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European and Mediterranean Plant Protection Organization
Organisation Européenne et Méditerranéenne pour la Protection des Plantes PM 7/98 (3)

Diagnostics
Diagnostic

PM 7/98 (3) Specific requirements for laboratories preparing accreditation for a plant pest diagnostic activity

Specific scope

This guideline includes specific quality management requirements for laboratories preparing for accreditation according to the ISO/IEC Standard 17025 *General requirements for the competence of testing and calibration laboratories* (2005, references to relevant parts of ISO/IEC Standard 17025 are included[1]). It should be noted that in EPPO standards the verb 'should' carries the highest level of obligation.

Specific approval and amendment

First approved in 2009-09.
First revision approved in 2014-04.
A second revision was prepared to incorporate the conclusions and recommendations of the Workshop on Flexible Scope 2017-06-26/28.
Second revision approved 2018-09.

1. Introduction

Development of quality management systems (also referred to as management systems or quality systems) and accreditation have become a concern for many laboratories in the EPPO region. EPPO Standard PM 7/84 *Basic requirements for quality management in plant pest diagnostic laboratories* was first adopted in 2007. PM 7/84 describes the basic requirements for supporting laboratories conducting plant pest diagnostics in designing their quality management system. PM 7/98 includes additional requirements for laboratories applying for accreditation. It is based on the ISO/IEC Standard 17025 *General requirements for the competence of testing and calibration laboratories* (ISO/IEC, 2005) and should be used together with PM 7/84. Until recently, laboratories usually applied for accreditation for only a small number of pests/test/matrix combinations for which they carry out routine testing and not for all pests which they are likely to test for. Many laboratories, however, need to extend their scope to cover more of their regular diagnostic activities.

Accreditation against ISO/IEC Standard 17025 is granted by national accreditation bodies, so it is important that laboratories develop good communication procedures and establish regular contact with their national accreditation body throughout the process.

This document does not deal with health and safety matters. Laboratory practices should conform to national health and safety regulations.

2. Terms and definitions

Definitions of terms used in this standard are included in PM 7/76 *Use of EPPO diagnostic protocols*.

In this Standard, 'test' refers to the application of a method to a specific pest and a specific matrix. Most test results are given in qualitative terms (the test is positive or negative or undetermined). It is recognized that some tests will generate quantitative data (e.g. optical density for ELISA, number of cells for IF, C_t values for real-time PCR, measurements for morphological features, etc.). However, in most cases such quantitative data is used to assign a qualitative result to the test (positive/negative/undetermined). Methods concerned include the following: bioassay methods, biochemical methods, fingerprint methods, isolation/extraction methods, molecular methods, morphological and morphometric methods, pathogenicity assessment, and serological methods.

As stated in PM 7/84: 'In the context of a plant pest diagnostic activity, results of one or more tests can be combined to contribute to a diagnosis'

Terminology varies between different international standards. A comparison table is available at https://upload.eppo.int/download/221odbcdc6308

[1]A new ISO 17025 was approved on 2017-12-13 and its implementation will be required by the end of 2019. A review of this EPPO Standard is planned in January 2019 to consider the need for revision. However, the risk-based process included in the new ISO Standard is already included in this revised version of PM 7/98.

3. Scope of accreditation: fixed and flexible scope

A laboratory can be accredited for different scopes, fixed and/or flexible.

A fixed scope defines clearly and unambiguously the range of tests covered by the laboratory's accreditation (e.g. IF test for the detection of *Clavibacter michiganensis* subsp. *sepedonicus* on potato tubers). However, a fixed scope does not readily allow new or modified tests to be added to a laboratory's scope, even when the competence of the laboratory in performing and validating related tests has already been evaluated by an accreditation body. Any change in the tests included in a fixed scope of accreditation is allowed after appropriate assessment and decision of the accreditation body. Although applications for an extension to the scope can be made at any time, the timescales involved may actually prevent quick reactions to a client's demands. Consequently, the concept of flexible scope has been developed. A flexible scope of accreditation allows a laboratory to report the results of certain tests as accredited prior to an audit by the accreditation body. Requirements for both types of scope are provided in sections 4 and 5, with additional requirements for flexible scope being given in section 6. Descriptions of scope are provided in *Guideline for the formulation of scopes of accreditation for laboratories* (ILAC-G18, 04/2010).

4. Management requirements (ISO 17025, 2005, point 4)

The laboratory should establish, implement and maintain a quality management system covering all facilities and activities in the scope of the accredited plant pest diagnostic activities.

The quality management system should describe the facilities and activities covered (including details of customers and pests which are tested for). It should be documented, and the quality documents should be archived (see also below). More details on the requirements for quality management systems can be found in the respective section of PM 7/84. It should be noted that PM 7/84 covers all plant pest diagnostic activities, including the non-accredited ones.

4.1. Commitment of top management (ISO 17025, 2005, point 4.2.3)

Top management (e.g. the institute manager) should commit to bringing into effect the goals of quality management and to continually improve the effectiveness of its quality management system. Management should also provide evidence of this commitment.

4.2. Continuous improvement and management reviews (ISO 17025, 2005, points 4.10 and 4.15)

The laboratory's quality management system should be reviewed periodically by top management to ensure its continuing suitability and effectiveness and to introduce necessary changes or improvements. A continuous improvement programme should be implemented by the laboratory. In-house mechanisms and external evaluation can provide the necessary information. Internal mechanisms include:

- the definition of quality objectives and adequate quality indicators (e.g. delivery of results on time or improved customer feedback); these should be reviewed by the management
- a plan of participation in proficiency tests or test performance studies
- the definition of a succession plan (e.g. to cover retirement or long-term absence of personnel) to ensure appropriate competence in the laboratory (optional)
- the organization of staff meetings to
  - plan preventive actions and assess corrective actions for their effectiveness
  - analyse thoroughly the results of audits (internal/external)
  - evaluate complaints and their corrective actions, etc.
  - identify training needs
  - collect suggestions for improvement by the staff
- procedures for seeking feedback from customers
- analysis of trends (technical or management)
- evaluation and updating of quality documents on a regular basis.

Assessments by external audits and results of interlaboratory comparisons (proficiency tests and/or test performance studies) are also important elements for continuous improvement.

5. Technical requirements (ISO 17025, 2005, point 5)

5.1. General (ISO 17025, 2005, point 5.1)

See PM 7/84, section 4.1.

5.2. Personnel (ISO 17025, 2005, point 5.2)

See PM 7/84, section 4.1.1.

5.3. Accommodation and environmental conditions (ISO 17025, 2005, point 5.3)

See PM 7/84, section 4.1.2.

5.4. Diagnostic tests (ISO 17025, 2005, point 5.4)

5.4.1. General (ISO 17025, 2005, point 5.4.1)
See PM 7/84 section 4.1.3.1.

5.4.2. Selection of tests (ISO 17025, 2005, point 5.4.2)
The laboratory should select tests that are suitable according to the circumstances of use (see EPPO Standard PM 7/

76 *Use of EPPO diagnostic protocols*). Tests described in the legislation (e.g. European Union or national legislation) are mandatory for the countries concerned. If no test is mandatory, tests published as international, regional or national standards should preferably be used. Whenever such tests are not available, or whenever performance could be improved, laboratory-developed or adapted tests can be considered.

The laboratory should ensure that it selects the latest valid edition of a test, unless it is not appropriate or possible to do so[2] . When necessary, the test description should be supplemented with additional details to ensure consistent application in the laboratory.

Tests used under accreditation should be validated. Validation is carried out to provide objective evidence that the test is suitable for the circumstances of use. If the test is not validated, it should undergo a validation process within the laboratory (see section 5.4.4). When a validated test is already available, the laboratory should provide objective evidence (verification) that it can operate the test according to the established performance characteristics (see section 5.4.5).

Before performing a validation or verification process, the laboratory should perform a risk analysis as described in section 5.4.3 to identify the extent of validation and/or verification to be performed.

5.4.3. Risk analysis before performing validation and/or verification (Fig. 1)

To identify which performance criteria need to be evaluated, and to what extent, the laboratory should conduct a risk analysis for each performance criterion to identify those that are critical in order to obtain a reliable result from the test. This risk analysis should be documented by the laboratory (see Appendix 1) and the choices made should be justified. The general process for risk analysis is described below and in part A of Fig. 1.

