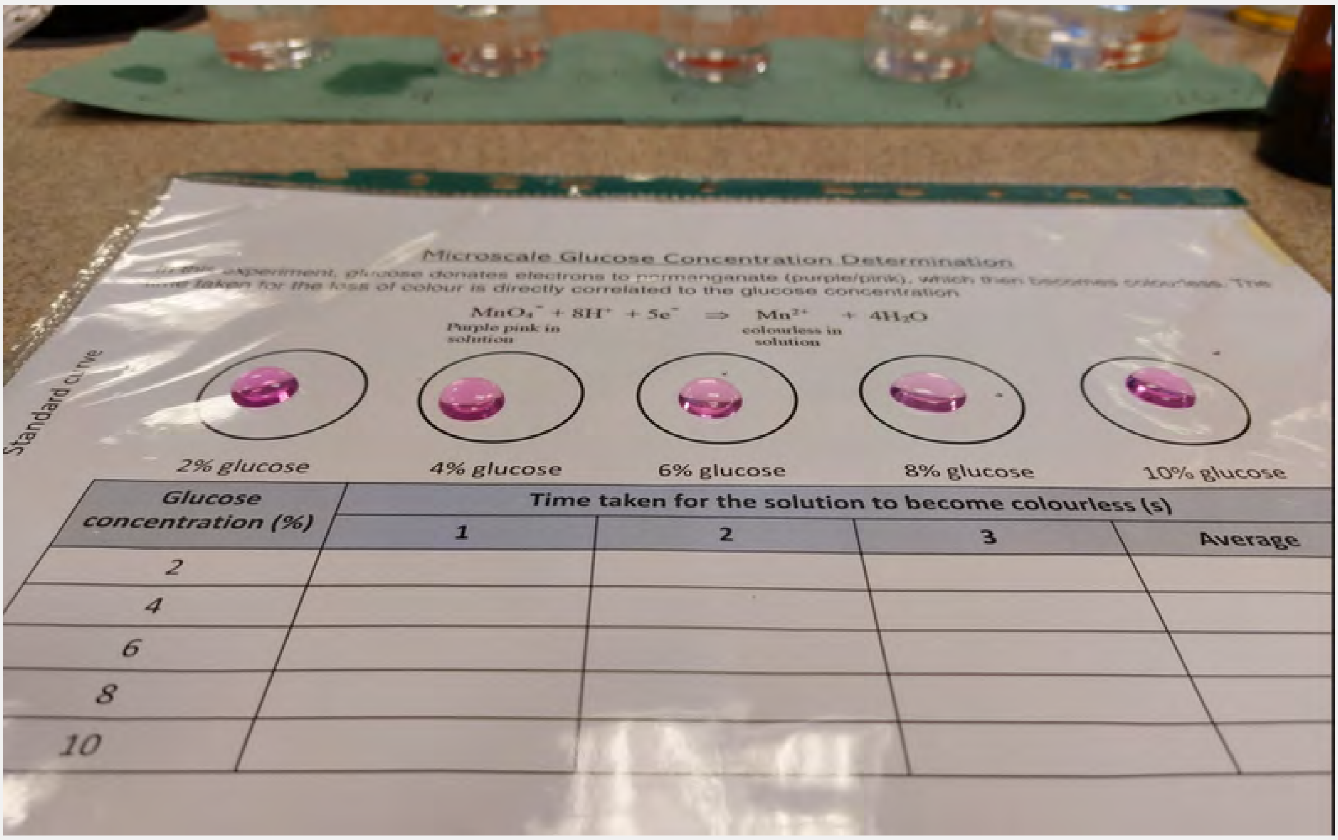
Construct of standard curve (glucose concentration versus time taken to decolourise).

The general trend: as the glucose concentration increases, the time taken to decolourise will decrease.



Glucose concentration (%)	Time taken for permanganate to decolourise (s)
2	500
4	360
6	279
8	184
10	128

Example data set that would be expected for this range of standard glucose concentrations.



STEP 6 - TESTING THE UNKNOWN SOLUTIONS

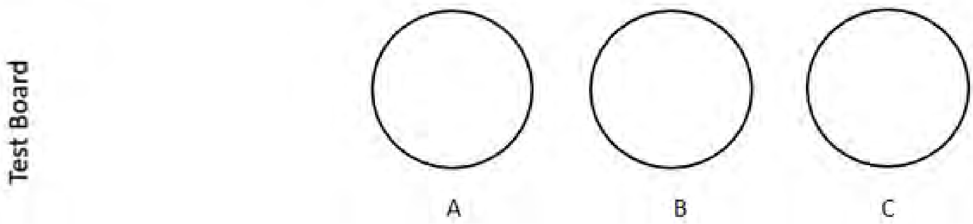
To the "Test Board", add:

- **6 drops of unknown solution** (the appropriate solution to each reaction circle, e.g. test sample A, B and C).

- **3 drops sulfuric acid** to each reaction circle.

Microscale Glucose Concentration Determination

Now you have performed the experiment with solutions of known glucose concentration, try out a series of solutions (A, B and C) of unknown glucose concentration. Can you work out their glucose concentration from your standard curve?



Solution	Time taken for the solution to become colourless (s)				Estimated glucose concentration (%)
	1	2	3	Average	
A					
B					
C					

STEP 7

To initiate the reaction, add 1 drop of potassium permanganate to each reaction circle and mix using a cocktail stick.

STEP 8

Record the time required for the pink colour of permanganate to disappear.

STEP 9

Use the standard curve to determine the glucose concentration of the unknown.