Subcommittee on Plant Health Diagnostics

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

Development of National Diagnostic Protocols
- Procedures for Authors

VERSION NUMBER	V7
STATUS	Endorsed
ISSUE DATE	June 2019
REVIEW DATE	June 2024
ISSUED BY	Executive Officer
AUTHOR	DPWG

CONTENTS

1. INTRODUCTION

1.1 Scope

The *Development of National Diagnostic Protocols – Procedures for Authors* (SPHD RS No.2) is a SPHD Reference Standard providing guidelines for authors on the development of diagnostic procedures/protocols (refer to SPHD RS No.1 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***.

1.2 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 SPHD for approval and Plant Health Committee endorsement, and when approved and endorsed, the document will be recognised as a National Diagnostic Protocol (NDP).

If an approved IPPC protocol exists, this should be used unless it is shown that the NDP has improved procedures for Australian conditions.

1.3 Review

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

1.4 Reference Standards

All SPHD Reference Standards can be found on the NPBDN website (https://www.plantbiosecuritydiagnostics.net.au/resources/#). On the Resource page search for the term 'Reference Standard'

1.5 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. 2006. ISPM No. 27 *Diagnostic Protocols for Regulated Pests*. Food and Agriculture Organisation for the United Nations, Rome.

IPPC. 2011. *Procedural Manual.* Food and Agriculture Organisation for the United Nations, Rome. September 2011.

IPPC. 2012. ISPM No. 5 Glossary of Phytosanitary Terms. FAO, Rome.

2. SPHD PROCEDURE FOR THE DEVELOPMENT OF NATIONAL DIAGNOSTIC PROTOCOLS

2.1 Introduction

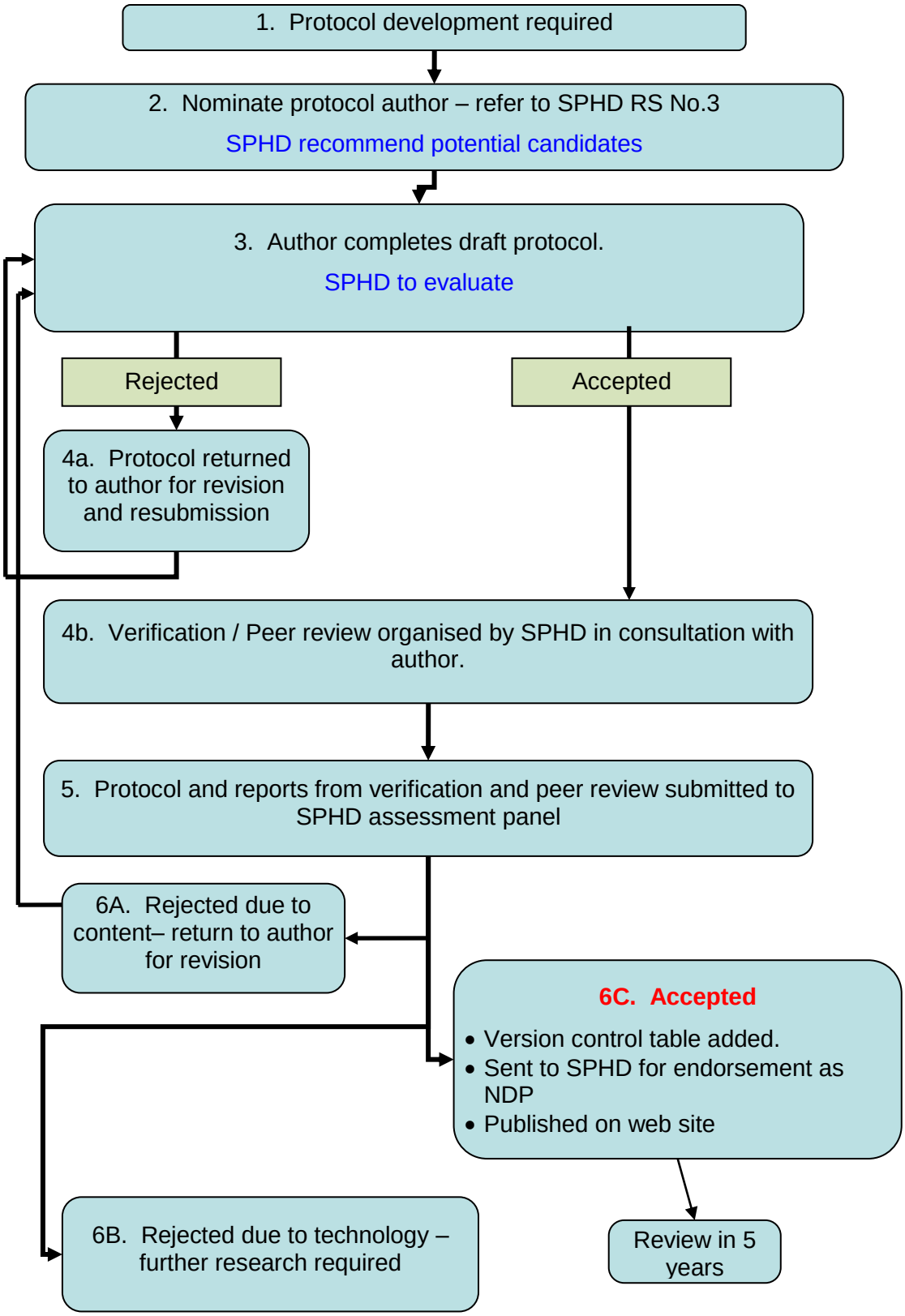
The SPHD Procedure for the Development of NDPs has been adapted from the IPPC Procedural Manual, Section 3.6.3. *Technical Panel to Develop Diagnostic Protocols for Specific Pests (IPPC 2011).*

