EUROPEAN AND MEDITERRANEAN PLANT PROTECTION ORGANIZATION
ORGANISATION EUROPEENNE ET MEDITERRANEENNE POUR LA PROTECTION DES PLANTES

24-29613[1]

Instruction to authors on the format and content of a diagnostic protocol

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GENERAL

In general authors should prepare their texts according to the Instructions for Authors for *Bulletin OEPP/EPPO Bulletin* (https://www.eppo.int/RESOURCES/eppo_publications/eppo_bulletin) from the very first draft.

Each protocol should contain all the information necessary for the named pest to be detected and positively identified (for some pests the scope may be more specific, i.e. identification of the "named pest" on specific hosts). Protocols for diagnosis (detection and identification) may be used in different circumstances that may require tests with different characteristics. Diagnostic protocols provide the minimum requirements, which may be a single test or a combination of tests, for reliable diagnosis of the relevant pests. Diagnostic protocols also provide additional tests to cover the full range of circumstances. The protocol should follow the headings (introduction, identity, detection, identification, reference material, reporting and documentation, performance characteristics, further information, feedback on this Diagnostic Protocol, protocol revision, acknowledgements and references) but may include additional sub-headings appropriate to the pest and its diagnosis. A template is available (see document **24-29614**). Related pests should preferably be covered in the same protocol.

Each illustration (photographs, diagrams and drawings) should be sent as a separate file; preferably PNG or TIF (or JPEG for photographs, GIF for drawings). The minimum resolution is 300 dpi. Authors are encouraged to upload their own illustration of symptoms or of morphological characteristics of specific bodies in the EPPO Global Database (https://gd.eppo.int/ following the instructions that are given in the section *How to submit new photos?*). Authors of diagnostic protocols should verify and mention copyrights for publication of illustrations that they add to the Diagnostic Protocol. Previously unpublished illustrations should be cited as "Courtesy: J Dow, if available institute (country alpha 2 ISO code)". Figures should be numbered preferably by order of appearance in the text and referred to as 'Figure X'. If possible, all abbreviations used in the figures should be explained in the legend of the figure.

Note that in EPPO style, the imperative is not used in the main text ("Take samples"…). According to the context one can write for example "samples should be taken" or "samples are taken", or "samples may be taken". However, in Appendices the imperative may be used and should then be used consistently. "Must" is not used.

Definitions of terms that should be used in diagnostic protocols are included in PM 7/76.

Use SI units and abbreviations and a full stop for decimal points (e.g. 1.5 µL).
When names of expert are used record them as follows "Family name" 'first name initial(s)' with no dot between the initials e.g. Smith IM

Latin names should be used throughout the protocol. According to the EPPO style for Standards, the first time a name is mentioned, genus and species should be written in full. For the next occurrences, the genus should be abbreviated. Authorities should be given for arthropod species the first time the scientific name is mentioned in the Standard. No reference is needed in the reference Section. Common names for hosts can be mentioned after first use of the Latin name e.g. *Solanum lycopersicum* (tomato). Check the preferred name in the EPPO Global Database and indicate to the EPPO Secretariat any discrepancy.

[1] Former versions 23-28446, 23-28350, 22-28014, 22-27776, 22-27609, 22-27462, 21- 27142, 21-26498, 20-26415, 20-25769, 20-25607, 19-25253, 19-24902, 19-24547, 18-24334, 18- 23648, 18- 23588, 17-22367 16- 22137, 16-21547, 15-21171, 15- 21171; 15-20820, 14-19833, 13- 19130, 12- 18298, 12-18078, 12-17367 11- 17244, 11- 17149, 11-16814, 11- 16544, 10-16428, 10- 15763.

WARNING

Diagnostic protocols should not instruct NPPOs on measures or actions to be taken on the basis of the diagnosis. Terminology used (in particular in flow diagrams) should be carefully considered to avoid any confusion with phytosanitary actions taken by the NPPO (in particular for the use of the term 'confirmed').

