Investigations with *ELISA*

using the SAPS *ELISA* kit for *Botrytis*

Technical Guide

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Note on units

The standard unit used by scientists for measuring a very small volume of solution is the microlitre (μl). For consistency, and to avoid confusing users of this kit, we have therefore used microlitres (μl) and millilitres (ml) in this publication and in the *Student Guide*.

1000 μl = 1 ml (= 1 cm^3)
1000 ml = 1 litre (= 1 dm^3)

Safety and liability

The materials including antibodies in this kit are supplied 'as is' and neither SAPS, SASA nor the University of Oxford give any warranty as to their fitness for purpose, quality or otherwise.

The attention of teachers and technicians in schools and colleges using the kit is drawn to the General Guidance on Safety (page 4 of this Technical Guide*) and to the Safety Notice and safety information on pages 4, 10 and 11. Students, teachers and technicians should ensure that the employer's risk assessment has been carried out before attempting any practical work.*

Copyright

For further information and a HELPLINE contact:

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Further information about SAPS can be found at: http://www-saps.plantsci.cam.ac.uk or at Homerton College, Cambridge CB2 2PH. Tel: 01223 507168.

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Mrs Leslie Wright, Arthur Mellows Village College, Peterborough, Cambridgeshire

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General guidance on safety

(See also Safety Information on pages 4, 10 and 11)

Your attention is drawn to the following advice regarding safety adapted, with permission, from the *School Science Review*.

For all practical procedures used in this kit, we have attempted to check that:

- all recognised hazards have been identified
- appropriate precautions are suggested
- where possible the proposed procedures are in accordance with commonly adopted model risk assessments
- if a special risk assessment is likely to be necessary this has been pointed out.

Teachers should, however, be aware that errors and omissions can be made, and that different employers adopt different standards. Before doing any practical activities, teachers should, therefore, always check their employer's own risk assessment. In particular, any local rules issued by their employer MUST be obeyed, whatever is recommended here.

It is assumed (unless the content dictates otherwise) that:

- practical work is conducted in a properly equipped and maintained laboratory
- any mains-operated equipment is properly maintained
- care is taken with normal laboratory operations
- good laboratory practice is observed when chemicals or living organisms are handled
- eye protection is worn whenever the risk assessment requires it
- students are taught safe techniques for such activities as heating chemicals or smelling them, and for handling microorganisms.

Readers requiring detailed guidance are referred to the following publications.

Hazcards (CLEAPSS, 1995 or 1998 update)
Topics in safety, 2nd edition (ASE, 1988)
Safeguards in the school laboratory, 10th edition (ASE, 1996)
Safety in science education (DFEE, 1996)
Preparing COSHH risk assessments for project work in schools (SSERC, 1991)
Hazardous chemicals: a manual for science education (SSERC, 1998)

For further advice on safety, you may wish to contact the following.

ASE, College Lane, Hatfield AL10 9AA, tel: 01707 267411
CLEAPSS, Brunel University, Uxbridge UB8 3PH, tel: 01895 251496
SSERC, St Mary's Building, 23 Holyrood Road, Edinburgh EH8 8AE, tel: 0131 558 8180

Introduction

This *Technical Guide* is part of the SAPS *ELISA* kit for *Botrytis*. It should be read in conjunction with the *Student Guide* which has been written to accompany the kit. The *Student Guide* provides a detailed practical protocol for using the materials and equipment in the kit, together with relevant background material to help students to gain an understanding of the theoretical aspects of the topic and some suggestions for further reading. The *Technical Guide* is intended for teachers and technicians and provides the necessary information for

- preparing for the practical sessions
- suggestions for further investigative work which could be undertaken by students
- useful references and other resources relevant to the immunological techniques described

The two booklets complement each other and both are required to give the complete supporting material for the SAPS *ELISA* kit. From time to time, the *Technical Guide* may be revised and information about the updated versions will be available on the SAPS website at:

http://www.saps.org.uk

or from the *HELPLINE (tel: 0131 244 8867, fax: 0131 244 8940, email: craig.douglas@sasa.gsi.gov.uk)*

Watch the website for further information about using the kit and carrying out investigations with it. Details and suggestions may be posted on the website from time to time, before a revised version of the *Technical Guide* is produced.

