International Plant Protection Convention
Protecting the world's plant resources from pests

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INSTRUCTIONS TO AUTHORS
DIAGNOSTIC PROTOCOLS FOR REGULATED PESTS

These instructions are based on International Standard for Phytosanitary Measures ISPM 27.Diagnostic protocols for regulated pests and are compiled to provide more specific explanatory guidance for authors of diagnostic protocols.

(Revised by the Technical Panel on Diagnostic Protocol (TPDP) in July 2016)

Food and Agriculture Organization of the United Nations

CONTACTS

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[1] Full link address: https://www.ippc.int/core-activities/standards-setting/expert-drafting-groups/technical-panels/technical-panel-diagnostic-protocols

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DIAGNOSTIC PROTOCOLS FOR REGULATED PESTS - INSTRUCTIONS TO AUTHORS

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These instructions are based on International Standard for Phytosanitary Measures ISPM 27 (*Diagnostic protocols for regulated pests*) and are compiled to provide more specific explanatory guidance for authors of diagnostic protocols (DPs). Authors are encouraged to study ISPM 27 to ensure that the DP is consistent with the standard. A template for DPs and guidelines on their format are also given.

Additional guidance for drafting groups has been developed to satisfy a demand for consistency in content, structure, semantics, terminology and presentation of IPPC standard setting documents: IPPC style guide for standards and meeting documents and in the *IPPC Procedure Manual for Standard Setting*.

1. GENERAL CONSIDERATIONS

1.1 Minimum requirements for reliable diagnosis of regulated pests

Under the heading titled ISPM 27 states:

Diagnostic protocols may be used in different circumstances that may require methods with different characteristics. Examples of such circumstances grouped according to an increased need for high sensitivity, specificity and reliability are:

- routine diagnosis of a pest widely established in a country
- general surveillance for pest status
- testing of material for compliance with certification schemes
- surveillance for latent infection by pests
- surveillance as part of an official control or eradication programme
- pest diagnostic associated with phytosanitary certification
- routine diagnosis for pests found in imported consignments
- detection of a pest in an area where it is not known to occur
- cases where a pest is identified by a laboratory for the first time
- detection of a pest in a consignment originating in a country where the pest is declared to be absent.

The ISPM also states:

> Diagnostic protocols provide the minimum requirements for reliable diagnosis of regulated pests. This may be achieved by a single method or a combination of methods. Diagnostic protocols also provide additional methods to cover the full range of circumstances for which a diagnostic protocol may be used. The level of sensitivity, specificity and reproducibility of each method is indicated where possible. NPPOs may use these criteria to determine the method or combination of methods that are appropriate for the relevant circumstances.

This means that the minimum requirement usually is applicable to one of the first indents (e.g. routine surveillance). Authors should provide information for the National Plant Protection Organization (NPPO) to make decisions on the methodology required for the relevant circumstances.

If necessary, DPs may describe more than one method to take into account the varying capabilities of laboratories and the situations for which the methods are applied. Such situations include diagnosis of different developmental stages of pests, which require different methodologies, as well as the degree of certainty required by the NPPO. For some purposes a single method may be sufficient, for others a

combination of methods may be necessary. This applies both to the minimum requirements for a diagnosis and where additional requirements are necessary (such as where a high degree of certainty in the diagnosis is required). In cases where morphological methods can be reliably used but appropriate molecular methods have been developed, the latter should be presented as alternative or supplementary methods.

1.2 Other general considerations

DPs are published as annexes to ISPM 27 (*Diagnostic protocols for regulated pests*). They describe procedures and methods for the detection and identification of pests that are regulated by Contracting Parties of the International Plant Protection Convention (IPPC) and relevant for international trade. They are addressed to diagnosticians/diagnostic laboratories performing official tests as part of phytosanitary measures. The DPs provide guidance on the diagnosis of specified pests. Information is provided on the specified pest, its taxonomic status and the methods to detect and identify it. As indicated in Section 1.1, DPs contain the minimum requirements for reliable diagnosis of the specified pest and provide flexibility to ensure the methods are appropriate for a range of circumstances of use.

DPs may cover a species, taxa below species level, several species within a genus, or an entire genus, for example where several species within a genus are regulated pests.

Authors should draft DPs in accordance with the requirements given in the main text of ISPM 27.

General guidelines on the format of DPs are appended. By using these guidelines, authors will help ensure consistency between DPs and facilitate processing of draft DPs. These guidelines will be consolidated as more DPs are developed. Authors are also invited to refer, as a model, to the first DP (for *Thrips palmi*).

- **Appendix 1:** provides a template that should be used for drafting DPs.
- **Appendix 2:** provides general guidelines on the formatting of DPs. is included in
- **Appendix 3:** A check list for authors of diagnostic protocols.
- **Appendix 4:** Some general considerations on the concept of combinations methods in diagnostic protocols.
- **Appendix 5:** Template tables for description of Polymerase chain reaction (PCR), Reverse transcriptase Polymerase chain reaction (RT-PCR) or PCR - Restriction fragment length polymorphism (PCR-RFLP) reactions

DPs are drafted by a group of authors called an editorial team co-ordinated by a lead author and overseen by a discipline lead from the Technical Panel on Diagnostic Protocols (TPDP). The editorial team, including the lead author, is recommended by the TPDP discipline lead and approved by the entire TPDP. To ensure global coverage of the protocol and to facilitate adoption, authors should consult relevant experts from different regions outside of the editorial team prior to submission of final drafts to the TPDP. A cover note giving the list of experts/countries that have written and reviewed the draft, and any main discussion points that have arisen and been resolved should be included (see Appendix 1).

DPs are reviewed on a regular basis (every 5 years unless a specific issue was raised). Authors should be aware that this will be done.

2. DEFINITIONS[2]

- Pest Diagnosis: The process of detection and identification of a pest.

[2] To be modified, if definitions are changed in the document on QA terms (under development).

- Reproducibility**:** Ability of a test method to provide consistent results when applied to aliquots of the same sample tested in different conditions.
- Sensitivity (also known as analytical sensitivity): Smallest amount of the target that can be detected reliably (target may include live organisms, antibodies, nucleic acids).
- Specificity (also known as analytical specificity): Characteristics of a test as concerns its performance with regard to cross-reactions with non-target (false positives) or lack of reaction with target (e.g. subgroups or individuals of the pest) (false negatives).

3. METHODOLOGY

Each DP should contain the methods and guidance necessary for the named pest(s) to be detected and positively identified by an expert (i.e. an entomologist, mycologist, virologist, etc.). Authors should select methods on the basis of their sensitivity, specificity and reproducibility, also taking into account the availability of equipment, the expertise required for these methods and their practicality (for example, ease of use, speed and cost). Only methods of relevance for diagnostics should be indicated in the protocol.

All methods should be described separately in a consistent manner with sufficient detail (including equipment, reagents and consumables) to be able to perform the test without further reference to the literature. However, common laboratory procedures do not need to be detailed in the text. Brand names should not be given unless they are technically necessary and directly affect the result of the diagnosis (see also below). If the method is based on a commercial kit it is not necessary to repeat the manufacturer's instructions, which can be referred to. DPs should not be written in the form of standard operating procedures but should provide sufficient detail to allow NPPOs to develop such procedures. However, the TPDP in its June 2015 meeting decide that the tables for description of PCR, RT-PCR or RFLP reactions should be included in the draft DPs. Where appropriate, reference may be made to methodology described in other adopted DPs annexed to the ISPM 27.

Where units of measurement are indicated (e.g. temperature, pH, etc.), a precise value should be indicated only if it is critical to the method (e.g. an analysis has to be performed at exactly 15 °C). In other cases, either a range of values should be given, or the word "approximately" be used before the value.

Validation data

For all methods, information on their sensitivity, specificity and reproducibility, and specifications from multi-laboratory validation trials (e.g. ring tests) (when available) should be included. These data, as far as possible, should be quantitative, but in the absence of quantitative data, qualitative information may be provided. For each method, if any element of the validation data is not available (e.g. sensitivity), it should be mentioned in the method description, in order to clearly indicate that this element has not simply be omitted.

Brand names

The names of particular brands of chemicals, reagents and equipment should, as far as possible, be avoided and a correct designation or description of the chemical, reagent or equipment shall be given rather than a trade name (brand name).

A standard paragraph under section "Detection and Identification" on the use of brand names should be added to the DPs, before the first mention to a brand name, as follows:

> In this diagnostic protocol, methods (including reference to brand names) are described as published, as these define the original level of sensitivity, specificity and reproducibility achieved. The use of names of reagents, chemicals or equipment in these diagnostic protocols implies no approval of them to the exclusion of others that may also be suitable. Laboratory procedures presented in the protocols may be adjusted to the standards of individual laboratories, provided that they are adequately validated.

Brand names should only be included when the brand is considered to affect the level of specificity, sensitivity and/or reproducibility quoted in the diagnostic protocol. If it is known that only one chemical, reagent and/or equipment is currently available, that is suitable for the successful application of the protocol. If this is the case, the brand name may be given in the text but shall be associated with a footnote as follows:

FOOTNOTE:

"In this diagnostic protocol, methods (including reference to brand names) are described as published, as these defined the original level of sensitivity, specificity and/or reproducibility achieved. The use of names of reagents, chemicals or equipment in these diagnostic protocols implies no approval of them to the exclusion of others that may also be suitable. Laboratory procedures presented in the protocols may be adjusted to the standards of individual laboratories, provided that they are adequately validated."