Tests can be characterized based on the following performance criteria:

analytical sensitivity
analytical specificity (inclusivity and exclusivity)
selectivity
repeatability
reproducibility.

In addition, robustness may indicate the degree of insensitivity of the test to deviations in the implementation, circumstances and quality of the materials (e.g. the age and condition of samples, different reagents) which occur in practice. Robustness is often included in reproducibility. A separate evaluation of the robustness will often not be necessary as it is done as part of the development of a test.

The scope of the test, for example detection and/or identification of organism *x* in matrix *y* by method *z*, should be identified. The following points could be considered as input for a risk analysis.

Intended use of the test (examples of questions and factors to consider

- description of the intended use (screening, confirmation, on-site testing)
- whether or not an expected level of performance or a specific intended use (on a contaminated area for screening, on specific hosts, cost of analysis) is required by the customer
- the impacts of the results (e.g. outbreak or survey in non-contaminated area)
- the level of statistical significance (e.g. level of confidence in the test) needed (impact on budget, repetitions, . . .)
- the hosts which should be considered for validation in the case of a wide host range or polyphagous pest
- the matrices that should be tested
- if there are specific species/strains or populations to be detected (e.g. specific species/strains or population present in the country)
- the possible cross-reactions that have to be considered (e.g. consider local conditions to define species to be tested for cross-reactions, such as specific populations, species, or hosts present in an area) and the cross-reactions that can be accepted
- the performance characteristics required for analytical sensitivity and analytical specificity (inclusivity and/or exclusivity)
- the level of flexibility needed for the use of the test (e.g. a network of laboratories as end users).

Constraints of the laboratory (examples of questions and factors to consider)

- the availability of reference material (pest related, matrix related)
- the level of flexibility needed for the use of the test (e.g. diversity of equipment available such as a PCR machine with rotor or plate)
- the availability of resources to perform the validation (budget, staff, equipment/reagents)
- time constraints.

Review of validation data available (examples of questions and factors to consider)

- are validation data available for the same test and/or similar tests that could be transferable (e.g. EPPO database on diagnostic expertise, validation section http://dc.eppo.int/validationlist.php, publications)
- tests included in EPPO Diagnostic Protocols are not all validated; however, EPPO Panels on Diagnostics considered that those presented in Appendix 2 give appropriate confidence with regard to repeatability and reproducibility.

Review of altered conditions (examples of questions and factors to consider)

- sustainability of supply of the reagents/chemicals
- change of reagents
- change of equipment.

[2]A laboratory may continue to use a previous version of a test if it is still appropriate for the circumstances of use.

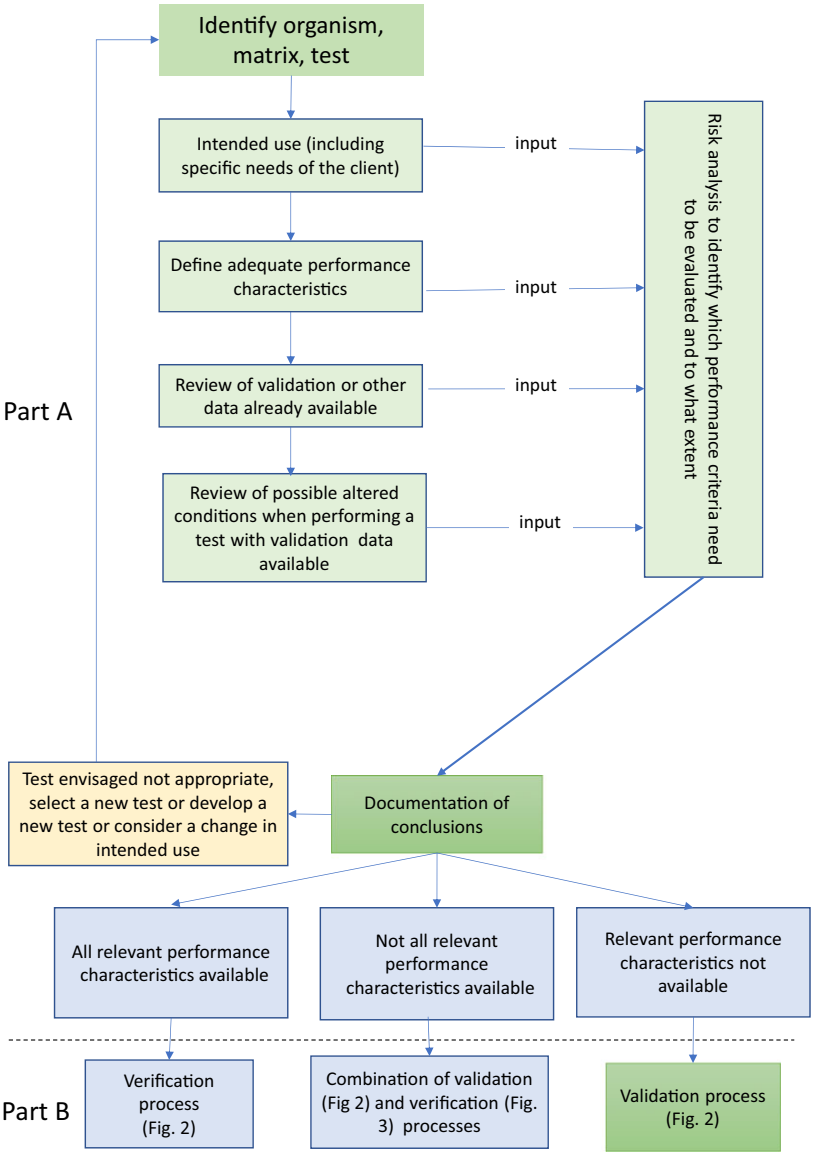

Fig. 1 Outline of the process for preparation for accreditation of a plant pest diagnostic test (including risk analysis). [Colour figure can be viewed at wileyonlinelibrary.com]

The output of risk analysis is shown in part B of Fig. 1.

At the end of the risk analysis the laboratory will have either identified and documented the extent of validation (see section 5.4.4) and/or verification (see section 5.4.5) to be performed or will need to select or develop a new test.

Examples of output of the risk analysis are presented below:

- If the data from a test using the same method is transferable from a test for another pest (consider if matrices are comparable) this could mean that the extent of validation can be reduced (e.g. for selectivity, repeatability, reproducibility); for example, experience with real-time PCR for Flavescence dorée would allow the extent of validation for repeatability and reproducibility for Bois Noir to be reduced.
- If the quantity of target in the sample is not a limiting factor, the extent of validation for analytical sensitivity can be reduced; for example identification on pure culture by PCR, as long as there is no inhibition effect (excess of matrix).
- If the test cannot distinguish between genera or species within a genus then inclusivity and exclusivity evaluations can be reduced (e.g. nematode extraction methods are not specific for one species or one genus).
- Reagents: the choice of reagent can be critical for the performance of a test. A change of reagent (or lots/-batches of reagent) or reagent supplier may influence the performance of a test. In such a case, a verification of the performance of the reagent should be done.
- Validation after significant change: if the laboratory makes a significant change to a validated test (e.g. testing outside the original scope) this 'new' test has to be validated. If a minor change to a validated test is made by the laboratory, a judgement as to whether

such a change requires validation or verification should be made and documented. Any change should be authorized by an appropriate person, and if relevant the customer and the accreditation body should be informed.

5.4.4. Validation of tests (ISO 17025, 2005, point 5.4.5) (Figs 1, part B, and 2)

5.4.4.1. Validation of tests other than morphological and morphometric tests. As mentioned in ISO 17025, 'the laboratory shall record the results obtained and the procedure used for the validation'.

The general process for validation is described below (see also Fig. 2).

The validation procedures are described here and the details given in Tables A1–A6 in Appendix 3 should be regarded as general guidance according to which a test can be validated. Figures given in those tables are based on the validation experience of experts from EPPO panels dealing with diagnostics. Test performance studies can be a valuable part of the validation process.