Before using the kit or carrying out any practical work, students, teachers and technicians in schools and colleges are advised to read the Safety Notice below and to ensure that the employer's risk assessment has been carried out. Further safety guidance is given on pages 10 and 11 in this *Technical Guide*.

SAFETY NOTICE

- All work done by students must be supervised
- Observe good laboratory practice
- Wipe up spills. Wash skin if splashed
- If you are asthmatic, wear a face mask because fungal spores may be released into the air during the experiment
- If you have sensitive skin, wear gloves throughout the procedure

Take care not to contaminate reagents – use a clean pipette or pastette for each solution. It may be useful to label these to prevent cross contamination.

You must take particular care with the TMB (tetramethylbenzidine) liquid substrate solution which contains TMB (harmful), methanol (toxic) and dimethyl sulphoxide (irritant). You should therefore avoid contact with skin and eyes.

You may dispose of the very small volumes of TMB by washing it down the sink with excess water.

⏩ The *ELISA* system – some further background information

The *Student Guide* provides relevant background information about the *E*nzyme-*L*inked *I*mmuno*S*orbent *A*ssay (*ELISA*) as an immunological technique, with an indication of how it is used in medicine and scientific research. The SAPS *ELISA* kit uses the plate-trapped *ELISA* system. It has been developed so that it can give meaningful results in a relatively short time-scale, say within a 2 hour practical session. In some investigational work, it may be desirable to allow longer times to obtain the results. This *ELISA* kit has been developed with the monoclonal antibody BC-12.CA4, which detects any species of *Botrytis*, including *Botrytis cinerea*. Antigens are obtained by taking washings from the fungal culture, or extracts from other source material to be tested. Investigations with the kit could be extended by using other monoclonal antibodies, which detect different fungal species. The second antibody is a commercial antibody, raised in goats, and it binds to the Fc region of murine (mouse) antibodies. A horseradish peroxidase enzyme has been conjugated to this goat anti-mouse antibody. Similarly other second antibodies could be used, some requiring different substrates. Some suggestions relating to this are given in the section *Extensions for the kit*.

All *ELISA* tests are conducted in polystyrene microtitre wells to which either the antigen or an antibody is passively adsorbed. There are three main *ELISA* formats. In the **plate-trapped antigen *ELISA*** (PTA-*ELISA*), the soluble antigen or target microorganism is incubated in microtitre wells at room temperature in an appropriate buffer. The antigen solution is then washed out using a saline buffer. These wells are incubated first with the specific monoclonal antibody, and then with the second antibody-enzyme conjugate followed by the chromogenic enzyme substrate, with saline washes containing *Tween 20* between each incubation step. The intensity of the colour which develops is determined. In research and commercial laboratories, absorbance values can be read using an *ELISA* plate reader with a filter of appropriate wavelength. Alternatively, estimates can be made using a colour chart, as supplied with the SAPS *ELISA* kit. In 'direct assays', the enzyme is conjugated directly to the primary antibody, whereas in 'indirect assays', a second antibody is used and this is generally a commercial antibody raised in another animal species, that recognises and binds to the primary antibody. Alkaline phosphatase and horseradish peroxidase are commonly used as the reporter enzymes.

In the **double antibody sandwich-*ELISA*** (DAS-*ELISA*), the antigen is captured between two antibodies. The test samples are incubated in microtitre wells, pre-coated with one antibody and then incubated with a second antibody that may or may not be directly conjugated to a reporter enzyme. The two antibodies forming the sandwich are usually different - one being a monoclonal antibody and the other a polyclonal antibody.

Competition *ELISA*s (c-*ELISA*) are commonly used for the detection of small molecules, such as pesticides and mycotoxins, and for assays requiring high levels of sensitivity. Test samples are pre-incubated with a very dilute solution of the specific antibody before exposure to antigen-coated wells. If the test sample contains the suspect antigen, it blocks all the antibody binding sites and prevents any antibody binding to the antigen on the pre-coated wells. However, if the test sample does not contain the homologous antigen, it does not block the antibody binding sites and, therefore, the antibody is free to bind to the antigen on the coated wells. Thus, at the end of a c-*ELISA*, *presence* of colour indicates a negative result and *absence* of colour is a positive result. In these assays, the amount of antigen needed to inhibit 50% binding of the antibody is usually determined and denoted as the I_{50} value.