If in the DP there is more than one mention to a brand name, the second mention (and the sub sequential mentions) to a brand name shall be associated with the footnote number with the full text (e.g. If the first mention to a brand name is footnote 1, the subsequent mentions to brand names should be accompanied by the same footnote number, i.e., footnote number 1).

Controls

Description of all the controls mentioned must be provided, and the minimum requirements for controls should be indicated.

Guidance on positive and negative controls and reference material should be included in each of the tests. Methods where the inclusion of appropriate controls is essential (e.g. enzyme-linked immunosorbent assay [ELISA]) should be indicated. Sources and specifications of controls and reference materials (e.g. catalogue numbers of bacterial reference strains) should be provided.

In the case of a high risk of aerosol contamination, and for specific pest, please consider if instructions should be provided to monitor possible cross contamination, e.g. compare the sequences of positive controls and positive samples.

As the minimum requirements for controls vary from test to test and from pest to pest, each DP drafting group, by the time of definition of the minimum requirements of which tests should be performed for diagnosis, would also decide the minimum requirements for control. The preparation of a guidance document with options for each pest group (i.e. each discipline) is under progress.

Methods

Authors should provide information and guidance on methods that either singly or in combination lead to diagnosis of the pest. However, DPs should not instruct NPPOs on the methods to use. Guidance should be provided on the interpretation of results, in particular the criteria for the determination of a positive or negative result for each method. In most cases, interpretation of results may be made within the section for each method. In some cases, a specific section may be needed (for example, for molecular methods). In case of conventional PCR the sample is considered negative, when a band of the expected size is not produced, regardless of other non-specific bands.General elements on combination of methods are provided as Appendix 4 for information. When methods are cross-referred to in different parts of the DP, it may be useful to indicate the section number where the method is fully described.

It is not necessary to include all methods which have been reported for a particular pest, only those which are reliable, currently available and considered to be of use for the purposes described in ISPM 27.

If several methods are needed for the diagnosis, and / or if many alternative methods are included, a flow diagram may be presented. It should show the different alternative methods allowing to reach the minimum requirements for the diagnostic. Where relevant, it should present the alternative methods for specific circumstances (e.g. symptomatic fruit, asymptomatic fruit). The diagram should indicate the

reliability of each method or combination of methods. It is not intended to be a decision-making tree but is intended to assist NPPOs in determining which method(s) are appropriate for use under different circumstances. It should not refer to different scenarios/situations of use of the diagnostic protocols, i.e. interception etc. When authors conclude that a combination of methods is needed, the reasons should be provided. The flow diagram should be accompanied by some explanation in the text, indicating the methods available and their advantages. The flow diagram can first be referred to before methods are described. Each method mentioned in the flow diagram should be accompanied by a cross-reference to the section number where this method is described.

When several methods are mentioned, their advantages and disadvantages should be given (e.g. duration of the test, cost, availability of reagents, requirements for specialized knowledge or equipment, limited validation data available such as covering only some populations of an organism) as well as the extent to which the methods or combinations of methods are equivalent.

Since the use of loop-mediated isothermal amplification (LAMP) may require licensing from specific countries, when it is included in the diagnostic protocol, the following footnote has to be included for every mention of LAMP:

FOOTNOTE:

"When using LAMP on a regular basis in an area which has a patent system such as Japan (Patent Nos. 3,313,358, 3,974,441 and 4,139,424), the United States of America(US6,410,278, US6,974,670 and US7,494,790), the European Union (Nos. 1,020,534, 1,873,260, 2,045,337 and 2,287,338), China (ZL008818262), the Republic of Korea (Patent No, 10-0612551), Australia (No. 779160), and the Russian Federation (No. 2,252,964), it is necessary for users to receive a license from Eiken Chemical Co., Ltd. before use."

Illustrations

If illustrations (e.g. photographs or line drawings) are essential to the diagnosis, they should be included in the protocol (detailed guidance in Appendix 3). Line drawings, if included, should be sufficient for diagnosis. If original illustrations are included, the author should be named. In addition, photographs, that provide additional information but are not essential for the diagnosis may be posted on the IPP. In some cases links may be provided to other web sources for photographs. With regards to possible copyrights, the discipline leads are required to submit the information to the IPPC Secretariat, and the Secretariat will contact authors to obtain any relevant permission to use the photographs or other illustrations. This ensures a proper record of any permission granted to use the illustrations.

4. STRUCTURE AND CONTENT OF A DIAGNOSTIC PROTOCOL

It is not possible to provide standardized content of DPs. Adopted DPs can be found at https://www.ippc.int/core-activities/standards-setting/ispms. DPs should follow the layout of section 2 of ISPM 27 and should be arranged into the following sections, numbered as follows:

(1) Pest information
(2) Taxonomic information
(3) Detection
(4) Identification
(5) Records
(6) Contact points for further information
(7) Acknowledgements
(8) References

Each section should be divided into sub-sections as required (especially the detection and identification sections) and both sections and sub-sections should be numbered. Appendix 1 provides a template that should be used for drafting DPs.

An index of the sections should be included at the start of the DP and the pages of the DP numbered. As DPs themselves will be annexes to ISPM 27, they should not have annexes or appendices.

Important note: all data in DPs should be publically available. Authors should in particular be aware that any material that may be developed specifically for the purpose of the DP, for example keys or photos of characters, will be made publically available during the development process.

4.1 Pest information

Authors should provide brief information on the pest (generally less than one page of type-written text), including, where appropriate, its life cycle, morphology, variation (morphological and/or biological), relationship with other organisms, host range (in general), effects on hosts, present and past geographic distribution (in general, not country-by-country), mode of transmission and dissemination (vectors and pathways). It is not necessary to include specific details about the epidemiology of the disease or its management.

Supplementary information, such as detailed information on the pest's geographic distribution or hosts, should not be included except when directly relevant for diagnosis. The DP is not intended to be a pest data sheet but reference to such data sheets/databases should be provided when publicly available and considered to provide useful background information. For examples see adopted DPs

All general information on the pest (biology, hosts, etc.) should be under this section, and not under other sections of the protocol.

Authority and dates of taxonomy information should be included. If 1 or 2 authors, full names of authority should be given. If more than 2 authors, abbreviate for the first authors last name and have "et al." References should be included in the reference section. The title of the diagnostic protocol once adopted should include the authority.

4.2 Taxonomic information

Under this section, the correct scientific name, authority and date (no authority/date is required for viruses and viroids) should be given and an overview of the relevant taxonomic hierarchy as appropriate to the type of pest (e.g. Domain, Kingdom, Phylum, Order, Family, Genus, Species, relevant below species taxon). Mention the references used for the scientific names indicated in this section.

Include synonyms and relevant former names (these may be taxonomically incorrect but relevant in relation to the literature) as appropriate. Only important synonyms should be mentioned, listed by chronological order. If there are other synonyms, a reference to a publication listing them can be added.

For fungi, the teleomorph name should be used; teleomorph synonyms may be included as appropriate. The anamorph name and its synonyms and macro- or micro-conidial states (as relevant) should also be presented under synonyms. For viruses, internationally recognized acronyms should be included.

The English common names widely used in international scientific literature should also be included. If possible and available, indicate a reference giving common names in other languages (but do not include common names in other languages in this section).

For fungi a reference to Mycobank may be included under Reference.

4.3 Detection

As stated in ISPM 27, this section provides information and guidance on:

- the plants, plant products or other articles capable of harbouring the pest.

- 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 part(s) of the plant, plant products or other articles on/in which it may be found.

- the developmental stages of the pest that may be encountered, 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 discovering 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.

- viability of the pest.

The ISPM also states that *guidance is also provided on resolving possible confusion with similar signs and/or symptoms due to other causes.*

Methods for detection may be interpreted differently depending on the type of pest being considered. For example, detection of an insect may relate to observation of individuals or signs of damage in consignments, whereas detection methods for bacteria may involve culturing extracts of suspected plant material on differential or semi-selective medium.

When a detection method may also be used for identification, it is recommended that it is described in the detection section (see 4.4. for the details to be provided for methods) and then referred to in the following identification section. Any comments about its use for detection or identification should be included in the relevant section. Methods that detect a group of pathogens rather than a specific pathogen should be described in the detection section.

Sampling in protocols refers to sampling for laboratory analysis, not to sampling for inspection of a commodity. For seed/grain, it might be acceptable to give more details. Sampling procedures for inspectors and inspectors' instructions on recognition of the pest from signs and symptoms should not be included but only essential information for diagnosis should be given. Procedures for inspectors are likely to be covered in an inspection manual. Additional information on the sample that may be relevant for proper diagnosis should be provided (e.g. minimum sample size, storage conditions).

In some cases where methods can be used for both detection and identification (e.g. virology) the methods should be described in the Detection section and cross-referenced in the Identification section.

