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SAGTEVRUGTEPLANTVERBETERINGSVERENIGING
DECIDUOUS FRUIT PLANT IMPROVEMENT ASSOCIATION

DPA F-1-1

TEST LABORATORIES

(As amended on 22 November 2016)

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1. REGISTRATION OF TEST LABORATORIES

- 1.1 Test laboratories must be registered with the Department of Agriculture (DoA) and must comply with the requirements of the Plant Improvement Act, before registration at the DPA can take place.
- 1.2 PIO's must inform the DPA of the laboratories used for every test regarding pathogens, insects or viruses, as prescribed by the Scheme.
- 1.3 If a laboratory is not registered at the DPA to conduct tests regarding specific pathogens, insects or viruses, the PIO concerned, should apply for registration of such a laboratory.
- 1.4 Laboratories applying for registration, must be evaluated by the designated panel using in accordance with the instructions as set out in Section 2. The panel must submit a report to the Technical Committee and the Board for approval.
- 1.5 Tests regarding the Scheme, can only be performed by laboratories acting on behalf of a PIO, which is registered at the DPA to perform the specific tests.
- 1.6 Registration of laboratories at the Board is subjected to the equipment used and the competence of the personnel who conducts the tests at the time of registration. Any change or improvement with regards to the equipment used or knowledge of the personnel, must be reported to the Board.
- 1.7 Registered laboratories must have the necessary documented literature, to be able to identify different fungi and bacteria.
- 1.8 Registered laboratories must have the requirements for approved ELISA and PCR suppliers with regards to virus tests conducted at that facility.
- 1.9 All equipment used, must be kept in good condition and service records must be readily available regarding the calibration of analytical balances and other equipment, as part of general laboratory practice.
- 1.10 All registered laboratories must be audited by DoA for the prescribed ELISA tests.

2. DIAGNOSTIC TEST EQUIPMENT REQUIREMENTS

To register a laboratory must have the following diagnostic testing equipment with regard to viruses, pathogens and insects:

2.1. Viruses

2.1.1 ELISA tests

- pH meter
- Scale (0-300g; 2 decimal)
- Incubator (37°C)
- Electronic ELISA-plate reader (405 nm)

2.2. Fungi

- Laminar flow cabinet
- Autoclave
- Scale (0-300g; 2 decimal)
- pH meter
- Growth-chamber or incubator for isolations (23 – 25°C)
- Stereo microscope (40X)
- Light microscope (1000X)
- Latest published ID keys for the relevant organisms, must be available in the laboratory.

2.3. Bacteria

- Laminar flow cabinet
- Autoclave
- Scale (0-300g; 2 decimal)
- pH meter
- Growth-chamber or incubator for isolations (23 – 25°C)
- Light microscope (100X)
- Latest published ID keys for the relevant organisms, must be available in the laboratory.

2.4 Nematodes

- Sieves:
 - Stack of three stainless steel or copper sieves
 - 4mm sieves
 - 90µm polyethylene-supporting sieves
- Stereo microscope (16X, 25X)
- Light microscope
- Temperature controlled oven. (40°C)
- Desiccator
- Reference plates for copies.

2.5 Insects

- Stereo microscope (40X)
- Latest published ID keys for the relevant organisms, must be available in the laboratory.

3. PLANT IMPROVEMENT REQUIREMENTS REGARDING VIRUS ELIMINATION

3.1. Heat therapy and Tissue culture

- Heat therapy-unit (40°C)
- Laminar flow cabinet
- Autoclave
- Scale (0-300g;)
- pH meter
- Growth-chamber or incubator (23 – 25°C)
- Stereo microscope (40X)

3.2. Somatic embryogenesis and Tissue culture

- Laminar flow cabinet
- Autoclave
- Scale (0-300g; 2 decimal)
- pH meter
- Growth-chamber or incubator (23 – 25°C)

3.3. Glasshouse

- Nucleus units must comply with requirements according to the scheme.

4. ELISA TESTS

4.1 Introduction

ELISA is an acronym for “Enzyme Linked Immuno-Sorbent Assay.” It is the most commonly used serological test for the detection of plant viruses.

There are two types of ELISA tests generally employed:

1. DAS ELISA (Double Antibody Sandwich)
2. Indirect ELISA test

4.2 DAS ELISA method

This method is used for tracing most plant viruses and may be summarised as follows:

- (a) Pure IgG against a specific virus is captured on the surface of an ELISA slide.
- (b) Homogenous virus particles are bound by the IgG, while non-specific particles are washed away.
- (c) E-IgG (enzyme conjugate) is added and attaches to the bound virus particles. Excess E-IgG is washed away.