The binding site on the monoclonal antibody, involved in the recognition of the *Botrytis* antigen, is almost certainly a carbohydrate that may or may not be part of a glycoprotein. Binding is inhibited by rhamnose and arabinose, indicating that the antigen contains rhamnose and arabinose. Research is currently being undertaken to determine more

information about the binding site.

For further information on the *ELISA* technique, preparation of antibodies and *Botrytis* infections, see the *Student Guide*.

⏩ Using *ELISA* in teaching

As stated in the *Student Guide*, use of a plant pathogen in the kit avoids most of the restrictions associated with work with human pathogens. However, the principles explored are the same. They illustrate the important use of monoclonal antibodies in diagnostics in medicine and veterinary medicine as well as for plant diseases.

In teaching, practical work with the *ELISA* techniques provides useful support for students to gain an understanding of topics relating to monoclonal antibodies in human health and disease as well as its application to an understanding of plant diseases.

The SAPS *ELISA* kit is appropriate for use with post-16 students of biology and biotechnology, including those on courses in Advanced Subsidiary (AS) and Advanced (A) GCE, Scottish Higher Still and equivalent vocational courses. It could also be useful to illustrate diagnostic immunological techniques for students in further and higher education. Once the basic procedure has been practised, the technique can then be used in a range of individual investigations or projects. Some suggestions for investigations are given in the *Student Guide*, with further ideas in this *Technical Guide* (on page12).

The section 'Teaching and topic links' (pages 14 to 16 of this *Technical Guide*) refers to specifications and syllabuses offered by the awarding bodies in England, Wales and Northern Ireland, and in Scotland. This section outlines areas of content relevant to and which can be linked with this *ELISA* kit.

In addition, practical work carried out with the *ELISA* kit offers opportunities for development of **Key Skills** (England and Wales). Some suggestions for activities that could provide evidence of achievement in Communication, Information Technology, Working with Others and Problem Solving are given on page 16 of this *Technical Guide*.

Kit components and preparation for the practical work

⏩ Kit components

In each kit, the quantities of equipment and other materials provided together with the instructions given are appropriate for about 10 students if they work together in pairs.

For satisfactory results, fresh solutions and cultures are needed as well as chemically clean plastic or glassware and other equipment. Individual replacement items are not available separately.

Checklist of equipment and materials supplied in the kit

The items marked with an asterisk (*) should be stored in a refrigerator (but not frozen) as soon as you receive them. This will give them a shelf life of 6 to 8 weeks.

6 x cultures of the fungus *Botrytis cinerea* (in tubes on agar slope) *
1 x 4 ml[1] first antibody, a monoclonal antibody (MAb) – BC-12.CA4 *
5 x 10 µl[1] second antibody, a commercial polyclonal antibody, conjugated to a horseradish peroxidase enzyme (Ab-EC). *
(These may look empty, but the antibody is there!)

1 x 0.5 ml *Tween 20* in small plastic tube
3 tablets of PBS (phosphate buffered saline)
1 x TMB (tetramethylbenzidine) – substrate for the enzyme in brown dropper bottle *
(*see SAFETY NOTICE on page 4 and further safety information on page 10*)

1 x sterile tube - labelled 'uninoculated'
1 x sterile tube - labelled 'inoculated'
5 x large disposable plastic tubes (~30 ml) - for the PBST (see page 8)
20 x small disposable plastic tubes (~ 6 ml) - 5 for the PBS, 5 for the fruit sample, 5 for the fruit filtrates, 5 for the fungal filtrates
15 x 1 ml sterile pipettes
25 x pastettes (deliver 20 µl droplets)
8 x microstrips (each strip has 4 microwells)
10 x microcups - 5 labelled MAb (monoclonal antibody), 5 labelled Ab-EC (antibody-enzyme conjugate)
10 x muslin squares for filtering the fruit and fungal extracts
1 x colour chart (for quantitative estimations - see page 9)

5 x *Student Guide*
1 x *Technical Guide*

Additional items required (not supplied with kit)

paper towels
beakers (for waste)
marker pens
glass rods
small volume of sterile water
face masks (for asthmatic students)
forceps
gloves
frozen raspberries

Rack to hold equipment ('blu tac' or something similar can be used to hold small items of equipment on the bench)

[1] There are 1000 µl in one millilitre (ml). See Note on units on page 1.