The use of vendor and brand names should be avoided unless extremely necessary for the test performance. One paragraph at the beginning of the Detection section should be included to cover all mentions of brand names (see previous section "3. Methodology" on "brand names"). The generic wording should be:

In this diagnostic protocol, methods (including reference to brand names) are described as published, as these define the original level of sensitivity, specificity and reproducibility achieved. The use of names of reagents, chemicals or equipment in these diagnostic protocols implies no approval of them to the exclusion of others that may also be suitable. Laboratory procedures presented in the protocols may be adjusted to the standards of individual laboratories, provided that they are adequately validated.

4.4 Identification

In this section, in addition to a description, authors should provide information and guidance on methods that either used alone or in combination lead to the identification of the pest. Methods for quick, presumptive indications of identity (which will later need to be confirmed) may also be included.

Any method that is specific to identification should be described in this section. Where some methods that might be used for identification are already described, the description should not be repeated, but cross-reference should be made to the relevant subsections in the Detection section.

Methodologies used in DPs are based on morphological, morphometric or biological characteristics of a pest, or on biochemical and/or molecular properties (see ISPM 27). Morphological characteristics may be investigated directly or may only be examined after culturing or isolation of the pest. This may also be required for biochemical and/or molecular assays. Where culturing or isolation procedures are necessary components of methods, details should be provided.

Where appropriate, methods for isolation of pests from asymptomatic plants or plant products (such as tests for latent infection) should be given as well as methods for extraction, recovery and collection of pests from plant or other material. Methods should similarly be provided for direct identification of pests using biochemical or molecular tests on asymptomatic material.

ISPM 27 states:

> For morphological and morphometric identifications, details are to be provided, as appropriate, on:
>
> - methods to prepare, mount and examine the pest (such as for light microscopy, electron microscopy and measurement techniques).
>
> - identification keys (to family, genus, species).
>
> - descriptions of the morphology of the pest or of its colonies, including illustrations of diagnostic characters, and an indication of any difficulties in seeing particular structures.
>
> - comparison with similar or related species.
>
> - relevant reference specimens or cultures.

Guidance should be provided on resolving possible confusion with similar and related species or taxa.

For molecular methods, details should be provided, as appropriate, on:

- the target sequence (e.g. target gene, amplicon size and location) and reaction conditions (e.g. oligonucleotide sequence, enzyme source and thermal cycler).
- nucleic acid extraction and purification (e.g. tissue sources, extraction and purification methods, and nucleic acid concentration.
- reverse transcription (e.g. reaction volume, concentration and volume of constituents, denaturation and incubation temperatures).
- polymerase chain reaction (e.g. reaction volume, concentration and volume of constituents, thermocycling conditions).
- restriction analysis (e.g. DNA preparation, reaction volume, concentration and volume of constituents, denaturation and incubation conditions).

  minimum controls (a standard text on controls for molecular methods is under development: The issue of controls was discussed in the 2016-07 TPDP meeting in Jamaica. The TPDP is developing a document with options for each pest group (i.e. each discipline).

Elements regarding the preservation of specimen, especially for entomology, should be included if necessary. Under the section identification, guidance should be given on short- and long-term preservation (where relevant).

In the case of diagnostic protocols for insects or nematodes, consider presenting the main characters for the diagnostic in a table (see *Thrips palmi*).

In the case of diagnostic protocols for plants, if there is no specific difficulty for identifying plants of the species concerned using a key, the text may simply give a reference(s) to suitable key(s).

4.5 Records

In this section, authors should refer to section 2.5 of ISPM 27 which lists the records required to be kept. There is no need to repeat section 2.5, only records that are required in addition to those detailed in ISPM 27 should be listed in the DP. However, in addition, authors should include a description of appropriate evidence of results where other NPPOs may be adversely affected by the results of the diagnosis and therefore the records and evidence of the results of the diagnosis should be retained for at least one year.

Standard text to be used:

Records and evidence should be retained as described in section 2.5 of ISPM 27 (*Diagnostic protocols for regulated pests*).

In cases where other contracting parties may be affected by the results of the diagnosis [, in particular in cases of non-compliance (ISPM 13 (*Guidelines for the notification of non-compliance and emergency action*)) and where [the pest, name of pest] is found in an area for the first time,] the following records and evidence and additional material should be kept for at least one year in a manner that ensures traceability: [the original sample, larvae and adults, preserved or slide-mounted specimens, culture(s) of the pest, [RNA, DNA] extracts, printed tissue sections and/or spotted plant extracts on paper or nylon membranes, PCR amplicons or test materials (e.g. photographs [of distinctive taxonomic structures, fungal structures, symptoms and signs], ELISA plate results printouts and photographs of gels].

4.6 Contact points for further information

In this section, authors, in cooperation with the discipline lead, should provide contact details (full name, address, e-mail, telephone, facsimile, etc.) of organizations or individuals with particular expertise on the pest(s), which may be consulted regarding any questions on the DP. These contacts must agree to act in this capacity prior to their inclusion in the DP.

It might be useful to have a global coverage when possible, or at least contacts in several regions. However the center of excellence might be in one region, and contacts from one region only might be indicated in this case. In general, it is preferable to avoid mentioning two contacts from the same country, except if they have very specific expertise and no contact is available elsewhere. The Secretariat can also be mentioned, in case none of the contact points can be reached.

Wording from ISPM 27 on requests for revision to the DP should also be added (see below and in Appendix 1).

Standard text to be used:

Further information on this protocol can be obtained from:

[name of institutes and contacts in the format: Unit, institute, complete mailing address, country (full name of expert; e-mail; tel. +XX etc.; fax: +XX etc.)].

A request for a revision to a diagnostic protocol may be submitted by national plant protection organizations (NPPOs), regional plant protection organizations (RPPOs) or Commission on Phytosanitary Measures (CPM) subsidiary bodies through the IPPC Secretariat (ippc@fao.org), which will in forward it to the Technical Panel on Diagnostic Protocols (TPDP)."

4.7 Acknowledgements

In this section, the name (initials) and address of the experts who wrote the first draft of the DP are given, together with those of any others who made major contributions. This list should be finalized in consultation between the lead author and the discipline lead. The inclusion of names in the acknowledgements should be at the discretion of the discipline lead in consultation with the lead author. In instances where these experts are the same individuals as those listed in the preceding section, the

details should be cross-referenced. Only those significantly involved in the development of the draft should be included in this section.

If drawings or illustrations were produced especially for the protocol, they can be acknowledged here. In addition, special contributions may be mentioned here, for example those experts that made extensive comments on the draft or when the draft protocol made extensive use of work done by others (e.g. ring-testing).

Standard text to be used:

The first draft of this protocol was written by [initials, family name (unit, institution, country, (see preceding section))]. In addition, the following experts were significantly involved in the development of this protocol [initials, family name (unit, institution, country, (see preceding section))].

4.8 References

ISPM 27 states:

> References to accessible scientific publications and/or published laboratory manuals are given that may provide further guidance on the methods and procedures contained in the diagnostic protocol.

In this section, relevant references to scientific publications and published laboratory manuals cited in the text should be given. The references should be kept to a minimum and should concern the diagnosis of the pest and species with which the pest may be confused, its symptomatology 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, although reference may be made to key publications which review this information, e.g. pest data sheets. The number of references included will vary between DPs, but preferably the list should include fewer than 40 references. See the guidelines in the Appendix 2 to these Instructions to authors for the format of references.

In its November meeting 2014[3], the Standard Committee agreed to add the following standard paragraph to refer to the ISPMs cited in the drafts: "*The present standard also refers to other International Standards for Phytosanitary Measures (ISPMs). ISPMs are available on the IPP at https://www.ippc.int/core-activities/standards-setting/ispms ''.*

[3] 2014 November SC meeting report: https://www.ippc.int/core-activities/standards-setting/standards-committee

APPENDIX 1 OF INSTRUCTIONS TO AUTHORS: STANDARDIZED TEMPLATE FOR DIAGNOSTIC PROTOCOLS

(TPDP, June 2013 Noted by SC May 2015. Revised by TPDP June 2015)

This standardized template is intended to help authors of diagnostic protocols (DPs) when drafting an IPPC diagnostic protocol. The *Instructions to authors* contain information and guidance on the content and formatting of protocols, as well as combination of methods in DPs. Required text is provided in black. Text to be completed by the author and guidance on how to complete it is between square brackets with guidance in italics. Text for completion by the Secretariat or TPDP lead is in square brackets and highlighted in grey. Examples are in boxes in pale green. Authors may use this file to write their draft protocol and can then remove all italics, boxed and highlighted text. A checklist for authors is included as Appendix 3 of the *Instructions to authors*, to cross-check the content of the draft once written.