NOTES ON INDIVIDUAL HEADINGS

1. Introduction

A few introductory sentences on the pest and its importance should be provided. Authors of diagnostic protocols should not write a long introduction incorporating information which **are not of diagnostic significance**. Information on first records, relationship with other organisms, host range, effects on hosts, or geographical distribution should be cross-checked with existing EPPO information such as EPPO Global Database. **If new information is found by the author, they should inform the EPPO Secretariat of the necessity to update the EPPO data package.**

If several tests can or should be combined (see sections detection and identification), (a) flow diagram(s) should be prepared. This flow diagram should define the sequence of steps and indicate for each step which tests can be chosen. This flow diagram is intended to provide an overview of the diagnostic process and may not cover all possible scenarios. When a flow diagram is included a standard sentence should be added at the end of the introduction section (see Template).

2. Identity

Name: correct scientific name, with authority
Other scientific names:
Acronym: for viruses
Taxonomic position:
EPPO Code:
Phytosanitary categorization: A1 or A2 quarantine pest for EPPO countries; EU annex; or equivalent

The EPPO Global Database should be checked for its content related to taxonomy, scientific name and synonyms. Any discrepancy should be reported to the EPPO Secretariat.
In 2014 at the Workshop on EPPO Diagnostic Protocols organised in the framework of the European Mycology Network experts decided that for fungi the Amsterdam Declaration on Fungal Nomenclature 'one fungus one name' should be implemented in EPPO diagnostic protocols. Authors are consequently encouraged to implement this Declaration when preparing diagnostic protocols. Priority is usually given to the oldest name however it is recognized that there are some exceptions where a case-by-case decision which is made by the International Commission on the Taxonomy of Fungi. Lists of names are being produced. Current names can be found at index Fungorum (www.indexfungorum), Mycobank (www.mycobank.org) and USDA (http://nt.ars.grin.gov/fungaldatabases/), Genera of fungi database (http://www.generaoffungi.org). It is noted that there may be discrepancies between the databases and that it takes some time for these to be updated so it is recommended to check more than one of these databases and to check the most recent literature.
A note on the use of the nomenclature of the International Committee on Taxonomy of Viruses and on the progressive transfer to a binomial nomenclature is also included for viruses.

3. Detection

The following indications should be provided as appropriate.

- Indicate the commodities on which the pest can be found.
- Describe the symptoms (characteristic features, difference with symptoms from other causes, similarities with symptoms from other causes)
- Explain how to discover the pest in the commodity (e.g. visual, hand lens), in particular in which part of the plant (or other matrix) it will be found, and where it will not be found. Indicate which developmental stages of the pest may be encountered. Sampling methods, depending on likely concentration and distribution of the pest should be indicated. Sampling of places of production (fields, orchards, forest plots…) is not covered in EPPO Diagnostic protocols.
- Describe procedures for extracting, recovering, and collecting the pest from the samples of plants, plant products or other articles or for demonstrating the presence of the pest in the plants, plant products or other articles. PM

7/119 *Nematode extraction* provides procedures for nematode extraction and should be cross-referred to in pest specific diagnostic protocols for nematodes.

- Describe procedures to culture and isolate the pest.
- Describe bacterial/fungal colony morphology.
- Describe screening tests for detecting the presence of the pest in symptomatic and asymptomatic plant material or other materials (e.g. soil or water), such as ELISA tests or culturing on selective media (the information to be provided for media is presented in Appendix 1).

Illustrations of symptoms on the plant and plant product are considered helpful.
Provide information on possible confusion with similar signs and symptoms due to other biotic and abiotic causes.
When a test allowing both detection and identification of a pest is available, it should be mentioned in both sections.
When quick, presumptive indications of identity (which will later need to be confirmed) exist (e.g. colony characteristics), they should be mentioned.

4. Identification

In this section, the means of identification that leads to an unequivocal diagnosis is described; it may be composed of several steps and based on different tests. As a general rule, the protocol should recommend one or a few particular means of identification which are considered to have advantages (of reliability, ease of use, speed, cost etc.) over other tests. If the recommended tests require equipment and expertise that are not widely available, other tests should be described.

When morphological identification is recommended details should be provided, as appropriate, on:

- procedures to mount and examine the pest (e.g. light microscope, electron microscope)
- description of the morphology of the insect/nematode, or of fungal/viral structures, with indication of difficulties in seeing particular structures
- identification keys if necessary (to family, genus, species as appropriate)
- illustrations (drawings or photographs, black-and-white or colour) as appropriate, especially of diagnostic morphological characters

Measurements should be given as follows: maximum range outside and common range in the middle e.g. (3.5–) 12.5 (–33.5). When different names exist for the same structure, one should consistently be used in the protocol. The other ones can be mentioned in parenthesis at first use.