Definitions of terms, acronyms and abbreviations used are contained in the SPHD RS No.1: Glossary of Terms and *ISPM No. 5 Glossary of Phytosanitary Terms (*IPPC 2012*).*

2.2 Procedures

1. A suitable expert/author approved by SPHD will lead the development of a diagnostic protocol either by adapting an existing diagnostic protocol or by developing a new diagnostic protocol as required. The author uses the Instructions to Authors for NDPs for guidance (SPHD RS No.2 Section 3) and if needed additional instructions may also be given by DSWG. A proforma is provided in Section 4.
2. A draft protocol should be submitted to SPHD for approval.
3. The protocol is evaluated by SPHD and may be returned to the author if considered not to contain sufficient detail.
4. Peer review of the diagnostic protocol is undertaken by a Plant Health Expert approved by SPHD (in accordance with SPHD RS No. 4).
5. Verification of the diagnostic protocol is undertaken by an Independent Laboratory approved by SPHD (in accordance with SPHD RS No. 4).
6. The diagnostic protocol and the associated peer review and verification reports are submitted to SPHD for approval by the Assessment Panel (in accordance with SPHD RS No. 3).
7. The diagnostic protocol is either endorsed by SPHD, or returned to the author, applicant or person nominated by SPHD with comments for further work required before resubmission.
8. Once endorsed, acknowledgment of the reviewer(s), verifying laboratory and original authors of the protocol is included with a citable ISBN.
9. The endorsed diagnostic protocol is published on the SPHD website.

Figure 1. Protocol development flowchart



3. INSTRUCTIONS TO AUTHORS OF NATIONAL DIAGNOSTIC PROTOCOLS

3.1 Introduction

These instructions to authors of NDPs are based on the ISPM No. 27 *Diagnostic Protocols for Regulated* Pests (IPPC 2006) and IPPC *Procedural Manual* section 3.6.3 (*Technical Panel to Develop Diagnostic Protocols for Specific Pests* (TPDP)) and Annex 11 (*TPDP Guidance, Part 1: Guidelines on Formatting of Diagnostic Protocols).*

Definitions of terms, acronyms and abbreviations used are contained in the SPHD RS No.1: *Glossary of terms* and the *ISPM No. 5 Glossary on Phytosanitary Terms* (IPPC 2012).

