

Subcommittee on Plant Health Diagnostic Standards

SPHDS Reference Standard No. 2 (SPHDS RS No. 2)

Development of Diagnostic Protocols - Technical Procedures

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INTRODUCTION

Scope

The *Development of Diagnostic Protocols – Technical Procedures* (SPHDS RS No.2) is a SPHDS Reference Standard providing guidelines on the development of diagnostic procedures/protocols (refer to SPHDS RS No.1, 2007 for definitions). In this document the term “diagnostic protocol” will infer reference to either a diagnostic procedure or a diagnostic protocol. The Reference Standard has been developed to standardise and incorporate relevant information in diagnostic protocols for the identification of emergency plant pests in Australia.

Purpose

The purpose of this Reference Standard is to provide guidelines and instructions to authors for the development of a diagnostic protocol. Once developed, the diagnostic protocol will be submitted to SPHDS for approval and Plant Health Committee endorsement, and when approved and endorsed, the document will be recognised as a National Diagnostic Protocol (NDP).

Review

The SPHDS RS No.2 will be reviewed every three years. The review will be implemented by the Diagnostic Standards Working Group (in consultation with the Subcommittee) for expert input. Changes to the Standard are subject to the approval of SPHDS members for adoption, and endorsement by the Plant Health Committee.

Reference Standards

All of the SPHDS Reference Standards can be found on the SPHDS website www.daff.gov.au/sphds

References

IPPC. 1997. ISPM No. 6 *Guidelines for Surveillance*. Food and Agriculture Organisation for the United Nations, Rome.

IPPC. 2001. ISPM No. 13 *Guidelines for the Notification of Non-compliance and Emergency Action*. Food and Agriculture Organisation for the United Nations, Rome.

IPPC. June 2008. *Procedural Manual*. Food and Agriculture Organisation for the United Nations, Rome.

IPPC. 2006. ISPM No. 27 *Diagnostic Protocols for Regulated Pests*. Food and Agriculture Organisation for the United Nations, Rome.

PART A

SPHDS PROCEDURE FOR THE PRODUCTION OF NATIONAL DIAGNOSTIC PROTOCOLS

Introduction

The SPHDS *Procedure for the Production of National Diagnostic Protocols* has been adapted from the IPPC *Procedural Manual*, Section 12.2.5: *Procedures Related to the Technical Panel on Diagnostic Protocols* (Nov 2008).

Definitions of terms, acronyms and abbreviations used are contained in the SPHDS RS No.1: *Glossary of terms*.

Procedures

1. Annual work programme

- 1.1 The Diagnostic Standards Working Group (DSWG) annually identifies priorities for the development of diagnostic protocols. Guidance from Plant Health Australia, the SPHDS and any other requests for reviews and amendments to a diagnostic protocol (that have been received by SPHDS/Plant Health Committee members) should be taken into account when submitting a work program to the SPHDS Executive Officer.
- 1.2 SPHDS reports annually through its Chair to Plant Health Committee (PHC). This report includes achievements and the proposed work program.

2. Nominations of Experts

- 1.1 The SPHDS Executive Officer calls for nominations of Experts for development or review of a diagnostic protocol.
- 2.2 The list of nominated Experts is submitted to SPHDS for acceptance.
- 2.3 The chosen Experts are invited to participate in the development or review of the diagnostic protocol(s) by open process.

3. Development of new diagnostic protocols (see part C)

- 3.1 A suitable expert/author will lead the development of a diagnostic protocol either by adapting an international diagnostic protocol, if it exists, or by developing a new diagnostic protocol (in accordance with SPHDS RS No.2, Part B) as required. The author uses the "Instructions to authors" for guidance (SPHDS RS No.2, Part B) and additional instructions may also be given by DSWG, if needed.
- 3.2 A draft protocol should be submitted to SPHDS for approval before the author is awarded initial funds to validate the procedures overseas (in accordance with SPHDS RS No.2, Part C).
- 3.3 Peer review of the diagnostic protocol is undertaken by a Plant Health Expert approved by SPHDS (in accordance with SPHDS RS No. 4).
- 3.4 Verification of the diagnostic protocol is undertaken by an Independent Laboratory approved by SPHDS (in accordance with SPHDS RS No. 4).
- 3.5 The diagnostic protocol and the associated peer review and verification reports are submitted to SPHDS for approval by the Assessment Panel (for approval processes refer to SPHDS RS No. 3).
- 3.6 The diagnostic protocol is either approved by SPHDS and submitted to PHC for endorsement, or returned to the author, applicant or person nominated by SPHDS with comments for further work required before resubmission.

