



General Certificate of Education
Advanced Level Examination
June 2011

Biology

BIO6T/P11/TN

Unit 6T A2 Investigative Skills Assignment

Teachers' Notes

Confidential

A copy should be given immediately to the teacher(s) responsible for
GCE Biology

Teachers' Notes**CONFIDENTIAL**

These notes must be read in conjunction with *Instructions for the Administration of the Investigative Skills Assignment: GCE Biology* published on the AQA Website.

The effect of ammonium hydroxide on the time taken for chloroplasts to decolourise DCPIP

Candidates will use a chloroplast suspension and the blue dye DCPIP to investigate electron transfer during the light-dependent reaction and collect raw data. In addition, they will investigate whether the rate of electron transfer is affected by ammonium hydroxide. Ammonium hydroxide is used as a readily available substance to mimic the effects of weed killer. The investigation will include a simple demonstration of the effect of ammonium hydroxide. However, because of the potential for variability with chloroplast suspensions, the time required with ammonium hydroxide could make this investigation unmanageable. To avoid this problem, data are provided for this part of the investigation only. A statistical analysis will therefore require both the data collected by the candidate individually and the data provided for all candidates.

In essence, this is a study of the Hill reaction but candidates are not expected to be aware of this, or have any knowledge of substances that might affect electron transfer.

Materials

In addition to access to general laboratory equipment, each candidate needs

- 8 spinach leaves
- measuring cylinder (50 cm³ or 100 cm³)
- access to a blender (or liquidiser)
- muslin or suitable material, such as, J-cloths for filtering (enough to make 3 layers)
- filter funnel
- 1 beaker to hold torn spinach leaves and isolation medium / chloroplast suspension (beaker 1)
- 1 large beaker (500 cm³ or 400 cm³) to hold small beaker, ice and 5 test tubes (beaker 2)
- 1 small beaker (100 cm³) (beaker 3)
- ice to half fill large beaker
- 9 test tubes (or 5 if you ask candidates to rinse out and reuse their tube X)
- test tube rack
- 60 cm³ cold isolation medium (see **Technical Information**)
- 60 cm³ cold DCPIP solution (see **Technical Information**)
- 10 cm³ cold ammonium hydroxide solution (1.5 mol dm⁻³)
- 15 cm³ cold distilled water
- 2 x 5 cm³ syringes
- 4 x 1 cm³ syringes
- piece of aluminium foil large enough to completely wrap one test tube
- lamp (bulb wattage is not crucial)
- marker pen
- timer

Managing the investigation

The spinach leaves should be left in the light for a few hours before the investigation begins, but do not allow the leaves to become hot.

Candidates will require access to a blender, but only for a few minutes each. If there is a limited supply, the centre should control the use of the apparatus and communication between candidates at such a time. If a blender is shared, it should be rinsed out with isolation medium after each use. You should ensure that sufficient isolation medium is available for rinsing. You could choose to ask candidates to do the rinsing after their turn. 50 cm³ was found to be a large enough volume to cover the blades of the blender used in the trial and should be sufficient for most blenders. The volume could be increased (but not decreased) if necessary.

Alternatively, to save time, candidates could take their beaker **1** to the teacher or a member of the technical team, who would blend the contents of the beaker and return the blended mixture to the candidate. Candidates do not have to use the blender themselves.

Both the isolation medium and DCPIP solution should be prepared beforehand using the phosphate buffer solution as detailed in **Technical Information**. The centre should produce appropriate volumes as required for its number of candidates. All solutions, and the supply of distilled water, should be kept in a cool place or refrigerated until the investigation is about to begin. The muslin or other material provided for filtering (J-cloth is a suitable substitute) must be large enough to cover the top of the filter funnel and be gathered up to allow squeezing of the contents. In the trials, three layers of muslin were used. There is no need for centrifugation at any stage in this method.

This investigation was successfully trialled using both spinach and baby spinach leaves obtained from a supermarket. Typically it took 1 to 2 minutes for DCPIP to decolourise in the presence of the chloroplast suspension. If necessary, the DCPIP can be diluted to half strength to ensure the colour change does not take more than 3 minutes. When ammonium hydroxide was also present, the colour change took longer. In some trials this was about 3 minutes but this varied considerably with different chloroplast suspensions. This explains why data will be supplied for this part of the investigation.

The materials supplied allow candidates to collect 5 sets of data which will be considered sufficient for a statistical analysis in this investigation. This is to reduce the time required to collect data.

Technical Information

Phosphate buffer solution (per 500 cm³)

Dissolve 4.48 g Na₂HPO₄·12H₂O and 1.7 g KH₂PO₄ in 500 cm³ distilled water.
Keep cool until required.

Isolation medium (per 250 cm³)

Dissolve 34.23 g sucrose and 0.19 g KCl in phosphate buffer solution and make up to 250 cm³ with phosphate buffer solution. Keep cool until required.

DCPIP solution (per 250 cm³)

Dissolve 0.01 g DCPIP and 0.93 g KCl in phosphate buffer solution and make up to 250 cm³ with phosphate buffer solution. Keep cool until required.

Ammonium hydroxide solution (1.5 mol dm⁻³)

Keep cool until required.

Candidates **must not** be given information about an ISA assessment until one week before Stage 1. One week before sitting Stage 1 of the ISA, teachers may give candidates the following information.

The investigation will be about photosynthesis.

In addition you will also need to understand the following topics

- cell organelles
- factors affecting crop yield.

There **must be** no further discussion and candidates **must not** be provided with any further resources to prepare for the assessment.

In this investigation, teachers must not give candidates the following information

- whether an end point is reached or not.

Teachers should

- show candidates how to use the blender and rinse it, if this is required.
- show candidates how the muslin or material chosen for filtering is layered or folded, placed over the top of a filter funnel and then squeezed to obtain the chloroplast suspension
- remind candidates that they only have to perform the experiment with tube **Y** once
- tell candidates that they must use the data supplied for the experiment with ammonium hydroxide in their statistical analysis.