Authors can use the proforma in Section 4 of this reference standard and also refer to NDPs on the NPBDN website (http://plantbiosecuritydiagnostics.net.au/resource-hub/priority-pest-diagnostic-resources) for examples.

2.2 Instructions

3.1.1 General requirements

A protocol should only contain information directly relevant to the accurate taxonomic identification of the organism.

Diagnostic protocols provide the minimum requirements for diagnostic procedures and methods for the detection and identification of plant pests. Information is provided on the 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 multiple genera of related pests.

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

The document should include page numbers and text font should be Cambria size 11 (see *Style Checklist for Authors* in Section 4).

3.1.2 Images and illustrations

Any images not copyright to authors must have written permission from the copyright owner, or be cited if from a published document, for use in the protocol.

All figures must be numbered and the figure caption include the country or region where images were taken and name of the copyright holder.

Images and illustrations must be appropriate, in focus, and of sufficient resolution. Authors should follow the requirements outlined in the style guide (Section 5).

3.1.3 Definitions

Pest detection in a diagnostic protocol is the information needed to detect the pest in the host material so it can then be identified.

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

3.1.4 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. 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 relevant for the definitive diagnosis should be included. Additional techniques which are more suitable for high throughput diagnostics (i.e. the ability to process large numbers of samples in a short time period) which could be used in a surveillance situation can be included in an appendix.

3.1.5 Structure and content of a diagnostic protocol

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

Contents

1. Introduction
2. Taxonomic information
3. Detection
4. Identification
5. Contact points for further information
6. Acknowledgements
7. References
8. Appendices (optional)
9. Diagnostic procedures to support surveillance

1. 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 should be referenced. This information may include: host range, effect on hosts and brief description of mode of transmission and dissemination (e.g. vectors).

Host range should be entered in list format: for long hosts lists this can be included in the appendix.

No geographical information, control or risk assessment (entry, spread, establishment potential or consequences of entry) should be included.

Other relevant information can be included in the appendix, e.g. life cycle, full details of transmission and dissemination.

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. ***If an IPPC protocol is available, and the NDP has improved procedures, the NDP should be used in preference. The NDP should clearly state the reason, and the date of the IPPC diagnostic protocol being referenced.***

2. Taxonomic information

The correct scientific name and authority should be given and an overview of the relevant taxonomic hierarchy (Kingdom, Phylum, Class, 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. Internationally recognised acronyms should be included.

For protocols that detect and identify groups of organisms (e.g. several species within a genus, several genera within a family, etc), the scope of the protocol should be clearly defined. Where possible, the taxonomic rules used to define this group should be included as well as evidence via referred papers that demonstrate the diagnostic capacity of the protocol. If appropriate a list of organisms that have been detected and identified using the diagnostic protocol can be included in an appendix.

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.

3. Detection

Detection is the information needed to detect the pest in or on the host material so it can then be identified. It is NOT the procedures used for identification.

However for some pests, particularly invertebrates, 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:

- The part(s) of the plant, plant products or other articles on/in which it may be found;
- The likely occurrence of the pest associated with developmental stages of the host(s), climatic conditions and seasonality;
- The signs or symptoms associated with the pest including illustrations/images;
- Differences or similarities with signs and/or symptoms from other pests/causes including illustrations/images;
- The developmental stages of the pest that may be encountered, together with their likely distribution on/in the plants/plant products or other articles;
- 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, e.g. age of the sample, chemical sprays applied to the crop prior to sampling, sample storage (sampling procedures for surveillance should not be included); and
- Detection procedures for extracting, recovering, and collecting the pest from the plants. These 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.
- If the pest is vector borne, include available detection methods in the vector.
- High throughput sequencing (HTS) can be used as a diagnostic procedure. Guidelines/standards for its use are currently in development. Until these are endorsed by SPHD, HTS data should be used cautiously, and the diagnosis confirmed by other currently used techniques.