The author should also specify if specialized expertise is generally needed for identification of the pest and if confirmation by a specialist is particularly recommended (at least for a first identification or in case of doubt) or if a complementary method should be performed (e.g. PCR, sequencing).

General instructions for both detection and identification tests

- Editorial instructions

The drafting team should select the tests to be recommended in EPPO diagnostic protocols among all the tests available in literature and in laboratories (see below). Detailed instructions on how to perform recommended tests are given in numbered Appendices at the end of the Standard. Reference to other tests can be given in relevant sections of the Standards.

When tests already described in other protocols are recommended for the detection of a pest, the authors should not describe them again, but cross-refer as appropriate. When the test is adapted from another published test the original reference should be given.

Each test should be separately described (e.g. ELISA, electrophoresis, PCR, real-time PCR, RFLP, sequencing) if relevant in a specific appendix. Guidelines for the information to be included for the description of buffers and media are presented in Appendix 1. Guidelines for information to be included in a Diagnostic Protocol for molecular PCR tests are presented in Appendix 2. Guidelines for information to be included in a Diagnostic Protocol for HTS tests are presented in Appendix 3. Guidelines for information to be included in a Diagnostic Protocol for pathogenicity tests and tests on indicator plants are presented in Appendix 4. Standards describing procedures for performing methods exist such as PM 7/97 on *Indirect Immunofluorescence test for plant pathogenic bacteria;* PM 7/100 on *Rep-PCR tests for identification of bacteria,* PM 7/101 on *ELISA tests for plant pathogenic bacteria. PM 7/125 ELISA tests for viruses, PM 7/126 Electron microscopy in diagnosis of plant viruses, PM 7/129 DNA barcoding as an identification tool for a number of regulated pests, PM 7/153 Mechanical inoculation of test plants.* Authors are requested to refer to these general Standards when appropriate.

When measurements (e.g. temperature, speed, time…) are given when describing a test, the author should consider if it can be affixed with "approximately".
Tests require the inclusion of appropriate controls for an unequivocal conclusion. Since quarantine pests are being considered, it may not always be possible to obtain a sample for a positive control, and an alternative may be suggested (e.g. repeated tests, confirmation by other methods). Guidance should also be provided on possible confusion with similar and related species or taxa.
The test or combination of tests which result in positive diagnosis should be specified.
When relevant, the diagnostic procedure should be briefly described (e.g. extraction from symptomatic material, presumptive diagnostics with a screening test isolation from…" (see recently published protocols for reference).

- Selection of the tests to be included in a diagnostic protocol

Tests recommended in diagnostic protocols should preferably meet the basic criteria listed below in order to limit the number of tests recommended:

- The diagnostic laboratories in the EPPO region should have some experience with the tests.
- The tests should be validated and performance characteristics should be provided in the relevant appendices that describe the tests.. The following performance characteristics should be considered when selecting a test: analytical sensitivity, analytical specificity, repeatability and reproducibility (and where appropriate analytical selectivity). When available, information on diagnostic sensitivity and diagnostic specificity should also be provided. Further information on performance characteristics are given in PM 7/98 *Specific requirements for laboratories preparing accreditation for a plant pest diagnostic activity*. When tests have undergone a test performance study, this should be indicated, and the results of this test performance study should be included. As a basic requirement, tests should be repeatable.
- The tests (description and validation data) should be publicly available (e.g. published in a scientific publication, in a database, on a website).

Tests included in Diagnostic Standards are usually based on different methods to take into account the capabilities of laboratory (e.g. equipment) and the circumstances of use (e.g. throughput needs). When several tests are recommended, their advantages and disadvantages should be given. When several tests using the same method (e.g. PCR on a specific region) are being considered for inclusion in a diagnostic protocol the author should make a judgement of the overall performance characteristics in order to choose the tests which perform better. Priority for description in Appendices of the protocol should be given to tests that have undergone a test performance study (assuming the results of the study were adequate). If the author does not feel able to make a judgement between tests they can all be included with a note to the relevant Panel to request assistance in making this judgement. The following template table can be used to list the tests and facilitate the selection of tests: https://docs.google.com/spreadsheets/d/15NvtCYws9yd68GPKIzns-Ec_KrUyTArk/edit?usp=sharing&ouid=116130151617642844813&rtpof=true&sd=true.

