Bulletin OEPP/EPPO Bulletin (2014) **44** (2), 117–147 ISSN 0250-8052. DOI: 10.1111/epp.12118

European and Mediterranean Plant Protection Organization
Organisation Européenne et Méditerranéenne pour la Protection des Plantes PM 7/98 (2)

Diagnostics
Diagnostic

PM 7/98 (2) 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* (references to relevant parts of ISO/IEC Standard 17025 are included). It should be noted that in EPPO standards the verb 'should' carries the highest level of obligation.

Specific approval and amendment

First approved in 2009–09.
Revision approved in 2014–04.

1. Introduction

Development of quality management systems (also referred to as management systems or quality systems) and accreditation have become a concern for many laboratories in the EPPO region. A Standard PM 7/84 *Basic requirements for quality management in plant pest diagnosis laboratories* was adopted in 2007. PM 7/84 describes basic requirements to assist laboratories conducting plant pest diagnosis in designing their quality management system. PM 7/98 includes additional requirements for laboratories applying for accreditation. It is based on the ISO/IEC Standard 17025 *General requirements for the competence of testing and calibration laboratories* (ISO/IEC, 2005) and should be used together with PM 7/84. Laboratories usually apply for accreditation only for a small number of pests for which they carry out routine testing, and not for all pests on which they are likely to perform a diagnosis.

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

This document concerns the quality of a diagnosis and does not specifically deal with health and safety matters. However, laboratory practices should conform to national health and safety regulations.

2. Scope of accreditation: fixed scope and flexible scope

Historically, the accreditation of laboratories has usually been based on a fixed scope which should define clearly and unambiguously the range of tests covered by the laboratory's accreditation (e.g. immunofluorescence test for the detection of *Ralstonia solanacearum* on potato tubers). However, this 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. Although applications for an extension to scope can be made at any time, the timescales involved may actually prevent quick reactions to client's demands. Consequently the concept of flexible scope has been developed.

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 (*Requirements for the Accreditation of Flexible Scopes* EA-2/15, 2008). Examples of situations where the need for flexible scope may arise are:

- Optimisation of a given test
- Modification of an existing test to broaden its applicability (e.g. to deal with new matrices)
- Inclusion of a test equivalent to the one that is already covered by accreditation.

The concept of flexible scope encompasses a degree of flexibility which is usually agreed in consultation with the accreditation body. Nevertheless, it should be noted that this degree of flexibility has a varying interpretation at the national level. The experience in plant pest laboratories so far is that flexible scope is helpful to allow 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 demonstrate that tests are valid, suitable for circumstances of use and are performed competently and consistently. If the laboratory decides to report a test as accredited and an audit later identifies problems with the procedures used, results may not be valid and all diagnosis reports issued may have to be withdrawn. Therefore, experience with a fixed scope accreditation is valuable before a laboratory applies for flexible scope, as all requirements of the ISO/IEC Standard 17025 have to be fulfilled in both cases. Nevertheless, a laboratory may already be accredited for activities other than plant pest diagnostics. Experience with a fixed scope accreditation in another activity may be sufficient for the direct application for a flexible scope for plant pest diagnostic activities.

3. Terms and definitions

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

In this Standard, 'test' refers to the application of a method to a specific pest and a specific matrix. All test results in laboratories performing tests for quarantine pests 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 values for real-time PCR, measurements for morphological features, etc.). However, such quantitative data is used to assign a qualitative value to the test result (positive/negative/undetermined). 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.

4. Management requirements (ISO 17025 point 4)

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

The quality management system should describe the facilities and activities covered (including details of the customers and tested pests). The quality system should be documented and the quality documents should be archived (see also below).

Management system of the laboratory: see same section of PM 7/84.

4.1 Commitment of top management (ISO 17025 point 4.2.3)

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

4.2 Continuous improvement and management reviews (ISO 17025 point 4.10 and 4.15)

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

- The definition of quality objectives and adequate quality indicators (e.g. on-time result delivery or improved customer feedback). These should be reviewed by the management
- The organisation of staff meetings to:
  - Plan preventive actions and assess corrective actions for their effectiveness
  - Analyse internal audits results thoroughly
  - Evaluate complaints and their corrective actions, etc.
  - Identify training needs
  - Collect propositions for improvement by the staff
- Procedures for seeking feedback from clients.
- Analysis of trends (technical or management)

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

5. Technical requirements (ISO 17025 point 5)

5.1 General (ISO 17025 point 5.1)

See same section of PM 7/84.

5.2 Personnel (ISO 17025 point 5.2)

See same section of PM 7/84.

5.3 Accommodation and environmental conditions (ISO 17025 point 5.3)

See same section of PM 7/84.

5.4 Diagnostic methods (ISO 17025 point 5.4)

5.4.1 General (ISO 17025 point 5.4.1)

The laboratory should use appropriate methods and procedures for all tests within its scope. These include sampling where relevant, handling, transport, storage, preparation and testing of samples. It is expected that diagnostic laboratories will have an understanding of the biology of organisms and take this into account when sub-sampling and/or when preparing the sample for analysis, including handling of the sample. Purchased supplies, reagents and consumable material should be appropriate for the intended use.

All instructions, standards, technical manuals, information provided by the manufacturer and reference data relevant to the work of the laboratory should be kept up-to-date and made readily available to personnel.

5.4.2 Selection of tests (ISO 17025 point 5.4.2)

The laboratory should use diagnostic tests that are suitable according to the circumstances of use (see EPPO Standard PM 7/76 *Use of EPPO diagnostic protocols*). Tests described in the legislation (e.g. European Union or national legislation) are mandatory for the countries concerned. If no test is mandatory, tests published as international, regional or national standards should, preferably, be used. Whenever such tests are not available or whenever performance could be improved, laboratory-developed or adapted tests can be considered.

The laboratory should ensure that it uses the latest valid edition of a test, unless it is not appropriate or possible to do so.[1] When necessary, the test description should be supplemented with additional details to ensure consistent application in the laboratory.