Sub headings should be included as appropriate, but it is not necessary to include all dot points as subheadings.

All illustrations/images should be of sufficient quality and size when printed to show required features and the country or region where images were taken must also be provided. All text and

images must be appropriately cited and correctly acknowledged. Authors must not violate intellectual property or copyright conventions.

Images for symptoms should:

- Show typical symptoms, including key characteristics such as necrosis, wilting, sporulation, bacterial ooze, fruiting bodies, etc.
- Clearly show the part of the plant exhibiting the symptom.
- Show correct composition and correct distance for the given symptoms.
- Be shown in context (close up, macro, symptomatic plant vs. non symptomatic plant(s).
- Include a point of reference such as an image scale, a coin or any other common object for size comparison, if not of a recognisable whole plant part.

4. 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. ***The minimum requirements for identification must be clearly stated.*** Diagnostic procedures for quick, presumptive indications of identity (which will later need to be confirmed) may also be included. Each diagnostic procedure should be described separately.

For all the diagnostic procedures, information should be provided on their accuracy, specificity and reproducibility, and specifications from multi-laboratory validation trials (if available). For protocols that detect and identify more than one organism information should be provided that clearly defines the scope of the protocol. If the list of organisms is extensive, this data can be presented as a table in an appendix. This table should include if relevant, key diagnostic characters to differentiate each organism.

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 material 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 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 or molecular properties.

Methodologies based on morphological or morphometric characteristics of a pest.

Details should be provided, as appropriate, on:

- Methods for extracting, recovering, and collecting the pest from the plants, plant products or other articles, including isolation and culture of the pest and duration of the process;
- Methods to prepare, mount and examine the pest (such as for light microscopy, electron microscopy);
- Identification keys (to family, genus, species and others), with references;
- Descriptions of the morphology of the pest, or of its colonies, including gender of the pest, illustrations of diagnostic characteristics, morphometric data and an indication of any difficulties in seeing particular structures;
- Comparison with similar or related species; and

- Relevant reference specimens or cultures, if available.

Images/illustrations for identification should:

- Be appropriately cited and correctly acknowledged. Authors must not violate intellectual property or copyright conventions.
- Include either macro or micro images to show appropriate diagnostic features used in identification and where necessary anointed with arrow, circle or similar to highlight the specific feature.
- Include scale bar or appropriate point of reference (coin, ruler, etc).

Images/illustrations for invertebrate identification should also:

- Include images of the whole invertebrate and appropriate life stages.
- Include habitus (how the organism looks in the field) if not shown in symptoms images.
- If the NDP covers more than one species, show the relevant characters for each of the taxa being compared.

Methodologies based on biochemical or molecular properties.

- For biochemical or molecular identifications, each method (e.g. serology, ELISA, electrophoresis, PCR, real-time PCR and RFLP) 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. Standard methods for DNA cloning and sequencing do not need to be described.
- Where DNA extraction methods used are not standard methods, reasons must be given for their use over a standard kit extraction method. Details of kit methods should not be included unless they are modified. Any modification must be justified.
- 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 or group-specific PCR amplification, sequencing of the amplicon is mandatory for confirmation of a suspected EPP. At least one reference sequence must be nominated and made available in a publically available 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 or the appropriate reference 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.

5. Contact points for further information

In this section, authors should provide contact details of institutes and individuals (Australian and/or international) with particular expertise on the pest(s), who may be consulted regarding any questions or for confirmatory diagnosis. Contact details should include address, email and phone.

The author should seek permission from the experts to include their details.

6. Acknowledgements

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

7. References

In this section, references cited in the protocol must be included. Other references to scientific publications, published laboratory manuals and web sites can be included in a separate section if they directly relate to detection or identification.

Use referencing style from *Australasian Plant Pathology* or *Journal of Australian Entomology Society*.

8. Appendices

Appendices may be used to provide relevant information such as life cycle, full details of transmission and dissemination.

9. Diagnostic procedures to support surveillance

This section provides information on the in-field and laboratory procedures utilized in the screening, detection or identification of plant pests in a surveillance situation.

