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Diagnostics
Diagnostic

PM 7/76 (5) Use of EPPO Diagnostic Standards

Specific scope

This Standard describes the purpose and use of EPPO Diagnostic Standards. Definitions used in these Standards are given in Appendix 1. This Standard is based on ISPM 27 (IPPC, 2006).

Specific approval and amendment

Approved in 2006-09.
Revised in 2010-09, 2014-09, 2016-11 and 2018-09.

1. Introduction

This Standard is designed to be used in conjunction with the pest-specific EPPO Diagnostic Standards of series PM 7 on diagnostics.

While pest-specific Diagnostic Standards are concerned with the diagnosis of pests and describe different tests (or combinations of tests) that can be used to detect and identify them, this Standard describes why tests used for the diagnosis of pests may differ according to the laboratory and circumstances of use, and provides definitions for terms used in these Diagnostic Standards (see Appendix 1). It also includes elements about communication between laboratories and their customers and general requirements for laboratories.

This Standard is consequently mainly designed for:

- diagnosticians and other laboratory personnel.
- persons responsible for submitting samples for diagnosis.
- National Plant Protection Organizations (NPPOs) which are responsible for deciding on phytosanitary actions based on the outcome of diagnosis.

2. EPPO Diagnostic Standards

2.1 Objectives and content of EPPO Diagnostic Standards

EPPO Series PM 7 Standards include two types of Standards: horizontal and pest-specific Standards (https://gd.eppo.int/standards/PM7/):

- Horizontal Standards include Standards related to quality assurance issues, Standards on how to perform specific methods, such as ELISA tests for plant pathogenic bacteria and electron microscopy in the diagnosis of plant viruses
- Pest-specific Diagnostic Standards are related to (a) specific pest(s) and provide the guidance necessary for an expert or competent staff who are specifically trained to detect and positively identify (a) pest(s) by a single test or combination of tests. Pest-specific Diagnostic Standards usually include more than one method to take into account the capabilities of laboratories and the circumstances of use (see section 4). These Standards are based on the scientific literature and the experience of EPPO experts. Information is provided on the pest, its taxonomic status and the tests to detect and identify it. In cases where morphological tests can be reliably used but appropriate molecular tests have been developed, the latter are presented as alternatives to provide flexibility to laboratories or as possible additional confirmatory tests to cover problematic life stages or incomplete specimens. The tests included in pest-specific Diagnostic Standards are selected on the basis of their performance characteristics (see section 3), which should be indicated in the Standard. In general, it is preferable that a confirmatory test should be done with a test based on a different biological principle or, for molecular tests, a second test that targets a different part of the genome. This is particularly important for critical cases (see section 4). As a basic requirement, tests should give repeatable and reproducible results. In addition, other factors such as ease of use, availability of equipment, expertise required for these tests and practicality (e.g. speed and cost) are taken into account. When the combination of several tests is recommended, flow diagrams are provided (in more recent pest-specific Diagnostic Standards).

2.2 Users of EPPO Diagnostic Standards

EPPO Diagnostic Standards 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 (EPPO, 2016). Results of the pest diagnosis may trigger phytosanitary action by the NPPO.

Laboratories may identify pests upon the request of NPPOs, growers or traders, referred to hereafter as customers.

The selection of tests requires communication with the customer (see section 5).

3. Characteristics of tests or combination of tests

Diagnostic tests have different levels of analytical sensitivity (including risks of false-negative results), analytical specificity (including risks of false-positive and false-negative results), speed and cost. These elements are taken into account by the customer and the laboratory when choosing a test for the diagnosis of a pest in specific circumstances of use.

The reliability of a test is a combination of the performance characteristics of the individual test, obtained from validation studies, their associated uncertainty and the experience of the laboratories with the tests (years of experience and number of samples tested) or information obtained when performing verification studies. More information on how to perform validation and verification is provided in PM 7/98 *Specific requirements for laboratories preparing accreditation for a plant pest diagnostic activity* (EPPO, 2018). Validation data is not available for all tests that are currently widely used in plant pest diagnostic laboratories. Lack of validation data is, in particular, often the case for high-throughput tests such as ELISA. However, there is often a long period of experience of use of such tests and it is usually possible for the laboratory to qualify the reliability of such tests (e.g. based on the number of years of experience, the number of samples tested, the use of controls and participation in proficiency tests). It is nevertheless recognized that performance characteristics allow a better understanding of the reliability of the tests. Often, multiple tests are used to increase the overall confidence in the diagnosis.

It should also be noted that:

- as experience has built up with molecular tests, confirmation by culturing is no longer a standard requirement in pest-specific Diagnostic Standards
- morphological identification does not always require confirmation by another test.