For some pests there is little experience in the EPPO region, however, the Panel on Diagnostics and Quality Assurance recognizes that diagnostic protocols for such pests are useful. When full description of these tests is not possible because information is not fully available (e.g. to prepare master mix tables for molecular tests) the information available should be provided but it should be flagged in the protocol that a full description is not possible.

5. Reference material

The author should indicate from where reference material (see PM 7/76) can be obtained. Reference to sequences in gene banks should be given when the author is confident about species identity verification. For example, EPPO-Q-bank (https://qbank.eppo.int/) includes sequences for properly documented species and strains present in collections. *Additional note: authors are encouraged to deposit reference material in international reference collections where applicable to help future users of EPPO protocols.*

For **headings 6 to 11** of a diagnostic protocol, see the template for diagnostic protocols (document 24-29614).

12. References

Only references cited in the text should be included.

References in the bibliography should be set out as required for publication in the EPPO Bulletin (see https://onlinelibrary.wiley.com/page/journal/13652338/homepage/forauthors.html). The names of periodicals should be given unabbreviated. Article titles may be translated into French or English within square brackets, and the language of origin stated. If the abstract is available in French or English this may be stated. Foreign-language titles of books and pamphlets can be left in the original language. The last date of access to websites should be stated (e.g. 01 Jan 2014).

Appendix 1

Instructions to describe buffer and medium preparation

Recipes for buffers and media should be given in an Appendix and presented according to the format presented below (see also the example provided in the template).

The recipe should include:

- The name of the buffer or medium and a recent reference, and when a modified medium is used also the reference of the modified version
- Ingredients usually in grams per Liter (L),
- Quantity of distilled or demineralized water (H_2O)
- pH, if applicable

When media are supplemented with antibiotics, quantities should be provided and the CAS number or purity should be indicated as well as the number of units. A description of how they should be dissolved and when they should be added should be provided.

The agar should be indicated as "*microbiological grade agar*" except if a very specific type of agar must be used, in this case it should be specified. Brand names should not be provided unless the use of a specific brand is needed.

Duration and conditions of storage should be provided after the media recipe (e.g.: store the prepared medium at 2-8°C; prepared plates should be stored at 2-8°C in the dark; shelf life of stock and of ready to use medium 7 days at 4 +/- 2°C; use freshly prepared media; store prepared mixture in the dark at <15°C). If applicable, the author should also specify if conditions are different for ready to use plates.

As medium sterilization conditions are often generic, the specification should be given at the beginning of the Appendix.
If special sterilization conditions are required in certain cases e.g. sterilization by filtration, it should be noted after the media recipe. Specify when pH should be adjusted and antibiotics added (before or after sterilization).

Appendix 2: Guidelines for information to be included in a Diagnostic Protocol for PCR Tests

These guidelines are designed to ensure that the Diagnostic Protocols give the requisite information for reliable reproduction of the polymerase chain reaction (PCR) step in molecular analyses. These guidelines do not require details to be given on how to perform analyses of the amplicons produced by the PCR such as gel electrophoresis, except in situation where specific conditions are required to obtain a clear separation of nucleic acid fragments. However, it includes information required for nucleic acid extraction and purification, as this is a prerequisite for PCR. Also, to enable identification for a large range of organisms using basic molecular technology, it includes minimum information for the set up of reverse transcription reactions and restriction enzyme analyses. These guidelines are designed to introduce a strict structure of the presented information with the aim to ease understanding of the test.
Different PCR-based tests may be distinguished, conventional PCR (including RT-PCR, IC-RT-PCR, PCR-RFLP, nested PCR), real-time PCR (probes based Taqman®, SYBR® Green) and other nucleic acid based methods (e.g. LAMP). The minimum information to be provided for the different PCR-based tests are listed.

Please note that the name q-PCR is not used in EPPO Standards as most tests so far are qualitative and not quantitative.

The information required for each test type is separated into four sections:

Section 1: General Information – general information on the nucleic acid source and preparation, on the gene(s) if applicable/known and amplicon(s) under investigation, and on the reaction constituents, including all details important for reproducibility of results.

