Bulletin OEPP/EPPO Bulletin (2017) **47** (1), 7–9 ISSN 0250-8052. DOI: 10.1111/epp.12365

Diagnostics
Diagnostic

PM 7/76 (4) Use of EPPO diagnostic protocols

Specific scope

This Standard describes the purpose of EPPO diagnostic protocols for regulated pests and includes definitions used in these protocols.

Specific approval and amendment

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

Introduction

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

While individual EPPO Standards are concerned with the diagnosis of individual pests and describe different tests that can be used to detect and identify a pest, 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 protocols.

This Standard is mainly designed for:

- persons responsible for writing diagnostic protocols
- persons responsible for submitting samples for diagnosis
- persons responsible for documenting and reporting on diagnosis
- NPPOs which are responsible for deciding on phytosanitary actions based on the outcome of diagnosis.

Purpose of EPPO diagnostic protocols

EPPO diagnostic protocols for pests are intended to be used by official[1] laboratories (hereafter laboratories) to detect and identify the pests in the EPPO and European Union lists and other pests of potential phytosanitary concern. The diagnostic protocols are based on the experience of EPPO experts and the scientific literature. Information is provided on the pest, its taxonomic status and the tests to detect and identify it.

Diagnostic protocols are suitable for use in different situations; for example, where laboratories may not have the same technical or operational capabilities, where diagnosis of different development stages may require different methodologies, or where the NPPO may require a specific degree of certainty, in particular when a decision on phytosanitary action has to be made. EPPO diagnostic protocols contain the tests and guidance necessary for (a) pest(s) to be detected and positively identified by an expert or competent staff who are specifically trained. The range of tests included provides flexibility to ensure that these are appropriate for the circumstances of use. Laboratories may identify pests upon request of NPPOs, growers or traders, referred to hereafter as clients.

Suitability of the test according to the circumstances of use

The diagnosis of a pest may be done in the framework of:

- routine survey(s) for 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[2] 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
- detection of a pest for the first time by a laboratory
- detection of a pest in a consignment originating in a country where the pest is declared to be absent.

[1]The definition of 'official' in the IPPC Glossary of phytosanitary terms is 'Established, authorized or performed by a National Plant Protection Organization' (IPPC, 2016)

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

Diagnostic tests have different levels of analytical sensitivity, analytical specificity, speed and cost. These elements are taken into account by the NPPO when choosing a test for the diagnosis of a pest in specific circumstances of use. Because phytosanitary action may be taken on the basis of a diagnosis, risks of false positives or false negatives have to be taken into account when deciding on the suitability of a test or combination of tests for a specific circumstance. This may involve communication between laboratory experts and persons in charge of management decisions in NPPOs.

Some specific circumstances of use are illustrated below.

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 choose a single test for which the probability of false positives or false negatives is not critical, leading only to the conclusion that it is highly probable that the pest is present in the sample. The NPPO may decide to take phytosanitary action on the basis of this laboratory result. In other 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 specificity, repeatability and reproducibility may be required. The NPPO may decide to wait for the outcome of a range or series of 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) a 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. Laboratories work on samples submitted by clients. As a general rule, the laboratory performing the diagnosis should adapt its procedures to achieve the degree of certainty requested by the client submitting the sample.

Consequences for the content of diagnostic protocols

Diagnostic protocols provide the guidance necessary for (a) pest(s) to be detected and positively identified by a single test or combination of tests. Diagnostic protocols provide tests to cover the full range of situations (e.g. capability of laboratories, circumstances of use). In cases where morphological tests can be reliably used but appropriate molecular tests have been developed, the latter are presented as alternative or additional confirmatory tests so as to provide flexibility to laboratories and to cover problematic life stages or incomplete specimens. The tests included in diagnostic protocols are selected on the basis of their level of analytical sensitivity (including risks of false negative results), analytical specificity (including risks of false positive and negative results), repeatability and reproducibility, which should be indicated in the protocol. In general, it is preferable that a confirmatory test should be done with a test based on a different biological principle. This is particularly important for critical cases. As a basic requirement, tests should give repeatable 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. NPPOs should use these criteria to determine the test or combination of tests that are appropriate for the relevant circumstances.

Provisions that apply to all diagnostic protocols

- 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. Quality control standards should be applied to minimize administrative and other errors, especially concerning labelling and documentation
- Laboratory tests may involve the use of chemicals or apparatus which present a certain hazard. In all cases, local safety procedures should be strictly followed
- Use of names of chemicals or equipment in these EPPO Standards 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 and that proper controls are included.

Definitions and explanations of terms used in EPPO Standards on diagnostics

Certified reference material	Reference material derived from a source that certifies the authenticity of the material. Preferably material should 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

(continued)

Table *(continued)*

Interlaboratory comparison	Organization, performance and evaluation of measurements or tests on the same or similaritems by two or more laboratories in accordance with predetermined conditions (i.e. proficiency testing or test performance studies)
Pest (IPPC, 2015)	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, 2016)	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 specimensThe 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, 2016)	A quarantine pest or regulated non-quarantine pest
Repeatability	Level of agreement between replicates of a sample tested under the same conditions
Reproducibility	Ability of a test to provide consistent results when applied to aliquots of the same sample tested under different conditions (time, persons, equipment, location etc.)
Robustness of a test	The extent to which altered test conditions (e.g. temperature, volume) 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	Smallest amount of target that can be detected reliably (this is sometimes referred to as '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	Proportion of infected/infested samples testing positive compared with results from an alternative test (or combination of tests). Sensitivity = true positives/(true positives + false negatives)
Specificity*	
Analytical specificity	Performance of a test with regard to cross-reactions with non-target. Further details on the procedures to determine analytical specificity are given in PM 7/98 *Specific requirements for laboratories preparing accreditation for a plant pest diagnostic activity*
Diagnostic specificity	Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from an alternative test (or combination of tests). Specificity = true negatives/(true negatives + false positives)
Test	The application of a method to a specific pest and a specific matrix. Methods include: bioassay methods, biochemical methods, fingerprint methods, isolation/extraction methods, molecular methods, morphological and morphometric methods, pathogenicity assessment, and serological methods
Test performance study (also referredto 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. It should be noted that other Standards have been developed in the framework of ISO that also include definitions (e.g. ISO 16140 *Microbiology of food and animal feeding stuffs – Protocol for the validation of alternative methods*).

Reference

IPPC (2016) *Glossary of Phytosanitary Terms. ISPM no. 5*. IPPC Secretariat, FAO, Rome (IT). https://www.ippc.int/static/media/files/publication/en/2016/01/ISPM_05_2015_En_2016-01-11_Reformatted.pdf [accessed on 20 September 2016]