A laboratory preparing for accreditation should only use validated tests. If this is not the case, tests should undergo a validation process within the laboratory (see 5.4.3). When a validated test is used, the laboratory should provide objective evidence that it can operate the test according to the established performance characteristics (see 5.4.4).

Tests providing all performance criteria are considered in this document as 'fully validated tests' and are referred to as 'standard methods' in ISO 17025.

5.4.3 Validation of tests (ISO 17025 point 5.4.5)

Validation is carried out to provide objective evidence that the test is suitable for the circumstances of use (see EPPO Standard PM 7/76 where the different intended uses are described). Test performance studies can be a valuable part of the validation process.

5.4.3.1 Validation of tests other than those based on morphological and morphometrical methods.

General

A test is considered fully validated when it provides data for the following performance criteria: analytical sensitivity, analytical specificity, repeatability and reproducibility. Depending on the scope of the test, selectivity may also need to be determined. The laboratory may also need to produce additional data on analytical specificity depending on local circumstances (e.g. likelihood of false reactions with closely-related organisms not included in the original data). Once these data are provided, these tests are considered validated. If values for the test performance criteria are not available or accessible (e.g. published sources or summary sheets for validation data available in the EPPO Database of Diagnostic Expertise[2]), the laboratory should produce the missing data or justify why they could not be produced. When values for performance criteria are partly available, the laboratory should verify that it can perform the test according to them (see 5.4.4).

Tests included in EPPO Diagnostics protocols are not all validated. 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 Appendix 1 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.

The regular revision programme of EPPO Diagnostic Protocols includes the addition of validation data.

Validation process

As mentioned in ISO 17025 'the laboratory shall record the results obtained and the procedure used for the validation'.

The general process for validation is described below (see also Fig. 1). More detailed guidance is given in Tables 4–9 in Appendix 5.

- Identify the scope of the test e.g. detection and/or identification of organism x in matrix y by method z considering any specific requirement related to the circumstances of use of the test (for examples, see PM 7/76).
- Consider the technical requirements to determine analytical specificity, analytical sensitivity, selectivity, reproducibility and repeatability performance characteristics, by consulting the guidelines in Tables 4–9 as required. Then define the type and constitution of samples needed for the validation. Validation is to be performed with reference material (see definition in PM 7/84), including artificially infested samples 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 characteristics or in a combined test setup. It is advised to follow the steps described in Fig. 1.

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

[2] http://dc.eppo.int/

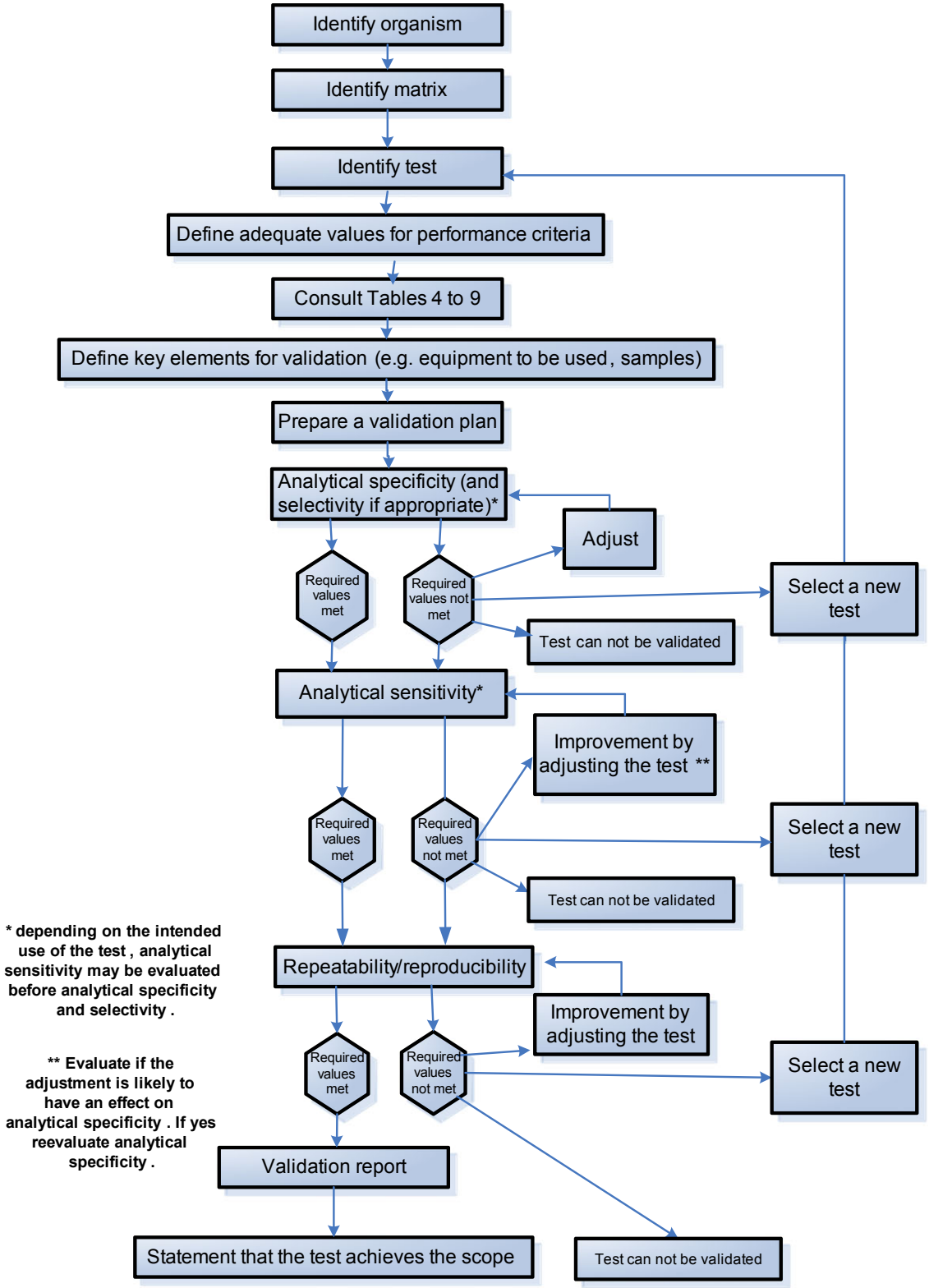

Fig. 1 Validation process.