The validation process:

- consider the output of the risk analysis and define a validation plan
- consider the technical requirements to determine analytical sensitivity, analytical specificity (inclusivity and exclusivity), selectivity, reproducibility and repeatability performance values by consulting the guidelines in Tables A1–A6 as required. Then define the type and constitution of samples needed for the validation. Validation is to be performed with reference material (see definition in PM 7/76) and/or spiked samples. When using cultures or isolates for biological tests, care should be taken that they have a proven virulence
- plan and perform the validation for individual performance criteria or in a combined test setup (see Fig. 2):
  - present the results in a validation report with a conclusion on whether the test meets the requirements identified (see Appendix 1)
  - performance characteristics are met: the test is validated
  - performance characteristics are not met:
    - adjust the test and perform new evaluation for the relevant performance criteria
    - if the test cannot be adjusted, test cannot be validated for the originally intended use (in specific cases adaptation of the intended use of the test may be considered).

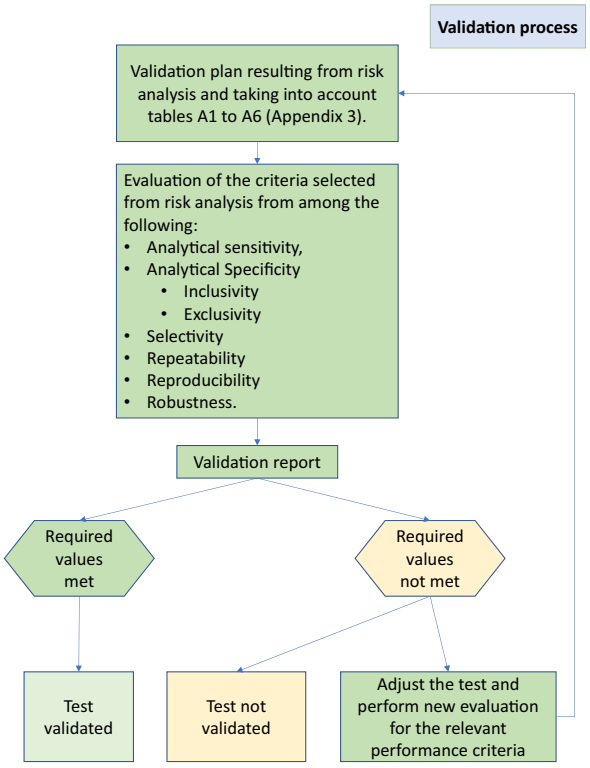

Fig. 2 Validation process. [Colour figure can be viewed at wileyonlinelibrary.com]

A comparison of a test (A) with a validated test (B) is an alternative means of validation which may be suitable in certain situations (see Appendix 4). This can only demonstrate that, for example, test A is as good as the validated test B with respect to selected performance criteria.

Additional information

Collected data and results of laboratory-performed validations (in particular related to reproducibility), as well as results of inter-laboratory comparison, can also provide an indication of the robustness of the test.

5.4.4.2. Validation for morphological and morphometric tests. It is acknowledged that the procedures for morphological and morphometric tests are ultimately a judgement based on expert opinion. Validation may therefore not follow the same procedures as for the other tests. Guidance for the validation of morphological and morphometric tests is given in Appendix 5. This guidance is applicable for these methods irrespective of the field in which they are used (entomology, nematology, mycology, botany, etc.). The laboratory should be able to justify the selection of morphological or morphometric tests made, in particular for those not described in international standards or peer-reviewed journals.

5.4.5. Verification of the performance of the laboratory to undertake a specified test (ISO 17025, 2005, point 5.4.2, second paragraph, last sentence)

5.4.5.1. Verification process for tests other than morphological and morphometric tests. General

Verification provides objective evidence that the laboratory is competent to perform a validated test according to the relevant performance characteristics. Verification can also be done by successfully participating in a proficiency

Table 1. Guidance on the verification of performance criteria

Performance criteria	Verification method
Analytical sensitivity	Analyse at least eight samples* at the established limit of detection (for viruses, viroids and phytoplasmas this should be at a low level) This can be combined with repeatability/ reproducibility
Analytical specificity (inclusivity and exclusivity)	Select a few of the most relevant targets (e.g. different strains, populations) for inclusivity and non-targets* for exclusivity. Tests should be performed with medium levels of organisms
Repeatability	Perform at least three simultaneous tests on the same material with low levels of the target*
Reproducibility	As for repeatability but at different moments, when possible with different operators, and when relevant with different equipment

*Artificial subsamples created from one sample can be used.

test or test performance study, provided that these allow the requirements in Table 1 to be fulfilled.

The general process for verification is described below (see also Fig. 3).

Verification process:

- consider the outcome from the risk analysis and prepare a verification plan. Perform the validated test as described or with minor changes to take into account local conditions (e.g. suppliers of reagents or equipment, unless it is specifically required in the validated test) to evaluate whether the laboratory meets the performance characteristics from the validation data (see guidelines in Table 1). Selectivity generally does not need to be verified; however, for example for serological methods, selectivity may need to be verified to evaluate the impact of different batches of antisera)
- plan and perform the verification for individual performance criteria or in a combined test setup
- present the results in a verification report with a conclusion on whether or not the laboratory meets the requirements identified
  - performance values are met: the laboratory can perform the test
  - performance values are not met:
    - if deviation from conditions described in the validated test affects the test results, investigate the reasons for this deviation. Correct, verify the test again or validate if required following the procedure described in the section 'Validation of tests'
    - investigate whether the minor changes that have been introduced in the test are the cause. If this is not the case, seek external guidance (e.g. contact the author of the test). Adjustments should then be made and the relevant steps repeated. If other reasons for deviation have been observed (e.g. staff errors) corrective action should be taken and documented
    - if the cause cannot be understood or modifications cannot be made to allow performance values to be met, the laboratory cannot operate the test according to the established performance criteria.

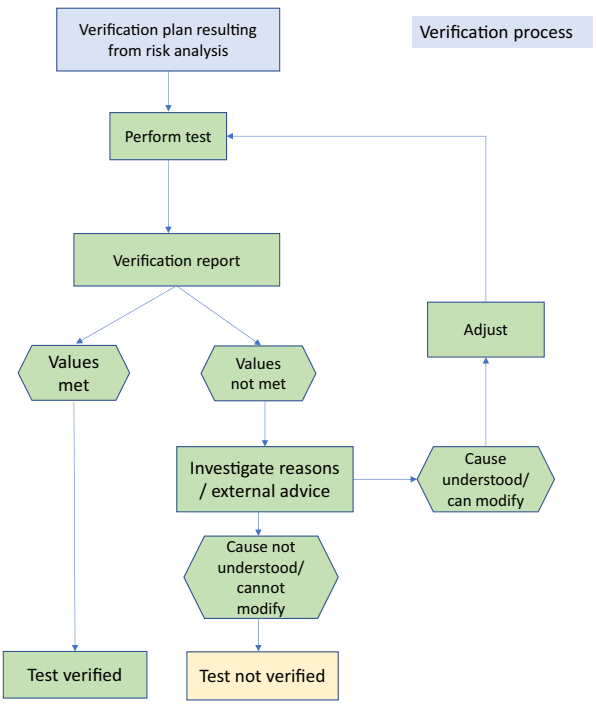

Fig 3 Verification process. [Colour figure can be viewed at wileyonlinelibrary.com]

5.4.5.2. Verification process for morphological and morphometric tests. The laboratory should confirm that it can properly carry out the validated morphological and/or morphometric identification. Such verification can be achieved by taking part in a proficiency test or by having a number of samples identified in the laboratory and then confirmed by an independent specialist or another validated test (e.g. PCR, sequencing).

5.4.6. Final result

Use of a 'Statement on test validation and/or verification' form (Appendix 1) can be valuable for summarizing the results.

5.4.7. Uncertainty of measurement (ISO 17025, 2005, point 5.4.6)

The laboratory should attempt to identify the factors influencing the uncertainty of a test such as staff, equipment and biological properties (i.e. serotypes, pathotypes). Repeatability and reproducibility will provide information on the level of uncertainty of the test result. Whenever possible, appropriate measures should be taken to control this uncertainty. If no measures are taken, the reasons for this should be recorded and the client should be made fully aware of the uncertainty surrounding the test.

