

*ISPM No. 27*



**INTERNATIONAL STANDARDS FOR  
PHYTOSANITARY MEASURES**

**ISPM No. 27**

***DIAGNOSTIC PROTOCOLS FOR REGULATED PESTS***

**(2006)**

Produced by the Secretariat of the International Plant Protection Convention





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## ENDORSEMENT

This standard was endorsed by the Interim Commission on Phytosanitary Measures in April 2006.

## INTRODUCTION

### SCOPE

This standard provides guidance on the structure and content of the International Plant Protection Convention (IPPC) diagnostic protocols for regulated pests. The protocols describe procedures and methods for the official diagnosis of regulated pests that are relevant for international trade. They provide at least the minimum requirements for reliable diagnosis of regulated pests.

### REFERENCES

- Determination of pest status in an area*, 1998. ISPM No. 8. FAO, Rome.  
*Export certification system*, 1997. ISPM No. 7. FAO, Rome.  
*Glossary of phytosanitary terms*, 2006. ISPM No. 5. FAO, Rome.  
*Guidelines for a phytosanitary import regulatory system*, 2004. ISPM No. 20. FAO, Rome.  
*Guidelines for inspection*, 2005. ISPM No. 23. FAO, Rome.  
*Guidelines for pest eradication programmes*, 1998. ISPM No. 9. FAO, Rome.  
*Guidelines for surveillance*, 1997. ISPM No. 6. FAO, Rome.  
*Guidelines for the notification of non-compliance and emergency action*, 2001. ISPM No. 13. FAO, Rome.  
*International Plant Protection Convention*, 1997. FAO, Rome.  
*Pest reporting*, 2002. ISPM No. 17. FAO, Rome.  
*Requirements for the establishment of areas of low pest prevalence*, 2005. ISPM No. 22. FAO, Rome.  
*Requirements for the establishment of pest free areas*, 1996. ISPM No. 4. FAO, Rome.  
*Requirements for the establishment of pest free places of production and pest free production sites*, 1999. ISPM No. 10. FAO, Rome.  
*The use of integrated measures in a systems approach for pest risk management*, 2002. ISPM No. 14. FAO, Rome.

### DEFINITIONS

Definitions of phytosanitary terms used in the present standard can be found in ISPM No. 5 (*Glossary of phytosanitary terms*).

### OUTLINE OF REQUIREMENTS

This standard sets the framework for the content of diagnostic protocols, their purpose and use, their publication and their development. Diagnostic protocols for specific regulated pests are included as annexes to this standard.

Information relevant for diagnosis is provided in the diagnostic protocol on the specified regulated pest, its taxonomic position, and the methods to detect and identify it. Diagnostic protocols contain the minimum requirements for reliable diagnosis of the specified regulated pests and provide flexibility to ensure that methods are appropriate for use in the full range of circumstances. The methods included in diagnostic protocols are selected on the basis of their sensitivity, specificity and reproducibility, and information related to these factors is provided for each of these methods.

Detailed information and guidance for the detection of pests is provided on, for example, signs and/or symptoms associated with the pest, illustrations (where appropriate), developmental stages of the pest, and methods for detecting the pest in a commodity, as well as methods for extracting, recovering and collecting the pests from plants. Information and guidance for the identification of pests includes detailed information on morphological and morphometric methods, methods based on biological properties, and methods based on biochemical and molecular properties of the pest. Furthermore detailed guidance is provided on the records that should be kept.

Diagnostic protocols are intended to be used by laboratories performing pest diagnosis as part of phytosanitary measures. They are subject to review and amendment to take into account new developments in pest diagnosis. The standard also provides guidance on how these protocols will be initiated, developed, reviewed and published.

## BACKGROUND

Proper pest detection and pest identification are crucial for the appropriate application of phytosanitary measures (see for example ISPM No. 4: *Requirements for the establishment of pest free areas*; ISPM No. 6: *Guidelines for surveillance*; ISPM No. 7: *Export certification system*; ISPM No. 9: *Guidelines for pest eradication programmes*; and ISPM No 20: *Guidelines for a phytosanitary import regulatory system*). In particular, contracting parties need proper diagnostic procedures for determination of pest status and pest reporting (ISPM No. 8: *Determination of pest status in an area*; ISPM No. 17: *Pest reporting*), and the diagnosis of pests in imported consignments (ISPM No. 13: *Guidelines for the notification of non-compliance and emergency action*).

