DOI: 10.1111/epp.12780

EPPO STANDARD - DIAGNOSTICS

PM 7/98 (5) 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* (2017, 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 and is the equivalent of 'shall' in the ISO Standard. This Standard should be used in conjunction with PM 7/76 *Use of EPPO diagnostic protocols.*

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. Third revision approved 2019–09. Fourth revision approved in 2021–09.

Authors and contributors are given in the Acknowledgements section.

1 | INTRODUCTION

Many laboratories in the EPPO region establish quality management systems (also referred to as management systems or quality systems) and apply for accreditation. Two EPPO Standards have been developed on these topics. The Standard PM 7/84 *Basic requirements for quality management in plant pest diagnostic laboratories* was adopted in 2007 and revised in 2018 (EPPO, 2018a). It describes basic requirements to support laboratories conducting plant pest diagnostics in designing their quality management system. PM 7/98 (current Standard) describes requirements for laboratories applying for accreditation. Compared to its previous versions, which included cross-references to PM 7/84, it is now a standalone document. It reflects the requirements of the revised ISO/IEC Standard 17025 *General requirements for the competence of testing and calibration laboratories* (ISO/IEC, 2017). The main addition compared to the previous version of PM 7/98 is a more comprehensive guidance on risk management.

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.

This is now possible with accreditation under a flexible scope. In this Standard, scopes of accreditation are described in Section 3 and requirements for flexible scope in Section 6.

Accreditation against the 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* (EPPO, 2018b).

In this Standard, 'test' refers to the application of a method to a specific pest and a specific matrix. The methods concerned include the following: bioassay methods, biochemical methods, fingerprint methods, isolation/extraction methods, molecular methods, morphological and morphometrical methods, pathogenicity assessment and serological methods. Most test results are given in qualitative terms (test 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 or C_q values for real-time PCR, measurements for morphological features, etc.). However, such quantitative data in most cases are used to assign a qualitative result to the test (positive/negative/undetermined).

[1]A new ISO 17025 was approved on 13 Dec 2017 and its implementation will be required by 01 Dec 2020.

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 maintained by the EPPO Secretariat and the Panel on Diagnostics and Quality Assurance is available at https://upload.eppo.int/download/221odbcdc6308.

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. immunofluorescence test for the detection of *Clavibacter 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 by 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 clients' 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 scopes are provided in Sections 4 and 5, whereas additional requirements for flexible scope are in Section 6. Descriptions of scopes are provided in *Guideline for the formulation of scopes of accreditation for Laboratories* (ILAC-G, 18:04/2010).

4 | MANAGEMENT REQUIREMENTS

The laboratory should establish, implement and maintain a quality management system that is capable of supporting and demonstrating the consistent achievement of the requirements of this Standard and assuring the quality of the laboratory results. Two options for management systems are provided in ISO 17025, 2017, the management system is either established in accordance with ISO 9001 (8.1.3 Option B of the ISO Standard) or following the requirements described in points 8.2 to 8.9 of ISO 17025 (8.1.2 Option A). The choice of options by the laboratory should be discussed with the accreditation body. Option A is addressed in this EPPO Standard.

In this section, management requirements covered in Sections 4 to 8 of ISO 17025 are described.

4.1 | General requirements (based on ISO 17025, 2017, point 4)

- Laboratory activities should be undertaken impartially, structured and managed to safeguard impartiality.
- Risk to impartiality should be identified and prevented, e.g. possible conflicts of interest between personnel and activities performed.
- Confidentiality of results to the customer and of customer's information is guaranteed. However, findings of regulated pests or new pests and associated customer information should be reported to the NPPO (including a requirement for customers from other countries to report such findings to the NPPO of their country). The customers will be notified of the information provided.

4.2 | Structural requirements (based on ISO 17025, 2017, point 5)

- The laboratory should be a legal entity, or a defined part of a legal entity, that is legally responsible for its laboratory activities. A government laboratory is deemed to be a legal entity on the basis of its governmental status.
- The laboratory should identify management that has overall responsibility for the laboratory and have personnel who have the authority to carry out their duties.
- Appropriate resources are available to conduct the plant pest diagnostic activity, for example personnel, facilities and equipment (see also Section 5, Technical requirements).
- The facilities and all activities should be described, including temporary or mobile facilities and the customer's facilities (laboratories at border inspection points or onsite testing). This should be documented, and the quality documents should be archived (see also below).
- Responsibilities and tasks of personnel are clearly defined (e.g. by organizational flow charts) and appropriately assigned.
- Procedures and instructions are documented to the extent necessary to ensure consistent application of the laboratory activities and the validity of the results.
- The laboratory management (e.g. the institute manager) should commit to bringing into effect the goals of quality management with effective communication within the laboratory and with customers, and to continually improving the effectiveness of its quality management system.

4.3 | Resource requirements (based on ISO 17025, 2017, point 6)

Requirements for personnel, facilities and equipment are presented in Sections 5.2, 5.3 and 5.4, respectively, of this EPPO Standard.

Requirements for externally provided products and services (based on ISO 17025, point 6.6) are provided below.

- Purchased supplies (e.g. equipment, reagents) and services (calibration services, proficiency testing services, testing services, e.g. sequencing) should be appropriate for the intended use, based on an established assessment procedure (ISO points 6.6.2 and 6.6.3).
- Any subcontracted testing work should be authorized in accordance with EPPO Standard PM 7/130 *Guidelines on the authorization of laboratories to perform diagnostic activities for regulated pests*.
- The laboratory should inform the customer when specific laboratory activities under the scope of accreditation are performed by external providers and gain the customer's approval (ISO point 7.1.1. c).

4.4 | Process requirements (based on ISO 17025, 2017, point 7)

- A process is in place to review requests, tenders and contracts for their feasibility (including the selection of tests), and this review should be documented.
- When a test is requested by a customer, the laboratory should inform the customer when it is considered to be inappropriate. Deviations requested by the customer should not impact the integrity of the laboratory or the validity of the results.
- A process is in place to deal with complaints.
- A process is in place to record, analyse and correct any deviation from procedures or requirements of the customer.
- Laboratories should have access to the data and information necessary to perform laboratory activities. The information management systems should be protected from unauthorized access and safeguarded against tampering and losses.
- The laboratory information management system should be validated (commercial software can be considered as sufficiently validated). Whenever there are any changes, including laboratory software configuration or modifications to commercial off-shelf software, they should be authorized, documented and validated before implementation.

4.5 | Management systems requirements (based on ISO 17025, 2017, point 8)

ISO 17025 point 8 provides details on the management system requirements. Some additional notes are provided below.

- The management system should document the policies and objectives of the laboratory to fulfil the requirements of ISO 17025. Examples of policies are staff recruitment and training, and purchase of material. Examples of objectives are to include, improve or maintain competence, to increase the number of tests per year, and to meet demands of customers or changes in equipment.

The laboratories should consider the risks and opportunities, and define actions to address these.

4.6 | Risk management

The revised ISO Standard 17025 places more emphasis on risk-based thinking leading to risk management. The Panel on Diagnostics and Quality assurance recommends that to perform risk management, the processes and objectives of the laboratory should be identified at operational (e.g. performing testing) and strategic (e.g. staff management) levels. The risk and opportunity analysis should be conducted to identify the critical points of the processes using tools such as strength, weaknesses, opportunities and threats (SWOT) analysis, mind mapping or the failure mode and effects analysis (FMEA) tool. The risk management should be proportional to the potential impact on the test validity and the effectiveness of the actions should be evaluated. The Supporting Information for this Standard presents more details on the risk management illustrated with examples (see Supporting Information S1. For readers looking at the paper or pdf version of this Standard, please see the html version to access this.).

The risk analysis before performing validation or verification is described in Section 5.4.3.

A risk analysis for non-conforming work should be performed to determine the risk level of the non-conforming work and if there is an impact on previous activities and test results. Based on the results of the risk analysis, subsequent action should be taken (e.g. acceptability of the non-conformity or recall of the test results and notification to the customer). Information on non-conforming work should be recorded.

5 | TECHNICAL REQUIREMENTS (ISO 17025, 2017, POINTS 6 AND 7)

5.1 | General

These technical requirements include resource and process requirements (ISO 17025, 2017, points 6 and 7).

Many factors determine the reliability of the test results. These factors include:

- Personnel
- Facilities and environmental conditions
- Plant pest diagnostic tests
- Equipment
- Reference materials/cultures
- Sampling
- Sample handling.

5.2 | Personnel (ISO 17025, 2017, points 5.2 and 6.2)

The laboratory management should define and ensure the competence and expertise of those who perform each specific stage of the plant pest diagnostic activity and their competence to use the equipment. The laboratory management should also ensure that the laboratory personnel, whatever their status (e.g. students, staff seconded from another organization), carry out their work in an impartial manner and respect confidentially requirements.

Personnel performing specific tasks should be qualified on the basis of appropriate education, training, experience and/or demonstrated skills (see examples in Appendix 1). Staff undergoing training should be appropriately supervised and authorized. Staff records should be maintained, including records concerning the date on which authorization and/or competence to perform a specific task is confirmed, and training records. A procedure should be put into place to review, ensure and monitor competence; this is especially critical after long absences.

5.3 | Facilities and environmental conditions (ISO 17025, 2017, point 6.3)

Laboratory facilities should enable correct performance of the plant pest diagnostic activities. Depending on the type of testing being performed, different steps of plant pest diagnostic activities may be combined in a working area, provided that necessary precautions are taken to avoid cross-contamination resulting from samples, reference materials and facilities (see Appendix 2). Specific guidance on handling quarantine organisms has been developed (see Table 1 in EPPO Standard PM 3/64 *Intentional import of live organisms that are plant pests or potential plant pests*) and specific regulations may apply in countries, e.g. Regulation (EU) 2016/2031[2] (EU, 2016).

A laboratory usually comprises testing facilities and ancillary facilities (entrances, corridors, storage rooms, toilets, archives, etc.). Separate locations or clearly separated/designated working areas are recommended for the following:

- Reception of samples
- Preparation of samples (segregate location for samples likely to be highly contaminated or powdery, e.g. soil samples, plants infected by fungi, insects or mites, tubers with soil)
- Testing of samples
- Storage of samples
- Appropriate disposal of material and waste
- Maintenance of reference materials/cultures
- Preparation and storage of media, buffers and reagents.