DRAFT ANNEX to ISPM 27– [Pest name] [(Topic number)] (*Add the scientific name of the pest and authority where required (no authority should be listed for viruses and viroids). Note: the year of naming is not relevant in the title. Secretariat will add the topic number of the subject from the List of topics for IPPC standards*)

Examples
DRAFT ANNEX to ISPM 27- *Tilletia indica* Mitra (2004-014)
DRAFT ANNEX to ISPM 27– *Potato spindle tuber viroid* (2006-022)
DRAFT ANNEX to ISPM 27– *Erwinia amylovora* (Burrill) Winslow *et al.* (2004-009)

Status box
(*Include the table below and complete relevant parts. Secretariat and TPDP lead to complete additional parts as appropriate.*)

Date of this document	[to be completed by the Secretariat]
Document category	Draft new annex to ISPM 27:2006 (*Diagnostic protocols for regulated pests)*
Current document stage	[to be completed by the Secretariat]
Origin	Work programme topic: [Topic (date of addition by CPM)] Original subject: [Name (number)]
	Example Work programme topic: Fungi and fungus-like organisms, CPM-1 (2006) Original subject: *Tilletia indica* / *T. controversa* (2004-014)
Major stages	[to be completed by the Secretariat]
Consultation on technical level	The first draft of this diagnostic protocol was prepared by: [first name, FAMILY NAME of lead author (unit, institute, city, ISO code of country) and co-authors] (*List the lead author and co-authors – complete addresses are not needed, but the unit, institute, city, country should be mentioned)*
	Example Dominie Wright (Department of Agriculture and Food of Western Australia, Perth, Australia); Guiming Zhang (Laboratory of Plant Inspection and Quarantine, Shenzhen Entry-Exit Inspection and Quarantine Bureau, Shenzhen City, China).
	(*Add, as appropriate, name of all experts who, although not part of the initial DP drafting group, contributed to the drafting or commented on the draft, as follows:*) - In addition, [names of experts (first name, family name (unit, institute, city, country))] [was/were] significantly involved in the development of this protocol.

	- This protocol has been commented upon by: [names of experts (first name, family name (unit, institute, city, country))] (*Also, other relevant information can be mentioned here, for example*:) - This draft protocol was adapted from a protocol originally drafted by: [names of experts (first name, family name (unit, institute, city, country))] - It was presented at the [e.g. conference/symposium on (name, place), date], and further comments were provided by: [names of experts (first name, family name (unit, institute, city, country))]
Main discussion points during development of the diagnostic protocol [to be updated throughout DP development]	[to be completed by the TPDP lead] • • (*Note: Especially after experts have been consulted at early stages of development, the cover note should indicate substantial comments that were not incorporated in the draft. Include as bullet points)*
Notes	[to be completed by the Secretariat]

Contents *[to be added later]*

This section, the IPPC editor and formatter will adjust later.

Section on endorsement

The first section of the standard should be added as follows:

Adoption

This diagnostic protocol was adopted by the Commission on Phytosanitary Measures in ----. [to be completed after adoption]

1. PEST INFORMATION

[Insert pest information text] *(See section 4.1 of the Instructions to authors*)

Example. *Thrips palmi*

Thrips palmi Karny (Thysanoptera: Thripidae) is a polyphagous plant pest, especially of species in the Cucurbitaceae and Solanaceae. It appears to have originated in Southern Asia and to have spread from there during the latter part of the twentieth century. It has been recorded throughout Asia and is widespread throughout the Pacific and the Caribbean. It has been recorded locally in North, Central and South America and Africa. For more general information about *T. palmi*, see EPPO/CABI (1997) or Murai (2002); online pest data sheets are also available from the Pests and Diseases Image Library (PaDIL, 2007) and EPPO (EPPO, 2008).

The species causes economic damage to plant crops both as a direct result of its feeding activity and from its ability to vector tospoviruses such as *Groundnut bud necrosis virus*, *Melon yellow spot virus* and *Watermelon silver mottle virus*. It is extremely polyphagous, and has been recorded from more than 36 plant families. It is an outdoor pest of, amongst others, *Benincasa hispida*, *Capsicum annuum*, *Citrullus lanatus*, *Cucumis melo*, *Cucumis sativus*, *Cucurbita* spp., *Glycine max*, *Gossypium* spp., *Helianthus annuus*, *Nicotiana tabacum*, *Phaseolus vulgaris*, *Pisum sativum*, *Sesamum indicum*, *Solanum melongena*, *Solanum tuberosum* and *Vigna unguiculat*a. In glasshouses, economically important hosts are *Capsicum annuum*, *Chrysanthemum* spp., *Cucumis sativus*, *Cyclamen* spp., *Ficus* spp., Orchidaceae and *Solanum melongena*. The thrips may be carried on plants for planting, cut flowers and fruits of host species, as well as on or associated with packing material, and in soil.

Thrips palmi is almost entirely yellow in coloration (Figures), and its identification is hampered by both its small size (1.0–1.3 mm) and its great similarity to certain other yellow or predominantly yellow species of *Thrips*.

2. TAXONOMIC INFORMATION

(Use the standardised text below and see section 4.2 of the Instructions to authors). Note: Species names are always italicised, family and other names are not (apart from family names for viruses and viroids, which are italicised).

Name: [Scientific name, authority and date]
Synonym (*or*) Synonyms: [Scientific name, authority and date.]
(*delete as appropriate)*
Taxonomic position: [insert taxonomic information]
Common name (*or)* Common names: [English common name(s), and reference, where available, to names in other languages]
(*delete as appropriate*)
Reference: [for fungi a reference to Mycobank may be included]

Examples - Insects

Name: *Thrips palmi* Karny, 1925
Synonyms: *Thrips gossypicola* Ramakrishna & Margabandhu, 1939
Taxonomic position: Insecta, Thysanoptera, Terebrantia, Thripidae
Common name: melon thrips

Name: *Trogoderma granarium* Everts, 1898
Synonyms: *Trogoderma khapra* Arrow, 1917
Trogoderma koningsbergeri Pic, 1933
Trogoderma afrum Priesner, 1951
Trogoderma granarium ssp. *afrum* Attia and Kamel, 1965
Taxonomic position: Insecta: Coleoptera: Dermestidae.
Common names: khapra beetle (English)

Examples – Virus and viroids

Name: *Plum pox virus* (acronym PPV)
Synonym: Sharka virus
Taxonomic position: *Potyviridae*, *Potyvirus*
Common names: Sharka, plum pox.

Examples – Bacteria

Name: '*Candidatus* Liberibacter solanacearum' (Liefting et al., 2009)
Synonym: '*Candidatus* Liberibacter psyllaurous' (Hansen et al., 2008)
Taxonomic position: Bacteria, Proteobacteria, Alphaproteobacteria, Rhizobiales, Rhizobiaceae, Candidatus Liberibacter
Common names: zebra chip (English), zebra complex (English), psyllid yellows (English)

3. DETECTION

[Insert text on detection of the pest] *(See sections 3 and 4.3 of the Instructions to authors)*

After the main heading, **3. Detection**, *insert introductory paragraphs, and organise the methods using the structure below. The headings should be used as required (numbering for illustrative purposes only). It is not possible to provide standardized text in this section, but examples can be found in adopted protocols.*

*Where detection and/or identification methods are different for plants with symptoms and plants without symptoms, consider separating 3. into "*3.1 Detection in symptomatic plants*" and "*3.2 Detection in asymptomatic plants*", and use the structure below for each of them.*

Where the use of brand name:

a) include the standard paragraph before the first mention, as below:

> In this diagnostic protocol, methods (including reference to brand names) are described as published, as these define the original level of sensitivity, specificity and reproducibility achieved. The use of names of reagents, chemicals or equipment in these diagnostic protocols implies no approval of them to the exclusion of others that may also be suitable. Laboratory procedures presented in the protocols may be adjusted to the standards of individual laboratories, provided that they are adequately validated.

b) in the first mention to a brand name, include the following footnote:

FOOTNOTE: "The use of this specific brand in this diagnostic protocol implies no approval of them to the exclusion of others that may also be suitable. This information is given for the convenience of users of this protocol and does not constitute an endorsement by the CPM of the chemical, reagent and/or equipment named. Equivalent products may be used if they can be shown to lead to the same results."

c) If in the DP there is more than one mention to a brand name, the second mention (and the sub sequential mentions) to a brand name shall be associated with the footnote number with the full text (e.g. If the first mention to a brand name is footnote 1, the subsequent mentions to brand names should be accompanied by the same footnote number, i.e., footnote number 1).

3.1 Symptoms

3.2 Sampling and sample preparation [symptomatic and asymptomatic material]

[Insert text on sampling and sample preparation]

(If methods for preparation of material are generic for all methods, it may be appropriate to include text on preparation of material in a general section at the beginning. Alternatively, if preparation of material relates to a group of methods it may be appropriate to include text associated with each type of methodology. Otherwise, where preparation of material is specific to a method, it should be included with the method description. See also section 4.3 of the Instructions to authors.)

3.3 Isolation [and culturing/growing] [from symptomatic material /from asymptomatic material]

3.3.1 [Name of method] e.g. Enrichment isolation

3.3.2 [Name of method] etc.

3.4 Biological detection

3.5 Serological detection

3.5.1 Preparation of material

(*If relevant, see note at 3.2*)

3.5.2 [Name of method] e.g. Double antibody sandwich indirect enzyme linked immunosorbent assay (ELISA)

3.5.3 [Name of method] e.g. Immunofluorescence (IF)

3.6 Molecular detection

3.6.1 Preparation of material

(*If relevant, see note at 3.2*)

3.6.2 Nucleic acid extraction

3.6.3 [Name of method] e.g. Conventional reverse transcription-polymerase chain reaction using the primers of Verhoeven et al. (2004)

3.6.4 [Name of method] e.g. Immunocapture reverse transcription-polymerase chain reaction

3.6.5 Controls for molecular tests

[Insert the following standardized text]

For a reliable test result to be obtained the following controls should be considered for each series of nucleic acid isolations, amplification of the target pest or target nucleic acid depending on the test used and the level of certainty required. As a minimum, for [method name] the [name minimum controls, e.g.

positive nucleic acid control, internal control and negative amplification control (no template control)] should be used.