National Plant Protection Organizations (NPPOs) have produced diagnostic protocols for regulated pests in order to adequately fulfil responsibilities according to Article IV of the IPPC (1997), in particular regarding surveillance, import inspections and export certification. In response to the need for regional harmonization, several Regional Plant Protection Organizations (RPPOs) have developed a significant number of regional diagnostic standards. This underlines the need for international harmonization, and those national and regional standards may form the basis for international protocols. Subsequently, the ICPM, at its Sixth session in 2004, recognized that there was a need for international diagnostic protocols within the framework of the IPPC and approved the formation of a Technical Panel on Diagnostic Protocols (TPDP) for that purpose.

## PURPOSE AND USE OF DIAGNOSTIC PROTOCOLS

The purpose of harmonized diagnostic protocols is to support efficient phytosanitary measures in a wide range of circumstances and to enhance the mutual recognition of diagnostic results by NPPOs, which may also facilitate trade. Furthermore these protocols should aid the development of expertise and technical cooperation, and they may also be relevant to the accreditation and/or approval of laboratories.

In addition to the methods included in the diagnostic protocols presented in the annexes to this standard, NPPOs may use other methods for diagnosing the same pests (for example based on bilateral agreements). The protocols and their components annexed to this ISPM are considered to have the status of an ISPM or part thereof (see section 3 of this ISPM and article X of the IPPC). Therefore, contracting parties should take into account, as appropriate, these diagnostic protocols when using or requiring the use of diagnostic methods in particular where other contracting parties may be affected.

Diagnostic protocols describe procedures and methods for the detection and identification of regulated pests that are relevant to international trade.

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.

Diagnostic protocols are intended to be used by laboratories performing pest diagnosis. Such laboratories may be established under or may be authorized by the NPPO to perform these activities in such manner that the results of the pest diagnosis may be considered as part of a phytosanitary measure of the NPPO.

The main elements of the procedure for the development of diagnostic protocols are presented in Appendix 1.

## REQUIREMENTS

### 1. General Requirements for Diagnostic Protocols<sup>1</sup>

Each protocol contains the methods and guidance necessary for the regulated pest(s) to be detected and positively identified by an expert (i.e. an entomologist, mycologist, virologist, bacteriologist, nematologist, weed-scientist, molecular biologist) or competent staff that are specifically trained.

The methods included in diagnostic protocols are selected on the basis of their sensitivity, specificity and reproducibility. In addition, the availability of equipment, the expertise required for these methods and their practicability (for example ease of use, speed and cost) are taken into account when selecting methods for inclusion in the diagnostic protocol. Usually these methods and their associated information should also be published. It may be necessary that some methods are validated before inclusion in the protocols. Such validation may include, for example, the use of a set of known samples, including controls, prepared to verify sensitivity, specificity and reproducibility.

Each diagnostic protocol usually describes more than one method to take into account the capabilities of laboratories and the situations for which the methods are applied. Such situations include diagnosis of different developmental stages of organisms, which require different methodologies, the need for an alternative diagnostic technique because of uncertainties of the initial diagnosis, as well as varying requirements for the level of sensitivity, specificity and reliability. For some purposes a single method may be sufficient, for other purposes a combination of methods may be necessary. Each protocol contains introductory information, information on the taxonomic position of the pest, methods for detection and identification of the pest, records to be kept, and references to appropriate scientific publications. In many cases a wide range of supplementary information is available which may support diagnosis, for example geographical distribution of the pest and host lists, but diagnostic protocols focus on the critical methods and procedures for pest diagnosis.

The aspects of quality assurance and in particular the reference materials that are required by diagnostic protocols (such as inclusion of positive and negative controls or collection of specimens) are specifically indicated in the corresponding section of the protocol.