This section is optional in the initial development of an NDP, but should be included if possible.

Details are outlined in section 3.1.6.

3.1.6 Section 9. Diagnostic procedures to support surveillance

Diagnostics to support surveillance provides information on the in-field and laboratory procedures utilized in the screening, detection or identification of plant pests in a surveillance situation. This information is intended for use by diagnostic laboratories and diagnosticians, however they may also be used in the development of a surveillance plan. These procedures are to be used to support surveillance activities and are NOT to be used as definitive diagnostics in an initial detection.

These procedures cannot be developed as a stand-alone document, but must be part of a NDP.

Structure and content

Section 9 should be arranged as per the protocol template provided with the following sections (with additional sub-headings as appropriate):

Contents

9.1 Introduction

This section should be no greater than 400 words.

Authors should provide information on the scope and types of tests included. For each test, authors should provide guidance on:

- the best use of the test;
- number of samples able to be tested at one time;
- time from receipt of sample to result.

This information could be presented in a table:

Test	Outcome	Identification confidence	Deployment	Time	Throughput
Test 1	To species	Low (<90%)	Field	>1 d	Medium (100s)
Test 2	To genus	Medium (90-99%)	Field/Laboratory	>1 h	Medium (100s)
Test 3	To subspecies	High (99%+)	Laboratory	>1 week	Low (10s)
Test 4	To species	High (99%+)	Laboratory	< 1 week	High (1000s)

9.2 Sampling

Authors should provide any information for sampling that is critical to the diagnosis. The aim is to provide information for the most useful sample for the diagnostician to run the required test.

If the sample requirements are specific to the test, they should be included in the test information. Any generic sampling information should be included here.

However this information may also be used to develop the surveillance plan, so the language should be clear and understandable by non-specialists. Be brief, but be specific: for example “wrap sample in paper”: should the paper be wet, damp, or dry? Paper towel or newspaper?

Sampling information may include, but is not restricted to;

- Type of sample, e.g. plant part, healthy vs diseased, live vs dead, adult vs juvenile, etc
- What not to sample, eg no soil with sample
- Size of the sample, eg number, weight, volume
- When to sample, e.g. best time of the year, growth stage of the crop, persistence in absence of host
- When not to sample, eg if surveillance is necessary at a non-ideal time of year, can we still sample
- Sample integrity, e.g. whether specific sampling methods or surveillance hygiene procedures may damage the specimen and make it unsuitable for diagnosis
- Preservation of sample, e.g. storage media, temperature, length of time sample can be left in storage, etc
- Any specific requirements for transport of sample
- Any information on hosts, e.g. definite non-hosts, symptomatic hosts, asymptomatic hosts, etc
- Any data that the diagnosticians may need from surveillance teams to assist with the diagnostic procedure, e.g. host, stage of growth, number of leaves in sample, chemical applications that affect sample quality, etc

Sub headings should be included as appropriate, but it is not necessary to include all dot points as subheadings.

It would be preferable if photographs are provided to illustrate the above. All illustrations/images should be of sufficient quality and size when printed to show required features and the country or region where images were taken must also be provided. All text and images must be appropriately cited and correctly acknowledged. Authors must not violate intellectual property or copyright conventions.

Sampling information could be supplied in a table. Plant example:

Specification	**Description**
Sample type	Plant tissue that shows typical symptoms. Very important to include the interface between healthy and diseased tissue. If present, include tissue with bacterial ooze For sampling asymptomatic plants, younger material including floral parts and shoots should be selected as they are more easily colonised.
What not to sample	Older non symptomatic plant tissue
Sample size	X cm of tissue, y number of floral parts/shoots
When to sample	The best time to sample is during the growing season when symptoms are most visible. This will depend on the kind of host and geographic location. Ooze most likely to be present in the morning when air humidity is high and temperature low.