- Present the results in a validation report with a conclusion on whether the validated test meets the requirements identified.
- Use of a 'Statement on test validation' form can be valuable and may be presented as in Appendix 3.

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 2). This can only demonstrate that e.g. the test A is as good as the validated test B with respect to selected performance criteria.

The validation procedures described here (and in particular the explanations given in Tables 4–9 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. The extent of the validation is a balance between costs, risks and what is technically feasible. Deviations from this guidance may be necessary depending on pest/matrix combination. In such case, reasons for deviation should be documented. It is not possible to provide a detailed description for each combination in this document.

Validation after significant change

If the laboratory makes a significant change to a 'fully 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 authorised by an appropriate person and if relevant the customer and the accreditation body should be informed.

Additional information

Collected data and results of laboratory-performed validations (in particular related to reproducibility), as well as results of inter-laboratory comparison (proficiency testing), can also provide an indication of the robustness of the test, i.e. to what extent different reagents or altered test conditions affect the established test performance values. Data on diagnostic sensitivity and diagnostic specificity can also be generated by comparison to (an) alternative test(s).

5.4.3.2 Validation for morphological and morphometrical methods.

It is acknowledged that the procedures for morphological and morphometrical methods 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 methods is given in Appendix 6. 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 methods made, in particular for those not described in international standards or peer-reviewed journals.

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

Verification is the process required to provide objective evidence that a laboratory is competent to perform a selected fully validated test for the intended use.

5.4.4.1 Verification process for tests other than those based on morphological and morphometrical methods.

General

Verification provides objective evidence that the laboratory is competent to perform a fully validated test according to its established performance characteristics. Verification can also be done by participating in a proficiency test or test performance study, provided that these allow the requirements in Table 1 to be fulfilled.

Additional information

Choice of reagent can be critical for the performance of a test. A change of reagent (or lots/batches of reagent) or reagent supplier may influence the performance of a test. In such a case, a verification of the performance of the reagent should be done either by comparison with the reagent previously used or according to Table 1.

Verification process

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

- Perform the validated test as described or with minor changes to take account of 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 criteria values from the validation data (see guidelines in Table 1). Selectivity does not need to be verified.

Table 1 Guidance on the verification of performance criteria

Performance criteria	Verification method
Analytical Sensitivity	Analyse at least eight samples* at the established limit of detection (for viruses, viroids and phytoplasma this should be at low level). This can be combined with repeatability/ reproducibility.
Analytical Specificity	Select a few of the most relevant targets (e.g. different strains) and non-targets*. 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.

*Artificial subsamples created from one sample can be used.

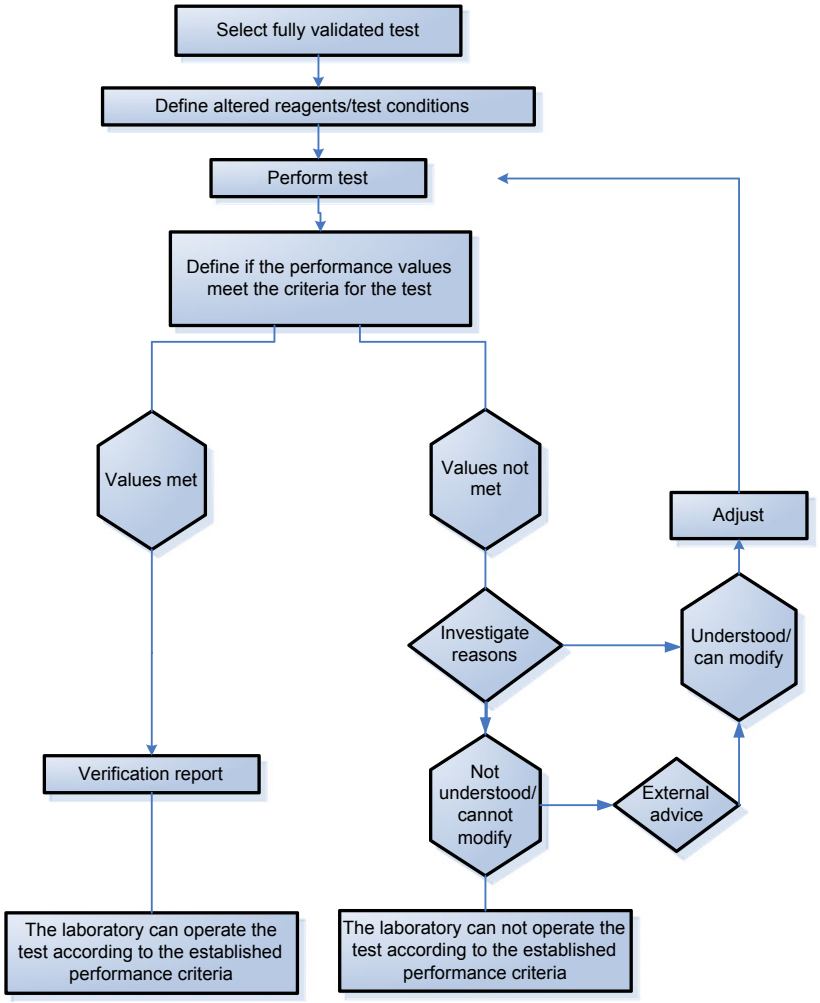

Fig. 2 Verification process.

- 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 'Validation of tests'. If performance values are not met, 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 for deviation have been observed (e.g. staff errors) corrective action should be taken and documented.
- Results of verification test:
  - A) Performance values are met: the laboratory can apply the test for routine use and a verification report stating that the requirements were or were not met should be produced. Use of a 'Statement on test verification' form can be valuable and may be presented as in Appendix 4.
  - B) Performance values are not met: the laboratory cannot operate the test according to the established performance criteria.