(*The rest of this section should provide a brief description of the controls. The minimum controls should be listed first, in the same order as they are named previously. Additional controls, if any, should be at the end. For each control, give additional details as necessary, e.g. specific controls named in individual methods in the protocol, etc.*)

Positive nucleic acid control

This is used to monitor the efficiency of the test method (apart from the extraction) [and with RT-PCR, the amplification]. [Description of the controls, e.g. Pre-prepared (stored) viroid nucleic acid, whole genome amplified DNA or a synthetic control (e.g. cloned PCR product)] may be used.

Internal control

For [method name(s)], plant internal controls [name(s) of gene(s) e.g. House Keeper Gene (HKG) such as COX or NAD] should be incorporated into the protocols to eliminate the possibility of PCR false negatives due to extraction failure, nucleic acid degradation or the presence of PCR inhibitors. Preferably the internal control primers should be used [add details, e.g. in a duplex reaction with the pospiviroid/PSTVd primers].

(*Add any qualifying information e.g. difficulties that may be encountered, effects on sensitivity, notes on the part of the assay that the gene acts as a control for e.g. with RT-PCR assays. Also examples of successful use of internal controls if known or relevant and not already referred to in the method descriptions in other sections.*)

When the internal control [name of gene] is not mentioned in the description of a PCR method, the laboratory should choose an internal control and validate it.

Negative amplification control (no template control)

This is necessary with conventional and real-time RT-PCR to rule out false positives due to contamination during the preparation of the reaction mix. PCR grade water that was used to prepare the reaction mix is added at the amplification stage.

Positive extraction control

This is used to ensure that nucleic acid from the target is of sufficient quantity and quality and that the target is detected. Nucleic acid is extracted from infected host tissue or healthy plant tissue that has been spiked with the target.

The positive control should be approximately 1/10 of the amount of [type of material e.g. leaf tissue] used per plant for the [RNA/DNA] extraction. (*Add any other relevant elements, on e.g. adjustments to quantity, amounts of control material to use for different bulking rates etc. and if this control is not detected, provide guidance on repeating tests or adjusting the bulking rate until reliable detection is achieved.*)

In the case of a high risk of aerosol contamination, and for specific pest, please consider if instructions should be provided to monitor possible cross contamination, e.g. compare the sequences of positive controls and positive samples.

Negative extraction control

This is used to monitor contamination during nucleic acid extraction and/or cross-reactions with the host tissue. This requires nucleic acid extraction and subsequent amplification of uninfected host tissue. It is recommended to include multiple controls when large numbers of positives are expected.

3.6.6 Interpretation of results from [Name of methods]

(*Insert as a separate section only if necessary*)

4. IDENTIFICATION

[Insert text on identification methods] *(See Section 3 and 4.4 of the Instructions to authors*)

It is not possible to provide standardized text in this section, but examples can be found in adopted protocols.

After the main heading, **4. Identification**, *insert introductory paragraphs, and use the structure below. Use the following headings as required (numbering for illustrative purposes only).*)

4.1 Morphological identification (Note: for insects, fungi, nematodes, plants)

4.1.1 Preparation of [developmental stage e.g. larvae, adults, seeds, plant material, teliospores] for examination (If necessary, normally for insects.)

4.1.2 Isolation [and culturing/growing] of [name of pest]

4.1.2.1 [Name of method] e.g. Germination of teliospores, Germination of similar Tilletia species

4.1.3 Identification of [developmental stage e.g. larvae, adults of] [family, genus, name of pest]

4.1.4 [Differentiation of / morphological comparison with] [developmental stage e.g. larvae, adults of][family, genus, name of pest] from similar species

[Insert simple key, table or text with relevant details]

4.1.5 Discriminating features of [developmental stage e.g. larvae, adults, name of pest] [of family, genus, name of pest]

[Insert checklist of key diagnostic features]

(*Add additional sections (and renumber) depending on the level of discrimination e.g. family, genus, species*.)

e.g. Table 1: Family Thripidae – shared characteristics

Body part	Characteristic
Antennae	seven or eight segments (occasionally six or nine)
	segments III–IV have emergent sense cones (sensoria)
Forewings (if fully developed)	usually slender, with two longitudinal veins each bearing a series of setae
Abdomen – female	with a serrated ovipositor, which is turned downwards at the apex
Median sternites – male	with or without glandular areas

Examples of structure of 4. Identification - ***Thrips palmi (morphological section only)***

General introductory paragraphs

4.1 Morphological identification of the adult thrips
4.1.1 Preparation of thrips for microscopic examination
4.1.2 Identification of the family Thripidae
Table 1: Family Thripidae – shared characteristics
Table 2: Genus *Thrips* – shared characteristics, adult specimens
4.1.4 Identification of *Thrips palmi*
4.1.4.1 Morphological characteristics of *Thrips palmi*

	Table 3: A list of morphological characteristics that collectively distinguish *Thrips palmi* from other species in the genus *Thrips*
4.1.4.2	Comparison with similar species (species that are yellow without darker body markings, or predominantly yellow, or sometimes yellow)
	Table 4: Simplified checklists of the diagnostic features for quick recognition: (a) the genus *Thrips*; (b) *Thrips palmi* (See Figure 4 for the location of the various features.)

4.2 Biological identification of [name of pest, strains, pathotypes]

(*For subsequent sections (Biological identification, Serological identification and Molecular identification) follow the same structure as in given in section 3. In addition sections on Identification using Nutritional and enzymatic tests or Biochemical identification methods may be required. If some elements are already described adequately in 3 (e.g. preparation of material, nucleic acid extraction, specific methods), do not repeat but cross-refer to the relevant subsection number.*)

4.2.1 Pathogenicity tests

4.3 Serological identification

4.3.1 Preparation of material

(*If relevant, see note at 3.2*)

4.3.2 [Name of method] (insert new section for each method)

4.4 Molecular identification

4.4.1 Preparation of material

(*If relevant, see note at 3.2*)

4.4.2 Nucleic acid extraction

4.4.3 [Name of method] (insert new section for each method)

4.4.4 Controls for molecular tests

[Insert standardized text from 3.6.5 with appropriate modification] Insert this section only if necessary i.e. if controls used for detection tests are different to those for identification.)

4.4.5 Interpretation of results from [Name of methods]

(*Insert text only if necessary and if interpretation of results is different when methods are used for identification rather than detection.*)

5. RECORDS

(*Include the following standardized text:*)

Records and evidence should be retained as described in section 2.5 of ISPM 27.

(*Add additional paragraph(s) as required in individual DPs. For example:)*

In cases where other contracting parties may be affected by the results of the diagnosis[, in particular in cases of non-compliance (ISPM 13:2001, *Guidelines for the notification of non-compliance and emergency action*) and where [the pest, name of pest] is found in an area for the first time,] the following records and evidence and additional material should be kept for at least one year in a manner that ensures traceability: [the original sample, larvae and adults, preserved or slide-mounted specimens, culture(s) of the pest, [RNA, DNA] extracts, printed tissue sections and/or spotted plant extracts on paper or nylon membranes, PCR amplicons or test materials (e.g. photographs [of distinctive taxonomic structures, fungal structures, symptoms and signs], ELISA plate results printouts and photographs of gels].

(*Additional specific text may be added. For example details on of sample and records may be required e.g. storage temperature (at −80 °C or freeze-dried and stored at room temperature) or culture conditions (e.g. mycelium from broths or mycelial plugs from agar plates can be stored frozen at −80 °C). Guidance may be included on handling isolates shown to have different molecular or biological characteristics compared to previously recorded isolates (e.g. offered to a national pest herbarium). Also, if there is evidence of any of the tests described failing to detect an isolate, authors may propose that details should be sent to the IPPC Secretariat.*

In some cases, records of the number of positive subsamples and the estimated number of [telio]spores detected in each positive subsample may need to be kept and, for fungi, records of colony morphology, especially any pigmentation and growth rate under defined conditions, may need to be kept.)

6. CONTACT POINTS FOR FURTHER INFORMATION

(*Add the following standardized text. See section 4.6 of the* Instructions to Authors.)

Further information on this protocol can be obtained from [name of institutes and contacts in the format: Unit, institute, complete mailing address, country (full name of expert; e-mail; Tel +XX etc.; Fax: +XX etc.)].

A request for a revision to a diagnostic protocol may be submitted by national plant protection organizations (NPPOs), regional plant protection organizations (RPPOs) or Commission on Phytosanitary Measures (CPM) subsidiary bodies through the IPPC Secretariat (ippc@fao.org), which will be forwarded it to the Technical Panel on Diagnostic Protocols TPDP.

Examples

Faculty of Horticultural Science, Department of Plant Pathology, Corvinus University, Villányi út 29-43, H-1118 Budapest, Hungary (Laszlo Palkovics, e-mail: laszlo.palkovics@uni-corvinus.hu; tel.: +36 14825438; fax: +36 14825023).