- (d) An enzyme substrate is added to trace the bound E-IgG and produce a yellow colour reaction.

4.3 Indirect ELISA method

The indirect ELISA method does not vary much from the DAS ELISA method, except that two antibody incubations follow after the addition of the plant sap.

- (a) Virus antibody A is added to the slide and binds on the surface area.
- (b) Plant sap is added.
- (c) Addition of virus antibody B.
- (d) Anti-B globulin is added ('goat anti-rabbit IgG').
- (e) Addition of enzyme substrate.

4.4. General ELISA protocol

- (a) Protocol as prescribed by the supplier must be followed.
- (b) In the case of nucleus plant material, tests must be performed in duplicate.
- (c) In the case of foundation and mother plant material single tests can be performed, provided that slides and reagents are preheated to incubation temperature as prescribed by the supplier.

[as amended 25 March 2009]

4.5 ELISA for non-quarantine viruses

4.5.1 APPLES

VIRUS	ABBREVIATION	SUPPLIER
Apple chlorotic leafspot trichovirus	ACLSV	Bioreba
Apple stem grooving capillovirus	ASGV	Bioreba
Apple mosaic ilarvirus	ApMV	Bioreba

4.5.2 PEARS

VIRUS	ABBREVIATION	SUPPLIER
Apple chlorotic leafspot trichovirus	ACLSV	Bioreba
Apple stem grooving capillovirus	ASGV	Bioreba

4.5.3 CYDONIA

VIRUS	ABBREVIATION	SUPPLIER
Apple chlorotic leafspot trichovirus	ACLSV	Bioreba
Apple stem grooving capillovirus	ASGV	Bioreba

4.5.4 STONE FRUIT

VIRUS	ABBREVIATION	SUPPLIER
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Apple chlorotic leafspot trichovirus	ACLSV	Bioreba
Apple mosaic ilavirus	ApMV	Bioreba
Prune dwarf ilarvirus	PDV	Bioreba
Prunus necrotic ringspot ilarvirus	PNRSV	Bioreba

5 PCR TESTS

5.1 Introduction

The polymerase chain reaction (PCR) is a technique that enables the specific amplification and hence detection of target DNA sequences from complex mixtures of nucleic acid. A combination of short, specific primers and thermostable DNA polymerases are used to amplify the target sequence, through repeated cycles of denaturation, reannealing, and DNA synthesis at high temperatures, allowing an exponential increase in the amount of the DNA of interest. By addition of a reverse-transcription (RT) step, PCR can also be applied to cDNA generated from RNA templates. Its extreme sensitivity and high specificity make it an unparalleled technique for the detection and characterization of rare messages, including viral infections that are difficult to detect and diagnose by serology or electron microscopy.

5.2 RT-PCR

The method is used for the detection of most plant viruses and can be summarized as follows:

- a) Appropriate plant samples are taken from which RNA is purified. Various methods are available for this step and can be determined by the service provider.
- b) RNA is subjected to quality control, and high-quality RNA is used for the diagnostic test.
- c) Primers are designed to bind to the genomes of specific viruses or virus groups. The distance (in base pairs) between primers determines the size of the amplification product (amplicon).
- d) RNA is converted into complementary or copy DNA (cDNA) in a reverse transcription (RT) reaction.
- e) The cDNA is then amplified using primers in a polymerase chain reaction (PCR) if the virus is present in the sample.
- f) The product of a positive RT-PCR can be determined in two ways:
 - i. The amplified DNA can be separated on an agarose gel under electric current and then appears as a band on the gel, the size of which can be determined if it is stained (EtBr) and exposed to UV light.

- ii. The RT-PCR can also be set up in a real-time PCR machine where the amplification of DNA is monitored in real-time on a computer.

5.3 PCR tests for Scheme viruses and viroids

VIRUS	ABBREVIATION
Apple chlorotic leafspot trichovirus	ACLSV
Apple mosaic ilavirus	ApMV
Apple stem grooving capillovirus	ASGV
Apple stem pitting virus	ASPV
Prune dwarf ilarvirus	PDV
Prunus necrotic ringspot ilarvirus	PNRSV
Plum viroid I	PVd-I