When not to sample	Sampling in winter on dormant plant material is difficult as cankers may not be visible.
Sample integrity	Sample on same day as dispatch, otherwise store in a cool place (preferably not a fridge as low temperatures can cause problems in isolating)
Sample preservation	Samples should be wrapped in damp paper towel in a plastic bag. Samples should be processed as soon as possible after collection but can be stored at ~8^{o}C for up to 14 days before processing.
Sample transport	Important to ensure temperature of samples remain at or below 20^{o}C during transport to laboratory. Ensure samples not in transit over the weekend.
Host specific sampling	There are no differences between hosts
Additional sample info	

Invertebrate example

Specification	**Description**
Sample type	Crawler life stage
What not to sample	Above ground symptomatic leaf material
Sample size	N/A
When to sample	Summer to early autumn (December-February)
When not to sample	Other times of the year
Sample integrity	
Sample preservation	Bottle of 80 % ethanol
Sample transport	Place samples in an esky (or appropriate holding container). Transport as quickly as possible to the laboratory
Host specific sampling	N/A – grapevine only host
Additional sample info	

9.3 In field test

In field test can be used to optimise sampling, reduce samples requiring laboratory testing or provide definitive diagnosis. Therefore, in field tests do not necessarily need to achieve definitive identification of the pest.

In field tests can include image based diagnostics (eg remote diagnostics, good photos), on-line tools and any other procedure that can inform sample triage.

If no in-field tests are available authors should note this here.

Authors should provide the specificity and sensitivity of the tests, including percentage of false negatives/positives where available. Field tests should have been validated in the field and not be purely laboratory based. Tests should have internal controls.

Authors should provide interpretation of results including what samples from the field test should be sent to the laboratory for further confirmation.

If the in-field tests are commercially available kits, the source is required, and an indication of whether they require specific expertise to run.

9.4 Laboratory tests

In this section, authors should provide information and guidance on diagnostic procedures that support surveillance activities.

If the procedure is already described in the NDP it should be referenced and not rewritten. However any amendments or additional information required for use in a surveillance context should be included here.

Authors should provide guidance on potential throughput ie. time to complete a certain number of samples (e.g. platform number, time an experienced entomologist can key out a sample, time to extract DNA, etc). If there is a potential to bulk samples, the number of samples that can be bulked should be stipulated, e.g. 16 leaves, 10,000 seeds, 4 invertebrate legs (this information could be provided in the table in the introduction). Authors should also stipulate the limitations to bulking, as analytical sensitivity and specificity may be impacted by bulking.

For all the diagnostic procedures not already in the NDP, authors should follow the guidelines in section 3.1.5.

4. PROFORMA FOR NATIONAL DIAGNOSTIC PROTOCOL.

Instructions to Authors

- This template serves as a guideline to authors in the production of a diagnostic protocol.
- This proforma can be developed as a stand-alone word document, or the author can obtain a copy of the NDP protocol master from the EO.
-
- Instructions from the SPHD Reference Standard No.2 – *Development of National Diagnostic Protocols –Procedures for authors* are not repeated in this template. Authors should familiarise themselves with the requirements of Reference Standard No. 2 prior to completion of the report.
- Various components (headings) of the template may relate only to specific groups of plant pests or diseases. Authors may select or add headings/sub-headings relevant to their pest organism.
- 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.
- Please follow the style guide. Images and illustrations must be appropriate, in focus, and of sufficient resolution. Authors should follow the requirements outlined in the style guide (Section 5).
- Table of Contents must reflect the contents of your report.
- An electronic copy must be sent to the SPHD Executive Officer:

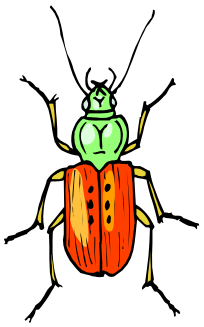
  Email: sphd@agriculture.gov.au

A protocol should only contain information directly relevant to the accurate taxonomic identification of the organism.