Although in most cases tests used for plant pest diagnosis provide qualitative results, these qualitative results may be based on measurement (morphometric data, counting of cells). This measurement may be just one part of the diagnostic process, but if this is critical for a final result its uncertainty should be estimated. Two examples of laboratory reports identifying critical points in the process are provided in Appendix 6.

5.5. Equipment (ISO 17025, 2005, point 5.5)

See section 4.1.4 of PM 7/84.

5.6. Reference materials (ISO 17025, 2005, point 5.6.3.2)

See section 4.1.5 of PM 7/84.

5.7. Sampling (ISO 17025, 2005, point 5.7)

See section 4.1.6 of PM 7/84.

5.8. Sample handling (ISO 17025, 2005, point 5.8)

See section 4.1.7 of PM 7/84.

5.9. Ensuring the quality of test results (ISO 17025, 2005, point 5.9)

See section 4.2 of PM 7/84.

5.10. Reporting the results (ISO 17025, 2005, point 5.10.3)

See EPPO Standard PM 7/77 *Documentation and reporting on a diagnosis*.

6. Additional requirements for flexible scope

A flexible scope of accreditation allows a laboratory to undertake certain tests and to report the results as accredited, even though these tests are not explicitly stated in the laboratory's scope, but in a specific list of tests under accreditation (*EA requirements for the accreditation of flexible scopes* EA-2/15, 2008). Examples of situations where the need for flexible scope may arise are:

- fast addition/deletion of tests under accreditation to answer urgent demands
- optimization of a given test
- modification of an existing test to broaden its applicability (e.g. to deal with new matrices, similar pests)
- inclusion of a test equivalent to one already covered by accreditation.

The concept of flexible scope encompasses a degree of flexibility which is usually agreed in consultation with the accreditation body. Nevertheless, it should be noted that this degree of flexibility is subject to a varying interpretation at the national level. The experience in plant pest diagnostic laboratories so far is that flexible scope is helpful as it allows a laboratory to be accredited for new tests prior to an audit by the accreditation body. However, it places more responsibility on the laboratory to manage its scope of accreditation, to demonstrate that tests are valid, suitable for the circumstances of use and are performed competently and consistently. If the laboratory decides to report a test as accredited and an audit later identifies problems with the procedures used, results may not be valid and all reports issued based on that test may have to be withdrawn. Therefore, experience with a fixed scope accreditation is valuable before a laboratory applies for flexible scope, as all requirements of the ISO/IEC Standard 17025 have to be fulfilled in both cases. Nevertheless, experience with a fixed scope accreditation in another activity may be sufficient for the direct application for a flexible scope for plant pest diagnostic activities. A laboratory should contact the accreditation body to discuss the possible options.

EA-2/15(2008) defines the requirements for accreditation of flexible scope, including the following elements:

- a clear definition of the extent and the limits of the flexible scope
- a procedure for the management of the scope (see Fig. 4 as an example and details provided below). Appropriate documents should be developed to ensure the traceability when the procedure is applied
- a list of tests included in the flexible scope is required and must be maintained by the laboratory
- definition of the frequency and the means to inform the accreditation body of changes to flexible scope
- information should be available to the client that the test is performed under flexible scope at the contract review stage.

The flow diagram in Fig. 4 outlines the procedure for the management of flexible scope and includes the requirements stated in EA-2/15. Before adding a new test in the scope, the laboratory should first confirm that this test fits with the current scope of accreditation. If not, the laboratory should communicate with its accreditation body to extend its scope of accreditation before including this new test.

One of the most important steps of the procedure is the authorization to add or delete a test from the accreditation scope. This responsibility should be clearly defined.

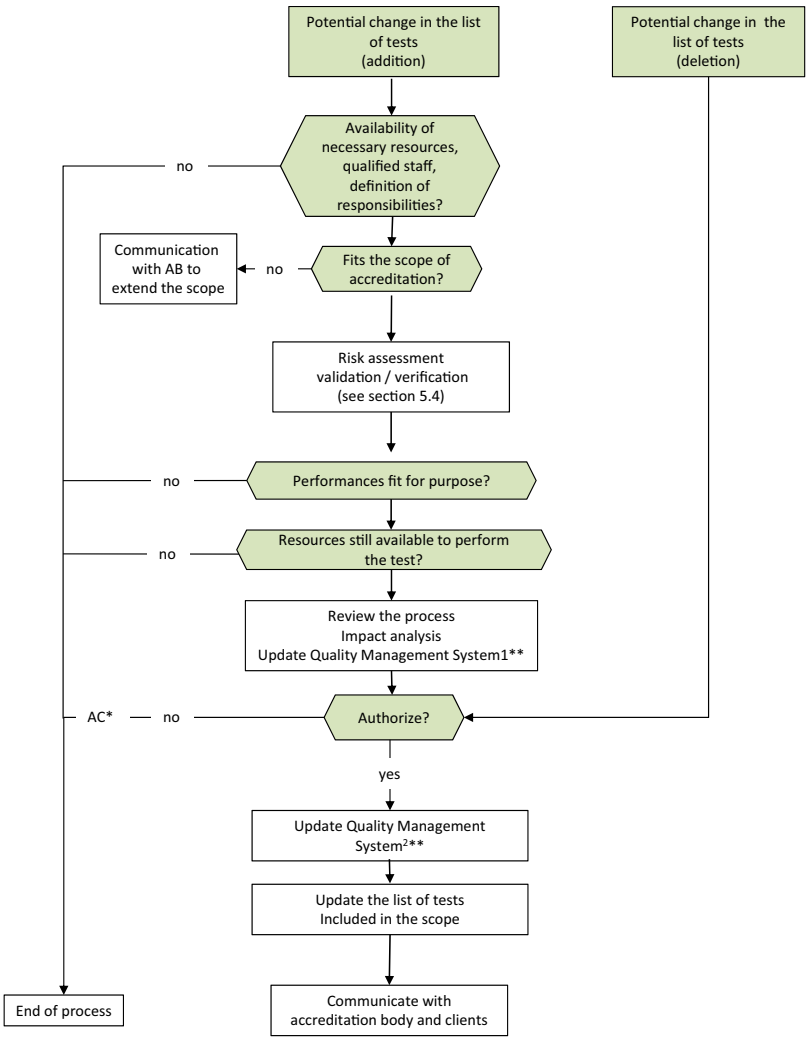

Fig. 4 Example of a procedure for the management of a flexible scope (AB, accreditation body). [Colour figure can be viewed at wileyonlinelibrary.com]

Before authorizing the update of the list of accredited tests, the laboratory should review the process leading to addition/deletion of a specific test to the scope of accreditation by examining the relevance and the completeness of the documentation (e.g. forms are duly completed). Auditing of the process can serve as a review.

The laboratory should also conduct an impact analysis to define the consequences for laboratory activities (e.g. appropriate resources, plans to adjust its organization, modification of the quality management system). The quality management system should be updated. This may include revision of documents (e.g. standard operational procedures), update of internal quality controls (e.g. controls, participation in proficiency testing), maintenance of competence (e.g. training of staff) and updating the audit programme.

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References (for the latest version of EPPO Standards, please consult EPPO Global Database or the EPPO website)

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Appendix 1 – Statement on test validation and/or verification

Test name:

Scope of test:

Intended use of the test:

Summary of the risk analysis:

Performance criteria	Selected for validation	Selected for verification	Not selected	Where to find information
Analytical sensitivity				
Analytical specificity: inclusivity				
Analytical specificity: exclusivity				
Selectivity				
Repeatability				
Reproducibility				
Robustness				

Report on validation

Additional comments

Documentation for the validity of the test and the requirements that the test should meet are available in the laboratory. Documentation includes laboratory books and other information as indicated below, which shows how procedures have been validated in this study:

Performance criteria	NA	A	B	C	D	Where to find documentation
Analytical sensitivity						
Analytical specificity: inclusivity						
Analytical specificity: exclusivity						
Selectivity						
Repeatability						
Reproducibility						
Robustness						

NA, not applicable; A, data from own laboratory experiments; B, data from inter-laboratory comparison; C, information from manufacturers; D, external information (literature, etc.); other information (optional).