Department of Agriculture and Food Western Australia, Biosecurity & Research Division, Plant Biosecurity Branch, Entomology Unit, 3 Baron-Hay Court, South Perth, WA 6151, Australia (Andreas Szito, -e-mail: aszito@agric.wa.gov.au; tel: +61 8 9368 3248, +61 8 9368 3965; fax: +61 8 9368 3223, +61 8 9474 2840).

Pest and Disease Identification Team, The Food and Environment Research Agency, Sand Hutton, York YO41 1LZ, United Kingdom. (Dom Collins; e-mail: dom.collins@fera.gsi.gov.uk; tel: +44 1904 462215; fax: +44 1904 462111).

7. ACKNOWLEDGEMENTS

(*Add the following standardized text indicating the experts that first drafted the text and those that made significant contributions. If the address was already mentioned in section 6, add "(see preceding section)"*)

The first draft of this protocol was written by [initials, family name (unit, institution, country, (see preceding section))]. In addition, the following experts were significantly involved in the development of this protocol [initials, family name (unit, institution, country, (see preceding section))].

(as relevant, use standardized text below – See section 4.7 of the Instructions to authors)

[Line drawings, Illustrations] for Figure [number] were produced by [name and address of expert]. The methods included in the protocol were ring tested by [names of experts or project and date] financed by [name of country organization and date].

(if relevant add other acknowledgements as necessary – see examples below)

Example

The first draft of this protocol was written by M. Cambra, IVIA, Spain (see preceding section); N.L. Africander, Department of Agriculture, Forestry and Fisheries, Private Bag X 5015, Stellenbosch, 75999, South Africa; L.

Levy, USDA, USA (see preceding section); S.L. Lenardon, IFFIVE-INTA, Cno. 60 Cuadras Km 51/2, Córdoba X5020ICA, Argentina. In addition, the following experts were significantly involved in the development of this protocol: A. Olmos and N. Capote, IVIA, Spain (see preceding section); G. Clover, Plant Health & Environment Laboratory, Ministry of Agriculture and Forestry, PO Box 2095, Auckland 1140, New Zealand; and D. Wright, Plant Health Group, Central Science Laboratory, Sand Hutton, York YO41 1LZ, United Kingdom. Line drawings for Figure 5 were produced by S. Kobro, Norwegian Crop Protection Institute, Norway.

Additional acknowledgements:

Tilletia indica [from draft DP]

The basis of this protocol was originally drafted by A.J. Inman, K.J.D. Hughes and R.J. Bowyer (2003), Food and Environment Agency, York, UK. That protocol was ring-tested in different European laboratories (Riccioni *et al*., 2002), and has formed the basis of the EPPO protocol PM 7/29(1) (EPPO, 2004).

The protocol has been enhanced by D.G. Wright, Department of Agriculture and Food, Western Australia, Australia; K.J.D Hughes, Food and Environment Agency, Sand Hutton, York, United Kingdom; and Guiming Zhang, Laboratory of Plant Inspection and Quarantine, Shenzhen City, China. V. Cockerell, Science and Advice for Scottish Agriculture, Edinburgh (United Kingdom) reviewed the protocol.

Erwinia amylovora [from draft DP]

Most techniques described were ring tested in a DIAGPRO project financed by the EU, in an EUPHRESCO project in 2009, and in a Spanish project in 2010.

PSTVd [from draft DP]
Thanks are due to S.L. Nielsen (Denmark), L. Seigner, S. Winter, M. Wassenegger (Germany), H. Koenraadt (The Netherlands), A. Fox, T. James, W. Monger, V. Mulholland (UK) for helpful comments during development of this protocol.

8. REFERENCES

[Insert references]

The following standard text should be provided before listing the refences:

"The present standard also refers to other International Standards for Phytosanitary Measures (ISPMs). ISPMs are available on the IPP at https://www.ippc.int/core-activities/standards-setting/ispms"

(*Provide a list of scientific references and other publications referred to in the protocol (see 4.8 in the* Instructions to Authors*)*

9. FIGURES

[Insert figures if necessary]

(*See section 3 in the* Instructions to Authors, as well as Appendix 3.)

Examples of figure legends
Figure 1: *Thrips palmi*, female (left) and male (photo: A. J. M. Loomans, PPS, Wageningen, the Netherlands; scale bar = 500 µm = 0.5 mm)
Fig. 5.11(a), (b): Abdominal tergite IX (dorsal), two pairs of campaniform sensilla (scale bar: 30 µm)
Figure 2: *Trogoderma granarium*: (A) adult, female; (B) comparison of shape of female (left) and male (right); (C) young larva; (D) mature larva. Scale bar: (A), (B), (D) = 2 mm; (C) = 1 mm. ((A), Tomasz Klejdysz, Instytut Ochrony Roślin - Państwowy Instytut Badawczy, Poznań, Poland; (B), (D), Ya.B. Mordkovich and E.A. Sokolov, All-Russian Plant Quarantine Centre, Bykovo Russia); (C), Cornel Adler, Julius Kűhn-Institut; (JKI) Germany))
Figure 1. Flow diagram showing the process to be used for the detection and identification of *Tilletia indica* in seed and grain samples

APPENDIX 2 OF INSTRUCTIONS TO AUTHORS: GUIDELINES ON FORMATTING OF DIAGNOSTIC PROTOCOLS

General guidelines on formatting of ISPMs are given in the "Administrative guidelines for the structure of standard-setting documentation" in the IPPC Procedural Manual, which can be found on the internet on the IPP (https://www.ippc.int/index.php?id=159891).This Appendix partly uses these guidelines but also gives additional recommendations that are specific to DPs. A standardized format for protocols is also under development.

1. FIRST PAGE

The first page should contain:

- a reference to ISPM 27 (*Diagnostic Protocols for Regulated Pests*) (i.e. "Annex to ISPM 27")
- the title of the draft protocol
- a cover note in the format of Appendix 1, indicating experts/countries that have written and reviewed the draft, and any main discussion points that have arisen and been resolved.
- a table of contents, listing all numbered headings and subheadings. At the drafting stage, the table of contents should be in the protocol, but it is not necessary to indicate page numbers.

2. MAIN TEXT

Numbered headings and sub-headings

Individual sections are detailed in the instructions on formatting of ISPMs above. Headings, sub-headings and further subdivisions should be numbered with Arabic numbers, for example: 1.1, 1.2.1, 1.3.2.2, etc.

Titles of level one (1., 2. etc) have a capital letter at the beginning of each word. Other numbered titles have only one capital letter at the beginning of the title.

Use of illustrations and tables

All illustrations (i.e. photographs, line drawings, flow diagram) and tables should be numbered with Arabic numbers and should be referred to in the text.

Figures/tables and text should match, i.e. all figures/tables should be referred to in the text, or should not be in the protocol. If a figure refers to several separate elements/characters, these elements should also be cross-referred to in the text. The flow diagram should indicate, for each method, the section number under which it is described.

For reason of file size, all complete figures (i.e. with images/captions/associated text) should not be in the main text of the protocol, but should be provided to the discipline lead as a separate Word file. Tables should remain with the text of the protocol.

All photographs, or specially drafted or reproduced illustrations should have an attribution. The text may be small type size and oriented vertically at the side of a photograph or it may be included in the caption of an illustration.

Illustrations should be of a sufficient quality for printing. A high quality file of each illustration should be provided, separately from the text, to the IPPC Secretariat. Detailed guidance is provided below:

(1) Ensure that images (photographs, diagrams, etc.) have a resolution of 300 dpi for sharp printing, and that the printed image is clear, illustrative for the purpose and of sufficiently high quality.

(2) Reduce images (at 300 dpi) to the smallest final dimensions that convey the necessary information in the image (5-8 cm is considered as a good width for most illustrations). If full page illustration is needed, maximum width is 16 cm)

(3) Crop all unnecessary parts of the image

(4) Ensure all texts concerning the image (explanatory detail with arrows or call-outs etc) is part of the caption and/or are linked together (A lot of separate boxes with details of identification of image number and insect parts poses a great risk of error.)

(5) At a late stage of development (when member comments are integrated and the protocol is being prepared for adoption, i.e. once the figures will not change anymore), also provide all figures/photographs as separate TIF or JPG files (compliant with a, b, c above), so that they can be further processed to achieve the optimal file size and quality.

Use of footnotes

Use of footnotes should be limited to increase readability of the text. If footnotes are nevertheless needed, they should be numbered with Arabic numbers. Note. A separate footnote is needed at each mention of a brand name (see section 3).

Terminology

- Phytosanitary terms should be used according to the most recent version of the ISPM 5: *Glossary of phytosanitary terms.*
- The general dictionary reference for English ISPMs is the Oxford English dictionary.
- Use organize, authorize and recognize (and not organise, authorise or recognise).
- Use website and not Web site or Website.