4. Circumstances of use of test or combination of tests

Diagnostic Standards may be used in different circumstances that may require tests or combinations of tests with different characteristics. Examples of such circumstances, presented according to an increased need for confidence in the outcome of the diagnosis, are given in ISPM 27 and are presented in the bullet points below:

- routine survey(s) for the 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 infestations[1] 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 circumstances of use described in the latter three bullet points are considered in this Standard as critical cases where additional confidence in the outcome of the diagnosis will be required. The detection of a pest in a consignment declared to have been submitted to a phytosanitary treatment is also considered to be a critical case.

5. Communication with customers

5.1 General communication with customers

Laboratories work on samples submitted by customers. The customer should be made aware that the confidence in the outcome of a diagnosis depends on the size of the sample and the procedures used for sampling consignments or places of production or area-wide surveillance. Guidance for sampling is given in ISPM 31 and many EPPO Standards from the series PM 3 (inspection procedures) and PM 9 (national official regulatory control systems).

As a general rule, the laboratory performing the diagnosis should try to adapt its procedures to achieve the degree of confidence requested by the customer submitting the sample. This requires communication between the customers and the laboratory. Details on communication between the laboratory and the NPPO are specifically described below.

5.2 Importance of communication between the laboratory and the risk managers on test selection

As test(s) results may trigger phytosanitary action (with significant financial, social and environmental consequences) it is important that as much relevant

[1]Note that according to the IPPC Glossary of phytosanitary terms, infestation includes infection.

information as possible is provided to the risk managers of the NPPO to help them make an informed decision. Communication between laboratory experts and risk managers is therefore needed to determine in advance the test(s) that are appropriate for the relevant circumstances (see section 4). In particular, the laboratory should communicate with the risk managers on the level of uncertainty of a test result (risks of false positives or false negatives, see below) taking into account the performance characteristics of the selected test. This should also include the provision of recommendations to risk managers of the NPPO regarding the use of a combination of tests rather than an individual test. The selection of tests should ideally be included in contingency, inspection or survey plans.

Specific circumstances of use have been listed in section 4, and some are further illustrated below with regard to the selection process.

In circumstances such as routine diagnosis or surveillance, speed and cost may be more critical than the level of analytical sensitivity or analytical specificity. The NPPO may decide to select a single test for which the risk of false positives or false negatives is considered acceptable. The NPPO may decide to take phytosanitary action on the basis of this laboratory result. In some cases (detection of a pest in an area where it is not known to occur, detection of a pest for the first time in a laboratory), tests with a high level of analytical sensitivity, analytical specificity, repeatability and reproducibility may be required. The NPPO may decide to wait for the outcome of further tests before taking phytosanitary action. Nevertheless, it should be noted that in specific situations (e.g. import of a suspected consignment of plants for planting into a pest-free area) the NPPO may decide to take phytosanitary action against a pest without a final confirmation of its identity by the laboratory if such an event would pose a high risk to an area. In such situations, the consequences of having a false-positive result of the preliminary test or a false-negative result of the confirmatory test have to be evaluated.

It is noted that some NPPOs establish multidisciplinary technical advice teams, emergency committees or contingency planning teams facilitating exchanges between risk managers, inspectors and laboratories, and this is recognized as important means of improving communication.

5.3 Communication between laboratories and the customers on test results

5.3.1 Inconclusive tests

When a test result is inconclusive, it is essential that the laboratory should communicate with the customer about the source of uncertainty of the test result (e.g. presence of the target at the limit of detection, poor quality of samples); resampling may be considered.

5.3.2 Viability of the pest

When certain tests are used (such as molecular or serological), demonstration of the viability of the pest is not always possible as only nucleic acids or proteins are detected. Some pests cannot be cultured or are difficult to culture, and some tests that can be used to demonstrate viability (e.g. bioassays) have a lower analytical sensitivity than other laboratory tests. Imported consignments (e.g. for seeds, fruits) or wood packaging material (WPM) may be treated to kill the pest of concern, and this treatment is mentioned on the phytosanitary certificate or certified by the presence of the IPPC mark on the WPM. This raises the question of what should be done when a pest is detected in a consignment that has been officially declared as treated. When a phytosanitary action may result from the test result, a discussion between the laboratory and the NPPO is needed to allow the NPPO to evaluate the risk and consequences of accepting a consignment infested with viable pests or refusing a consignment with only non-viable pests.