	Where to find documentation
Diagnostic sensitivity	
Diagnostic specificity	

Report on verification

Description of changes

Documentation for the verification of the test and the requirements that the test should meet are available in the laboratory. Documentation includes laboratory books and other information which shows how the verification has been performed in this study.

Performance criteria	Performance characteristics obtained	Meet requirements of the validated test (yes/no)	Where to find documentation
Analytical sensitivity			
Analytical specificity: inclusivity			
Analytical specificity: exclusivity			
Repeatability			
Reproducibility			

On the basis of the above statement the validity and/or verification of the test is judged suitable for the scope of the test.

Person responsible for carrying out the test

Name in block capitals:

Signature, date:

Authorizing person

Name in capital letters:

Signature, date:

Appendix 2 – List of tests included in EPPO Diagnostic Protocols that are widely used

A survey carried out in 2008 and repeated in 2013 on the use of tests included in EPPO Diagnostic Protocols (Petter & Suffert, 2010) showed that those presented in this Appendix are widely used. Consequently, EPPO Panels on Diagnostics considered that these tests give appropriate confidence with regard to repeatability and reproducibility. A laboratory implementing these tests should at least produce or collect validation data regarding analytical sensitivity and analytical specificity.

Tests must have been used in a minimum of two laboratories and for a minimum of eight samples in each laboratory to be considered widely used. Please note that molecular tests include DNA extraction.

Since the surveys, several protocols have been updated and new tests added. However, only the tests included in protocols at the time of the surveys are listed here as we do not have data on the frequency of use of the tests in the subsequently revised protocols.

The list of tests included in EPPO Diagnostic Protocols that are widely used is provided as Appendix S1 in the online Supporting Information.

Appendix 3 – Tables giving detailed guidance for the validation process by field (bacteriology, botany, entomology, mycology, nematology, virology and phytoplasmology)

Instructions for the use of the tables[3]

Comment on the figures

Figures given in these tables are based on the validation experience of experts from EPPO Panels dealing with diagnostics. Deviations from this guidance may be possible or necessary depending on pest/matrix combination. Validation for morphological and morphometric methods for all fields are described in Appendix 6.

General note on analytical sensitivity

Whenever possible, the limit of detection (as defined in PM 7/76) of a test should be determined. Nevertheless, this limit cannot always be established absolutely while detecting plant pests. There are organisms that cannot be cultured (obligate pathogens), which cannot be quantified (fungi), which are only present in the plant or which cannot be purified (e.g. phytoplasmas). For this reason, exact concentrations of these organisms cannot or can hardly ever be established accurately and so estimates have to be used. Even with those that can be purified (many bacteria and viruses), the concentration can only be estimated (e.g. cfu or mg mL^{-1}). This estimation is often based on an indirect measurement. Where applicable, serial dilutions should be carried out until an end point is achieved.

General note on replicates

The number of replicates (given in the tables below) does not refer to the number of technical repetitions (e.g. duplicate/triplicate reactions which are carried out as standard for ELISA tests or real-time PCR runs).

[3]It should be noted that during country consultation comments were received on these. The Panel on Diagnostics and Quality Assurance considered these during their 2018 meeting. They considered that it was not possible to address the changes proposed at this stage (in addition comments were made for some disciplines only and these tables have been designed to be as harmonized as possible). It was concluded that these comments will be addressed during the next revision. A consultation of the specialized Panels will be organized to gather feedback about the relevant tables and tables will also be prepared for proteomic methods.

Bacteriology

Table A1. Bacteriology (see also the instructions for the use of the tables)

Method for extraction of target organism from a matrix (isolation of the target)	
Extraction is always validated by a test	
Analytical sensitivity	The method should permit the extraction/isolation of a sufficient quantity of the target organism to allow it to be cultured or analysed further. Perform extractions from at least three samples with high/medium/low levels of target Samples may consist of known infected material or samples may be produced by adding infected material with known cell density of the target bacterium to the matrix (detection of latent infection or contamination)
Analytical specificity	This parameter is not applicable. Extraction of the target organism from a sample is by definition non-specific
Selectivity	This parameter is not applicable. Extraction of the target organism from a sample is by definition non-selective
Repeatability	Use at least one sample with a low concentration of target and make at least three subsamples (extractions). Assess extraction efficiency by the relevant test method. If consistent results are not obtained, additional samples should be extracted
Reproducibility	As for repeatability, but with different operator(s) if possible, on different days and with different equipment when relevant
Molecular methods (e.g. PCR, real-time PCR, LAMP)	
This step also includes methods for the isolation of DNA from the matrix	
Analytical sensitivity	Analyse at least three series of spiked sample extracts with a range of 10^1–10^6 cells of the target organism per mL. Preferentially, this is done by making decimal diluted cell suspensions of the target bacterium in the sample extracts. Determine the lowest cell density giving a positive test result If consistent results are not obtained after three series, then additional series should be prepared and tested Analytical sensitivity refers to a specific set of test parameters which should be stringently defined and standardized, for example the brand of PCR reagents (in particular DNA polymerase) and PCR cycle conditions
Analytical specificity	Inclusivity: analyse strains of the target bacterium covering genetic diversity, different geographical origins and hosts Exclusivity: analyse a set of non-target bacteria, in particular those associated with the matrix For both inclusivity and exclusivity use cell suspensions of pure cultures at approximately 10^6 cells per mL. For non-targets, the concentration of nucleic acid should be high enough to maximize the possibility of cross reaction but remain realistic. In addition, the test results can be supported by in silico comparison of probe/primer sequences to sequences in genomic libraries
Selectivity	Determine whether variations in the matrix (e.g. by using different hosts of the same family, different cultivars of the host plant) affect the test performance
Repeatability	Analyse at least three replicates of spiked sample extracts with a low concentration. If consistent results are not obtained, additional replicates should be prepared and tested
Reproducibility	As for repeatability, but with different operator(s) if possible, on different days and with different equipment when relevant
Serological methods (IF and ELISA)	
Analytical sensitivity	Analyse at least three series of spiked sample extracts with a range of 10^2–10^6 cells of the target organism per mL. Preferentially, this is done by making decimal diluted cell suspensions of the target bacterium in the sample extracts. Determine the lowest cell density giving a positive test result at the working dilution of the antiserum/antibodies If consistent results are not obtained after three series, additional series should be prepared and tested Analytical sensitivity refers to a specific set of test parameters which should be stringently defined and standardized, e.g. the number of microscope fields to read in the IF test and the OD threshold in the ELISA test
Analytical specificity	Inclusivity: define specificity of antibodies on strains of the target bacterium covering genetic diversity, different geographical origins and hosts. Exclusivity: define specificity of antibodies on a set of non-target bacteria, in particular those associated with the matrix For both inclusivity and exclusivity use cell suspensions of pure cultures at approximately 10^6 cells per mL and use antiserum/antibodies at their working dilution
Selectivity	Determine whether variations in the matrix (e.g. by using different hosts of the same family, different cultivars of the host plant) affect the test performance
Repeatability	Analyse at least three replicates of spiked sample extracts with a low concentration. If consistent results are not obtained, additional replicates should be prepared and tested
Reproducibility	As for repeatability, but with different operator(s) if possible and on different days and with different equipment when relevant

(continued)