[2]The relevant articles in this Regulation are 60 to 64.

Different activities can be separated by time. The work area should be appropriately disinfected between different samples and/or activities. Specific requirements are mentioned in Appendix 2.

The laboratory should be appropriately equipped to ensure proper storage, testing and containment of samples.

Access to the laboratory should be restricted to authorized personnel, who should be aware of the intended use of a particular area and restrictions imposed on working in such areas.

The laboratory should monitor, control and record environmental conditions where they may influence the quality and reliability of the test results. Failures resulting from deviating environmental conditions should be documented and corrective actions recorded (see Appendix 2).

Measures should be taken to ensure good housekeeping in the laboratory. Space should be sufficient to allow work areas to be kept clean and tidy. Clothing appropriate to the testing being performed should be worn, especially when working in microbiological and molecular laboratories.

5.4 | Diagnostic tests

5.4.1 | General

The laboratory should use appropriate tests and procedures for all analyses performed within its scope (ISO 17025, 2017, point 7.2.1.1). These include sampling, handling, transport, where relevant, storage, preparation and testing of samples. It is expected that plant pest diagnostic laboratories will have an understanding of the biology of organisms and take this into account when subsampling and/or preparing the sample for testing. Equipment, reagents and consumable materials should be appropriate for the intended use.

All instructions, standards, technical manuals and reference data relevant to the work of the laboratory should be kept up to date and made readily available to personnel. Deviation from tests should occur only if documented, technically justified, authorized by an appropriate person and accepted by the customer (ISO 17025, 2017, point 7.2.1.7).

5.4.2 | Selection of tests

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 (ISO 17025, 2017, points 7.2.1.1 and 7.2.1.4).

The laboratory should ensure that it selects the latest valid edition of a test, unless it is not appropriate or possible to do so.[3] When necessary, the test description should be supplemented with additional details to ensure consistent application in the laboratory (ISO 17025, 2017, point 7.2.1.3).

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 that it can operate the test according to the established performance characteristics (see Section 5.4.5) (ISO 17025, 2017, points 7.2.1.5 and 7.2.2).

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

To identify which performance criteria need to be evaluated, and to what extent, the laboratory should conduct a risk analysis for each performance criterium to identify critical ones in order to obtain a reliable result of the test. This risk analysis should be documented by the laboratory (see Appendix 3) and the choices made should be justified. The general process for risk analysis before performing validation and/or verification is described below and in Figure 1, part A.

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. age and condition of samples, different reagents) that occur in practice. Robustness often is included in the reproducibility. A separate evaluation of the robustness will often not be necessary as it is part of the development of a test.

The scope of the test, e.g. 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.

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

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

- Description of the intended use (screening, on-site testing, confirmation of a previous test result).
- Did the client express a specific expected level of performance or a specific intended use (on a contaminated area for screening, on specific hosts, cost of analysis)?
- What are the impacts of the results (e.g. outbreak or survey in non-contaminated area)?
- Which statistical significance (e.g. level of confidence in the test) is needed (impact on budget, repetitions etc.)?
- In the case of a wide host range or polyphagous pest, which hosts should be considered for validation?
- Which matrices should be tested?
- Are there specific species/strains or populations to be detected (e.g. specific species/strains or population present in the country)?
- Which possible cross-reactions 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)? Which cross-reactions can be accepted?
- Which performance characteristics for analytical sensitivity and analytical specificity (inclusivity and/or exclusivity) are needed?
- What level of flexibility is needed for the use of the test (e.g. network of laboratories as end users)?

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

- What is the availability of reference material (pest related, matrix related)?
- What level of flexibility is needed for the use of the test (e.g. diversity of equipment available such as a PCR machine with rotor or plate)?
- What is the availability of resources to perform the validation (budget, staff, equipment/reagents)?
- What are the 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, but EPPO Panels on Diagnostics considered that those presented in Appendix 4 give appropriate confidence regarding 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.

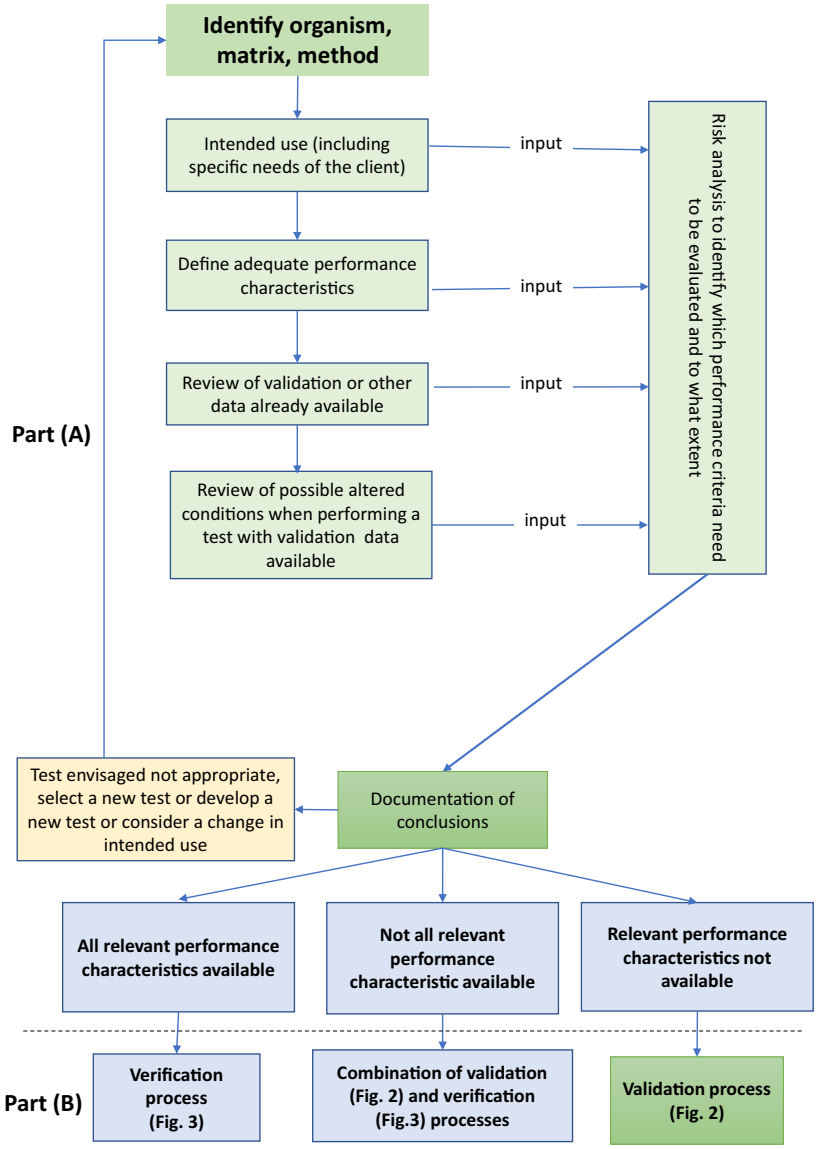

FIGURE 1 Outline of the process for preparation for accreditation of a plant pest diagnostic test (including risk analysis)

Output of risk analysis (Figure 1, part B).

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.

- Transferable skills: 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). 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.
- Analytical sensitivity: If the quantity of target in the sample is not a limiting factor, the extent of validation for analytical sensitivity can be reduced. Example: Identification on pure culture by PCR, as long as there is no inhibition effect (excess of matrix).
- Analytical specificity: If the test cannot distinguish between genera or species within a genus, then inclusivity and exclusivity evaluations can be reduced. Example: Nematode extraction methods are not specific for one species or one genus.
- Reagents: If the choice of reagents is 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, 2017, point 7.2.2) (Figure 1, part B and Figure 2)

5.4.4.1 Validation of tests other than morphological and morphometrical tests

As mentioned in ISO 17025, 2017 'the laboratory shall record [among other factors] the validation procedure used [...] and the results obtained' (point 7.2.2.4).

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

The validation procedures described here and the details given in Table A2–A7 (in Appendix 5) should be regarded as general guidance according to which a test can be validated. Figures given in these 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.

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 Table A2–A7 as required. Then define the type and composition 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 Figure 2).
- Present the results in a validation report with a conclusion on whether the test meets the requirements identified (see Appendix 3).
  - Performance characteristics are met: the test is validated.
  - Performance characteristics are not met:
    - Adjust the test and perform a new evaluation for the relevant performance criteria.
    - If the test cannot be adjusted, the test cannot be validated for the originally intended use (in specific cases adapting the intended use of the test may be considered).

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 6). This can only demonstrate that, for example, test A is as good as 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 interlaboratory comparisons, can also provide an indication of the robustness of the test.

5.4.4.2 Validation for morphological and morphometrical tests

It is acknowledged that the procedures for morphological and morphometrical tests are ultimately a judgement based on expert opinion. Validation therefore may not follow the same procedures as for the other tests. Guidance for the validation of morphological and morphometrical tests is given in Appendix 7. This guidance is applicable for these methods irrespective of the field they are used in (entomology, nematology, mycology, botany, etc.). The laboratory should be able to justify the selection of morphological or morphometrical 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, 2017, point 7.2.1.5)

5.4.5.1 Verification process for tests other than morphological and morphometrical 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 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 Figure 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, but for serological methods, for example, 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.