### 2. Specific Requirements for a Diagnostic Protocol

Diagnostic protocols are arranged according to the following sections:

- Pest information
- Taxonomic information
- Detection
- Identification
- Records
- Contact points for further information
- Acknowledgements
- References.

#### 2.1 Pest information

Brief information is provided on the pest, 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 geographical distribution (in general), mode of transmission and dissemination (vectors and pathways). When available, reference to a pest data sheet should also be provided.

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<sup>1</sup> The following general provisions apply to all diagnostic protocols:

- Laboratory tests may involve the use of chemicals or equipment which present a certain hazard. In all cases, national safety procedures should be strictly followed;
- Use of names of 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.

## 2.2 Taxonomic information

This section provides information on the taxonomy of the pest involved and includes:

- name (current scientific name, author and year (for fungi, teleomorph name if known ))
  - synonyms (including former names)
  - accepted common names, anamorph name of fungi (including synonyms)
  - acronym of viruses and viroids
- taxonomic position (including information on subspecies classifications where relevant).

## 2.3 Detection

This section of the diagnostic protocol 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 the pest may be found
- the developmental stages of the pest that may be detected, together with their likely abundance and distribution on/in the plants/plant products or other articles
- the likely occurrence of the pest associated with developmental stages of the host(s), climatic conditions and seasonality
- methods for detecting the pest in the commodity (e.g. visual, hand lens)
- methods for extracting, recovering and collecting the pest from the plants, plant products or other articles, or for demonstrating the presence of the pest in the plants, plant products or other articles
- methods for indicating the presence of the pest in asymptomatic plant material or other materials (e.g. soil or water), such as ELISA<sup>2</sup> tests or culturing on selective media
- viability of the pest.

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

## 2.4 Identification

This section provides information and guidance on methods that either used alone or in combination lead to the identification of the pest. When several methods are mentioned, their advantages/disadvantages are given as well as the extent to which the methods or combinations of methods are equivalent. A flow diagram may be presented if several methods are needed to identify the pest or many alternative methods are included.

Main types of methodologies used in diagnostic protocols include those based on morphological and morphometric characteristics, biological properties such as virulence or host range of a pest, and those based on biochemical and molecular properties. Morphological characteristics may be investigated directly or after culturing or isolation of the pest. Culturing and/ or isolation may also be required for biochemical and/or molecular assays. Details are provided when culturing or isolation procedures are necessary components of methods.

For morphological and morphometric identifications, details are 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 morphological diagnostic characteristics, and an indication of any difficulties in seeing particular structures
- comparison with similar or related species
- relevant reference specimens or cultures.

For biochemical or molecular identifications, each method (e.g. serological methods, electrophoresis, PCR<sup>3</sup>, DNA barcoding, RFLP<sup>4</sup>, DNA sequencing) is described separately in sufficient detail (including equipment, reagents and consumables) to perform the test. Where appropriate, reference may be made to methodology described in other diagnostic protocols annexed to this standard.

<sup>2</sup> Enzyme-Linked Immunosorbent Assay

<sup>3</sup> Polymerase Chain Reaction

<sup>4</sup> Restriction Fragment Length Polymorphism

In cases where more than one method can be used reliably, other appropriate methods may be presented as alternative or supplementary methods, e.g. where morphological methods can be used reliably and appropriate molecular methods are also available.

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

For all the methods included in this section, information is provided on their sensitivity, specificity and reproducibility, where relevant. Where appropriate, guidance is provided on positive and negative controls and reference material to be included in tests. Guidance is also provided on removing possible confusion with similar and related species or taxa.

Diagnostic protocols provide guidance on the criteria for the determination of a positive or negative result for each method or information necessary to determine if an alternative method be applied.

Those cases where the use of appropriate controls for a specific technique, including where relevant reference material, is essential are clearly indicated in the protocol. When appropriate controls are not available, other tests, preferably based on different identification principles, may increase the certainty of the identification. Alternatively, a sample, specimen or, where appropriate, an image should be sent to another laboratory with experience in diagnosis of the suspected pest and possessing the required control or reference materials. Specimen(s) or material for reference purposes should be properly preserved.