Plating methods: selective isolation	
Analytical sensitivity	Analyse at least three series of spiked sample extracts with a range of 10^1–10^6 cells of the target organism per mL. Preferentially, this is done by making decimal diluted cell suspensions of the target bacterium in the sample extracts. Determine the lowest cell density giving a positive test result If consistent results are not obtained after three series, additional series should be prepared and tested Analytical sensitivity refers to a specific set of test parameters which should be stringently defined and standardized, for example the brand of ingredients for the medium (in particular antibiotics and preparation of stock solutions) and incubation conditions
Analytical specificity	Inclusivity: define specificity of the culture medium on strains of the target bacterium covering genetic diversity, different geographic origin and hosts Exclusivity: define specificity for a set of non-target bacteria, in particular those associated with the matrix For both inclusivity and exclusivity use a cell suspension at approximately 10^6 cells per mL and analyse by dilution plating
Selectivity	Determine whether variations in the matrix (e.g. by using different hosts of the same family, different cultivars of the host plant) affect the test performance
Repeatability	Analyse at least three replicates of spiked sample extracts with a low concentration. If consistent results are not obtained additional replicates should be prepared and tested
Reproducibility	As for repeatability, but with different operator(s) if possible and on different days and with different equipment when relevant
Bioassay methods: selective enrichment in host plants	
Analytical sensitivity	Analyse at least three series of spiked sample extracts with a range of 10^2–10^6 cells of the target organism per mL. Preferentially, this is done by making decimal diluted cell suspensions of the target bacterium in the sample extracts. Determine the lowest cell density giving a positive test result. This implies isolation from test plants with or without symptoms of infection If consistent results are not obtained after three series, additional series should be prepared and tested Analytical sensitivity refers to a specific set of test parameters which should be stringently defined and standardized (e.g. stage of test plants, inoculation method and incubation conditions)
Analytical specificity	Inclusivity: define specificity of the bioassay on strains of the target bacterium covering genetic diversity, different geographical origins and hosts Exclusivity: define specificity for a set of non-target bacteria, in particular those associated with the matrix For both inclusivity and exclusivity use a cell suspension at approximately 10^6 cells per mL
Selectivity	Determine whether variations in the matrix (e.g. by using different cultivars including the most susceptible cultivar or cultivars) affect the test performance
Repeatability	Analyse at least three replicates of sample extracts with a low concentration and use the host plants determined in the specificity test. If consistent results are not obtained additional replicates should be prepared and tested
Reproducibility	As for repeatability but with different operator(s) if possible and on different days and with different equipment when relevant
Pathogenicity test	
Analytical sensitivity	This parameter is not relevant for the pathogenicity test, which is generally performed with cell suspensions of approximately 10^6 cells per mL. However, analytical sensitivity may be considered when inoculating in different growth stages of the host plant
Analytical specificity	Inclusivity: define specificity of the pathogenicity test on a set of strains of the target bacterium covering genetic diversity, different geographical origins and hosts Exclusivity: define specificity on a set of non-target bacteria, in particular those associated with the matrix. For both inclusivity and exclusivity use cell suspensions of approximately 10^6 cells per mL A positive result implies expression of symptoms and re-isolation of the target bacterium (Koch's postulates)
Selectivity	Determine whether using different cultivars of the host plant affects the test performance
Repeatability	Analyse at least three replicates of a set of strains of the target bacterium covering variability in identification tests and virulence. Use cell suspensions of approximately 10^6 cells per mL A positive result implies expression of symptoms and re-isolation of the target bacterium (Koch's postulates)
Reproducibility	As for repeatability but with different operator(s) if possible and on different days and with different equipment when relevant
Fingerprint methods: protein profiling, fatty acid profiling and DNA profiling	
Analytical sensitivity	Determine the minimum quantity of harvested bacteria from selected culture media needed to perform a reliable analysis Test parameters should be stringently defined and standardized (e.g. culture medium, stage of culture for harvesting of cells)
Analytical specificity	Inclusivity: define specificity of the fingerprint method on strains of the target bacterium covering genetic diversity, different geographical origins and hosts Exclusivity: define specificity for a set of non-target bacteria, in particular those associated with the matrix For both inclusivity and exclusivity provide markers for differentiation at subspecies or pathovar level For both inclusivity and exclusivity test results can be supported by in silico comparison with data in relevant databases
Selectivity	Not applicable
Repeatability	Analyse at least three replicates of the protein/fatty acid/DNA extract
Reproducibility	As for repeatability but with different operator(s) if possible and on different days and with different equipment when relevant

Botany

Table A2. Botany (see also the instructions for the use of the tables)

Method for extraction of target organism from a matrix	
Extraction is always validated by a test	
Analytical sensitivity	The method should be able to extract a sufficient quantity of the target organism to allow it to be analysed further. The percentage of invasive alien plant seeds that are recovered by the extraction method may be determined from a minimum of three samples
Analytical specificity	This parameter is not applicable. Extraction of the target organism from a sample is by definition non-specific
Selectivity	This parameter is not applicable. Extraction of the target organism from a sample is by definition non-selective
Repeatability	Use at least one sample with a low concentration of target and make at least three subsamples (extractions). Assess extraction efficiency by the relevant test method. If consistent results are not obtained, additional samples should be extracted
Reproducibility	As for repeatability, but with different operator(s) if possible, on different days, and with different equipment when relevant

Entomology

Table A3. Entomology (see also the instructions for the use of the tables)

Method for extraction of target organism from a matrix	
Extraction is always validated by a test	
Analytical sensitivity	The method should be able to extract a sufficient quantity of the target organism to allow it to be analysed further. The percentage of insects that are recovered by the extraction method may be determined from a minimum of three samples DNA extraction: validation is included in molecular methods validation
Analytical specificity	This parameter is not applicable. Extraction of the target organism from a sample is by definition non-specific
Selectivity	This parameter is not applicable. Extraction of the target organism from a sample is by definition non-selective
Repeatability	Not applicable
Reproducibility	Not applicable
Molecular methods (e.g. PCR, real-time PCR, LAMP)	
This step also includes methods for isolation of DNA from the matrix	
Analytical sensitivity	Prepare a certain number of individuals. This number varies depending on the genus, species and stage. Determine the minimum number of individuals or parts of individuals to be detected. Perform at least three experiments. If consistent results are not obtained after three series, additional series should be prepared and tested Analytical sensitivity refers to a specific set of test parameters which should be stringently defined and standardized, for example the brand of PCR reagents (in particular DNA polymerase) and PCR cycle conditions
Analytical specificity	Inclusivity: analyse a range of target organism(s), covering genetic diversity, different geographical origins and hosts Exclusivity: analyse relevant non-target organism(s), in particular those associated with the matrix. The concentration of nucleic acid should be high enough to maximize the possibility of cross-reaction but remain realistic For both inclusivity and exclusivity the test results can be supported by in silico comparison of probe/primer sequences to sequences in genomic libraries
Selectivity	Determine whether variations of the matrix (e.g. by using different cultivars of the host plant) affect the test performance
Repeatability	Analyse at least three replicates of sample extracts with a low concentration. If consistent results are not obtained, additional replicates should be prepared and tested
Reproducibility	As for repeatability, but with different operator(s) if possible, on different days, and with different equipment when relevant

Mycology

Table A4. Mycology (see also the instructions for the use of the tables)