*** These items should be stored in a refrigerator (but not frozen) as soon as you receive them.** *(See further information on pages 8 and 9.)*

⏩ Preparation of raspberries

Six raspberries should be removed from the freezer two days prior to carrying out the experiment. Fresh raspberries can be used if available.

Three of the raspberries are enclosed in the sterile tube labelled 'uninoculated' and left undisturbed at room temperature.

Three are treated as follows and then enclosed in the sterile tube labelled 'inoculated': Use a 1 ml pipette from the kit to add 1 ml sterile water to a *Botrytis* culture. Use the tip of the pipette to rub the surface of the culture gently to dislodge the spores. Then use the same pipette to inoculate the raspberries by putting this spore suspension onto the surface of the fruit. Seal the container and incubate at room temperature for two days.

When preparing the fruit filtrate (see 'Practical Procedures, *Student Guide*, page 6, step 3) do ***NOT*** use more than half a raspberry. If the fruit extract is too concentrated, plant molecules from the fruit bind preferentially to the walls of the wells, thus reducing the amount of *Botrytis* antigen that can become bound. This results in lower levels of binding to the antibodies and therefore lower colour intensities (i.e. paler blue in the wells) at the final stage. Thus the assay does not then reflect the true intensity of *Botrytis* infection.

⏩ Preparation of solutions

It is recommended that the chemicals are stored in the refrigerator and are not made up into solution until required. Allow solutions to reach room temperature before use.

PBS (phosphate buffered saline)

Dissolve each tablet in 200 ml of distilled or clean tap water. Dispense 5 ml to each of 5 clean small plastic tubes, labelled PBS. Provide 1 tube per pair of students. Use the rest to prepare PBST (*see PBST, below*).

PBST (phosphate buffered saline with *Tween 20*)

To make up PBST, use a 1 ml pipette from the kit to add 2 drops of *Tween* to the remaining volume (175 or 200 ml) of PBS (*see note above for PBS*). Transfer as required to the 30 ml large tubes, labelled PBST and provide 1 tube per pair of students. Replenish as necessary.

Tween is a detergent. It reduces the likelihood of nonspecific binding of antibodies and antigens to each other and to the plastic of the microwells.

TMB (tetramethylbenzidine) – substrate for the enzyme
(*see SAFETY NOTICE on page 4 and safety information on pages 10 and 11*)
6 ml is provided in dark brown dropper bottle. TMB is light sensitive. It is ready for use but keep it on the front bench and allow students to dispense it only under supervision. Each student adds 2 drops (80 µl) to each test well *after* removal of the second antibody and completion of washing in the final step.

TMB is the substrate for the enzyme and is converted to a blue colour by the second antibody-enzyme conjugate. *(See note on page 10 on testing for peroxidase activity.)*

Fungal culture and antibodies

N.B. All three of these require refrigeration, but do not freeze.

Culture containing *Botrytis cinerea*

This is provided in a tube, on an agar slope. Six cultures are included in the kit, which allows 1 culture for each group plus one culture to inoculate the fruit.

These cultures can be kept unopened for several weeks at 4 °C. Please check your employer's guidelines for regulations regarding maintenance of microorganisms.

The monoclonal antibody (MAb) – BC-12.CA4

4 ml is provided in a small tube, labelled 'Monoclonal antibody – BC-12.CA4'. Using a 1 ml pipette from the kit transfer 0.5 ml to each of 5 microcups, labelled appropriately. Provide 1 microcup per pair of students.