5.4.4.2 Verification process for morphological and morphometrical methods.

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.

5.4.5 Uncertainty of measurement (ISO 17025 point 5.4.6)

The laboratory should attempt to identify the factors influencing the uncertainty of a test such as staff, equipment and biological properties (i.e. serotypes, pathotypes). Repeatability and reproducibility will provide information on the level of uncertainty of the test result. Whenever possible, appropriate measures should be taken to control this uncertainty. If no measures are taken, the reasons for this should be recorded and the client should be made fully aware of 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 (morphometrical data, counting of cells). This measurement may be just one part of the diagnostic process, but if this is critical for a diagnosis its uncertainty should be estimated. Two examples of laboratory reports identifying critical points in the process are provided in Appendix 7.

5.5 Equipment (ISO 17025 point 5.5)

See same section of PM 7/84.

5.6 Reference materials (ISO 17025 point 5.6.3.2)

See same section of PM 7/84.

5.7 Sampling (ISO 17025 point 5.7)

See same section of PM 7/84.

5.8 Sample handling (ISO 17025 point 5.8)

See same section of PM 7/84.

5.9 Ensuring the quality of diagnosis (ISO 17025 point 5.9)

See same section of PM 7/84.

5.10 Reporting the results (ISO 17025 point 5.10.3)

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

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 (2008) EA-2/15 Requirements for the Accreditation of Flexible Scopes http://www.eurolab.org/docs/EA/EA-2_15.pdf [accessed on 16 September 2009]. European Association for Accreditation.

EPPO (2007) Basic requirements for quality management in plant pest diagnosis laboratories. *EPPO Bulletin* **37**, 580–588.

EPPO (2010) PM 7/76 (2) Use of EPPO diagnostic protocols. *EPPO Bulletin* **40**, 350–352.

EPPO (2006) PM 7/77 (1) Documentation and reporting on a diagnosis. *EPPO Bulletin* **36**, 459–460.

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.

ISO/IEC (2005) *Standard 17025. General requirements for the competence of testing and calibration laboratories*. Available on www.iso.org [accessed on 30 January 2014].

Petter F & Suffert M (2010) Survey on the use of tests mentioned in EPPO diagnostic protocols. *Bulletin OEPP/EPPO Bulletin* **40**, 5–22.

ISO (2003) *ISO 16140 Microbiology of food and animal feeding stuffs Protocol for the validation of alternative methods*. Available on www.iso.org [accessed on 30 January 2014].

Appendix 1 – List of tests included in EPPO Diagnostic protocols that are widely used

Based on the data from the laboratories responding to the EPPO surveys on the use of EPPO diagnostic protocols in 2007 and in 2013, tests must have been used in a minimum of two laboratories and for a minimum of eight samples in each laboratory to be considered widely used. Please note that molecular tests include DNA extraction.

Bacteriology

PM 7/20 (2) *Erwinia amylovora*

- o Asymptomatic plants – Direct isolation (on CCT, King's B and levan media)
- o Asymptomatic plants – Enrichment DASI ELISA
- o Asymptomatic plants – Enrichment followed by conventional PCR
- o Asymptomatic plants – Enrichment followed by PCR (Bereswill *et al.,* 1992)
- o Asymptomatic plants – Enrichment followed by Real-time PCR (Gottsberger, 2010)
- o Asymptomatic plants – Enrichment followed by Real-time PCR (Pirc *et al.,* 2009)
- o Asymptomatic plants – Enrichment isolation (on King's B or CCT)
- o Asymptomatic plants – isolation followed by Agglutination test
- o Asymptomatic plants – isolation followed by Hypersensitivity tests
- o Asymptomatic plants – isolation followed by IF
- o Asymptomatic plants – isolation followed by Pathogenicity test
- o Symptomatic plants – isolation followed by agglutination test
- o Symptomatic plants – isolation followed by biochemical tests
- o Symptomatic plants – Direct isolation (on CCT, King's B and levan media)
- o Symptomatic plants –DNA sequencing methods
- o Symptomatic plants – Enrichment DASI ELISA
- o Symptomatic plants – Enrichment isolation (on King's B or CCT)
- o Symptomatic plants – Fatty acid profiling
- o Symptomatic plants – Hypersensitivity tests
- o Symptomatic plants – IF
- o Symptomatic plants – Lateral flow devices
- o Symptomatic plants – Nested-PCR (Llop *et al.,* 2000)
- o Symptomatic plants – Pathogenecity test
- o Symptomatic plants – PCR (Bereswill *et al.,* 1992)
- o Symptomatic plants – PCR (Gottsberger adapted from Obradovic *et al.,* 2007)
- o Symptomatic plants – PCR (Stoger *et al.,* 2006)
- o Symptomatic plants – PCR (Taylor *et al.,* 2001)
- o Symptomatic plants – Real-time PCR (Gottsberger, 2010)
- o Symptomatic plants – Real-time PCR (Pirc *et al.,* 2009)

PM 7/21 (1) *Ralstonia solanacearum*

- o Non-selective isolation
- o Selective isolation
- o IF
- o Bioassay
- o PCR
- o Biochemical characteristics
- o ELISA
- o Pathogenicity test
- o REP-PCR

PM 7/42 (2) *Clavibacter michiganensis* subsp. *michiganensis*

- o Sampling and screening of tomato nursery plantlets for latent infections according to Appendix 1
- o Seed extract – Dilution plating on non-selective media
- o Seed extract – Dilution plating on semi-selective media
- o Seed sample – Conventional-PCR (adapted from Pastrik & Rainy, 1999)
- o Seed sample – Direct PCR on IF positive seed extract (Pastrik & Rainy, 1999)

o Seed sample – FAP
o Seed sample – IF
o Seed sample – Pathogenicity test
o Symptomatic plant samples – Biochemical characteristics
o Symptomatic plant samples – Conventional-PCR (adapted from Pastrik & Rainy, 1999)
o Symptomatic plant samples – Dilution plating on non-selective media
o Symptomatic plant samples – Dilution plating on semi-selective media
o Symptomatic plant samples – Direct PCR on IF positive seed extract (Pastrik & Rainy, 1999)
o Symptomatic plant samples – FAP
o Symptomatic plant samples – IF
o Symptomatic plant samples – Pathogenicity test