Methods for quick, preliminary indications of identity (which will later need to be confirmed) may also be included in diagnostic protocols.

## 2.5 Records

This section provides information on the records that should be kept:

- scientific name of pest identified
- code or reference number of the sample (for traceability)
- nature of the infested material including scientific name of host where applicable
- origin (including the geographic location if known) of the infested material, and location of interception or detection
- description of signs or symptoms (including photographs where relevant), or their absence
- methods, including controls, used in the diagnosis and the results obtained with each method
- for morphological or morphometric methods, measurements, drawings or photographs of the diagnostic features (where relevant) and, if applicable, an indication of the developmental stage(s)
- for biochemical and molecular methods, documentation of test results such as photographs of diagnostic gels or ELISA printouts of results on which the diagnosis was based
- where appropriate, the magnitude of any infestation (how many individual pests found, how much damaged tissue)
- the name of the laboratory and, where appropriate, the name of the person(s) responsible for and/or who performed the diagnosis
- dates of collection of the sample, and of detection and identification of the pest.
- where appropriate, state of the pest, alive or dead, or viability of its development stages.

Evidence such as culture(s) of the pest, nucleic acid of the pest, preserved/mounted specimens or test materials (e.g. photograph of gels, ELISA plate printout results) should be retained, in particular in cases of non-compliance (ISPM No. 13: *Guidelines for the notification of non-compliance and emergency action*) and where pests are found for the first time (ISPM No. 17: *Pest reporting*). Additional items may be required under other ISPMs such as ISPM No. 8 (*Determination of pest status in an area*).

The period for which records should be kept depends on the purpose for which a diagnosis is made. In cases where other contracting parties may be adversely affected by the results of the diagnosis, records and evidence of the results of the diagnosis should be retained for at least one year.

## 2.6 Contact points for further information

Contact details of organizations or individuals with particular expertise on the pest(s) are provided; they may be consulted regarding details on the diagnostic protocol.

### **2.7 Acknowledgements**

The name and address of the experts who wrote the first draft of the diagnostic protocol are given, together with those of any others who made major contributions.

### **2.8 References**

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.

### **3. Publication of Diagnostic Protocols**

Diagnostic protocols are published as annexes to this ISPM and thus are individual publications under the IPPC framework with a specific publication and/or revision date. If appropriate, they may also form part of other ISPMs. The process of their adoption includes stringent review by internationally acknowledged scientists/experts for the relevant discipline.

An index to the annexes is provided as Appendix 2 [Appendix 2 will be added to the standard when protocols have been approved].

## APPENDIX 1

## MAIN ELEMENTS OF PROCEDURES FOR DIAGNOSTIC PROTOCOLS

**1. Development of Diagnostic Protocols**

The TPDP will commission an expert to lead the development of a diagnostic protocol by adapting, as appropriate, protocols that have already been approved by RPPOs, or other international or national organizations, or by developing a new diagnostic protocol. The diagnostic protocol will be developed further by a small group of experts selected by the TPDP and will then be submitted, in cooperation with the IPPC Secretariat, to the TPDP which, when satisfied with the content, will submit it to the Standards Committee.

**2. Review of Existing Diagnostic Protocols**

TPDP members will review the diagnostic protocols in their discipline on an annual basis or as determined by the TPDP. A request for a revision to a diagnostic protocol may also be submitted by NPPOs, RPPOs or CPM subsidiary bodies through the IPPC Secretariat ([ippc@fao.org](mailto:ippc@fao.org)), which will in turn forward it to the TPDP.

The TPDP will evaluate the request, identify those diagnostic protocols that require revision and oversee their revision. New methods should be at least equivalent to existing methods or provide a significant advantage for their worldwide application such as costs, sensitivity or specificity. Appropriate evidence should be provided to support any claims.

**3. Requests for New Diagnostic Protocols**

Requests for new diagnostic protocols, in addition to those identified in the work programme of the TPDP, should be sent by NPPOs, RPPOs or CPM subsidiary bodies through the IPPC Secretariat using a form for topics and priorities for standards, by 31 July of each year.