Method for extraction/isolation/baiting of target organism from a matrix	
Extraction is always validated by a test.	
Analytical sensitivity	The method should be able to extract/isolate/bait a sufficient quantity of the target organism to allow it to be cultured or analysed further. Whenever possible, determine by blending healthy and infected tissue the smallest amount of diseased tissues or features required to be plated or identified in order to perform a reliable analysis Extract/isolate/bait the target from at least three samples (naturally infected or artificially infected samples). This may include a washing procedure and membranes to trap spores
Analytical specificity	This parameter is not applicable. Extraction of the target organism from a sample is by definition non-specific
Selectivity	This parameter is not applicable. Extraction of the target organism from a sample is by definition non-selective
Repeatability	Use at least one sample with low concentration of target and make at least three subsamples (extractions). Assess extraction efficiency by the relevant test method. If consistent results are not obtained additional samples should be extracted
Reproducibility	As for repeatability, but with different operator(s) if possible, on different days, and with different equipment when relevant
Molecular methods (e.g. PCR, real-time PCR, LAMP)	
This step also includes methods for isolation of DNA from the matrix	
Analytical sensitivity	Determine the minimum quantity of target (e.g. number of conidia or weight of infected material in healthy material) from which a detectable amount of target DNA can be obtained. Perform at least three experiments with serial dilutions, preferably in host plant DNA. If consistent results are not obtained after three series, additional series should be prepared and tested Analytical sensitivity refers to a specific set of test parameters which should be stringently defined and standardized, for example the brand of PCR reagents (in particular DNA polymerase) and PCR cycle conditions
Analytical specificity	Inclusivity: analyse a range of target organisms covering genetic diversity, different geographical origins and hosts Exclusivity: analyse relevant non-target organisms (e.g. phylogenetically close fungi) that might be present in the sample and sample extract. The concentration of nucleic acid should be high enough to maximize the possibility of cross reaction but remain realistic For inclusivity and exclusivity the test results can be supported by in silico comparison of probe/primer sequences to sequences in genomic libraries
Selectivity	Determine whether variations of the matrix (e.g. by using different cultivars of the host plant) affect the test performance
Repeatability	Analyse at least three replicates of sample extracts with a low concentration. If consistent results are not obtained, additional replicates should be prepared and tested
Reproducibility	As for repeatability, but with different operator(s) if possible, on different days, and with different equipment when relevant
Serological methods: ELISA	
Analytical sensitivity	Determine the minimum quantity of target (e.g. number of conidia or weight of infected material in healthy material) from which a positive test resultant the working dilution of the antiserum/antibodies Analytical sensitivity refers to a specific set of test parameters which should be stringently defined and standardised (e.g. the OD threshold). If consistent results are not obtained after three series, additional series should be prepared and tested
Analytical specificity	Inclusivity: define specificity of antibodies on strains of the target organism covering genetic diversity, different geographical origins and hosts. Exclusivity: define specificity on a set of non-target organisms, in particular those associated with the matrix
Selectivity	Determine whether variations of the matrix (e.g. by using different cultivars of the host plant) affect the test performance
Repeatability	Analyse at least three replicates of sample extracts with a low concentration. If consistent results are not obtained, additional replicates should be prepared and tested
Reproducibility	As for repeatability, but with different operator(s) if possible, on different days, and with different equipment when relevant
Bioassay methods: pathogenicity test	
Analytical sensitivity	Determine the necessary quantity of matrix or matrix extract (e.g. grams of leaves, soil) to produce symptoms. Perform three experiments with five dilution series If consistent results are not obtained after three series, additional series should be prepared and tested Analytical sensitivity refers to a specific set of test parameters which should be stringently defined and standardized (e.g. stage of test plants, inoculation method and incubation conditions)
Analytical specificity	Inclusivity: define specificity of the bioassay on strains of the target fungi covering genetic diversity, different geographical origins and hosts Exclusivity: define specificity for a set of non-target fungi, in particular those associated with the matrix
Selectivity	Determine whether variations of the matrix (e.g. by using different cultivars of the host plant) affect the test performance
Repeatability	Analyse at least three replicates of sample extracts with a low concentration and use the host plants determined in the specificity test. If consistent results are not obtained, additional replicates should be prepared and tested
Reproducibility	As for repeatability, but with different operator(s) if possible, on different days, and with different equipment when relevant

Nematology

Table A5. Nematology (see also the instructions for the use of the tables)

Method for extraction of target organism from matrix	
Extraction is always validated by a test	
Analytical sensitivity	The method should be able to extract a sufficient quantity of the target organism to allow it to be analysed further. The percentage of nematodes that is recovered by the extraction method should be determined from a minimum of three samples
Analytical specificity	This parameter is not applicable. Extraction of the target organism from a sample is by definition non-specific
Selectivity	Determine whether variations of the matrix (e.g. type of soil for cyst extractors) affect the test performance
Repeatability	Use at least one sample with low concentration of target and make at least three subsamples (extractions). Assess extraction efficiency by the relevant test method
Reproducibility	As for repeatability, but with different operator(s) if possible, on different days, and with different equipment when relevant
Molecular methods (e.g. PCR, real-time PCR, LAMP)	
This step also includes methods for isolation of DNA from the matrix	
Analytical sensitivity	Prepare a number of individuals. This number varies depending on the genus, species and stage. Determine the minimum number of individuals or parts of individuals to be detected or identified Perform at least three experiments. If consistent results are not obtained after three series then additional series should be prepared and tested Analytical sensitivity refers to a specific set of test parameters which should be stringently defined and standardized, for example the brand of PCR reagents (in particular DNA polymerase) and PCR cycle conditions
Analytical specificity	Inclusivity: analyse a range of target organism(s), covering genetic diversity, different geographical origins and hosts Exclusivity: analyse relevant non-target organism(s), in particular those associated with the matrix. The concentration of nucleic acid should be high enough to maximize the possibility of cross-reaction but remain realistic For both inclusivity and exclusivity the test results can be supported by in silico comparison of probe/primer sequences to sequences in genomic libraries
Selectivity	Not applicable for nematodes if they have been previously isolated from the matrix. However, if the PCR test is used as a detection test, determine whether variations of the matrix (e.g. soil, plant) affect the test performance
Repeatability	Analyse at least three replicates of sample extracts with a low concentration. If consistent results are not obtained, additional replicates should be prepared and tested
Reproducibility	As for repeatability, but with different operator(s) if possible, on different days, and with different equipment when relevant
Biochemical methods (e.g. enzyme electrophoresis, protein profiling)	
This step also includes sample preparation	
Analytical sensitivity	Determine the minimum number of individuals to be detected to perform a reliable analysis with a minimum of three samples whenever possible. The smallest number of target individuals depends on the condition of the sample (good to very poor), the known intra-species variability, the difficulty to interpret features, etc. Test parameters should be stringently defined and standardized
Analytical specificity	Inclusivity: analyse a range of target organism(s) Exclusivity: analyse non-target genus and/or species
Selectivity	Not applicable
Repeatability	Analyse at least three replicates of sample extracts with a low concentration. If consistent results are not obtained, additional replicates should be prepared and tested
Reproducibility	As for repeatability, but with different operator(s) if possible, on different days, and with different equipment when relevant
Baiting or bioassay methods (including pathogenicity tests)	
Analytical sensitivity	Determine the minimum number of individuals needed to produce symptoms or multiply in plant material with at least three repetitions Analytical sensitivity refers to a specific set of test parameters which should be stringently defined and standardized (e.g. stage of test plants, inoculation method) If consistent results are not obtained, additional replicates should be prepared and tested
Analytical specificity	Inclusivity: define specificity of the bioassay on strains of the target organism covering genetic diversity, different geographical origins and hosts Exclusivity: define specificity for a set of non-target organisms, in particular those associated with the matrix
Selectivity	Determine whether variations of the matrix (e.g. by using different cultivars of the host plant) affect the test performance
Repeatability	Use at least three replicates with the minimum number of individuals required to establish a population and use the host plants determined in the specificity test. If used for a pathogenicity test, the three replicates should have the minimum number of individuals needed to produce symptoms. If consistent results are not obtained, additional replicates should be prepared and tested
Reproducibility	As for repeatability, but with different operator(s) if possible, on different days, and with different equipment when relevant

Virology and phytoplasmology

Table A6. Virology and phytoplasmology (see also the instructions for the use of the tables). This table covers viruses, viroids and phytoplasmas