4. Protocol review

- 4.1 Endorsed National Diagnostic Protocols/Procedures (NDP) will be assessed for currency by the Assessment Panel (refer to SPHDS RS No, 3) every three years or earlier if required.
- 4.2 The Assessment Panel may choose to engage other Plant Health Experts to assist with the process.
- 4.3 If updates are required, the Assessment Panel engages an Expert to develop an updated draft of the diagnostic protocol. If the changes are substantial, and include new procedures, the approval process will be undertaken as if this were a new protocol, including verification and/or peer review.
- 4.4 The revised draft is submitted to SPHDS for approval.
- 4.5 If approved, the reviewed diagnostic protocol is submitted to PHC for endorsement.

PART B

SPHDS INSTRUCTIONS TO AUTHORS OF NATIONAL DIAGNOSTIC PROTOCOLS

Introduction

These instructions to authors of National Diagnostic Protocols are based on the ISPM No. 27 *Diagnostic Protocols for Regulated Pests* (2006) and IPPC *Procedural Manual*, Section 12.2.5.2: *Instructions to Authors of Diagnostic Protocols* (Nov 2008).

Definitions of terms, acronyms and abbreviations used are contained in the SPHDS RS No.1: *Glossary of terms*.

Instructions

1. General considerations

Diagnostic protocols provide the minimum requirements for diagnostic procedures and methods for the detection and identification of plant pests. The diagnostic protocols provide guidance on the diagnosis of specified pests. Information is provided on the specified pest, its host and taxonomic status and the methods to detect and identify it based on the best available information.

Diagnostic protocols may cover a species, an intra-specific taxon, several species within a genus, or an entire genus (where several species within a genus are to be included).

If an approved IPPC protocol exists, this should be used as the basis for the NDP unless that protocol is proven not to work in Australian conditions.

Authors will be supplied with any information contained in the Plant Biosecurity Toolbox (PBT) (www.padil.gov.au/pbt/) to use as the initial information source for the NDP. If other information is needed to complete the NDP then it can be obtained from other sources.

Authors must ensure that all text and images are appropriately cited and correctly acknowledged. Authors must not violate intellectual property or copyright conventions.

The country or region where images were taken must also be provided.

2. Definitions

2.1 Pest detection in a diagnostic protocol, in accordance with ISPM No. 27, is defined as providing information and guidance on:

- the plants, plant products or other articles capable of harbouring the pest;
- the part(s) of the plant, plant products or other articles on/in which the pest may be found;
- the signs and/or symptoms associated with the pest (characteristic features, differences or similarities with signs and/or symptoms from other causes), including illustrations, where appropriate;
- the developmental stages of the pest that may be detected, together with their likely abundance and distribution on/in the plants/plant products or other articles;
- the likely occurrence of the pest associated with developmental stages of the host(s), climatic conditions and seasonality;
- methods for detecting the pest in the commodity (e.g. visual, hand lens)

- methods for extracting, recovering and collecting the pest from the plants, plant products or other articles, or for demonstrating the presence of the pest in the plants, plant products or other articles;
- methods for indicating the presence of the pest in asymptomatic plant material or other materials (e.g. soil or water), such as ELISA tests or culturing on selective media; and/or
- viability of the pest.

For all the methods included in this section, information should be provided on their sensitivity, specificity and reproducibility, where relevant. Where appropriate, guidance should be provided on positive and negative controls and reference material to be included in tests. Guidance should also be provided on resolving possible confusion with similar signs and/or symptoms due to other causes.

2.2 Pest identification in a diagnostic protocol is defined as the process of ascertaining the taxonomic identity of an organism; and

2.3 Pest diagnosis in a diagnostic protocol is defined as the process of detection and identification of a pest.

Methods for detection may be interpreted differently depending on the type of pest being considered. For example, detection of insects may relate to observation of individuals or signs of damage in consignments, whereas detection methods for bacteria may involve application of specific culturing methods.

3 Methodology

Each protocol should contain the diagnostic procedures and guidance necessary for the named pest(s) to be detected and positively identified by a diagnostician. Authors should select diagnostic procedures on the basis of their accuracy; sensitivity and reproducibility, also taking into account the availability of equipment, the expertise required for these procedures and their practicality (e.g. ease of use, speed and cost).