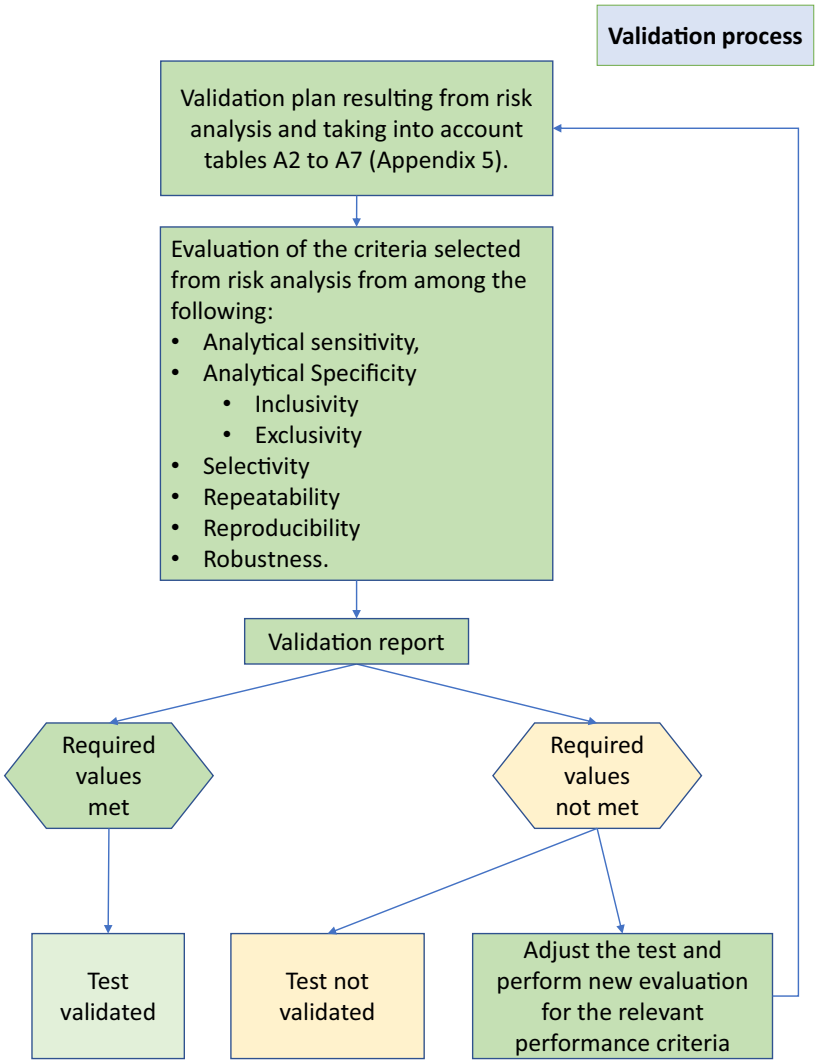

FIGURE 2 Validation process

TABLE 1 Guidance on the verification of performance criteria

Performance criteria	Verification method (artificial subsamples prepared from one sample may be used)
Analytical sensitivity	Analyse at least eight samples at the established limit of detection (for viruses, viroids and phytoplasma 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 at medium levels of organisms.
Repeatability	Perform at least three simultaneous tests on the same material with low levels of target.
Reproducibility	As for repeatability but at different moments, when possible with different operators and when relevant with different equipment.

- 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 test results, investigate the reasons for this deviation. Correct, verify the test again or validate if required following the procedure described in Section 5.4.4.
    - Investigate whether the minor changes that have been introduced in the test are the cause. If it is not the case, seek external guidance (e.g. contact the author of the test). Adjustments should then be made and relevant steps repeated. If other reasons

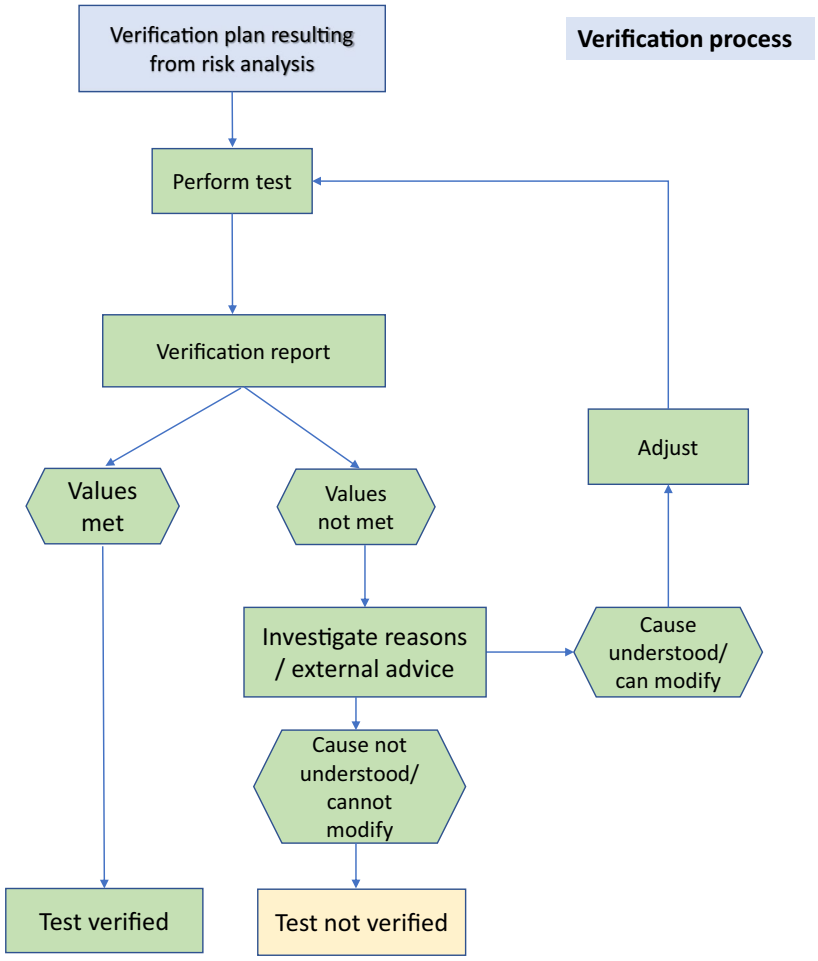

FIGURE 3 Verification process

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.

5.4.5.2 Verification process for morphological and morphometrical tests

The laboratory should confirm that it can properly carry out the validated morphological and/or morphometrical 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 3) can be valuable for summarizing the results.

5.4.7 | Evaluation of measurement uncertainty (ISO 17025, 2017, point 7.6)

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 (e.g. morphometrical data, number 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 8.

5.5 | Equipment (except reference material and data) (ISO 17025, 2017, point 6.4)

Note: In ISO 17025, equipment includes, but is not limited to, instruments (microscopes, thermocyclers, ELISA-readers, pipettes, etc.), software, measurement standards, reference materials and data, reagents (e.g. Mastermix, antisera, etc.) and consumables (e.g. pipette tips, slides, plates, tubes). In this EPPO Standard reference material and data are covered in point 5.6.

The laboratory should have access to the equipment required for testing and this should be operated by competent personnel. Equipment, whether under the laboratory's permanent control or not, should be listed and a programme should be documented and implemented for the handling, storage, transport, maintenance, verification, calibration and corrective action of key equipment which significantly affects the results.

Equipment that has been subjected to overloading or mishandling, or gives suspect results, or has been shown to be defective or outside specified limits should be taken out of service, clearly labelled or marked, and appropriately stored until it has been repaired and shown to perform correctly when appropriate or disposed of (e.g. for reagents, consumables). The laboratory should examine the effect of such deviation and initiate the management of non-conforming work procedures (see Section 4.6).

5.5.1 | Calibration and verification programmes (ISO 17025, 2017, points 6.4.7 and 6.5)

Frequency of calibration and verification should be planned and reviewed when necessary. This can be based on risk analysis (examples and recommendations can be found in Appendix 9). Calibrations may be performed in-house by using certified or appropriate reference material provided by a competent producer. Calibrations should have traceability to International System of Units (SI) whenever possible. Only qualified personnel should perform calibration and verification programmes, using procedures appropriate to the intended use. Calibration and verification may also be performed externally by specialized, competent companies.

Documents on external and internal calibration and verification of performance (including when the next calibration is due) should be maintained and made available within the laboratory. Equipment should be appropriately labelled (see Appendix 10).

5.5.2 | Maintenance of equipment

Up-to-date instructions on the use and maintenance of equipment (including any relevant manuals provided by the manufacturer) should be readily available for use by the appropriate laboratory personnel. Maintenance of essential equipment should be carried out at specified intervals preferably based on risk analysis as determined by factors such as the rate of use and age/complexity of the equipment, and this maintenance documented (see Appendix 1 for guidance on maintenance of equipment).

5.5.3 | Records (ISO 17025, 2017, point 6.4.13)

Records should be maintained for equipment significant to the tests performed. Depending on the type and sensitivity of equipment, and the conditions required by the manufacturer to ensure failure-free running, the records should include:

- The identity of the item of equipment, including software and firmware versions
- The current location
- The manufacturer's name and type identification
- The manufacturer's instructions
- Dates, results and copies of reports and certificates of all calibrations or verifications, adjustments, date of next calibration or verification where appropriate
- Maintenance carried out to date and the maintenance plan where appropriate
- Damage, malfunction and repair of the equipment.

5.6 | Reference material and reference data (ISO 17025, 2017, points 6.4, 6.5, 6.6 and 7.2.1.2)

Reference material provides essential traceability and is used, for example:

- To monitor performance of detection and identification tests
- To demonstrate the accuracy of results
- To calibrate or verify equipment
- To monitor laboratory performance
- To validate or verify tests
- To enable comparison of tests.

5.6.1 | Biological reference material

If possible, certified biological reference material should be used from which biological reference material, and subsequently working material, can be produced. Laboratories may also produce their own biological reference material from which working material is derived. To maintain confidence in the status of biological reference material, verification of identity and purity should be carried out according to defined procedures and schedules (including, as applicable, morphology, pathogenicity, virulence, antigenic properties, molecular properties, etc.). The laboratory should have procedures for safe handling, transport, storage and use of

biological reference material to prevent contamination or deterioration and to protect their integrity.

Working material derived from biological reference material (e.g. reference cultures) from an international collection should be kept separate from the original material.

5.6.2 | Other sources, including reference data

These include books, pictures, slides collections, morphological identification keys, scientific literature and sequence databases that can be used to support diagnosis. Reference data should be kept up to date and readily available.

5.7 | Sampling (ISO 17025, 2017, point 7.3)

Sampling is a procedure in which material is collected outside a laboratory to perform a test. A sample should be representative of the material under test and should be selected based on knowledge of the distribution of the pest to be detected. Such a representative sample may not always be available: if so, this should be documented. Sampling usually involves targeting symptomatic plants or plant parts.

Correct sampling is an operation that requires careful attention. Not all laboratories are involved in sampling. Laboratories involved in sampling should have a sampling process (both plan and procedure) for collecting samples to be followed whenever practicable. This process should address the factors to be controlled and be based on appropriate statistical tests.

The laboratory should have procedures for recording relevant data relating to sampling whether the process is performed by the laboratory staff or by the customer.