The monoclonal antibody may be pink or orange red in colour, depending on the batch. The colour should have no effect on the reaction.

The monoclonal antibody BC-12.CA4 recognises any species of *Botrytis* and can therefore be described as genus-specific. It does not recognise any other fungi, bacteria or molecules derived from plants.

Second antibody-enzyme conjugate (Ab-EC)

A very small volume (10 µl) of the concentrate is provided in each of the 5 screw capped Eppendorf tubes, labelled 'Ab-EC'. Using a 1 ml pipette from the kit, add 0.5 ml of PBST to each tube and **agitate well to mix**. Using the same 1 ml pipette, then transfer this 0.5 ml into one of the 5 appropriately labelled microcups. Repeat this procedure with the remaining 4 Eppendorf tubes as required. Provide 1 microcup per pair of students. *You should prepare enough for immediate use only.*

Warning – the container with the second antibody may look empty, but it is there!

Before use, check that the second antibody (Ab-EC) is active. To do this, use a clean pastette and mix together one drop of the *diluted* second antibody made up in PBST with one drop of TMB substrate solution. A colour reaction (blue) should occur if the enzyme is active. The TMB solution alone should be colourless. If there is no reaction, contact the *HELPLINE*.

Take great care not to cross contaminate the different solutions, TMB substrate solution is very sensitive.

The colour chart and quantitative estimate of *Botrytis*

The colour chart is provided to enable students to make quantitative estimates of the fungal antigen present in surface washings, as tested by the SAPS *ELISA* kit. The colours on the chart match those obtained when a dilution series of an extract from a known mass of freeze-dried mycelium, from a liquid culture of *Botrytis cinerea*, was tested by the same *ELISA* method. The numbers 2 to 128 on the chart are 'arbitrary *Botrytis* units', known as 'BcAg' and these represent increasing relative concentrations of the *Botrytis* antigen (and therefore of *Botrytis* in the original material). The zero (0) value is for the colourless solution, obtained with a blank, using PBS only and no *Botrytis* antigen.

Practical procedures, further advice and guidance, safety

Practical instructions

Detailed practical instructions to carry out the protocol are given in the 'Student worksheet' provided in the *Student Guide* (pages 5 to 8). An annotated flow chart, which includes diagrams of items of equipment used, is also provided. Teachers and technicians should read these notes in the *Technical Guide* in conjunction with the instructions in the *Student Guide*.

Testing plant material for peroxidase activity

Peroxidases are naturally present in some plant tissues and give a positive (blue) colour reaction with TMB. You can check the uninfected host tissue for peroxidases by adding 2 drops of TMB to an extract of the material. If a blue colour develops within 5 minutes, this indicates peroxidases are present and that the untreated material is unsuitable for direct use with this *ELISA* kit. To make it suitable you need to inactivate the peroxidase enzymes in the plant extract by boiling the extract for 5 minutes. Raspberries (fresh and frozen), strawberries and rose petals are suitable to use with the kit without the need to boil. Other material that could be used, but may need the boiling treatment, includes cucumber, tomato, lettuce, grapes and some varieties of apples.

Timing for the procedure

This practical protocol has been modified from a research protocol so that it can be undertaken with a class group within a 2 hour practical time slot. In the procedure, all the times given for the incubation steps should be considered *minimum* times.

Increased sensitivity can be achieved by extending the antibody incubation times, by up to 1 hour. For longer incubation times, e.g. overnight, keep the wells in a refrigerator. If longer incubation times are used for investigations, the times should be the same for all treatments.

Washing the wells

Make sure that the students wash the wells with PBST carefully and thoroughly at each step. This washing is important to remove unbound reagents. Inaccuracies occur if the washing is not done thoroughly. It is especially important to make sure that all the washing fluid is removed after the last washing step.

Safety information

General advice on safety in relation to the practical procedures is given on page 4 of this *Technical Guide*. Please also note the safety notice in the student worksheet in the *Student Guide*, which is repeated on page 4. You are advised to undertake your own risk assessment for the practical work being undertaken.

The notes below give additional advice regarding the use of use of TMB in the *ELISA* protocol and the disposal of material and equipment.