Section 2: Methods – methods on nucleic acid extraction and purification, reverse transcription, (real-time) PCR and RFLP, including details on reaction volumes, precise amounts and final concentrations per reaction required for the test as well as PCR run conditions. The guidance on information to be provided on reaction setup is separated in seven sub-sections,

- 2.1) Nucleic acid extraction and purification,
- 2.2) Reverse Transcription (RT; to produce cDNA from RNA),
- 2.3) Conventional PCR,
- 2.4) One step Reverse Transcription PCR,
- 2.5) Real-time Polymerase Chain Reaction – real-time PCR,
- 2.6) One step real-time Reverse Transcription Polymerase Chain Reaction – real-time RT-PCR,
- 2.7) Restriction Fragment Length Polymorphism (RFLP) Reaction.

Consult the relevant sub section for the test that are to be included in the protocol. Amounts of reagents should be indicated as the final concentration in mM, µM or nM. Enzyme amounts should be given in Units.; if crude or non-quantified DNA/RNA is used this should be noted. If using ready-made premixes or buffers only the final concentrations have to be indicated where applicable. The information is presented in an order that allows for easy assembly of the reaction.

Section 3: Essential Procedural Information - information that the authors regard as essential and that is not described in the earlier sections. All essential information not contained in the above sections but necessary for successful performance of the reaction according to the authors (especially where the window for a successful reaction is narrow) should be indicated in this section.

Section 4 Data on performance characteristics available - the validation data reported in that section should be produced using the procedure described in section 2. Validation data for slightly modified tests should be added to the EPPO Database on diagnostic expertise and should not be reported in that section except if the data is considered as informative and important by the authors (e.g. additional data on analytical specificity).

More detailed information is provided in the template.

Appendix 3: Guidelines for information to be included in a Diagnostic Protocol for HTS Tests

These guidelines are designed to ensure that the Diagnostic Protocols give the requisite information for reliable reproduction of HTS tests.

The information required for each test is separated into four sections:

Section 1: General Information – general information on the HTS test (scope, platform, targets, instruments…).

Section 2: Methods – description of the different steps of the HTS test including sample preparation, nucleic acid extraction, library preparation, sequencing and bioinformatic analysis.
Describe the protocol that was validated and stay factual.

Section 3: Essential Procedural Information - information that the authors regard as essential and that is not described in the earlier sections. This information, especially when critical for a successful test, should be indicated in this section. In particular, the relevant controls and how to interpret the results should be indicated in this section.

Section 4 Data on performance characteristics available - the validation data reported in this section should be produced using the procedure described in section 2. Validation data for slightly modified tests should be added to the EPPO Database on diagnostic expertise and should not be reported in this section except if the data is considered informative and/or important by the authors (e.g. additional data on analytical specificity).

More detailed information is provided in the template. Examples are provided in the supporting information of PM 7/151.

Appendix 4: Guidelines for information to be included in a Diagnostic Protocol for Pathogenicity Tests

Information needed for the description of pathogenicity tests or tests performed with indicator plants

- For pathogenicity tests, the name of the plant species and cultivar(s) to be used should be given (ideally this should be the same plant species and cultivar on which the pest was isolated, alternatively another plant known to express symptoms may be selected).
- For tests based on indicator plants, the name of the indicator plant species and cultivar(s) should be given (it may be the same plant species and cultivar on which the pest was isolated, or another plant species known to express symptoms).

The following points apply to both types of tests:

- Plant growth stage to be inoculated.
- If the plant should be in a specific condition at the time of inoculation (e.g. to increase the uptake of the pathogen), this should be mentioned.
- Number of test plants and the number positive and negative controls)
- Conditions of growth for test plants should be described (greenhouse, growth chamber…), including when appropriate temperature, light and humidity conditions. If conditions should differ before, during and after inoculation this should be mentioned. As for other tests, when temperatures are given these should be affixed with "approximately" when the author considers this is acceptable (when a given temperature is essential for the test to perform correctly this should be specified).
- Type and concentration of inoculation material (e.g. dry spores, bacterial suspensions…), type of structure to be used (e.g. conidia, ascospores, …) and mode of preparation.
- Method of inoculation of the test plants and controls.
- Description of symptom to be observed and whenever relevant minimum number/percentage of plants on which symptoms should be seen.
- Description of the frequency of observation of the test plants to detect symptoms, indication about the time needed for the first symptoms to appear and maximum time period for the observation.