5.7.1 | Records of sampling (ISO 17025, 2017, point 7.3.3)

Details of sampling should be recorded and communicated to the appropriate personnel. Records should include the following:

- Sampling procedure
- Date and time of sampling
- Data to identify and describe the sample (e.g. matrix, plant species, batch number, suspected pest)
- The name of the person who performed sampling
- The equipment used
- Environmental or transport conditions
- Sampling location
- Deviations, additions or exclusions from the documented sampling procedure.

5.8 | Sample handling (ISO 17025, 2017, point 7.4)

The laboratory should have procedures for safe transportation, receipt, handling, protection, storage, retention and/or disposal of samples, including all provisions necessary to protect the integrity of the sample and to protect the interest of the laboratory and the customer.

Subsampling by the laboratory prior to testing is considered to be part of the test. Subsampling should be designed taking into account uneven distribution of pests.

The laboratory should have a system for uniquely identifying samples. The system should be designed and operated to ensure that samples cannot be confused physically or when referred to in records or other documents. The system should, if appropriate, accommodate a subdivision of groups of samples and the transfer of samples within and from the laboratory. The identifier of a sample should be retained as long as this sample is retained by the laboratory. Suggested content for a form to identify a sample is presented in Appendix 12.

Plant pests may be sensitive to factors such as temperature or duration of storage and transport, so it is important to check and record the condition of the sample on receipt by the laboratory. If there is insufficient material in the sample or the sample is in poor condition due to physical deterioration, incorrect temperature, torn packaging or deficient labelling, or when a sample does not conform to the description provided, or if the test required is not described in sufficient detail, the laboratory should consult with the customer before deciding whether to test or refuse the sample. In any case, the facts and the results of discussions should be recorded.

Samples awaiting testing should be stored under suitable conditions to minimize changes to any pest populations present and to protect them from cross-contamination. Storage conditions should be defined and recorded when necessary. Where samples have to be returned to the customer, care is required to ensure that they are not damaged.

A procedure for the retention and disposal of samples should be written. Samples should be stored until the test results are obtained, or longer if required (e.g. for potential complementary analysis).

A laboratory should have procedures in place to treat samples after testing to conform to national or international regulations for quarantine and other plant pests. The procedures should also be designed to minimize the possibility of contaminating the test environment or materials. Further details on confinement conditions may be found in EPPO Standard PM 3/64 *Intentional import of live organisms that are plant pests or potential plant pests*.

5.9 | Ensuring the validity of test results (ISO 17025, 2017, point 7.7)

The validity of test results should be ensured at different levels, i.e. for each test and diagnostic process, as well as for global quality control of the laboratory.

Internal quality management consists of compliance with all the procedures undertaken by a laboratory for the continuous evaluation of its work. The main objective is to ensure the consistency of results day to day and their conformity with defined criteria. If analysis of data is found to deviate from the defined criteria, then appropriate action should be taken to prevent incorrect results from being reported. The interval between internal quality checks (defined in Table 2) will be influenced by the number of actual tests performed. Monitoring of test validity should be planned, reviewed and registered. Wherever possible positive/negative controls should be used: this should be the minimum level for quality control. A quality control programme may also consist of different checks, as described in Table 2.

A procedure should be in place for managing infrequently used tests. Operators' transferable skills may provide evidence of competence in tests based on the same method. Whenever possible, an external quality assessment (such as an external proficiency programme or proficiency tests) should be used to demonstrate competence. The validity of test results is influenced by both technical performance of personnel and test performance characteristics. If the validity of test results is called into question, it is important to be able to distinguish between these two. A test may demonstrate appropriate process control but poor diagnostic performance or vice versa.

5.10 | Reporting the results (ISO 17025, 2017, point 7.8)

See EPPO Standard PM 7/77 *Documentation and reporting on a diagnosis* (EPPO, 2019).

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, 2019). 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 or similar pests)
- Inclusion of a test equivalent to one already covered by accreditation.

TABLE 2 Internal and external quality checks (ISO 17025, section 7.7)

Elements of quality control programme	Level of control[a]
The use of reference material (e.g. target organisms, closely related organisms, non-target organisms which might be naturally present in a composite material), see section 5.6	First
Internal/endogenous control (e.g. COX, NAD5, 18S)	First
The use of artificially contaminated samples	First or second
Replicate testing using the same test (technical replicates or repeated testing)	First or second
Comparative testing of the same sample by different operators	First or second
Vertical audit[b] of records for a specific sample/analysis	Second or third
Blind testing by processing samples with known levels of pests between routine samples	Second
Comparison of results of different tests based on different biological principles	Second
Retesting of retained plant material or extracts thereof, water or soil samples and insect traps (within a predetermined suitable storage time and condition of the material before retesting)	Second
Trend analysis on first-, second- and third-line controls (e.g. positive controls, Shewhart chart or results from proficiency tests) including quantitative data	Second or third
Intra- or interlaboratory evaluation of documentation of the specific determinants on which diagnoses are based (in particular for visual determination of insects, nematodes and fungi)	Third
Interlaboratory comparisons (in particular proficiency tests)	Third
Supporting data (e.g. contra-expertise)	Third
Use of alternative instrumentation that has been calibrated to provide traceable results	First or third
Functional and intermediate check(s) of measuring and testing equipment	First or third

[a]First-line controls are used to monitor the actual performance of the test, second-line controls are used for the performance of a single operator within a laboratory and third-line controls evaluate the performance of the laboratory.

[b]Checking all steps of the diagnostic process for a particular sample.

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 has a varying interpretation at the national level. The experience in plant pest diagnostic laboratories so far is that a 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 and to demonstrate that tests are valid, suitable for circumstances of use, and 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 this test may have to be withdrawn. Experience with a fixed scope of accreditation is therefore 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 of 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 (2019) defines the requirements for accreditation of flexible scopes, 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 Figure 4 as an example and the details provided below). Appropriate documents should be developed to ensure traceability when the procedure is applied.
- A list of tests included in the flexible scope is required and maintained by the laboratory.

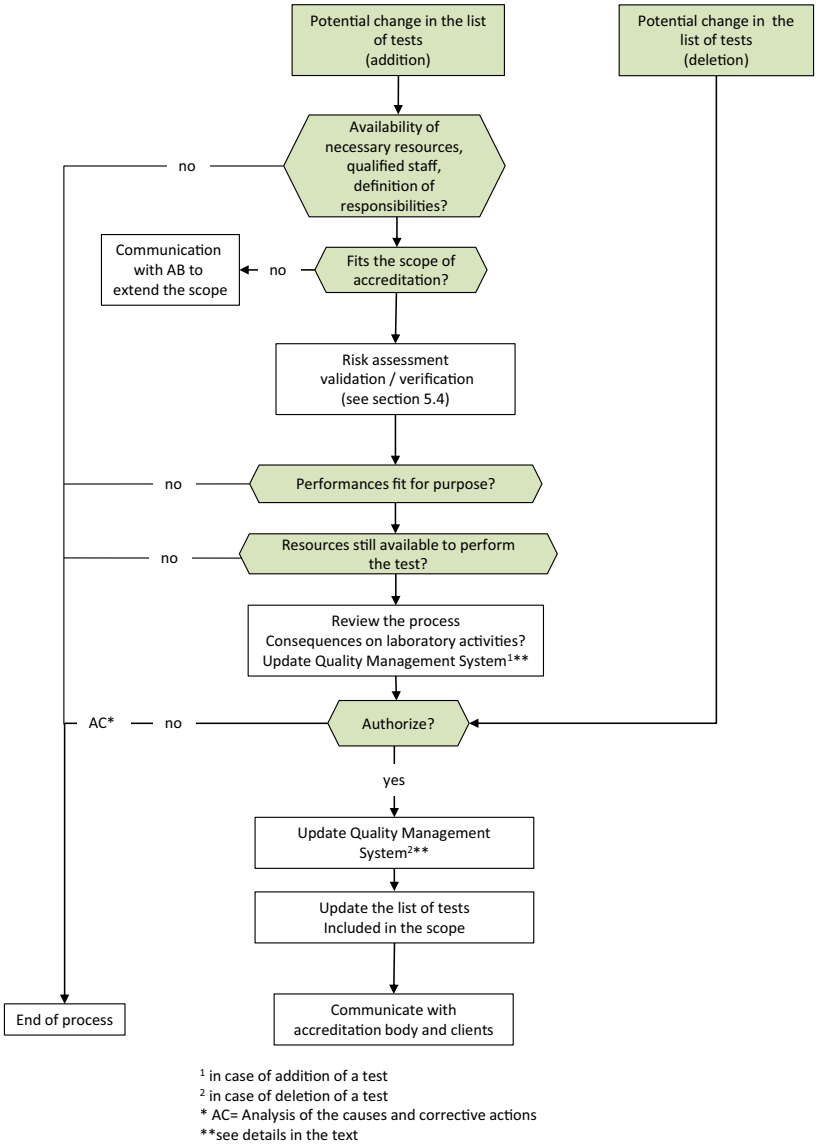

FIGURE 4 Example of a procedure for the management of flexible scope. AB, accreditation body

The frequency and means to inform the accreditation body of changes to the flexible scope should be agreed. Information should be available to the client that the test is performed under flexible scope at the contract review stage.

The flow diagram in Figure 4 outlines the procedure for management of the flexible scope and includes the requirements stated in EA-2/15. Before adding a new test to 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 in the procedure is the authorization to add or delete a test from the accreditation scope. This responsibility should be clearly defined.

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 the process can serve as a review.

The laboratory should identify the consequences for the laboratory activities (e.g. appropriate resources, plans to adjust its organisation, modification of the quality management system). The quality management system should be updated. This may include revision of documents (e.g. standard operation procedures), update of internal quality controls (e.g. controls, participation in proficiency testing), maintenance of the competence (e.g. training of staff) and updating the audit program.

7 | FEEDBACK ON THIS DIAGNOSTIC STANDARD

If you have any feedback concerning this Diagnostic Standard, please contact diagnostics@eppo.int.

8 | STANDARD REVISION

An annual review process is in place to identify the need for revision of Diagnostic Standards. Standards identified as needing revision are marked as such on the EPPO website. When errata and corrigenda are in press, this will also be marked on the website.