You should be aware of the following precautions when using TMB, the liquid substrate system. TMB (3,3' 5,5 tetramethyl-benzidine) is harmful and reported to be a *non-carcinogenic* analogue of benzidine. The liquid substrate system also contains methanol which is toxic and dimethyl sulphoxide which is an irritant.

- Care should be taken to avoid contact with skin and eyes and inhalation of fumes.
- Any student with a sensitive skin is advised to wear gloves. They use an eye dropper type of bottle to add 2 drops of the substrate to the microwells, so the risk of inhaling fumes is minimal.
- Small volumes of TMB may be washed down the sink with plenty of water. Wear gloves and safety spectacles for this operation to avoid contact with skin and eyes. This is

accepted as safe because of the small amounts of chemical in use.

Once the wells have been washed thoroughly, they may be discarded with the normal waste.

The amount of substrate used is not more than 80 µl per reaction, hence volumes of the substrate in use at any one time are very small. For example, with a maximum class size of 30, if each student has 4 wells then 320 µl x 30 students would give a total of 9.6 ml of substrate. As students often work in pairs or small groups, the total volume is reduced proportionally.

With the small volumes in use, the risk factor for students using TMB, as described in this protocol, is considered to be LOW.

Note - We consider that the hazards of TMB liquid substrate solution are no greater than those associated with many chemicals used in A level science or equivalent courses. The risks from these hazards are lower than those in much post-16 work because the quantities of TMB are very small. However, teachers and technicians should be aware that TMB (3,3',5,5 tetramethyl-benzidine) is not listed in the model (general) risk assessments commonly used in school science. Teachers and technicians should, therefore, consult their employers about the need for a special risk assessment. Appropriately affiliated schools and colleges in England and Wales may consult CLEAPSS and in Scotland they may consult SSERC.

Disposal of materials and washing up of equipment

Equipment, such as forceps, glass rods and reagent tubes, can be washed up in detergent in the usual way in the laboratory sink or equivalent. The detergent kills the spores from *Botrytis* and dilutes any reagents used. There is no need to autoclave cultures or other equipment which has been used, though teachers may do so if they wish. The risk factor with *Botrytis* is minimal (comparable with throwing away rotting vegetables or fruit from the domestic kitchen), which is one of the reasons why *Botrytis* is useful as teaching material.

Extensions for the kit – project work, ideas and tips

Some relatively straightforward suggestions have been given in the *Student Guide*, and this list provides more ideas which could be developed into projects or investigational work. In some cases it may be necessary to obtain other materials, such as a different monoclonal antibody. SAPS would welcome feedback or information relating to successful investigations, so that ideas can be shared with others using the SAPS *ELISA* kit. *You can keep in touch through the SAPS website (see page 4) or by contacting the SAPS Head Office at Homerton College, Cambridge.*

1. Heat denatures most proteins but not carbohydrates. Surface washings of the fungus could be heated by boiling for 5 minutes in a glass tube or polypropylene Eppendorf with a pierced lid suspended in boiling water before being used to coat wells. If the specific antigen recognised by BC-12.CA4 is heat stable it is, almost certainly, a carbohydrate or fungal glycoprotein.

2. Students could study the germination of spores of *B. cinerea* by making a suspension of spores in 0.5% glucose or sucrose in sterile water and then incubating them overnight at room temperature as drops on a glass slide or in microtitre wells (80 µl per well) . They could then carry out an *ELISA* with such wells using the same procedure as for wells coated with surface washings. (Germinating spores secrete antigens.)

3. Visual observations of spore structures and fungal development can be worthwhile. Testing of Koch's postulates could be undertaken to determine which of several fungi present may cause the infection symptoms.

4. There is a possibility that other monoclonal antibodies (MAbs) or second antibody-enzyme conjugates (Ab-ECs) could be obtained. For example, the MAb AF-CA2 recognises all species of *Penicillium* and *Aspergillus*. In addition, a different substrate could be used with the Ab-EC to enable students to extend the range of investigations.

References and further information

In addition to the references listed in the *Student Guide*, teachers and technicians may find the following references helpful.