PM 7/59 (1) *Clavibacter michiganensis* subsp. *sepedonicus*
o IF
o Fish
o PCR (Pastrik, 2000)
o Dilution plating on MTNA or RCP 88
o Bioassay
o Biochemical characteristics
o FAP
o Pathogenicity test
o Real-time PCR

PM 7/60 (1) *Panthoea stewartii* subsp. *stewartii*
o Dilution plating
o IF
o Cell staining
o FAP
o Morphology of colonies and Biochemical characteristics
o PCR (Coplin & Marjercak, 2002)

PM 7/65 (1) *Xanthomonas fragariae*
o DAS ELISA
o IF
o Isolation method 1
o PCR (Appendix 2)
o Biochemical and physiological identification – conventional tests
o Dilution plating after detached leaf assay
o Fatty Acid Profiling
o Isolation method 2

PM 7/96 (1) *Xylophilus ampelinus*
o Biochemical tests
o Direct isolation from symptomatic material on YPGA and/or NA
o ELISA
o Extraction from symptomatic leaves
o Extraction from symptomatic shoots – first method
o Real-time PCR Dreo *et al.* (2007)

PM 7/99 (1) *Clavibacter michiganensis* subsp. *insidiosus*
o Dilution plating of seed extracts on King's B supplemented with cycloheximide
o Extraction from seeds
o IF on pure cultures

PM 7/102 (1) *Curtobacterium flaccumfaciens* pv. *flaccumfaciens*
o Conventional PCR Tegli *et al.* (2002)
o Direct isolation with NBY and SSM
o Direct isolation with YPGA and SSM
o Extraction from asymptomatic seeds soaking method

o Morphological and biochemical characteristics of pure cultures
o Pathogenicity test method A

PM 7/110 (1) *Xanthomonas* spp. *(Xanthomonas euvesicatoria, Xanthomonas gardneri, Xanthomonas perforans, Xanthomonas vesicatoria)* causing bacterial spot of tomato and sweet pepper
o Biochemical characteristics
o DNA barcoding
o Extraction from seeds
o Extraction from symptomatic plant
o Fatty acid methyl ester analysis
o Hypersensitive reaction
o IF on pure cultures
o Isolation from seed on CKTM
o Isolation from seed on NA
o Isolation from seed on YDC
o Isolation from seed on YPGA
o Isolation from symptomatic plant on Wibrink's media
o Isolation from symptomatic plant on YGCA
o Pathogenicity test

Entomology

PM 7/3 (2) *Thrips palmi* (EPPO Standard replaced by Annex 1 of ISPM 27)
o Morphological identification

PM 7/11 (1) *Frankliniella occidentalis*
o Morphological identification

PM 7/12 (1) *Parasaissetia nigra*
o Morphological identification

PM 7/13 (1) *Trogoderma granarium*
o Morphological identification

PM 7/19 (1) *Helicoverpa armigera*
o Morphological identification

PM 7/38 (1) *Unaspis citri*
o Morphological identification

PM 7/35 (1) *Bemisia tabaci*
o Morphological identification

PM 7/36 (1) *Diabrotica virgifera*
o Morphological identification

PM 7/51 (1) *Aonidiella citrina*
o Morphological identification

PM 7/53 (1) *Liriomyza* spp.
o Morphological identification
o PCR RFLP

PM 7/55 (1) *Rhizoecus hibisci*
o Morphological identification

PM 7/56 (1) *Scirtothrips aurantii, Scirtothrips citri & Scirtothrips dorsalis*
o Morphological identification

PM 7/71 (1) *Opogona sacchari*
o Morphological identification

PM 7/74 (1) ***Popillia japonica***
o Morphological identification

PM 7/83 (1) ***Rhynchophorus ferrugineus*** **and** ***Rhynchophorus palmarum***
o Morphological identification

PM 7/104 (1) ***Ceratitis capitata***
o Morphological identification

PM 7/107 (1) ***Rhagoletis completa***
o Morphological identification

PM 7/109 (1) ***Epitrix cucumeris, E. similaris*** **and** ***E. tuberis***
o Morphological identification

Mycology

PM 7/15 (1) ***Ciborinia camelliae***
o Morphological identification *in vivo*

PM 7/17 (2) ***Guignardia citricarpa***
o Conventional PCR (Bonants *et al.*, 2003)
o Isolation on cherry decoction agar
o Isolation on oatmeal agar
o Isolation on potato dextrose agar
o Morphological identification
o Real-time PCR (van Gent-Pelzer *et al.*, 2007)
o Sequencing ITS 1 and 2 – following Conventional PCR (Bonants *et al.,* 2003)

PM 7/18 (2) ***Monilinia fructicola***
o Conventional PCR (Ioos & Frey, 2000)
o Isolation on potato dextrose agar
o Morphological identification

PM 7/27 (1) ***Puccinia horiana***
o Morphological identification *in vivo*

PM 7/28 (1) ***Synchytrium endobioticum***
o Detection of sporangia in soil
o Morphological identification
o Biossay for descheduling
o Pathotype identification – Field test
o Pathotype identification – Glynne-Lemmerzahl method
o Pathotype identification – Spieckermann method

PM 7/29 (2) ***Tilletia indica***
o Wash test for non-treated seed
o Isolation and germination of teliospores on water agar
o Morphological identification
o Wash test for treated seed

PM 7/45 (1) ***Cryphonectria parasitica***
o Isolation
o Morphological identification