ACKNOWLEDGEMENTS

This revision was initiated and worked on by the Panel on Diagnostics and Quality Assurance and finalized by an Expert Working Group composed of Ms Anthoine (ANSES, FR), Ms Bokuma (State Plant Protection Service, LV), Ms de Krom (NPPO-NRC, NL), Ms Ermakovich (Agricultural Research Centre, EE), Ms Santala (Finnish Food Safety Authority Research Department, FI) and Ms Weekes (Fera Science Limited, GB). The EPPO Secretariat would like to thank all the experts involved and in particular Ms Anthoine and her team from ANSES (FR) for the great support for the organization of the Workshop on the revision of PM 7/98 (Maisons-Alfort, FR, 2019-02-11/13) which provided input for the preparation of this revision. The final revision was reviewed by the members of the Panel on Diagnostics and Quality Assurance. The previous revision of PM 7/98 was prepared by an Expert Working Group composed of Ms Anthoine (ANSES, FR), Ms Baginska (PIORIN, PL), Ms Dreo (NIB, SI), Mr Inghelbrecht (ILVO, BE), Mr König (JKI, DE), Ms Reisenzein (AGES, AT), Ms Santala (Finnish Food Safety Authority Research Department, FI), Ms van der Blom (NPPO-NRC, NL), Ms Weekes (Fera Science Limited, GB) and Mr Werkman (NPPO-NRC, NL). It was reviewed by the EPPO Panel on Diagnostics and Quality Assurance. The EPPO Secretariat would also like to thank Ms Edema and her team from the National Reference Center (NL) for the great support for the organization of the Workshop on Flexible Scope which provided input for the preparation of this revision. It was reviewed by the members of the Panel on Diagnostics and Quality Assurance.

REFERENCES

AFNOR. (1995) *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*. Association Française de Normalisation, La Plaine Saint-Denis (FR).

EA (2019) *EA-2/15 Requirements for the Accreditation of Flexible Scopes*., http://www.european-accreditation.org/publication/ea-2-15-m accessed on 14 June 2019]. European Association for Accreditation.

EPPO (2019) PM 7/77 (2) Documentation and reporting on a diagnosis. *EPPO Bulletin* 49, 527–529.

EPPO (2018a) PM 7/84 (2) Basic requirements for quality management in plant pest diagnosis laboratories. *EPPO Bulletin* 48, 378–386.

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

EU (2016) Regulation (EU) 2016/2031 of the European Parliament of the Council of 26 October 2016 on protective measures against pests of plants, amending Regulations (EU) No 228/2013, (EU) No 652/2014 and (EU) No 1143/2014 of the European Parliament and of the Council and repealing Council Directives 69/464/EEC, 74/647/EEC, 93/85/EEC, 98/57/EC, 2000/29/EC, 2006/91/EC and 2007/33/EC. https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32016R2031 [last accessed 2021-06-21]

Hughes KJD, Griffin RL, Tomlinson JA, Boonham N, Inman AJ & Lane C (2006) Development of a one step real-time PCR assay for diagnosis of *Phytophthora ramorum*. *Phytopathology* 96, 975–981.

ILAC-G18:04 (2010) Guideline for the Formulation of Scopes of Accreditation for Laboratories, EA.

ISO (2016) ISO 16140–2 Microbiology of the food chain – method validation – Part 2. Protocol for the validation of alternative (proprietary) methods against a reference method. Available on www.iso.org [accessed on 01 Feb 2019].

ISO/IEC (2017) Standard 17025. General requirements for the competence of testing and calibration laboratories. Available on www.iso.org [accessed on 01 February 2018].
Petter F & Suffert M (2010) Survey on the use of tests mentioned in EPPO diagnostic protocols. *EPPO Bulletin* 40, 5–22.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

APPENDIX 1 - EXPERTISE AND COMPETENCE

An expert will have a combination of deep knowledge in a specific field, longstanding experience and particular cognitive skills.

A competent person will be able to demonstrate that he/she can perform a particular task successfully.

For example, for the selection of morphological or morphometrical methods expertise is required. For the use of the selected tests, the laboratory should confirm that its staff is competent to carry out the morphological and/or morphometrical identification.

Examples of factors to consider when evaluating expertise or competence can be found in Table A1.

TABLE A1 Examples of factors to consider when evaluating expertise or competence

Expertise (in a specific field)	Competence (for a particular task)
Education/training: diplomas/certificates	Education/training: diplomas/certificates
Peer evaluation	Interlaboratory comparison (in particular, proficiency testing)
Proven track record: successful outcomes	Blind samples
Measure of esteem, e.g. member of international Working Group or Panels, journal editor, reviewer, technical expert, keynote speaker, invited expert, technical assessor	Internal controls (including data trending where possible): validation data
Publications: relevant to the area of work	Contra-expertise inside or outside the laboratory
Annual review/validation	Audit (both internal and external)
Continued professional development (CPD) leading to a professional qualification (e.g. in the UK Royal Society of Biology, chartered biologists/plant health professional)	CPD

APPENDIX 2 - ENVIRONMENTAL MONITORING AND AVOIDANCE OF CONTAMINATION

The laboratory should ensure that environmental conditions, laboratory arrangements and working procedures are such as to minimize the risk of cross-contamination through air, surfaces, equipment, personnel, etc. Contamination can be minimized or avoided in the following ways:

- Laboratory equipment should not routinely be moved between different areas inside the laboratory.
- Where relevant a documented vector control programme should be in place.
- Reference materials/cultures should be stored in a separate location in the laboratory.
- Housekeeping and cleaning procedures should be defined, implemented and documented for both equipment and facilities.
- Hygienic working procedures (e.g. use of "sticky" mats when appropriate, use of gloves, disinfectants, filter tips for pipettes, disposable plastic ware) should be defined and implemented.
- Careful handling of samples.

The laboratory should monitor the quality of laboratory air and surfaces of relevant areas at regular intervals. The monitoring can be done by using air settlement plates (e.g. plate count agar or other appropriate non-selective plates), contact plates (for even surfaces) or swabbing (for other surfaces and equipment), and insect traps. Buffers exposed to air or surfaces can also be tested. For laboratories working on nematodes, the normal hygienic procedures ensure that contamination is avoided.

Specific additional requirements for molecular laboratories

- Dedicated molecular work areas should be organized for (a) nucleic acid extraction and purification, (b) preparation of Mastermix, (c) addition of sample to the Mastermix, (d) nucleic acid amplification and (e) analysis of

amplification products. It is highly recommended to have at least three distinct rooms. Preparation of Mastermix, nucleic acid extraction, and/or analysis of amplification products should not be performed in the same room.
- Dedicated equipment (including pipettes) should be used in each work area. Dedicated laboratory coats should preferably be used in each work area (at least a specific coat for Mastermix preparation) and gloves should be worn.
- Tubes containing amplification reaction products should not be opened within work areas used for nucleic acid extraction or Mastermix/reaction mixture preparation.
- UV PCR workstations are decontaminated at each use using UV light.

Specific guidelines for monitoring contamination with bacteria and fungi

To monitor for airborne and surface contamination air samplers, settle plates, contact plates or swabbing (see below) can be used weekly.

Air settlement plates, preferably three in each area to be monitored, should be exposed to air contaminants for a definite time (30 min recommended), closed and incubated for 3 days (at approximately 30℃) or 5 days (at room temperature). Contact plates should be exposed on the surfaces to be monitored for 15 s (recommended), closed and incubated as above.

The acceptable level of cfu/plate/area (background counts) for bacteria or fungal colonies should be defined by the laboratory according to the testing being carried out and according to the special requirements of the environment (e.g. clean rooms). Environmental monitoring should be documented, corrective actions described and performed if needed and recorded. Cleaning should be intensified if needed and new samples taken after corrective actions have been performed.

Specific guidelines for monitoring contamination with insects

Pests should be monitored using sticky plates.

APPENDIX 3 - 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						
Diagnostic sensitivity						
Analytical specificity: inclusivity						
Analytical specificity: exclusivity						
Diagnostic specificity						

Performance criteria	NA	A	B	C	D	Where to find documentation
Selectivity						
Repeatability						
Reproducibility						
Robustness						

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

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:
Authorising person
Name in block capitals:
Signature, date:

APPENDIX 4 - 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 regarding 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 nucleic acid extraction.

Since the surveys, several protocols have been updated and new tests added. However, only the tests that included 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 supporting information (see Supporting Information S2. For readers looking at the paper or pdf version of this Standard, please see the html version to access this.).

APPENDIX 5 - 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

Comment on the figures

The 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 morphometrical methods for all fields are described in Appendix 7.

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 per mL). This estimation is often based on an indirect measurement. Where applicable, serial dilutions should be carried out until an end point is achieved.

Analytical sensitivity refers to a specific set of test parameters which should be stringently defined and standardized, for example brand of PCR reagents (in particular DNA polymerase), PCR cycle conditions, the number of microscope fields to read in the IF test, the OD threshold in the ELISA test, brand of ingredients for the medium (in particular antibiotics and preparation of stock solutions) and incubation conditions, e.g. stage of test plants, inoculation method and incubation conditions.

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).

Bacteriology

When making dilution series of bacterial extracts users of the Standard should be aware that bacteria cells may cluster, therefore making dilution series less reliable. To limit this effect, extracts should be vortexed thoroughly.