Davey B (1989) *Immunology* Booklet No 5 in series entitled 'Biochemistry across the school curriculum, Guidance Notes for Advanced Biology'.
Biochemical Society (59 Portland Place, London W1N 3AJ. Tel 020 7580 5530.)

Dewey F.M., Cole L. (1996) Monoclonal antibody-based assays for detection and quantification of *Botrytis cinerea.* In *Diagnosis and Identification of Plant Pathogens*. Edited by Dene H.W., Adam G., Dickmann M., Frahm J., Manler-Machnik A. and van Halteren P. Kluver Academic, Netherlands.

Hames B.D., Hooper N.M., Houghton J.D. (1997) *Instant Notes in Biochemistry* Bios Scientific Publishers [ISBN 1-85996-265-3]

Lydyard P.M., Whelan A., Fanger M.W. (2000) *Instant Notes in Immunology* Bios Scientific Publishers [ISBN 1-85996-077-4]

Madigan M.T., Martinko J.M., Parker J. (1997) *Brock Biology of Microorganisms* Prentice Hall [ISBN 0-13-571225-4]

Laboratory Techniques in Immunology - The ELISA test. This is a 23 minute video produced by the University of Leeds in 1989.
Price £33.00 + VAT and £1.50 p&p. Order code: SELI 001V. For more information see: http://mediant.leeds.ac.uk/vtcatalogue/

Monoclonal Antibodies. This is a 24 minute video produced by Shotlist, The EBS Trust, 36-38 Mortimer Street, London W1N 7RB. Tel: 0171 765 4635, email: mail@ebstrust.u-net.com
Price: £35 + VAT.

Teaching and topic links, with Key Skills opportunities

The *ELISA* technique, as presented in the SAPS *ELISA* kit for *Botrytis,* has most relevance to post-16 students in Biology, Human Biology and Biotechnology, though it may be of interest to younger students in their study of Biology within a science course.

Within a teaching programme, key features of the *ELISA* kit can be used to illustrate

- antibody-antigen interactions
- the immune response
- applications and uses of monoclonal antibodies
- diagnostic techniques that are highly specific and widely used in research, medicine and other industries
- aspects of disease and infections caused by the plant pathogen *Botrytis cinerea*
- utilisation of enzymes as 'reporters' in biosensor systems
- use of small scale practical techniques, which are used extensively in laboratories at a higher level

In England, Wales and Northern Ireland, specifications introduced by the five awarding bodies for teaching in September 2000, conform to the QCA / ACCAC / CCEA Subject Criteria for Biology (published in 1999). In Scotland, changes made by SQA to Advanced Higher arrangements in Biology and in Biotechnology, introduced in summer 2000, are to be examined first in May 2001.

The table below summarises areas of content in these specifications in which teaching of topics could be linked to and enhanced by doing practical work with the *ELISA* kit. In some cases, particularly in the Scottish Advanced Higher specifications in Biology and in Biotechnology, suggested learning activities specify use of the *ELISA* technique or experiments with *Botrytis*. In addition, practical work with the *ELISA* kit could provide opportunities to develop and provide evidence of achievement in different Key Skills or Core Skills. Examples of possible activities appropriate for certain Key Skills requirements are also described.

Teaching topic	Awarding Body					
	AQA	Edexcel	OCR	WJEC	CCEA	SQA
Antibody-antigen interactions	✓	✓	✓	✓	✓	
Immune response	✓		✓			
Uses of monoclonal antibodies	✓		✓	✓		✓
Diagnostic techniques						✓
Aspects of disease	✓		✓	✓		
Botrytis and other plant diseases						✓
Enzymes in biosensors						
Practical techniques (*ELISA*)						✓