Diagnostic protocols should include more than one diagnostic procedure, if appropriate, to take into account the varying capabilities of laboratories. Diagnosis of different developmental stages of organisms may require different methodologies. For most purposes a combination of procedures will be necessary, however for some purposes a single procedure may be sufficient. In cases where morphological procedures can be reliably used, but appropriate molecular procedures have been developed, the use of both procedures must be described.

Authors should provide information and guidance on diagnostic procedures that either singly or in combination lead to diagnosis of the pest. When several procedures are mentioned, their advantages/disadvantages should be given as well as the extent to which the procedures or combinations of procedures are equivalent. If several procedures are needed for the diagnosis, and/or if many alternative procedures are included, a schematic flow diagram may be necessary.

Only methods of relevance for the diagnosis should be included.

4 Structure and content of a diagnostic protocol

4.1 Overall structure

Diagnostic protocols should be arranged with the following sections (with additional sub-headings as appropriate):

4.1.1 Contents Page

4.1.2 Introduction

- 4.1.3 Taxonomic information
- 4.1.4 Detection
- 4.1.5 Identification
- 4.1.6 Contact points for further information
- 4.1.7 Acknowledgements
- 4.1.8 References

4.2 Introduction

This section should be no greater than 400 words.

Authors should provide brief information on the pest that is directly relevant for diagnosis and where possible, should be referenced. This information may include: its appearance, relationship with other organisms, host range, effect on hosts, life cycle, mode of transmission and dissemination (eg vectors) and geographic distribution (in general – not by country).

Detailed information, if available, should be referenced but not included.

Authors should include information on whether other protocols are available, reference them, and comment on why they are not suitable, or how they are related to this protocol.

Geographic information on occurrence of plant pests in Australia should NOT be included, unless it refers to potential distribution, in this section or elsewhere in the document.

4.3 Taxonomic information

Under this subheading, the correct scientific name and authority should be given and an overview of the relevant taxonomic hierarchy (Kingdom, Phylum, Order, Family, Genus, Species, relevant subspecific taxon). Include synonyms and relevant former names (these may be taxonomically incorrect, but relevant in relation to the literature) as appropriate. For fungi, the teleomorph name should be used; teleomorph synonyms may be included as appropriate. The anamorph name and its synonyms (as relevant) should also be presented. For viruses and MLOs, internationally recognised acronyms should be included.

Any taxonomic disagreements should be stated along with appropriate references.

A taxonomic description can be included only if this information is not covered in detection.

4.4 Detection

Detection is the information needed to detect the pest in the host material so it can then be identified. It is NOT the procedures used for identification. However for some pests, particularly insects, there may be significant cross-over of information. If the information for detection is also the method used for identification, place the method in the identification section.

Authors should provide information and guidance on:

- 4.4.1 The plants, plant products or other articles capable of harbouring the pest;
- 4.4.2 The signs or symptoms associated with the pest (characteristic features, differences or similarities with signs and/or symptoms from other causes), including illustrations, where appropriate;
- 4.4.3 The part(s) of the plant, plant products or other articles on/in which it may be found;

- 4.4.4 The developmental stages of the pest that may be encountered, together with their likely concentration and distribution on/in the plants/plant products or other articles;
- 4.4.5 The likely occurrence of the pest associated with developmental stages of the host(s), climatic conditions and seasonality;
- 4.4.6 Methods for discovering the pest in the commodity (e.g. visual, hand lens);
- 4.4.7 Sampling procedures critical for the detection methods and the diagnostic procedures. Any aspects of the sample that may impact on the diagnostic procedure must be included, for example age of the sample, chemical sprays applied to the crop prior to sampling, sample storage.

Sampling procedures for surveillance should not be included in the main body of the protocol (this may be added as an appendix or be referred to as being a part of the national response plan if such a plan exists).
- 4.4.8 Methods for extracting, recovering, and collecting the pest from the plants, plant products or other articles or for demonstrating the presence of the pest in the plants, plant products or other articles.
- 4.4.9 Methods for demonstrating the presence of the pest in asymptomatic plant material or other materials (e.g. soil or water), such as ELISA tests, culturing on selective media or baiting.
- 4.4.10 The criteria for the determination of a positive or negative result for each method.