TABLE A2 Bacteriology (see also the instructions for the use of the tables)

Method for extraction of target bacterium from matrix Extraction is always validated in combination with a test	
Analytical sensitivity	The method should be able to extract a sufficient quantity of the target bacterium to allow it to be analysed further. Whenever possible, perform at least three experiments with serial dilutions of diseased tissues in the healthy sample selected. Alternatively, 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). If consistent results are not obtained after three experiments, additional experiments should be conducted.
Analytical specificity	This parameter is not applicable. Extraction of the target organism from a sample is per definition non-selective.
Selectivity	This parameter is generally not applicable. Extraction of the target organism from a sample is usually non-specific.
Repeatability	Use at least three replicates with a low concentration ideally obtained from a single sample. 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. DNA barcoding, conventional PCR, real-time PCR, LAMP This step also includes methods for the isolation of nucleic acids from the matrix	
Analytical sensitivity	Perform at least three experiments with healthy sample extracts spiked with a range of 10–10^6 cells of the target bacterium per mL. Preferentially, this is done by making decimal diluted cell suspensions of the target bacterium in the sample extracts. Determine the minimum cell density giving a positive test result. If consistent results are not obtained after three experiments, additional experiments should be conducted. For non-culturable bacteria, determine the maximum dilution of infected sample extract in the healthy sample extract giving a positive test result.
Analytical specificity	Inclusivity: analyse a range of strains of the target bacterium covering genetic diversity, different geographic origin and hosts. Exclusivity: analyse a set of relevant non-target bacteria (e.g. phylogenetically close species) and other bacteria 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, if cross reactions are observed at 10^6 cells per mL, lower and more realistic concentrations may be evaluated although the concentration should be high enough to maximize the possibility of cross reaction. *For both inclusivity and exclusivity, the results can be supported by in silico comparison of probe/primer sequences to sequences in genomic libraries.*

TABLE A2 (Continued)

Molecular methods, e.g. DNA barcoding, conventional PCR, real-time PCR, LAMP This step also includes methods for the isolation of nucleic acids from the matrix	
Selectivity	Not applicable for bacteria if they have been previously isolated from the matrix and kept in pure culture. Determine whether variations in the matrix (e.g. different age/conditions/part of plant material or different cultivars of the host plant) affect the test performance.
Repeatability	Analyse at least three replicates of sample extracts with a low concentration determined by the results from the analytical sensitivity experiments. 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	Perform at least three experiments with healthy sample extracts spiked with a range of 10–10^6 cells of the target bacterium per mL. Preferentially, this is done by making decimal diluted cell suspensions of the target bacterium in the sample extracts. Determine the minimum cell density giving a positive test result at the working dilution of the antiserum/antibodies. If consistent results are not obtained after three experiments, additional experiments should be conducted. For non-culturable bacteria, determine the maximum dilution of infected sample extract in the healthy sample extract giving a positive test result.
Analytical specificity	Inclusivity: define specificity of antibodies on strains of the target bacterium covering genetic diversity, different geographic origin and hosts. Exclusivity: define specificity of antibodies on a set of relevant non-target bacteria (e.g. phylogenetically close species) and other bacteria 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. For non-targets, if cross reactions are observed at 10^6 cells per mL, lower and more realistic concentrations may be evaluated although the concentration should be high enough to maximize the possibility of cross reaction.
Selectivity	Not applicable for bacteria if they have been previously isolated from the matrix and kept in pure culture. Determine whether variations in the matrix (e.g. different age/conditions/part of plant material or different cultivars of the host plant) affect the test performance.
Repeatability	Analyse at least three replicates of healthy sample extracts spiked with a low concentration determined by the results from the analytical sensitivity experiments. 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.
Plating methods: selective isolation For culturable bacteria only.	
Analytical sensitivity	Perform at least three experiments with healthy sample extracts spiked with a range of 10–10^6 cells of the target bacterium per mL. Preferentially, this is done by making decimal diluted cell suspensions of the target bacterium in the sample extracts. Determine the minimum cell density giving a positive test result. If consistent results are not obtained after three experiments, additional experiments should be conducted.
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 of the culture medium for a set of relevant non-target bacteria (e.g. phylogenetically close species) and other bacteria 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. different cultivars of the host plant, plant material in different condition (age or freshness) or materials collected from different environments (e.g. sewage vs irrigation water) affect the test performance.
Repeatability	Analyse at least three replicates of spiked sample extracts with a low concentration determined by the results from the analytical sensitivity experiments. 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.

TABLE A2 (Continued)

Bioassay: selective enrichment in test plants	
Analytical sensitivity	Perform at least three experiments with spiked sample extracts with a range of 10^2–10^6 cells of the target bacterium per mL. Preferentially, this is done by making decimal diluted cell suspensions of the target bacterium in the sample extracts. Determine the minimum 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 experiments, additional experiments should be conducted.
Analytical specificity	Inclusivity: define specificity of the bioassay on strains of the target bacterium covering genetic diversity, different geographic origin, and hosts. Exclusivity: define specificity for a set of relevant non-target bacteria (e.g. phylogenetically close species) and other bacteria 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. different age/conditions/part of plant material or different cultivars of the host plant) affect the test performance.
Repeatability	Analyse at least three replicates of sample extracts with a low concentration determined by the results from the analytical sensitivity experiments. 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.
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 geographic origin and hosts. Exclusivity: define specificity on a set of relevant non-target bacteria (e.g. phylogenetically close species) and other bacteria 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 variations in the matrix (e.g. different age/conditions/part of plant material or 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 as much as possible of the genetic diversity and virulence present inside this bacterium. 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). 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.

Fingerprint methods: protein profiling, fatty acid profiling and DNA profiling	
Analytical sensitivity	Perform at least three experiments. Determine the minimum quantity of harvested bacterial colony material grown on selected culture media to perform a reliable analysis. If consistent results are not obtained after three experiments, additional experiments should be conducted.
Analytical specificity	Inclusivity: define specificity of the fingerprint method on strains of the target bacterium covering genetic diversity, different geographic origin and hosts. Exclusivity: define specificity on a set of relevant non-target bacteria (e.g. phylogenetically close species) or other bacteria associated with the matrix. *For both inclusivity and exclusivity, provide markers (Main Spectra Profiles in the database) for differentiation at species, subspecies or pathovar level.* *For both inclusivity and exclusivity, 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 with a low concentration determined by the results from the analytical sensitivity experiments. 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 if possible, with different equipment.

Botany

TABLE A3 Botany (see also the instructions for the use of the tables)

Method for extraction of target plant from matrix Extraction is always validated in combination with another method	
Analytical sensitivity	The method should be able to extract a sufficient quantity of the target plant to allow it to be analysed further. Whenever possible perform at least three experiments to determine the percentage of invasive alien plant seeds that is recovered by the extraction method. If consistent results are not obtained after three experiments, additional experiments should be conducted.
Analytical specificity	This parameter is not applicable. Extraction of the target organism from a sample is per definition non-specific.
Selectivity	This parameter is generally not applicable. Extraction of the target organism from a sample is usually non-selective.
Repeatability	Use at least three replicates with a low concentration ideally obtained from a single sample. 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. DNA barcoding, conventional PCR, real-time PCR, LAMP This step also includes methods for isolation of nucleic acid from the matrix	
Analytical sensitivity	Perform at least three experiments. Determine the minimum quantity of the target from which a detectable amount of target DNA can be obtained. If consistent results are not obtained after three experiments, additional experiments should be conducted.
Analytical specificity	Inclusivity: analyse a range of specimens of the target plant(s), covering genetic diversity, different geographic origin and hosts. Exclusivity: analyse relevant non-target plant(s) (e.g. phylogenetically close plants). The concentration of nucleic acid should be high enough to maximize the possibility of cross reaction but remain realistic. *For both inclusivity and exclusivity, results can be supported by in silico comparison of probe/primer sequences to sequences in genomic libraries.*
Selectivity	Not applicable for plants as they are previously isolated from the matrix.
Repeatability	Analyse at least three replicates of sample extracts with a low concentration determined by the results from the analytical sensitivity experiments. 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.

Entomology (and acarology)

TABLE A4 Entomology and acarology (see also the instructions for the use of the tables)

Method for extraction of target arthropod from matrix Not applicable for insects and mites if they are received as individual specimens In other cases, extraction is always validated in combination with another method	
Analytical sensitivity	The method should be able to extract a sufficient quantity of the target arthropod to allow it to be analysed further. Whenever possible perform at least three experiments to determine the percentage of arthropods that is recovered by the extraction method. If consistent results are not obtained after three experiments, additional experiments should be conducted
Analytical specificity	This parameter is not applicable. Extraction of the target arthropod from a sample is per definition non-specific.
Selectivity	This parameter is generally not applicable. Extraction of the target arthropod from a sample is usually non-selective.
Repeatability	Use at least three replicates with a low concentration ideally obtained from a single sample. 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.

TABLE A4 (Continued)

Molecular methods e.g. DNA barcoding, conventional PCR, real-time PCR, LAMP This step also includes methods for isolation of nucleic acid 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 part of individuals to be detected or identified. Perform at least three experiments. If consistent results are not obtained after three experiments, additional experiments should be conducted.
Analytical specificity	Inclusivity: analyse a range of specimens of the target arthropod(s), covering genetic diversity, different geographic origin and hosts. Exclusivity: analyse relevant non-target arthropod(s) (e.g. phylogenetically close arthropods), 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 insects and mites if they are previously isolated from the matrix. However, if the molecular test is used as a detection test, determine whether variations in the matrix (e.g. different age/conditions/part of plant material or different cultivars of the host plant) affect the test performance.
Repeatability	Analyse at least three replicates of sample extracts with a low concentration determined by the results from the analytical sensitivity experiments. 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 (including oomycetes)

TABLE A5 **Mycology(including oomycetes)** (see also the instructions for the use of the tables)

Method for extraction/isolation/baiting of the fungus/oomycete from matrix Extraction is always validated in combination with another method.	
Analytical sensitivity	The method should be able to extract/isolate/bait a sufficient quantity of the target fungus/oomycete to allow it to be cultured or analysed further. Whenever possible, perform at least three experiments with serial dilutions of diseased tissues or artificially infected samples. This may also include washing procedures and membranes to trap spores. If consistent results are not obtained after three experiments, additional experiments should be conducted. Extract/isolate/bait the target from at least three samples (naturally infected or artificially infected samples). This may include washing procedure and membranes to trap spores.
Analytical specificity	This parameter is not applicable. Extraction of the target fungus/oomycete from a sample is in most cases non-specific and specific detection of the target fungus/oomycete is based on the subsequent method (e.g. morphological identification, PCR).
Selectivity	If relevant, determine whether variations in the matrix (e.g. type of soil, texture or organic matter content, host species, host tissue) may affect the performance of the test.
Repeatability	Use at least three replicates with a low concentration ideally obtained from a single sample. 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. DNA barcoding, conventional PCR, real-time PCR, LAMP This step also includes methods for isolation of nucleic acid from the matrix.	
Analytical sensitivity	Perform at least three experiments with serial dilutions, preferably in host plant tissue extracts. Determine the minimum quantity of target (e.g. number of conidia, weight of infected material in healthy material) from which a detectable amount of target DNA can be obtained. If consistent results are not obtained after three experiments, additional experiments should be conducted
Analytical specificity	Inclusivity: analyse a range of strains of the target fungus/oomycete covering genetic diversity, different geographic origin and hosts. Exclusivity: analyse relevant non-target fungi (e.g. phylogenetically closely related fungi/oomycete), 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 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 fungi if they have been previously isolated from the matrix and kept in pure culture. Determine whether variations in the matrix (e.g. different age/conditions/part of plant material or different cultivars of the host plant, types of soil) affect the test performance.

