

NATIONAL STANDARDS FOR PHYTO SANITARY MEASURES

NSPM: 27

Diagnostic protocols for regulated pests 2013

(This standard is approved by (the NPPO and) the Quarantine Committee of Nepal, chaired by the secretary of the Ministry of Agriculture Development on 1st December 2013 and, is submitted by National Plant Quarantine Program to the National Notification Authority to be notified to the WTO member states)

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1. Introduction

1.1 Scope

This National standard provides guidance on the structure and content of diagnostic protocols for regulated pests. The protocols describe procedures and methods for the official diagnosis of regulated pests that are relevant for international trade. The standard provides requirements for reliable diagnosis of regulated pests. NSPM preparation based on guidelines and recommendations developed within the framework of the IPPC. This standard also adopted the principles, recommendations and format of ISPM to achieve international harmonization of phytosanitary measures with the aim to facilitate trade.

1.2 References

IPPC. 1997. *International Plant Protection Convention*. Rome, IPPC, FAO.

ISPM 4. 1995. *Requirements for the establishment of pest free areas*. Rome, IPPC, FAO. [published 1996]

ISPM 5. *Glossary of phytosanitary terms*. Rome, IPPC, FAO.

ISPM 6. 1997. *Guidelines for surveillance*. Rome, IPPC, FAO.

ISPM 7. 1997. *Export certification system*. Rome, IPPC, FAO. [revised; now ISPM 7:2011]

ISPM 8. 1998. *Determination of pest status in an area*. Rome, IPPC, FAO.

ISPM 9. 1998. *Guidelines for pest eradication programmes*. Rome, IPPC, FAO.

ISPM 10. 1999. *Requirements for the establishment of pest free places of production and pest free production sites*. Rome, IPPC, FAO.

ISPM 13. 2001. *Guidelines for the notification of non-compliance and emergency action*. Rome, IPPC, FAO.

ISPM 14. 2002. *The use of integrated measures in a systems approach for pest risk management*. Rome, IPPC, FAO.

ISPM 17. 2002. *Pest reporting*. Rome, IPPC, FAO.

ISPM 20. 2004. *Guidelines for a phytosanitary import regulatory system*. Rome, IPPC, FAO.

ISPM 22. 2005. *Requirements for the establishment of areas of low pest prevalence.* Rome, IPPC, FAO.

ISPM 23. 2005. *Guidelines for inspection.* Rome, IPPC, FAO.

ISPM 27.2006. *Diagnostic protocols for regulated pests.*

1.3 Definitions

Definitions of phytosanitary terms used in the present standard can be found in ISPM 5 (*Glossary of phytosanitary terms*).

1.4 Outlines 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 will be developed base on this standard and will be 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 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.

2. Background

Proper diagnosis is crucial for the appropriate application of phytosanitary measures (see for example ISPM 4:1995, ISPM 6:1997, ISPM 7:1997, ISPM 9:1998 and NSPM: Guidelines for a phytosanitary import regulatory system). In international trade, contracting parties should agree on diagnostic procedures for determination of pest status and pest reporting (ISPM 8:1998; NSPM: Pest reporting), and the diagnosis of pests in imported consignments (ISPM 13:2001).

The laboratories of National Plant Protection Organization, Nepal are doing diagnostic works since many years. However, NPPOs of Nepal have not produced national standard on diagnostic protocols for regulated pests in order to adequately fulfill 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 national standard for diagnostic protocol. Plant Protection Division, Department of Agriculture recognizes that there is a need for national standard for diagnostic protocol and approves the formation of a Technical Panel on Diagnostic Protocol (TPDP).

3. Purpose and use of diagnostic proposals

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 will 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. The protocol will be helpful for the different laboratories working with the diagnostic work of pest of different crops. This protocol will be basis for developing protocols for specific pests of different crops important in 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 (NSPM-Sampling).

Diagnostic protocols provide the 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.

4. Requirement

4.1 General Requirements for Diagnostic Protocols

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 who 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. 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 checks, 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 finding 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.

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.

4. 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.

4.2.1 Pest information

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, dissemination (vectors and pathways), economic importance, and past history. When available, reference to a pest data sheet should also be provided.

4.2.2 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).

4.2.3 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 (Enzyme linked immunosorbent assay) tests or culturing on selective media viability of the pest.

For all methods included in this section, information is provided on their sensitivity, especially and reproductively, 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.

4.2.4 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 (Polymerase chain reaction), DNA barcoding, RFLP (Restriction fragment length polymorphism), 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. 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.

4. 2.5 Records

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

- Scientific name of pest identified
- Strain, biotype and other difference, if appropriate
- Code or reference number of the sample (for traceability)
- Nature of the infested material including scientific name of host, variety, stages of plant part affected, 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 and address 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.
- Sample preserved condition

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 13:2001) and where pests are found for the first time (NSPM: Pest reporting). Additional items may be required under other ISPMs such as ISPM 8:1998.

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.

4.2.6 Contact points for further information

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

4.2.7 Acknowledgements

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

4.2.8 References

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

5. Publication of Diagnostic Protocols

Diagnostic protocols are published as annexes to this NSPM 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.

6. Main elements of procedures for diagnostic protocols

6.1. Development of Diagnostic Protocols

Plant Protection Directorate, with the initiation of National Plant Quarantine Program (NPQP) should create a Technical Panel on Diagnostic Protocols (TPDP) for Nepal. The panel should commission an expert to lead the development of a diagnostic protocol by adopting as appropriate, protocols that have already been approved by RPPOs, or other international or national organizations or by developing a new protocol. Research institutes like Plant Pathology, Entomology of NARC and other institutes of Nepal have longtime experience on diagnostic work. The institutes should be involved to develop diagnostic protocol. Official mechanism that will share both work and opportunities among NPQP and other institutes should be developed. The diagnostic protocol should be developed further by a small group of experts selected by the TPDP, Nepal and will then be submitted to TPDP, Nepal.

6.2. 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, or subsidiary bodies through the NPQP, 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 national application such as costs, sensitivity or specificity. Appropriate evidence should be provided to support any claims.

6.3. 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, or subsidiary bodies through NPQP using a form for the topics and priorities for standards.

7. Request for Diagnosis to Laboratory

When diagnosis cannot be done at the inspection site or at field, the samples or specimens of pests, diseases or weeds should be sent to accredited laboratory for diagnosis. Following considerations should be made while sending samples/specimens to the laboratory:

- Preserve specimens in proper condition so that specimens reach in the laboratory in good condition without spoilage
- Use ice-box, if necessary in the summer when temperature is too high for transportation.
- Use sterilized hand-gloves and tools to collect specimens
- Collect specimens (plant parts with sign or symptoms) with least disturbance
- Collect good amount of specimens and send to the laboratory so that it can be used in duplicate
- Collect specimens use envelopes, preferably, “Nepali khams” to keep plant parts like leaves, stems and roots
- Do not use plastic envelopes to keep specimens
- If soil-borne diseases like nematodes or soil insects are suspected, collect soil from rhizosphere and keep in plastic bags. Do not seal plastic bags. Just staple the bag two or three places
- Provide detail information about crops and environments that will help for identification (narrowing down the process) examples; crop, variety, season, time of seeding/planting, time when disease(symptom) appeared, pattern of disease(uniform or patchy), critical cultural practices done before disease or insect observed like irrigation (flooding), spraying fungicide or insecticide or other chemicals, fertilizer application etc.
- If insects are observed, kill or demobilize insect properly and send to laboratory
- Provide, if available all stages of insects.