For all the methods and diagnostic procedures included in this section, information should be provided on their accuracy, specificity and reproducibility, and specifications from multi-laboratory validation trials (if available). Where appropriate, guidance should be provided in positive and negative controls and reference material to be included in diagnostic procedures. Sources and specifications (technical, commercial, collection entry codes) of controls and reference materials should be indicated. Where the inclusion of additional specific controls, including reference material, is essential, this must be indicated in the protocol.

Guidance should be provided on resolving possible confusion with similar signs and symptoms due to other causes. Guidance should also be provided on possible resolution of ambiguous results.

Detection methods should be described in sufficient detail that the user of the protocol will be able to apply the method without further reference to the literature. Where appropriate, reference may be made to methodology described in other diagnostic protocols annexed to this protocol.

4.5 Identification

In this section, authors should provide information and guidance on diagnostic procedures that either singly or in combination lead to the identification of the pest. Diagnostic procedures for quick, presumptive indications of identity (which will later need to be confirmed) may also be included (see Detection 4.4.8). Each diagnostic procedure should be described separately.

For all the diagnostic procedures included in the section, information should be provided on their accuracy, specificity and reproducibility, and specifications from multi-laboratory validation trials (if available). Guidance should be provided on positive and negative controls and reference material to be included in each of the procedures. Sources and specifications (technical, commercial, collection entry codes) of controls and reference materials should be indicated.

Guidance should be provided on the criteria for the determination of a positive or negative result for each method, and methods to resolve ambiguous results. Guidance should also be provided on resolving possible confusion with similar and related species or taxa.

Where appropriate, diagnostic procedures for detection of pests from asymptomatic plants or plant products should also be given.

Two main types of methodologies are included in diagnostic protocols, those which are based on morphological and morphometric characteristics and those based on biochemical and molecular properties.

4.5.1 Methodologies based on morphological and morphometric characteristics of a pest.

Details should be provided, as appropriate, on;

- 4.5.1.1 Methods to isolate and culture the pest, including duration of the process
- 4.5.1.2 Methods to prepare, mount and examine the pest (such as for light microscopy, electron microscopy);
- 4.5.1.3 Identification keys (to family, genus, species and others), with references;
- 4.5.1.4 Descriptions of the morphology of the pest, or of its colonies, including gender of the pest, illustrations of diagnostic characteristics, morphometric data and in indication of any difficulties in seeing particular structures;
- 4.5.1.5 Comparison with similar or related species, and
- 4.5.1.6 Relevant reference specimens or cultures if available.

4.5.2 Methodologies based on biochemical and molecular properties.

For biochemical or molecular identifications, each method (e.g. serology, ELISA, electrophoresis, PCR, real-time PCR, RFLP, and/or sequencing) should be described separately in sufficient detail (including equipment, reagents and consumables) to be able to perform the diagnostic procedures without further reference to the literature. Where appropriate, reference may be made to methodology described in other diagnostic protocols annexed to this standard.

Where DNA extraction methods used are not standard methods, reasons must be given for their use over a standard kit extraction method.

Where gene sequences used to develop the tests are not referenced, information on their stability, the alignment from which they are developed and the population sample used to develop them should be included.

Where a protocol is based on species-specific PCR amplification, sequencing of the amplicon is mandatory for confirmation of a suspected exotic pathogen. At least one reference sequence must be nominated and made available in an internationally recognised DNA database (e.g. GenBank, EMBL, DDBJ) and the expected level (%) of intra-specific sequence variation must be provided. Where used, data from International sequence databases must be derived from appropriately validated specimens and accession numbers must be included.

Where culturing or isolation are necessary components of these methods, details should be provided including the duration of the process if not previously included in 4.5.1.

4.6 Contact points for further information

In this section, authors should provide contact details of institutes or individuals with particular expertise on the pest(s), which may be consulted regarding any questions or for confirmatory diagnosis.

4.7 Acknowledgements

In this section, the name and address of the experts who wrote the first draft are given, together with those of any others who made major contributions.

Authors must ensure that all text and images are appropriately cited and correctly acknowledged. Authors must not violate intellectual property or copyright conventions. The country or region where images were taken must also be provided.

4.8 References

In this section, references to scientific publications and published laboratory manuals should be given. The references should concern the diagnosis of the pest and species with which the pest may be confused, its symptomology and methods for extraction, detection and identification. It is not necessary to include a complete list of references concerning geographic distribution, host lists, epidemiology and general biology unless these references provide additional information that may assist future reviews

PART C

DEVELOPMENT PROCESS FOR NATIONAL DIAGNOSTIC PROTOCOLS