TABLE A5 (Continued)

Molecular methods, e.g. DNA barcoding, conventional PCR, real-time PCR, LAMP This step also includes methods for isolation of nucleic acid from the matrix.	
Repeatability	Analyse at least three replicates of sample extracts with a low concentration. determined by the results from the analytical sensitivity experiments. 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	Perform at least three experiments with serial dilutions preferably in host plant tissue. Determine the minimum quantity of target (e.g. number of conidia, weight of infected material in healthy material) from which a positive test result can be obtained at the working dilution of the antiserum/antibodies. If consistent results are not obtained after three experiments, additional experiments should be conducted.
Analytical specificity	Inclusivity: define specificity of antibodies on strains of the target fungus/oomycete covering genetic diversity, different geographic origin and hosts. Exclusivity: define specificity of antibodies on a set of relevant non-target fungi/oomycetes (e.g. phylogenetically closely related species), in particular those associated with the matrix. For both inclusivity and exclusivity, use antiserum/antibodies at their working dilution.
Selectivity	Not applicable for fungi if they have been previously isolated from the matrix and kept in pure culture. Determine whether variations in the matrix (e.g different age/conditions/part of plant material, different cultivars of the host plant, types of soil) affect the test performance.
Repeatability	Analyse at least three replicates of sample extracts with a low concentration determined by the results from the analytical sensitivity experiments. 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: Pathogenicity test In mycology, pathogenicity tests are also called bioassays	
Analytical sensitivity	Perform at least three experiments with dilution series. Determine the necessary quantity of matrix or matrix extract (e.g. grams of leaves, soil) to produce symptoms. If consistent results are not obtained after three experiments, additional experiments should be conducted.
Analytical specificity	Inclusivity: define specificity of the bioassay on strains of the target fungus/oomycete covering genetic diversity, different geographic origin and hosts. Exclusivity: define specificity for a set of relevant non-target fungi/oomycetes (e.g. phylogenetically close species), in particular those associated with the matrix.
Selectivity	Determine whether variations in the matrix (e.g. different age/conditions/part of plant material or different cultivars of the host plant, types of soil) affect the test performance.
Repeatability	Analyse at least three replicates of sample extracts with a low concentration determined by the results from the analytical sensitivity experiments. 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 A6 **Nematology** (see also the instructions for the use of the tables)

Method for extraction of target organism from matrix Extraction is always validated in combination with another method.	
Analytical sensitivity	Whenever possible perform at least three experiments to determine the percentage of nematodes that is recovered by the extraction method. The method should be able to extract a sufficient quantity of the target nematode to allow it to be analysed further. Alternatively, perform at least three experiments with serial dilutions of infested tissues/soil or artificially infested samples. If consistent results are not obtained after three experiments, additional experiments should be conducted.
Analytical specificity	This parameter is not applicable. Extraction of the target nematode from a sample is per definition non-specific.
Selectivity	If relevant, determine whether variations in the matrix (e.g. type of soils for cysts extractors) may affect the test performance.
Repeatability	Use at least three replicates with a low concentration ideally obtained from a single sample. Assess extraction efficiency by the relevant test method. If consistent results are not obtained, additional samples should be extracted.

TABLE A6 (Continued)

Method for extraction of target organism from matrix Extraction is always validated in combination with another 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. DNA barcoding, conventional PCR, real-time PCR, LAMP This step also includes methods for isolation of nucleic acid from the matrix	
Analytical sensitivity	Perform at least three experiments. Prepare a number of individuals. This number varies depending on the genus, species, and stage. Determine the minimum number of individuals or part of individuals to be detected or identified. If consistent results are not obtained after three experiments, additional experiments should be conducted.
Analytical specificity	Inclusivity: analyse a range of populations of the target nematode(s), covering genetic diversity, different geographic origin and hosts. Exclusivity: analyse relevant non-target nematode(s) (e.g. phylogenetically close nematodes), 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 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 molecular test is used as a detection test, determine whether variations in the matrix (e.g. types of soil) affect the test performance.
Repeatability	Analyse at least three replicates of sample extracts with a low concentration determined by the results from the analytical sensitivity experiments. 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	Prepare a number of individuals. Determine the minimum number of individuals to be identified. Perform at least three experiments. If consistent results are not obtained after three experiments, additional experiments should be conducted. The minimum number of individuals depends on the condition of the sample (good to very poor), the known intraspecies variability, the difficulty to interpret features, etc.
Analytical specificity	Inclusivity: analyse a range of populations of target nematode(s) covering genetic diversity, different geographic origin and hosts. Exclusivity: analyse relevant non-target genus and/or species (e.g. phylogenetically close species), in particular those associated with the matrix. *For both inclusivity and exclusivity, results can be supported by in silico comparison with data in relevant databases.*
Selectivity	Not applicable.
Repeatability	Analyse at least three replicates of a sample with the minimum number of individuals determined by the results from the analytical sensitivity experiments. 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 (including pathogenicity tests)	
Analytical sensitivity	Perform at least three experiments. Determine the minimum number of individuals to produce symptoms or multiply in plants material. If consistent results are not obtained after three experiments, additional replicates should be prepared and tested.
Analytical specificity	Inclusivity: define specificity of the bioassay on populations/strains of the target organism covering genetic diversity, different geographic origin and hosts. Exclusivity: define specificity for a set of relevant non-target organisms (e.g. phylogenetically close species), in particular those associated with the matrix.
Selectivity	Determine whether variations of the matrix (e.g. different cultivars of the host plant) affect the test performance
Repeatability	Analyse at least three replicates of a sample with the minimum number of individuals determined by the results from the analytical sensitivity experiments. 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 A7 Virology and phytoplasmology (see also the instructions for the use of the tables) This table covers viruses, viroids and phytoplasmas

Molecular methods, e.g. DNA barcoding, conventional PCR, real-time PCR, LAMP This step also includes methods for isolation of nucleic acid from the matrix	
Analytical sensitivity (relative sensitivity)	Perform at least three experiments with serial dilutions of an infected sample tissue extract in healthy plant tissue extract. As the concentration of viruses, viroids and phytoplasmas is not known, determine the maximum dilution of the sample extract giving a positive result. If consistent results are not obtained after three experiments, additional experiments should be conducted.
Analytical specificity	Inclusivity: analyse a range of variants/strains of the target virus, viroid, phytoplasma covering genetic diversity, different geographic origin and hosts. Exclusivity: analyse relevant non-targets virus(es), viroid(s), phytoplasma(s) (e.g. phylogenetically close viruses/viroid(s) or phytoplasmas), in particular those that might be present 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 primer/probe sequences to sequences in genomic libraries.*
Selectivity	Determine whether variations in the matrix (e.g. different age/conditions/part of plant material or 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 print-ELISA, including sample preparation (not applicable for viroids)	
Analytical sensitivity (relative sensitivity)	Perform at least three experiments with serial dilutions of an infected sample tissue extract in healthy plant tissue extract. As the concentration of viruses, viroids and phytoplasmas is not known, determine the maximum dilution of sample extracts giving a positive result at the working dilution of the antiserum/antibodies. If consistent results are not obtained after three experiments, additional experiments should be conducted.
Analytical specificity	Inclusivity: define specificity of antibodies on variants/strains of the target covering genetic diversity, different geographic origin and hosts. Exclusivity: define specificity of antibodies on a set of (relevant) non-targets (e.g. phylogenetically close species), in particular those associated with the matrix. For both inclusivity and exclusivity, use antiserum/antibodies at their working dilution.
Selectivity	Determine whether variations in the matrix (e.g. different age/conditions/part of plant material or different cultivars of the host plant) affect the test performance.
Repeatability	Analyse at least three replicates of sample extracts with a low (relative) concentration determined by the results from the analytical sensitivity experiments. 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: plant test (e.g. mechanical or insect inoculation) and grafting	
Analytical sensitivity (relative sensitivity)	Perform at least three experiments with serial dilutions of an infected sample tissue extract in healthy plant tissue extract. As the concentration of viruses, viroids and phytoplasmas is not known, determine the maximum dilution to allow that virus/viroids and phytoplasmas to multiply in plants which may produce symptoms. If consistent results are not obtained after three experiments, additional experiments should be conducted. Not relevant for grafting.
Analytical specificity	Inclusivity: determine reactivity of the bioassay on variants/strains of the target covering genetic diversity, different geographic origin and hosts. Exclusivity: determine reactivity of the bioassay on a set relevant of non-targets (e.g. phylogenetically close species), in particular those associated with the matrix.
Selectivity	Determine whether variations in the matrix (e.g. different cultivars of the host plant) affect the test performance.
Repeatability	Analyse at least three replicates of sample extracts with an appropriate dilution determined by the results from the analytical sensitivity experiments. 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.

TABLE A7 (Continued)

Biochemical methods, e.g. electrophoresis, R-PAGE	
Analytical sensitivity (relative sensitivity)	Perform at least three experiments with serial dilutions of an infected sample tissue extract in healthy plant tissue extract. Determine the maximum dilution of sample extracts giving a positive result. If consistent results are not obtained after three experiments, additional experiments should be conducted. Test parameters should be stringently defined and standardized.
Analytical specificity	Inclusivity: investigate intraspecific variability by analysing a range of variant/strains of target virus or phytoplasma(s). This is not applicable for R-PAGE. Exclusivity: analyse a set of targets/nucleic acids/proteins/contaminants from a set of relevant non-targets (e.g. phylogenetically close species), in particular those associated with the matrix. *For both inclusivity and exclusivity, test results can be supported by in silico comparison with data in relevant databases.*
Selectivity	This parameter is generally not applicable. If relevant, 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 determined by the results from the analytical sensitivity experiments. 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 6 - 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 test A presents an advantage (e.g. speed, ease of use).

It is recognized that 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 the one determined for the validated test B.

Repeatability and reproducibility should also be evaluated for test A (see Appendix 5).

The comparison of 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 A8. Samples are processed with the two tests in parallel.