6. General requirements for laboratories using EPPO Diagnostic Standards

- Laboratory procedures should be adequate for the handling of quarantine pests (including positive controls), with particular reference to waste disposal facilities, and should respect the conditions of appropriate licences issued by the NPPO [see also PM 3/64 *Intentional import of organisms that are plant pests or potential plant pests* (EPPO, 2006)]. Quality control standards should be applied to minimize administrative and other errors, especially concerning labelling and documentation.
- Laboratory tests may involve the use of, or exposure to, chemicals, biological agents or apparatus which present a certain hazard. In all cases, local safety procedures should be strictly followed.
- In pest-specific Diagnostic Standards, tests (including reference to brand names) are usually described as published, as these define the original level of analytical sensitivity and analytical specificity, repeatability and reproducibility achieved.
- Consequently, use of names of chemicals or equipment in these EPPO Diagnostic Standards implies no approval of them to the exclusion of others that may also be suitable.
- Laboratory procedures presented in the Standards may be adjusted to the conditions (e.g. expertise, material, equipment) of individual laboratories, provided that they are adequately validated or verified and that proper controls are included.

References

EPPO (2006) PM 3/64 (1) Intentional import of organisms that are plant pests or potential plant pests. *Bulletin OEPP/EPPO Bulletin* **36**, 191–194.

EPPO (2018) PM 7/98 (3) Specific requirements for laboratories preparing accreditation for a plant pest diagnostic activity. *EPPO Bulletin* **48,** 117–147 (this issue).

EPPO (2016) PM 7/130 (1) Guidelines on the authorization of laboratories to perform diagnostic activities for regulated pests. *Bulletin OEPP/EPPO Bulletin* **46**, 538–540.

IPPC (2006) *Diagnostic Protocols for Regulated Pests.* ISPM 27 IPPC Secretariat. FAO, Rome (IT). https://www.ippc.int/en/publications/593/ [accessed on 2018-02-14]

IPPC (2017) *Glossary of Phytosanitary Terms*. ISPM no. 5. IPPC Secretariat. FAO, Rome (IT). https://www.ippc.int/en/publications/622/ [accessed on 26 March 2018]

Appendix 1 – Definitions and explanations of terms used in EPPO Diagnostic Standards

It should be noted that other Standards have been developed in the ISO framework that also include definitions (e.g. ISO 16140 *Microbiology of food and animal feeding stuffs – protocol for the validation of alternative methods* and ISO 17034 *General requirements for the competence of reference material producers*).

Certified reference material	Reference material derived from a source that certifies the authenticity of the material. The material should preferably come from an internationally recognized source such as a national reference collection. The material should have a unique identification code allowing traceability and the name of the person who certified its authenticity. Details of how the material was authenticated should also be supplied. If appropriate, information about its activity (e.g. pathogenicity, antigenic properties) under specified conditions should also be supplied along with any related uncertainty at a stated level of confidence
Diagnosis	The process of detection and identification of a pest (ISPM 27, 2006) (i.e. the interpretation of the result of a diagnostic process)
Interlaboratory comparison	Organization, performance and evaluation of measurements or tests on the same or similar items by two or more laboratories in accordance with predetermined conditions (i.e. proficiency testing or test performance studies)
Pest (IPPC, 2017)	Any species, strain or biotype of plant, animal or pathogenic agent injurious to plants or plant products
Proficiency testing	Establishing the competence of a laboratory in analysing defined samples using their established test (evaluation of the competence of the laboratory)
Quarantine pest (IPPC, 2017)	A pest of potential economic importance to the area endangered thereby and not yet present there, or present but not widely distributed and being officially controlled
Reference material	Material appropriate to the test and diagnosis being performed such as live cultures, infested plant material, DNA/RNA preparations, images of a diagnostic quality or mounted specimens. The reference material used should be documented and appropriate to the test and diagnosis being performed. It should be ensured that the material used is producing the features for which it was selected, for example expressing a desired antigen for use in serological diagnosis, or display specific physical features (e.g. sporulation) if used for morphological diagnosis
Regulated pest (IPPC, 2017)	A quarantine pest or regulated non-quarantine pest
Repeatability	The level of agreement between replicates of a sample tested under the same conditions
Reproducibility	The ability of a test to provide consistent results when applied to aliquots of the same sample tested under different conditions (e.g. time, persons, equipment, location)
Robustness of a test	The extent to which altered test conditions (e.g. temperature, volume, change of reagents) affect the established test performance values (e.g. analytical sensitivity, analytical specificity)
Selectivity	The extent to which variations in the matrix affect the test performance (matrix effect)
Sensitivity*	
Analytical sensitivity	The smallest amount of target that can be detected reliably (this is sometimes referred to as the 'limit of detection'). Further details on the procedures to determine analytical sensitivity are given in PM 7/98 *Specific requirements for laboratories preparing accreditation for a plant pest diagnostic activity*
Diagnostic sensitivity	The proportion of infected/infested samples testing positive compared with results from an alternative test (or combination of tests). Diagnostic sensitivity = true positives/(true positives + false negatives)