PM 7/66 (1) ***Phytophthora ramorum***
o Isolation from plant material with vegetable juice agar V8
o Isolation from plant material with P5ARP
o Isolation from plant material with P5ARP[H]
o Isolation from soil with P5ARP[H]
o Isolation from water with P5ARP[H]
o Conventional PCR (Wagner & Werres, 2003)

o Conventional PCR (Kox *et al.*, 2002)
o Conventional PCR (Lane *et al.,* 2003)
o Isolation from plant material with carrot juice agar (Kröber, 1985)
o Isolation from plant material with cherry decoction agar
o Isolation from plant material with carrot piece agar (CPA; Werres *et al.,* 2001)
o Isolation from plant material with dark carrot agar
o Isolation from plant material with PARB [H]
o Isolation from plant material with SNA
o Rhododendron leaf test for soil and water samples (Themann *et al.*, 2002) in combination with isolation onto CPA (Werres *et al.,* 2001)
o Real-time PCR (Hayden *et al.,* 2004)
o Real-time PCR (Hughes *et al.,* 2005)
o Sequencing of the ITS region

PM 7/85 (1) *Plasmopara halstedii*
o Bioassay
o Conventional PCR in seed (Ioos *et al.,* 2007)

PM 7/86 (1) *Diaporthe vaccinii*
o Morphological identification *in vitro* on MEA
o Morphological identification *in vitro* on PDA
o Morphological identification *in vivo*

PM 7/91 (1) *Gibberella circinata*
o Identification in plant issues except seeds by dual-labelled probe real-time PCR (Ioos *et al.,* 2009)
o Identification in pure cultures by conventional or SyBr green real-time PCR (Schweigkofler *et al.*, 2004)
o Identification in seeds by conventional or SyBr green real-time PCR (Schweigkofler *et al.,* 2004)
o Identification in seeds by dual-labelled probe real-time PCR (Ioos *et al.*, 2009)
o Isolation from plant tissue except seeds on DCPA
o Isolation from plant tissue except seeds on PDAS
o Isolation from seeds on DCPA and transfer to PDA and SNA
o Isolation from seeds on Komada's medium and transfer to PDA and SNA
o Morphological identification in pure culture

PM 7/92 (1) *Gremmeniella abietina*
o Morphological identification *in vitro* on other rich agar media
o Morphological identification *in vivo*

PM 7/93 (1) *Melampsora medusae*
o Morphological identification

PM 7/111 (1) *Fusarium foetens*
o Isolation from symptomatic plant on SNA supplemented with antibiotics followed by plating on semi selective media

PM 7/112 (1) *Phytophthora kernoviae*
o Baiting test soil
o Baiting test water
o Conventional PCR Schlenzig (2011)
o Isolation on CPA
o Isolation on semi-selective V8 agar
o Morphological identification
o Real-time PCR targeting a region of the ras-related protein (Ypt1) Schena *et al.* (2006)
o Sporulation induction Julius Kühn Institute
o Test targeting the ITS region Hugues *et al.* (2011)

Nematology

PM 7/4 (3) *Bursaphelenchus xylophilus*
o Extraction Bearmann funnel
o ITS RFLP PCR Burgermeister *et al.* (2009)

o Morphological identification

PM 7/40 (3) *Globodera rostochiensis* and *Globodera pallida*
o Bioassay (test A Bioassay – Method performed in Germany and Austria)
o Bioassay (test A Test of Reproduction- Method performed in Norway)
o Extraction by flask and paper strip methods
o Extraction Fenwick can
o Extraction Schuiling centrifuge
o Extraction Seinhorst elutriator
o Extraction Wye washer
o Hatching test A (method performed in Norway)
o Hatching test C (methods performed in France)
o Morphological and morphometrical identification
o Multiplex PCR test (Bulman & Marshall, 1997)
o Viability testing (Visual determination)

PM 7/41 (2) *Meloidogyne chitwoodi* and *Meloidogyne fallax*
o Extraction from roots incubation method
o Extraction from roots maceration/incubation/flotation Coolen (1979)
o Extraction from soil Baermann funnel
o Extraction from soil for large quantities adapted Baermann funnel
o Extraction from tubers (enzymatic digestion) Araya & Caswell-Chen (1993)
o Extraction from tubers (tissues mixed in a blender) extraction sieves or floatation (Viaene *et al.,* 2007)
o Morphology
o PCR RFLP Zijlstra *et al.* (1997)
o PCR Wishart *et al.* (2002)
o PCR Zijlstra *et al.* (2000)
o Real-time (TaqMan) PCR, Zijlsta & van Hoof (2006)

PM 7/87 (1) *Ditylenchus destructor* and *Ditylenchus dipsaci*
o Extraction from soil
o Extraction of *D. dipsaci* from seeds
o Morphological and morphometrical identification

PM 7/88 (1) *Radopholus similis*
o Extraction from plant material
o Extraction from soil
o Morphological and morphometrical identification

PM 7/89 (1) *Heterodera glycines*
o Morphological and morphometrical identification

PM 7/94 (1) *Hirschmanniella* spp.
o Extraction from roots
o Extraction from soil
o Morphological and morphometrical identification

PM 7/95 (1) *Xiphinema americanum sensu lato*
o Extraction from soil
o Morphological and morphometrical identification

PM 7/103 (1) *Meloidogyne enterolobii*
o Extraction from roots
o Extraction from soil
o Morphological and morphometrical identification

Phytoplasmology

PM 7/62 (1) *Candidatus Phytoplasma mali*
o Woody indicators

o ELISA
o Universal PCR (Lorenz *et al.*, 1995)
o Universal PCR (Lee *et al.*, 1998)
o AP- or 16SrX-group specific PCR (Lorenz *et al.*, 1995)
o AP-specific PCR (Jaraush *et al.*, 1995)

PM 7/63 (1) ***Candidatus Phytoplasma pyri***
o Universal PCR (Lorenz *et al.*, 1995)
o Universal PCR (Lee *et al.*, 1998)

PM 7/79 (1) ***Grapevine flavescence dorée phytoplasma***
o Multiplex nested-PCR (for simultaneous detection of flavescence dorée and bois noir)
o Direct generic PCR followed by nested generic PCR followed by RLFP
o Direct generic PCR followed by nested group-specific PCR