TABLE A8 Minimum number of samples required when comparing test A to a validated test B

	Level of organism		
Type of material	**Low/low (relative[a])**	**Medium/medium (relative[a])**	**High/high (relative[a])**
Isolates of pure cultures of target or samples spiked with target	10[b]	7[b]	5[b]
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		

The number of samples indicated in this table has been determined by comparison with published standards, e.g. ISO 16140 *Microbiology of food and animal feeding stuffs: Protocol for the validation of alternative methods* (ISO, 2016) 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).

[a]For virology and phytoplasmology.

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

Correlation between results obtained with the validated test B and test A should be evaluated for the different pest levels. Results can be presented as shown in Table A9 and relative performance characteristics calculated.

Positive deviation and negative deviation need to be analysed.

TABLE A9 Example of results from a correlation between a validated test B and test A

		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 (ISO, 2016).

APPENDIX 7 - VALIDATION OF MORPHOLOGICAL AND MORPHOMETRICAL TESTS USED IN, FOR EXAMPLE, ENTOMOLOGY, NEMATOLOGY, MYCOLOGY, BOTANY AND VIROLOGY

This guidance is based on the validation experience of experts from EPPO Panels dealing with diagnostics.[16]

Morphological identification is based on expertise (see Appendix 1). Expert judgment 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 the following:

- Morphological and morphometrical tests included in International Standards such as the IPPC Diagnostic Protocols and the EPPO Diagnostic Protocols
- Morphological and morphometrical 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 photographs (specimens and photographs should be identified and confirmed by an expert)
- Morphological and morphometrical 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 morphometrical 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 morphometrical identification.

APPENDIX 8 - 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 Supporting Information S3. For readers looking at the paper or pdf version of this Standard, please see the html version to access this.):

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 and B version 1b – Detection of phytoplasmas from 16SrV group (flavescence dorée) and 16SrXII group (bois noir)/Matrix: *Vitis* sp.

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

APPENDIX 9 - CALIBRATION OF EQUIPMENT AND VERIFICATION OF PERFORMANCE OF EQUIPMENT

Calibration

The information in Table A10 is provided for guidance purposes and the frequency will be based on the need, use, type and previous performance of the equipment (in particular in relation to the drift observed between calibrations).

TABLE A10 Recommendations and suggested frequencies for calibration of equipment

Type of equipment	Recommendation	Suggested frequency
Reference thermometers and reference thermocouples	(a) Full traceable re-calibration	(a) Every 7 years
	(b) Single point (at working temperature)	(b) Annually
Spectrophotometric equipment	Calibration	Annually
Calibration weight(s)	Full traceable calibration	Every 7 years
Microscopes	Traceable calibration of stage micrometer	Initially
Pipettes	Calibration	Annually
Autoclaves (for media preparation)	Calibration	Annually

Verification of performance

The information in Table A11 is provided for guidance purposes and the frequency will be based on the need, type, use and previous performance of the equipment. Monitoring frequency should be adapted to the conditions of the laboratory with a frequency being higher at the beginning and adapted later based on identified risk.

TABLE A11 Guidance on verification of performance of equipment

Type of equipment	Recommendation	Suggested frequency
Temperature-controlled equipment (incubators, baths, refrigerators, freezers, Berlese funnels, slide drying benches, etc.)	(a) Establish stability and uniformity of temperature	(a) Initially, and after repair, modification
	(b) Monitor temperature	(b) Daily/each use
Thermocyclers	Verification of efficiency	Annually
Spectrophotometers	Certified plate	Annually
Working thermometers, working thermocouples and data loggers	Check against reference thermometer at ice-point and/or working temperature range	Annually
Sterilizing ovens	(a) Establish stability and uniformity of temperature	(a) Initially and after repair/modification
	(b) Monitor sterilization	(b) Each use
Autoclaves (for destruction)	(a) Establish characteristics for typical loads/cycles	(a) Initially and after repair/modification
	(b) Establish stability and uniformity of temperature	(b) Annually
	(c) Monitor sterilization	(c) Each use
Chemical fume hood	(a) Establish performance	(a) Initially and after repair/modification
	(b) Filters and air flow monitoring	(b) Annually
Laminar air-flow cabinets and biosafety cabinets (microbiology)	(a) Establish performance	(a) Initially and after repair/modification
	(b) Check with sterility plates or swabbing	(b) Monthly
	(c) Filters and air flow monitoring	(c) Yearly
Growth chambers	(a) Monitor temperature, humidity and light	(a) Each use
	(b) Monitor for pests using sticky plates	(b) Monthly
pH meters	Adjust check using at least two buffers	Daily/first use
Balances	Check zero and reading against check weight	Daily/first use
Check weight(s)	Check against calibrated weight or check on balance immediately following traceable calibration	Annually

TABLE A11 (Continued)

Type of equipment	Recommendation	Suggested frequency
Stills, de-ionizers and reverse osmosis unit	(a) Check conductivity	(a) Before use
	(b) Check for microbial contamination	(b) Monthly if the treated water or the end-use product containing the treated water are not sterilized by autoclaving or filtration before use
Gravimetric diluters	(a) Check weight volume (weight) dispensed	(a) Daily
	(b) Check dilution ratio	(b) Monthly
Automatic media preparators	Check sterility using chemical and biological indicators	As recommended by manufacturer
Pipettors/pipettes	Check accuracy, fidelity and precision of volume dispensed	Regularly (to be defined by taking account of the frequency and nature of use, and depending on the drift observed)
Spiral platers	(a) Establish performance against conventional method	(a) Initially and annually
	(b) Check stylus condition and the start and end points	(b) Daily/each use
	(c) Check volume dispensed	(c) Monthly
Colony counters	Check against number counted manually	Annually
Anaerobic jars/incubators	Check with anaerobic indicator	Each use

Risk-based analysis examples for equipment

Examples of a risk-based analysis developed by a specific laboratory for two types of equipment are given in Supporting Information S1: Supporting information on process approach and risk management. For readers looking at the paper or pdf version of this Standard, please see the html version to access this.

APPENDIX 10 - EQUIPMENT: IDENTIFICATION AND LABELLING PROCEDURES

This example document suggests the information sufficient to clearly identify equipment.

Identification procedure

Each piece of equipment should be identified by a unique code, all of which should be recorded in a specific register. Different methods and codes can be used, and they will depend on the system implemented by the quality assurance department of each laboratory. The two following methods may be used:

- The identification code is composed of, for example, five alphanumeric characters: three letters referring to the equipment type and two numbers indicating the number in a series. Example: BAL02 represents the second (02) balance (BAL) in the laboratory. The main advantage of this coding method is that the code indicates the type of equipment to which it refers.
- The equipment is identified by a unique specific serial number. Example: material n°250, whatever it may be, is the 250th piece of equipment registered and identified in the laboratory. Although this system is very easy to apply, it is not possible to have an idea of the type of equipment concerned from its number.

Labelling procedure

Each piece of equipment should be permanently labelled with its unique code. This label should not be modified or removed, therefore it is often suggested that the equipment is etched with its unique code. The code should be positioned to be easily read without needing to handle the equipment. Care should be taken when etching equipment to avoid damaging it.

A temporary label may also mention the date when the next calibration, verification or maintenance is due.

APPENDIX 11 - GUIDANCE ON MAINTENANCE OF EQUIPMENT AND ENVIRONMENT

Table A12 is provided for guidance purposes and the frequency will be based on the need, use, type and previous performance of the equipment.

TABLE A12 Guidance on maintenance of equipment and environment

Type of equipment	Recommendation	Suggested frequency
Incubators (for microbiological purposes)	Clean and disinfect internal surfaces	Monthly
Incubators (for other than microbiological purposes)	Clean and disinfect internal surfaces	Every 3 months
Refrigerators, freezers, ovens	Clean and disinfect internal surfaces	Annually
Centrifuges	(a) Service	(a) Annually
	(b) Clean and disinfect	(b) After each use
Autoclaves	(a) Make visual checks of gasket, clean/drain chamber	(a) Regularly as recommended by manufacturer
	(b) Full service	(b) Annually
	(c) Safety check of pressure vessel	(c) Annually
Safety cabinets	Full service and mechanical check	Annually
Laminar air-flow cabinets	Service and mechanical check	As recommended by manufacturer
Microscopes	(a) Clean and full maintenance service	(a) Annually
	(b) Check eye-piece graticule	(b) Every 6 months
pH meters	Clean electrode	Before and after each use
Balances, gravimetric diluters	(a) Clean	(a) After each use
	(b) Service	(b) Annually
Stills	Clean and de-scale	As required (e.g. every 3 months)
De-ionizers, reverse osmosis units	Replace cartridge/membrane	As recommended by manufacturer
Anaerobic jars	Clean/disinfect	After each use
Media dispensers, volumetric equipment, pipettes and general service equipment	Decontaminate, clean and sterilize as appropriate	After each use
Spiral platers	(a) Service	(a) Annually
	(b) Decontaminate, clean and sterilize	(b) After each use
Mixers/blenders	Clean	Each use
Thermocyclers	General service	Annually
Growth chamber	Clean	After each use
Berlese funnels	Clean	Each use
Slide drying benches	Clean	Weekly
Laboratory	(a) Clean and disinfect working surfaces	(a) Daily and during use
	(b) Clean and disinfect floors, sinks and basins	(b) Weekly
	(c) Clean and disinfect other surfaces	(c) Every 3 months

APPENDIX 12 - SUGGESTED FORM FOR SAMPLE IDENTIFICATION (LABORATORY SHEET)

Sample record form

The example of sample record form shown below enables anonymous tracing of samples or batches of samples within a laboratory. A group of samples may be recorded as one batch when they arrive from the same client, are all of the same plant species or plant part and the same analysis is required.

Batch identification code (if appropriate):	
Plant species:	Purpose of sampling (e.g. import, control of outbreak, survey):
Analysis requested by the client:	Nature of the submitted material to analyse (e.g. plant part, isolated pest):
Name of the person receiving/recording the sample:	Date and if relevant time of sampling:
	Date of reception/recording:
	Suitability of the sample for testing:

Comments (e.g. urgent, type and name of applied pesticides etc.):

Sample identification codes	
Laboratory identification code (code given by the laboratory, unique to each sample)	Client's identification code (identification code given by the client, unique to each sample)

Analysis undertaken	
Analysis protocols (used by the laboratory)	Date and signature (of the operator responsible for choosing the relevant analysis protocol)

Report of the analysis sent	
Report number	Date and signature (of the operator responsible for sending the report)