(continued)

Specificity*	
Analytical specificity (comprises inclusivity and exclusivity, see below)	Further details on the procedures to determine analytical specificity (inclusivity and exclusivity) are given in PM 7/98 *Specific requirements for laboratories preparing accreditation for a plant pest diagnostic activity*
Inclusivity	The performance of a test with a range of target organisms covering genetic diversity, different geographical origin and hosts
Exclusivity	Performance of a test with regards to cross-reaction with a range of non-targets (e.g. closely related organisms, contaminants)
Diagnostic specificity	The proportion of uninfected/uninfested samples (true negatives) testing negative compared with results from an alternative test (or combination of tests). Diagnostic specificity = true negatives/(true negatives + false positives)
Method	Methods include: bioassay methods, biochemical methods, fingerprint methods, isolation/extraction methods, molecular methods, morphological and morphometric methods, pathogenicity assessment and serological methods
Test	The application of a method to a specific pest and a specific matrix
Test performance study (also referred to as ring tests or collaborative trials)	Evaluation of the performance of one or more tests by two or more laboratories using defined samples (evaluation of a test)

*Note that the analytical sensitivity and analytical specificity of a test are distinct from its diagnostic sensitivity and diagnostic specificity. Therefore, the terms 'sensitivity' and 'specificity' should always be used with the requisite adjectives.

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ADDENDUM

Addendum - PM 7/76 (5) Use of EPPO diagnostic protocols

An additional definition is to be added to the table in Appendix 1 – Definitions and explanations of terms used in EPPO Diagnostic Standards

Reference material	Material appropriate to be used for testing and diagnosis such as live cultures, (infested) plant material, nucleic acid, sequence data, images of a diagnostic quality or mounted (or unmounted) specimens. The reference material should be documented. It should be ensured that the reference material is producing the features for which it was selected, for example expressing a desired antigen for use in serological testing or being free from the target when used as a negative control, or reliably displaying specific physical features (e.g. sporulation, chaetotaxy) when used for morphological identification.

It was agreed to publish this update as an addendum.

REFERENCE

EPPO (2018) PM 7/76 (5) Use of EPPO diagnostic protocols. *EPPO Bulletin* 48(3), 373–377.

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ADDENDUM

Addendum – PM 7/76 (5) Use of EPPO diagnostic protocols

During the last meeting of the Panel on Diagnostics and Quality Assurance, the Panel discussed the revision of the definitions of diagnostic sensitivity and diagnostic specificity and the procedure for the selection of tests to be included in EPPO Diagnostic Protocols, and agreed on the following changes to be made to PM 7/76 (EPPO, 2018) before the Standard is revised in full.

In Section 2.1, the following text should be replaced (new text indicated in bold):

Current version:
The tests included in pest-specific Diagnostic Standards are selected on the basis of their performance characteristics (see section 3), which should be indicated in the Standard.

New version:
The tests included in pest-specific Diagnostic Standards are selected on the basis of their performance characteristics (see section 3) **usually obtained in EPPO diagnostic laboratories**, which should be indicated in the Standard. **However, for some pests there is little experience in the EPPO region and tests are selected and described based on literature. However, it should be noted that there may be some limitations in the use of such tests in official laboratory analysis.**

In Appendix 1, the following definitions should be replaced:

Current version:

Diagnostic sensitivity	The proportion of infected/infested samples testing positive compared with results from an alternative test (or combination of tests). Diagnostic sensitivity = true positives/(true positives + false negatives)
Diagnostic specificity	The proportion of uninfected/uninfested samples (true negatives) testing negative compared with results from an alternative test (or combination of tests). Diagnostic specificity = true negatives/(true negatives + false positives)

New version:

Diagnostic sensitivity	The proportion of infected/infested samples testing positive compared with the results from an alternative test (or combination of tests)[a] **or with the assigned values of samples.** Diagnostic sensitivity = true positives[b]/(true positives[b] + false negatives[b])
Diagnostic specificity	The proportion of uninfected/uninfested samples testing negative compared with results from an alternative test (or combination of tests)[a] **or with the assigned values of samples.** Diagnostic specificity = true negatives[b]/(true negatives[b] + false positives[b])

[a] **Performance characteristics should be available for the alternative test (or combination of tests).**

[b] **Note that true/false positives/negatives should be described as positive/negative agreement/deviation when comparing the results of a test with the ones of a (combination of) reference test(s).**

REFERENCE

EPPO (2018) PM 7/76 (5) Use of EPPO Diagnostic Standards, *EPPO Bulletin* 48, 373–377.