Scientific names

- Family names are italicized only for viruses and viroids (i.e. not for insects, bacteria etc.).
- Indicate the author after the first occurrence (in the text) of the Latin name of a pest.
- The species name should be written in full at its first occurrence, e.g. *Thrips palmi*, and shortened at others: *T. palmi*. If another species of the same genus are mentioned later in the text, it is not necessary to write the genus name in full, e.g. *T. flavus*. However, in cases where abbreviating the genus is confusing, the name can be given in full, for example if another genus starting with the same letter is mentioned in the same paragraph (example: "Hosts include *Triticum aestivum* (wheat) …*T.[Tilletia] indica* has been shown to infect other …).
- Latin names are italicized (but not spp., sp. etc.)
- Use Latin names for host plants (common names may be indicated between brackets at first occurrence if appropriate).

Measurement units

When measurement units are abbreviated, the standard abbreviation should be used, e.g.:

m	meter
s	second
W	watt
min	minute
litre	litre
ml	milliliter
µl	microliter

There should be a space between the number and the unit.

Other specific formatting

- Gene names are italicized when written in full, except the gene number (e.g. *NADH dehydrogenase* 5 gene)
- Acronyms should be written in full at the first mention.

Lists of items

See *Thrips palmi*.

List of references

References should be in alphabetical order.

References to other ISPMs and the IPPC are detailed in the procedural manual, but usually not needed in protocols. Regarding scientific references and other publications, some examples are given below. Attention is drawn to the fact that the total number of pages should be included for references to books.

Article in a journal or proceedings

Bhatti, J.S. 1980. Species of the genus *Thrips* from India (Thysanoptera). *Systematic Entomology*, 5: 109–166.

Brunner, P.C., Fleming, C. & Frey, J.E. 2002. A molecular identification key for economically important thrips species (Thysanoptera: Thripidae) using direct sequencing and a PCR-RFLP-based approach. *Agricultural and Forest Entomology*, 4: 127–136.

Kox, L.F.F., van den Beld, H.E., Zijlstra, C. & Vierbergen, G. 2005. Real-time PCR assay for the identification of *Thrips palmi*. *Bulletin OEPP/EPPO Bulletin*, 35: 141–148.

Mordkovich, Ya.B. & Sokolov, E.A. 2000. Выявление капрового жука в складских помещниях, *Защита и* карантин *растиений*, 12: 26–27 [in Russian].

Mound, L.A. & Morris, D.C. 2007. A new thrips pest of *Myoporum* cultivars in California, in a new genus of leaf-galling Australian Phlaeothripidae (Thysanoptera). *Zootaxa*, 1495: 35-45.

Books or conference proceedings

Mound, L.A. & Kibby, G. 1998. *Thysanoptera. An Identification Guide*. 2nd edition. Wallingford, UK, CAB International. 70 pp.

Nakahara, S. 1994. The genus *Thrips* Linnaeus (Thysanoptera: Thripidae) of the New World. USDA Technical Bulletin No. 1822. 183 pp.

Sakimura, K., Nakahara, L.M. & Denmark, H.A. 1986. A thrips, *Thrips palmi* Karny (Thysanoptera: Thripidae). Entomology Circular No. 280. Division of Plant Industry, Florida; Dept. of Agriculture and Consumer Services. 4 pp.

Section from a book

Barba, M., Hadidi. A., Candresse. T. & Cambra, M. 2011. Plum pox virus. In: A. Hadidi, M. Barba, T. Candresse & W. Jelkmann, eds. Virus and virus-like diseases of pome and stone fruits, Chapter 36. St. Paul, MN, APS Press. 428 pp.

EPPO/CABI. 1997. *Thrips palmi*. *In* I.M. Smith, D.G. McNamara, P.R. Scott & M. Holderness, eds. *Quarantine Pests for Europe*, 2nd edition. Wallingford, UK, CAB International. 1425 pp.

CD-Rom:

Moritz, G., Mound, L.A., Morris, D.C. & Goldarazena, A. 2004. Pest thrips of the world: visual and molecular identification of pest thrips (CD-ROM), Centre for Biological Information Technology (CBIT), University of Brisbane. ISBN 1-86499-781-8.

Article from proceedings

Murai, T. 2002. The pest and vector from the East: *Thrips palmi*. *In* R. Marullo, & L.A. Mound, eds. *Thrips and Tospoviruses: Proceedings of the 7th International Symposium on Thysanoptera*. Italy, 2–7 July 2001, pp. 19–32. Canberra, Australian National Insect Collection.

Internet documents or websites

EPPO. 2008. URL: http://www.eppo.org/ (accessed 17 June 2008).

PaDIL. 2007. Pests and Diseases Image Library. URL: http://www.padil.gov.au (accessed 18 Oct 2007.

USDA (United States Department of Agriculture). 2004. *Minimum sanitation protocols for offshore geranium cutting production*. APHIS-PPQ Pest Detection and Management Programs. 27 pp. Available at http://www.aphis.usda.gov/plant_health/plant_pest_info/ralstonia/downloads/ralstoniaworkplan.pdf (accessed January 2010).

APPENDIX 3 OF INSTRUCTIONS TO AUTHORS: CHECKLIST FOR AUTHORS OF DIAGNOSTIC PROTOCOLS

(TPDP November 2012, noted by SC May 2013. Revised by TPDP June 2013, noted by SC May 2015. Reviewed by TPDP June 2015.)

The headings of the protocol are as in Appendix 2 (Template for diagnostic protocols). Data used for DP are public available.

Title

- Use scientific name, with authority were relevant, scope indicated.

Consultation on technical level

- The detailed information provided in Appendix 2 (Standardized template for diagnostic protocols) is followed, and include a statement at the beginning of the protocol to indicate when it was drafted.

Pest information

- Geographic information is general, not by country, terms are carefully used (present, recorded, established etc).
- Latin name for the pest is used and include author(s) after the first occurrence of the name of the pest only.
- Latin names for hosts are used, italic on or below species level, higher ranks roman.
- All general information on the pest (biology, hosts, etc) is grouped in this section exclusively, max. 1 page. References to datasheets are included, no details on epidemiology or disease management.
- Subjective terms (e.g. (significant) economic impact) are avoided.

Taxonomic information

- Reference that is used for the names indicated in this section is mentioned.
- Scientific name is used throughout the protocol, common name is indicated only in this section once in English, reference to common names in other languages is advised.
- The relevant code of nomenclature is followed.
- No journal citations are given after names.
- Synonyms included are the important ones only, listed by chronological order.
- Species names are italic, higher ranks normal.
- Species are mentioned in full, genus is abbreviated at further occurrences, unless there might be a confusion with other generic names starting with the same letter.

Detection

- Text and flow diagram: both should be in line, text includes the steps/methods with their advantages and limitations, whether including a flow diagram is really essential is considered, minimum requirements are clearly indicated, the scope of the diagnoses is clearly defined.
- The flow diagram is not intended to be a decision scheme.
- Section dealing with the controls included.
- Combined detection/identification methods are described in this section, and refer to this under identification section.

- Sampling information is provided only for laboratory analysis, not for inspection (except for seed/grain testing, where relevant additional information could be provided).
- The necessary of the use of commercial kits/brand names is checked (i.e. use microtubes instead of eppendorf).
- common laboratory procedures, e.g. handling of samples, quarantine requirements, facilities, are not detailed in the text.
- All methods included are relevant for the diagnosis.
- The reasons for using a combination of methods are provided.
- Addition of a second method is evaluated following 1 (ISPM 27).
- When several methods are mentioned, their (dis)advantages are given (1).
- Method descriptions are not written as standard operation procedures.
- Reference to manufacturer's instructions in method descriptions are provided, the choices of manufacturers are explained or provided with a disclaimer.
- specificity data .
- Controls for the methods used.
- Test results performance studies.
- Specification of pH, temperature range or exact.

Identification

- Text and flow diagram: Both should be in line, text includes the steps/ methods with their advantages and limitations, whether including a flow diagram is really essential is considered, minimum requirements are clearly indicated, the scope of the diagnoses is clearly defined.
- The flow diagram is not intended to be a decision scheme.
- Guidance for interpreting sequencing results are provided.
- Relevant information on preservation is provided (kind of material, period).
- The reasons for using a combination of methods are provided.
- Results of performance criteria sensitivity, specificity and reproducibility, and data of ring tests are included or referred.
- Use of tables for morphological characters is considered.
- Control for the methods used.
- Interpretation of the results.
- Results of test performance studies.

Records

- Reference to section 2.5 of ISPM 27 is included, relevant additional records and evidence that should be maintained in case were other NPPO's may be involved are mentioned.

Contact points for further information

- Contacts are from several regions and appropriate, preference for one contact per country only
- The standard text provided in 3 is followed and consistent.

Acknowledgements

- Not yet harmonized, point of discussion in 3.

References

- The different type of references (journals/proceedings, books/conference proceedings, CD-Rom, internet documents or websites recognized and referred as indicated in 3.

- References with those mentioned in the text unambiguous, cross-checked.
- Author citations in text are consistent (e.g. (Smith *et al.*, 1996, Castlebury and Carris, 1999), the order is year of publication, followed by alphabethic in similar years.

Figures

- All illustrations included are necessary.
- Preference of use of line drawings or photographs has been considered.
- The numbers of illustrations are referred in the text at the right place.
- Tables are included in the text, and all other illustrations are kept in separate files..
- The size of the illustrations and captions are given according to 3.