Virology

PM 7/30 (2) ***Beet necrotic yellow vein benyvirus***
o ELISA

PM 7/31 (1) ***Citrus tristeza closterovirus***
o DAS-ELISA
o Tissue print-ELISA

PM 7/32 (1) ***Plum pox potyvirus***
o Biological testing (graft-inoculation of indicator plants)
o DAS-ELISA with 5B-IVIA or polyclonal antibodies
o IC-RT-PCR
o Co-PCR
o DASI-ELISA with 5B-IVIA universal monoclonal antibodies

PM 7/33 (1) ***Potato spindle tuber pospiviroid***
o DIG probe
o R-PAGE
o RT-PCR
o TaqMan

PM 7/34 (1) ***Tomato spotted wilt virus, Impatiens necrotic spot virus*** **and** ***Watermelon silver mottle virus***
o Mechanical inoculation of indicator plants
o TAS-ELISA
o RT-PCR
o Detection of TSWV in plants and individual thrips using real-time fluorescent RT-PCR (TaqMan)
o Lateral Flow Devices (LFD)

PM 7/50 (1) ***Tomato yellow leaf curl*** **and** ***Tomato mottle begomoviruses***
o TAS-ELISA
o PCR method 1 (Accotto *et al.,* 2000)
o DNA extraction method 1 (Accotto *et al.*, 2000)
o PCR/MPCR, Deng *et al.* (1994)

PM 7/67 (1) ***American plum line pattern virus (Ilarvirus)***
o DAS ELISA (Clark & Adams, 1977)
o Woody indicators

PM 7/113 (1) ***Pepino mosaic virus***
o Biossay mechanical inoculation from leaf or fruit extracts
o Biossay mechanical inoculation from seed extract
o DAS ELISA
o Extraction from seeds
o Real-time PCR Ling *et al.* (2007)

Appendix 2 – Procedure for validation of a test (A) by comparison with a validated test (B)

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

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

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

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

Perform three repetitions with the target organism and three with each of the non-target organisms as indicated in Table 2. Samples are processed with the two tests in parallel.

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, 2003)* and AFNOR XP V03-111 *Agricultural and food products analysis. Protocol for the intra-laboratory evaluation of an alternative method of qualitative analysis against a reference method* (AFNOR, 1995).

Correlation between results obtained with the validated test (B) and the test (A) should be evaluated for the different pest levels. Results can be presented as shown in Table 3 and relative performance characteristics calculated.

Table 2 Minimum number of samples required when comparing a test (A) to a validated test (B)

	Level of organism		
Type of material	Low/Low (relative*)	Medium/Medium (relative*)	High/High (relative*)
Isolates of pure cultures of target or samples spiked with target	10†	7†	5†
Isolates of pure cultures of non-target(s) or samples spiked with non-target(s)	–	22–44	–
Naturally contaminated sample with target organism	Adequate dilution series are prepared with 15 positive samples previously identified with the validated test (B) to reach the limit of detection of the validated test (B).		

*For virology and phytoplasmology.

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

Table 3 Example of results from a correlation between a validated test B and a 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%.

Appendix 3 – Statement on test validation

Test name

Scope of test

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	A	B	C	D	Where to find documentation
Analytical Sensitivity					
Analytical Specificity					
Selectivity (when relevant)					
Repeatability					
Reproducibility					

A = Data from own laboratory experiments.
B = Data from interlaboratory comparison.
C = Information from manufacturers.
D = External information (literature, etc.).

Other information (optional)

	Where to find documentation
Diagnostic Sensitivity	
Diagnostic Specificity	
Robustness (see 5.4.3)	

On the basis of the above statement the validity 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 capital letters:

Signature: Date:

Appendix 4 – Statement on test verification

Test name

Selected fully validated test

Scope of the fully validated test

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 test has been performed in this study.

Performance criteria	Performance criteria values obtained	Meet requirements of the fully validated test (yes/no)	Where to find documentation
Analytical sensitivity			
Analytical specificity			
Repeatability			
Reproducibility			

On the basis of the above statement of verification 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 capital letters:

Signature: Date:

Appendix 5 – Tables giving detailed guidance for the validation process by field (Bacteriology, Botany, Entomology, Mycology, Nematology, Virology & Phytoplasmology)

Instructions for the use of the tables

Comment on the figures

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

General note on analytical sensitivity

Whenever possible, the limit of detection (as defined in PM 7/76) of a test should be determined. Nevertheless, this limit cannot always be established absolutely while detecting plant pests. There are organisms that cannot be cultured (obligate pathogens), which cannot be quantified (fungi), which are only present in the plant or which cannot be purified (e.g. phytoplasmas). For this reason, exact concentrations of these organisms cannot or can hardly ever be established accurately and so estimates have to be used. Even with those which can be purified (many bacteria and viruses), the concentration can only be estimated (e.g. cfu's 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.

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

Table 4 Bacteriology (see also the instructions for the use of the tables)

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

(continued)

Table 4 *(continued)*

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

Botany

Table 5 Botany (see also the instructions for the use of the tables)

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

Entomology

Table 6 Entomology (see also the instructions for the use of the tables)

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

Mycology

Table 7 Mycology (see also the instructions for the use of the tables

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

Nematology

Table 8 Nematology (see also the instructions for the use of the tables)

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

Virology and Phytoplasmology

Table 9 Virology & Phytoplasmology (see also the instructions for the use of the tables). This table covers viruses, viroids and phytoplasmas

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

Appendix 6 – Validation of Morphological and Morphometrical methods used in e.g. Entomology, Nematology, Mycology, Botany

This guidance is based on the validation experience of experts from EPPO Panels dealing with diagnostics.[3]

For morphological identification, 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 by the experts as reference documentation to support the identification. As these documents or supporting information have been produced by specialists of the group(s) concerned, they are consequently considered as validated tests in the current standard.

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

- Morphological and morphometrical methods included in International Standards such as the IPPC Diagnostic Protocols and the EPPO Diagnostic Protocols.
- Morphological and morphometrical methods, 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 methods in common usage which are published in non-peer reviewed publications including electronic media (in particular for keys).