Specification or syllabus reference

Awarding body and specification	Relevant specification reference
AQA Biology / Biology (Human) A	**Module 2** – *Making Use of Biology*: section 11.1 - application of enzymes in biotechnological processes; section 11.5 principles of immunology **Module 3** – *Pathogens and Disease*: section 12.3 - principles of immunology; section 12.9 - monoclonal antibodies
AQA Biology B	**Module 7** (Option) – *Microbes and Disease*: section 15.12 - the immune response **Module 8** (Option) – *Behaviour and Populations*: section 15.17 - infectious disease
Edexcel Biology and Biology (Human)	**Unit 2B** – *Exchange, Transport and Reproduction*: section 2B.2 - blood and body fluids **Unit 2H** - *Exchange, Transport and Reproduction in Humans*: section 2H.2 - blood and body fluids
OCR Biology	**Module 2802** - *Human Health and Disease*: section 5.2.6 - immunity **Module 2805, component 04** – *Microbiology and Biotechnology*: section 5.8.5 - biotechnology in medicine
WJEC Biology	**Unit BI4** – *Biochemistry and Health*: section 4.7 Human defence mechanisms; section 4.8 Applications and contemporary issues
CCEA Biology	**Module 4** – *Coordination, Biochemistry and Ecosystems*: section 4.2 Immunity
SQA Biology AH	**Mandatory Unit** – *Environmental Biology* – section (*b*)3 i: Interactions between species **Optional Unit** – *Biotechnology* – section (*b*)3 ii: Monoclonal antibodies
SQA Biotechnology A	**Unit** – *Applications of biotechnological processes* – section 3i: Monoclonal antibodies

AQA (Assessment and Qualifications Alliance), Stag Hill House, Guildford, Surrey GU2 5XJ.
Tel: 01483 506506, Fax: 01483 300152. website address: www.aqa.org.uk

Edexcel, Stewart House, 32 Russell Square, London WC1B 5DN.
Tel: 020 7393 4444, Fax: 020 7393 4445. website address: www.edexcel.org.uk

OCR (Oxford Cambridge and RSA Examinations), 1 Hills Road, Cambridge CB1 2EU.
Tel: 01223 552552, Fax: 01223 552553. website address: www.ocr.org.uk

WJEC (Welsh Joint Education Committee), 245 Western Avenue, Cardiff CF5 2YX.
Tel: 029 2026 5000, Fax: 029 2057 5994. website address: www.wjec.org.uk

CCEA (Northern Ireland Council for the Curriculum Examinations and Assessment),
29 Clarendon Road, Belfast BT1 3BG.
Tel: 028 9026 1200, Fax: 028 9026 1234. website address: www.ccea.org.uk

SQA (Scottish Qualifications Authority), Hanover House, 24 Douglas Street,

Glasgow G2 7NQ
Tel: 0141 248 7900, Fax: 0141 242 2244
or at Ironmills Road, Dalkeith, Midlothian EG22 1LE.
Tel: 0131 663 6601, Fax: 0131 654 2664. website address: www.sqa.org.uk

Key Skills opportunities – *suggested activities*

The relevant Key Skill area is given in square brackets, after the suggested activity.

1. Interpret practical instructions (as presented in the *ELISA* booklet), including diagrammatic representation, on an unfamiliar topic
   [***communication** – selection and synthesis of information*]

2. Make a poster to explain, to a non-specialist, the events at a molecular level occurring in the wells when carrying out the *ELISA* technique
   [***communication** – presentation with images (of a complex subject)*
   ***information technology** – presentation of information for different purposes (including text, images, numbers)*]

3. Apply the *ELISA* technique to a practical investigation with unfamiliar material to detect or monitor the presence of *Botrytis* under different environmental conditions, and ensure that any results obtained are quantitative
   [***problem solving** – identification of the problem; production and comparison of different options in solving the problem, agree method of solution; planning and implementation of the selected method for solving the problem; evaluate the outcome*]

4. Get a group of students to make a presentation, to other students, which explores and then discusses the use of *ELISA* as a diagnostic technique, making reference to a range of different applications. The discussion can include the potential benefits of carrying out small-scale practical techniques in school or college laboratories (in post-16 studies)
   [***communication** – participation in group discussion (of a complex subject); presentation with images (of a complex subject); selection and synthesis of information (for extended documents and images)*
   ***information technology** – using different sources for information retrieval; how to exchange information to meet different purposes; presentation of information for different purposes (including text, images, numbers)*
   *working with others – **planning and participating in activities with others; agreement of targets and monitoring progress; production***]