Draft Diagnostic Protocol for
[Common name of pest (Taxonomic name of pest)]

Or:

[Taxonomic name of pest], the cause of [common name of pest]



[INSERT Image of plant pest/disease in colour]

Prepared by:

[Insert Date]

[Address of agency]:

Mailing address:
Website:
Ph #:
Fax. #:

[Department of Agriculture logo]	*[Agency logo if applicable]*

[The "Contents" table below is developed from the headings selected in the document. If you do not know how to use this function in word consult the help function in Word.

Contents can be updated by clicking on the contents heading, then the "update table" tab that will appear above it. Choose "Update entire table".]

Contents

1. INTRODUCTION

Separate each section as required with a "Page Break". Only use "Section break" when changes to formatting are required.

2. TAXONOMIC INFORMATION

3. DETECTION

Detection is the information needed to detect the pest in or on the host material so it can then be identified. It is NOT the procedures used for identification.

[These subheadings are examples – delete or include others as needed using Heading 2.]

3.1 Symptoms

3.2 Sampling

3.3 Methods

4. IDENTIFICATION

Information and guidance on diagnostic procedures that either singly or in combination lead to the taxonomically accurate identification of the pest.

The minimum requirements for identification must be clearly stated.

[These subheadings are examples – delete, amend or include others as needed using the appropriate heading level. Only heading levels 1, 2 and 3 are included in the contents]

4.1 Morphological or morphometric characteristics

4.1.1 Sub heading level 3 example

4.1.1.1 Sub heading level 4 example

Sub heading level 5 example

4.2 Biochemical or molecular properties.

5. CONTACT POINTS FOR FURTHER INFORMATION

6. ACKNOWLEDGEMENTS

7. REFERENCES

8. APPENDICES

9. DIAGNOSTICS PROCEDURES TO SUPPORT SURVEILLANCE

9.1 Introduction

9.2 Sampling

9.3 In field tests

9.4 Laboratory tests

9.5 Acknowledgements

9.6 References

5. STYLE CHECKLIST FOR AUTHORS

When writing the scholarship, the author should ensure the following style points are checked.

Overall	Font size minimum Cambria 11 or Arial 10	
	Formatting with headings numbered	
	Contents page generated from "Table of Contents"	
	Pages numbered	
	Appropriate image on title page	
Images	Numbered, captioned, geography and copyright included. Eg "**Figure 1** Ringspot symptoms on tree in Hungary (Photo J. Bloggs)" See further information below	
Introduction	Length <400 words	
	No risk or geographic information	
	Host range included as list	
Taxonomy	Hierarchy included	
References	Use style from *Australasian Plant Pathology* or *Journal of Australian Entomology Society*.	

5.1 Images or illustrations used in NDPs

- Format: for images use JPG or TIFF, for line art/graphics/logos can also use PNG.
- Resolution of images a minimum of 300 dpi at 100%.
- Resolution of line drawings a minimum of 600 dpi at 100%.
- Image size minimum of 500 KB, maximum 5Mb.
- Images in focus.
- Image files cropped as close to the actual image as possible. If this means a lot of cropping, do it in another program and save the cropped image at the required resolution.
- Images of high quality with good contrast of dark and light.

**Note: the resolution will change if you enlarge the image from the original. For example, if you double the size the resolution will be reduced to 150dpi. Pictures can be resized in programs like photoshop.*

Do not:

- Supply files that are optimized for screen use (e.g., GIF, BMP, PICT, WPG); the resolution is too low.
- Supply files that are too low in resolution.
- Supply files in which colours are not realistic, text is illegible, or images are pixilated.

5.3. Inserting images or illustrations in word docs.

Please do not try to be fancy with placing your images. It makes editing quite difficult.

- Insert the image complete, position left and wrap: “In line with text”.
- Enter the caption underneath.

Do not:

- Put images in frames or tables. If you want the picture to end up in a table, enter a note in the table such as “image 1 here” and provide as a separate file (preferred naming convention: pest name_FigX.jpg).
- Put multiple images on one line (ie side by side).

Web sites with useful information.

- https://authorservices.wiley.com/asset/photos/electronic_artwork_guidelines.pdf
- https://www.escienceediting.org/journal/view.php?number=15