Molecular methods (e.g. PCR, real-time PCR, LAMP)	
This step also includes methods for extraction of RNA/DNA from the matrix	
Analytical sensitivity (relative sensitivity)	Because the concentration of viruses, viroids and phytoplasmas is never known, determine the maximum dilution of RNA/DNA detected. Perform at least three experiments with serial dilutions. If consistent results are not obtained after three series, additional series should be prepared and tested Analytical sensitivity refers to a specific set of test parameters which should be stringently defined and standardized, for example the brand of PCR reagents (in particular DNA polymerase) and PCR cycle conditions
Analytical specificity	Inclusivity: analyse a range of targets covering genetic diversity, different geographical origins and hosts Exclusivity: analyse relevant non-targets, in particular those that might be present in the matrix. The concentration of nucleic acid should be high enough to maximize the possibility of cross-reaction but remain realistic For both inclusivity and exclusivity the test results can be supported by in silico comparison of primer/probe sequences to sequences in genomic libraries
Selectivity	Determine whether variations of the matrix (e.g. by using different cultivars of the host plant) affect the test performance
Repeatability	Analyse at least three replicates of sample extracts with a low (relative) concentration. If consistent results are not obtained, additional replicates should be prepared and tested
Reproducibility	As for repeatability, but with different operator(s) if possible, on different days, and with different equipment when relevant
Serological methods: ELISA and tissue, including sample preparation (not applicable for viroids)	
Analytical sensitivity (relative sensitivity)	Perform at least three experiments with serial dilutions of an infected sample in the healthy sample selected. Determine the highest dilution of sample extracts which could be detected Analytical sensitivity refers to a specific set of test parameters which should be stringently defined and standardized (e.g. the OD threshold in the ELISA test). If consistent results are not obtained after three series, additional series should be prepared and tested
Analytical specificity	Inclusivity: define specificity of antibodies on strains of the target covering genetic diversity, different geographical origins and hosts Exclusivity: define specificity of antibodies on a set of non-targets, in particular those associated with the matrix
Selectivity	Determine whether variations of the matrix (e.g. by using different cultivars of the host plant) affect the test performance
Repeatability	Analyse at least three replicates of sample extracts with a low (relative) concentration. If consistent results are not obtained, additional replicates should be prepared and tested
Reproducibility	As for repeatability, but with different operator(s) if possible, on different days, and with different equipment when relevant
Bioassay methods: plant test (mainly used as verification test for viruses or viroids but not for phytoplasmas) and grafting	
Analytical sensitivity (relative sensitivity)	Determine the maximum dilution of infected sample needed in the healthy sample to produce symptoms or multiply in plants. This is only an estimation of dilutions that can be used. Perform three series with dilution steps Analytical sensitivity refers to a specific set of test parameters which should be stringently defined and standardized (e.g. stage of test plants, inoculation method and incubation conditions). If consistent results are not obtained, additional replicates should be prepared and tested Not relevant for grafting
Analytical specificity	Inclusivity: define specificity of the bioassay on strains of the target covering genetic diversity, different geographical origins and hosts Exclusivity: define specificity of the bioassay on a set of non-targets, in particular those associated with the matrix
Selectivity	Determine whether variations of the matrix (e.g. by using different cultivars of the host plant) affect the test performance
Repeatability	Analyse at least three replicates of sample extracts with an appropriate dilution determined in the sensitivity test and select host plants on the basis of the results of the above performance criteria. If consistent results are not obtained, additional replicates should be prepared and tested
Reproducibility	As for repeatability, but with different operator(s) if possible, on different days, and with different equipment when relevant
Biochemical methods (e.g. electrophoresis, R-PAGE)	
Analytical sensitivity (relative sensitivity)	Perform at least three experiments with serial dilutions of infected sample in the healthy sample selected. Determine the highest dilution of sample extracts which could be detected Test parameters should be stringently defined and standardized
Analytical specificity	Inclusivity: investigate intra-specific variability Exclusivity: compare with relevant target/proteins/contaminants and show that differentiation can be made
Selectivity	Not applicable
Repeatability	Analyse at least three replicates of sample extracts with a low (relative) concentration. If consistent results are not obtained, additional replicates should be prepared and tested
Reproducibility	As for repeatability, but with different operator(s) if possible, on different days, and with different equipment when relevant

Appendix 4 – Procedure for validation of a test (A) by comparison with a validated test (B)

Comparison of a test (A) with a validated test (B) can be an appropriate validation procedure for situations when the analytical sensitivity or analytical specificity level of the validated test (B) is considered adequate and when the test (A) presents an advantage (e.g. speed, ease of use).

It is recognized that the test (A) may have a better sensitivity or specificity level than the validated test (B) and that the comparison will only provide the information that the sensitivity or specificity of test (A) is at least at the level of that determined for the validated test (B).

Repeatability and reproducibility should also be evaluated for the test (A) (see Appendix 4).

Comparison of the test (A) with the validated test (B) should be performed as follows:

Perform three repetitions with the target organism and three with each of the non-target organisms as indicated in Table A7. Samples are processed with the two tests in parallel.

Table A7. Minimum number of samples required when comparing a test (A) to a validated test (B)

	Level of organism		
Type of material	Low/low (relative*)	Medium/ medium (relative*)	High/ high (relative*)
Isolates of pure cultures of target or samples spiked with target	10†	7†	5†
Isolates of pure cultures of non-target(s) or samples spiked with non-target(s)	–	11–22	–
Naturally contaminated sample with target organism	Adequate dilution series are prepared with 15 positive samples previously identified with the validated test (B) to reach the limit of detection of the validated test (B).		

*For virology and phytoplasmology.

†The total number of samples of target(s) should be at least twice the number of non-target(s).

The number of samples indicated in this table has been determined by comparison with published standards, for example ISO 16140 *Microbiology of food and animal feeding stuffs. Protocol for the validation of alternative methods* (ISO, 2003) and AFNOR XP V03-111 *Agricultural and food products analysis. Protocol for the intra-laboratory evaluation of an alternative method of qualitative analysis against a reference method* (AFNOR, 1995).

Correlation between results obtained with the validated test (B) and the test (A) should be evaluated for the different pest levels. Results can be presented as shown in Table A8 and relative performance characteristics calculated.

Positive deviation and negative deviation need to be analysed.

Table A8. Example of results from a correlation between a validated test B and a test

		Validated test B		
		+	–	Total
Test A	+	69 (*PA*)	3 (*PD*)	72
	–	6 (*ND*)	12 (*NA*)	18
	Total	75	15	90

This table is adapted from Hughes *et al*. (2006). Numbers in this table are for demonstration purposes. PA (positive agreement), PD (positive deviation), ND (negative deviation), NA (negative agreement). Positive (+) and negative (–) results for 90 samples tested using both tests, illustrating diagnostic sensitivity (PA/(PA + ND)), diagnostic specificity (NA/(NA + PD)), and relative accuracy (PA + NA)/(PA + PD + ND + NA). Diagnostic sensitivity = 92%, Diagnostic specificity = 80%; Relative accuracy = 90%. It should be noted that relative accuracy is no longer referred to in the revised version of ISO 16140.

Appendix 5 – Validation of morphological and morphometric tests used in, for example, entomology, nematology, mycology and botany

This guidance is based on the validation experience of experts from EPPO Panels dealing with diagnostics.[4]

Morphological identification is based upon expertise (see PM 7/84). Expert judgement is usually based on the use of available documentation in the form of keys, original morphological descriptions, specimens and voucher photographs, which are recognized as reference documentation to support the identification. As these documents or supporting information have been produced by specialists of the group(s) concerned, they are consequently considered as validated tests in the current Standard.

Examples of documents or supporting information considered as validated tests in the current Standard include:

- morphological and morphometric tests included in international standards such as the IPPC Diagnostic Protocols and the EPPO Diagnostic Protocols
- morphological and morphometric tests, taxon reviews, descriptions, preferably including original articles, and keys published in peer-reviewed journals, preferably including original articles
- voucher specimens and type material (such as holotypes, paratypes, lectotypes and neotypes) and voucher pho-

t-

[1]A new ISO 17025 was approved on 2017-12-13 and its implementation will be required by the end of 2019. A review of this EPPO Standard is planned in January 2019 to consider the need for revision. However, the risk-based process included in the new ISO Standard is already included in this revised version of PM 7/98.

[4]Experience with accreditation for morphological and morphometric identification in a forensic laboratory was also taken into account.

ographs (specimens and photographs should be identified and confirmed by an expert)
- morphological and morphometric tests in common usage, which are published in non-peer-reviewed publications including electronic media (in particular for keys).

The laboratory should have the expertise to be able to select and justify the selection of morphological and morphometric methods made, in particular for those not described in international standards or peer-reviewed journals. The keys or other documentation may need to be reviewed to ensure they are relevant/appropriate for the intended use, for example to ensure inclusion of all necessary species (e.g. from different geographical regions/new species described).

As explained in section 5.4.5.2, the laboratory should confirm that it can properly carry out the morphological and/or morphometric identification.

Appendix 6 – Example of laboratory reports on the critical points in the diagnostic process and relating to uncertainty of measurement

The following examples of laboratory reports on the critical points in the diagnostic process and relating to uncertainty of measurement are provided as Supporting Information (see Appendix S2):

Report 1: Identification of critical points and estimation of the uncertainty of measurement (courtesy of the National Institute of Biology, Slovenia, 2012).

Report 2: Detection of Flavescence dorée (FD) and Bois Noir (BN) by real-time PCR. Validated method: French Method MOA006 parts A et B version 1b – Detection of phytoplasmas from 16SrV group (Flavescence dorée) and 16SrXII group (Bois Noir)/Matrix: *Vitis* sp.