General

- Appendices or annexes should not be included.
- Consistency of terminology.
- Right abbreviations of measurements units as indicated in 1, are used.
- Are there capabilities for every member country to apply the proposed methods?
- Are limitations of molecular techniques for quarantine pests considered?
- Are the limitations explicitly said: about countries, species, hosts (where the investigations were done)?
- Are the limitations of morphometric techniques said: as regional keys, immatures, anabiosis cases?
- Are reproducibility, sensitivity and specificity clearly expressed?
- If contacts for organization of validation, proficiency tests, etc. are known, are they provided?

APPENDIX 4 OF INSTRUCTIONS TO AUTHORS: COMBINATION OF METHODS IN DIAGNOSTIC PROTOCOLS - SOME GENERAL CONSIDERATIONS ON THE CONCEPT

Diagnostic methods are often used in combination with others in order to increase the sensitivity, specificity or reliability of the diagnosis. ISPM 27 provides in section 1 the following guidance on this:

"Diagnostic protocols may be used in different circumstances that may require methods with different characteristics. Examples of such circumstances grouped according to an increased need for high sensitivity, specificity and reliability are:

- routine diagnosis of a pest widely established in a country
- general surveillance for pest status
- testing of material for compliance with certification schemes
- surveillance for latent infection by pests
- surveillance as part of an official control or eradication programme
- pest diagnostic associated with phytosanitary certification
- routine diagnosis for pests found in imported consignments
- detection of a pest in an area where it is not known to occur
- cases where a pest is identified by a laboratory for the first time
- detection of a pest in a consignment originating in a country where the pest is declared to be absent.

For example, in the case of routine diagnosis, the speed and cost of a test method may be more relevant than sensitivity or specificity. However, the identification of a pest by a laboratory or in an area for the first time may require methods with a high level of specificity and reproducibility. The significance of the outcome of a diagnosis is often dependent on proper sampling procedures. Such procedures are addressed by other ISPMs (under preparation).

Diagnostic protocols provide the minimum requirements for reliable diagnosis of regulated pests. This may be achieved by a single method or a combination of methods. Diagnostic protocols also provide additional methods to cover the full range of circumstances for which a diagnostic protocol may be used. The level of sensitivity, specificity and reproducibility of each method is indicated where possible. NPPOs may use these criteria to determine the method or combination of methods that are appropriate for the relevant circumstances."

In particular relevant for "the combination of methods" is the following statement:

> "Diagnostic protocols provide the minimum requirements for reliable diagnosis of regulated pests. This may be achieved by a single method or a combination of methods."

The core decisions that are required in the case of each protocol are therefore:

> What is the minimum requirement for a reliable diagnosis?
>
> Is a combination of methods necessary to achieve this? If yes, which combination?

It is obvious and generally accepted, that the combination of methods may only be appropriate, if at least one of the core factors "sensitivity, specificity or reliability" are increased by the combination[4]. It is however also known, that some methods may provide a higher specificity than others (and therefore may be used as a 2nd method), while such method may not provide necessarily the same sensitivity as

[4] In some situations it may be decided to apply both or even more tests at the same time in parallel. This paper does not address this situation and the considerations that may lead to such decisions. In general the final characteristics of the parallel application of different methods is equates to the "sum" of the best characteristics of the relevant methods applied.

the first method (e.g. monoclonal versus polyclonal antibodies; bioassay versus PCR). In particular in such cases the priorities of the system applied (e.g. sensitivity, specificity or reliability) as required by the framework of the diagnosis (see list of examples in quotation from ISPM 27 above) need to be careful balanced. Pending the framework in which the diagnosis is applied a certain combination may not be appropriate while in others a combination may be required.

The template on the next page analyses the possible situations and provides an indication whether a combination of methods with certain characteristics may be appropriate in diagnostic protocols. This template may help authors of diagnostic protocols and the TPDP to follow a consistent approach when the necessity and appropriateness of combinations of methods in DPs are discussed.

In reality when methods are combined all factors are to be considered and the methods are selected according the needs of the individual situation.

In summary the following conclusion can be drawn:

(1) The addition of a second method is not recommended, if the 2nd method has a **lower sensitivity** or is **less reliable** than the first method. In these circumstances the combination increases the risk of contradicting results. Pending the mode of interpretation this may include the risk of "false negative results".

(2) The addition of a second method is generally not recommended or not appropriate, if the 2nd method provides a higher sensitivity, a lower specificity or a higher reliability than the 1st method unless some other reason supports this combination.

(3) The addition of a second method is recommended, if the second method provides a **higher specificity** than the first method. Such combination is often used, when the first method is cheaper or faster than the second one (screening method). In general **high costs and low speed** of methods are good reason to apply them as a second method only, if they provide on the other hand some advantages over the 1st method e.g. higher sensitivity, higher specificity or higher reliability.

How to apply this template:

1. Consider that the decision on the first method has already been taken. The second method is only applied if the result of the first method is positive. (*see also * below)*.
2. Consider the individual column assuming that the other factors/methods are equivalent.
3. Ask the question: Is the combination recommended? focusing on the 2nd method.

The classification "**Risk**" is used to express that the combination carries the risk of weakening a result already achieved by method 1. Such combination should be avoided in all circumstances.

The classification "**Not appropriate**" is used to express that in general the combination of such factors in the given order is not contributing to the results of a diagnosis. In some specific situations the combination may nevertheless considered to be appropriate.

	Sensitivity		**Specificity**		**Reliability**		**Costs**		**Speed**	
Method 1	higher	Lower	Lower	Higher	Lower	Higher	lower	higher	lower	higher
Method 2	lower	higher	Higher	Lower	Higher	Lower	higher	lower	higher	lower
Combination recommended ?	No ---------	No/yes -+-+-+-	Yes ++++	No/yes ----------	No/yes -+-+-+-	No --------	Yes ++++	No --------	No --------	Yes ++++
Reason	Risk of contradicting results and false negative interpretation	Generally not appropriate, unless sample is already suspected	Appropriate if other factors (speed, cost etc.) suggest this order	Generally not appropriate, unless 2nd method provides some other benefit (isolation)	Generally not appropriate, unless in a situation where a false negative result (of the 1st method) can be tolerated.	Risk of contradicting results and false negative interpretation	Appropriate if 2nd method provides some other benefit. Typical situation.	Not appropriate unless 2nd method provides some other benefit (isolation)	Not appropriate unless 2nd method provides some other benefit (isolation)	Appropriate, fast result

* In some situations it may be appropriate that the 2nd method is applied even if the result of the first test was negative. Such situations may occur where most test results are positive and only a few results are negative. This condition does not apply to import situations. Also when consignments for export are tested such situations - if they exist at all - are rare. Such situation may occur in some specific surveillance situations in a heavily infested area. The inclusion of this situation in this table would be very complex and is therefore not addressed by this.

APPENDIX 5 OF INSTRUCTIONS TO AUTHORS: TEMPLATE TABLES FOR DESCRIPTION OF PCR, RT-PCR OR PCR-RFLP REACTIONS

(TPDP July 2014, *prepared by TPDP discipline lead on Nematology. Noted by SC May 2015. TPDP revised in June 2015*)

Background

Authors are required to use in IPPC diagnostic protocols, and to be used as a quality check tool, tables that describe polymerase chain reaction (PCR), Reverse transcriptase Polymerase chain reaction (RT-PCR) OR PCR - Restriction fragment length polymorphism (PCR-RFLP) reactions.

Table 1. Mastermix composition template for PCR and cycling conditions for a final reaction of XX µL

Reagents	Final concentration	Comments
PCR grade water	N.A.	Quantity for total volume
PCR buffer (or provide individual reagent's concentration)	1 X	
dNTPs	µM	
Other component		Where relevant, specify
Primer (Forward)	µM	
Primer (Reverse)	µM	
Probe	µM	Applicable for real time PCR
Enzyme quantity (DNA polymerase)	U	
DNA (quantity/volume)		Specify unit
Cycling parameters		
Initial denaturation	°C min	
Number of cycles		
Denaturation	°C min	
Annealing	°C min	
Elongation	°C min	
Final elongation	°C min	
Expected amplicons		
Description		

Table 2. Master mix composition and template for one step RT-PCR and cycling conditions for a final reaction of XX µL

Reagents	Final concentration	Comments
PCR grade water	N.A.	Quantity for total volume
RT-PCR buffer (or provide individual reagent's concentration)	1 X	
dNTPs	µM	
Other component (e.g. $MgCl_2$)		Where relevant, specify
Primer (Forward)	µM	
Primer (Reverse)	µM	
Probe	µM	Applicable for real time PCR
Enzyme quantity (reverse transcriptase + polymerase)	U	

RNA (quantity/volume)		Specify unit
Cycling parameters		
cDNA synthesis	°C min	
Initial denaturation	°C min	
Number of cycles		
Denaturation	°C min	
Annealing	°C min	
Elongation	°C min	
Final elongation	°C min	
Expected amplicons		
Description		

Table 3. Master mix composition and template for RFLP and reaction conditions for a final reaction of XX µL

Reagents	Final concentration	Comments
PCR grade water	N.A.	Quantity for total volume
Enzyme buffer	1 X	
Other component		Where relevant, specify
Restriction enzyme quantity (name)	U	
PCR product volume	µL	
Reaction condition (temperature and duration)	°C min or h.	
Expected fragments		
Description		