As explained in 5.4.4.2, the laboratory should confirm that it can properly carry out the morphological and/or morphometrical identification.

The laboratory should be able to justify the selection of morphological and morphometrical methods made, in particular for those not described in international standards or peer-reviewed journals.

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

Appendix 7 – Example of laboratory reports on the critical points in the diagnostic process and relating to uncertainty of measurement

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

Scope	Diagnostics of phytoplasma
Method (name, protocol number)	Real-time PCR (02D-Pos43)
Harmful organism	FD and BN
Type of sample (description, protocol number)	grapevine, vectors, other host plants (02D-Pos10)
Remarks	/
Authorized analyst (signature, date)	Nataša Mehle, 6.7.2012
Other providers (signature, date)	/
Head of operations (signature, date)	

A step in the process	Possible impact on the result	Measures for reducing the uncertainty of measurement	Document that defines the measures
Sampling – type of a sample	Unequal distribution of phytoplasma in plant samples. Insects trapped on sticky plate traps are not suitable for the analysis.	The manner of sampling is not under our direct control; however, it is clearly specified. Upon reception of samples we verify if the sample is suitable for the analysis.	Yearly Program of special control of grapevine yellows (Op. Prev.: Program posebnega nadzora trsnih rumenic) 02D-Pos10
Sampling – time	Seasonal variability in phytoplasma concentrations in the samples.	The time of sampling is specified. Upon reception of samples we verify if the sample is suitable for the analysis (weather it has been sampled at the suitable period of time/season).	Yearly Program of special control of grapevine yellows (Op. Prev.: Program posebnega nadzora trsnih rumenic) 02D-Pos10
Sampling –unequivocal labeling of the samples	Equivocal labeling of the samples – the result of the analysis is recorded under the wrong sample.	The labeling of the samples is not entirely under our direct control; however, the labeling of the samples is clearly specified. Upon the reception of samples we verify if the client's label on the sample matches the label on the sampling report.	Yearly Program of special control of grapevine yellows (Op. Prev.: Program posebnega nadzora trsnih rumenic) 02R-Nav05
Transport of the sample to the laboratory	Long-term storing of the samples at high temperatures, as well as the freezing and thawing of the samples may cause damage to the samples, which consequently may result in a reduced possibility of detecting phytoplasma in these samples.	The means of delivery to the laboratory are specified. Upon the reception of samples we verify the status of the samples.	Yearly Program of special control of grapevine yellows (Op. Prev.: Program posebnega nadzora trsnih rumenic) 02D-Pos10
Reception of the sample	The sample has not been delivered to the responsible person – the analyses are not carried out on time or not at all.	All employees must be familiar with the instructions for reception of samples.	02R-Nav05; 02R-Sez07
Documenting the sample	Information about the delivered sample does not reach the responsible person – – the analyses are not carried out on time or not at all, or wrong analyses are being performed.	Precise instructions for recording of the newly delivered samples.	02R-Sez07, 02D-Nav01, 02D-Nav16

(continued)

Table *(continued)*

A step in the process	Possible impact on the result	Measures for reducing the uncertainty of measurement	Document that defines the measures
Storage of the sample	Long-term storing of the samples at high temperatures, as well as the freezing and thawing of the samples may cause damage to the samples, which consequently may result in a reduced possibility of detecting phytoplasma in these samples.	Designated place for storing of the samples.	02R-Nav05
Selection of tests	An unsuitable test has been performed	Precisely defined battery of tests and a well defined sequence of procedures upon performing the testing.	02D-Sez01, 02D-Pos10
Preparation of the sample for the analysis	Contamination among the samples.	The sample preparation and sample homogenization procedures are specified, including the manner of carrying out the work in order to prevent contamination among the samples. Only responsible authorized persons carry out these procedures.	02D-Pos06, 02D-Pos54, 02D-Pos43, 02R-Sez04
	Choosing an unsuitable part of the sample; insufficient homogenization of the sample.	Homogenization procedure (with simultaneously performed DNA extraction) is controlled using NKI and KE (KE is performed for each sample separately).	
Extraction	Choosing an unsuitable method for the extraction of DNA; an error during carrying out the extraction procedure; contamination during the extraction procedure.	A choice and performance of the extraction procedure are well specified. They are carried out only by responsible authorized persons. NKI is included in the extraction procedure. Adequacy of DNA extraction procedure is verified for every individual sample by amplification of an internal control (endogenous nucleic acid, KE).	02D-Pos06, 02D-Pos54, 02D-Pos43, 02R-Sez04
Performing the method – analysts?	Not familiar with the method – erroneous execution.	The method is carried out only by qualified and trained analysts.	02R-Sez04
Performing the method – impact of the inhibitors?	False negative results due to the presence of the inhibitors of the PCR reaction in DNA extract.	Testing of additional series of DNA dilutions when high presence of the inhibitors in DNA extracts is anticipated. DNA extraction is repeated using an alternative validated (different) extraction procedure.	02D-Pos43 02D-Pos06
Performing the method – impact of the matrix on the specificity?	New and unknown matrix – nonspecific reaction using oligonucleotide primers and probe.	For any new type of samples and/or findings (such as new hosts) we perform the additional confirmation tests.	02D-Pos10, 02D-Pos43
Performing the method – impact of the matrix on the sensitivity?	New and unknown matrix – decreased sensitivity of the method.	When low phytoplasma concentration is expected in the samples (due to the KE results), additional tests are performed using more concentrated DNA.	02D-Pos43
Performing the method – possibility of contaminations?	False positive results.	Inclusion of the internal controls: NTC 1, NTC 2.	02D-Pos48
		Well defined execution of the method that prevents possibility of contaminations.	02D-Pos48
		Individual steps of the analytic procedure are carried out in separate rooms.	02D-Pos18

(continued)

Table *(continued)*

A step in the process	Possible impact on the result	Measures for reducing the uncertainty of measurement	Document that defines the measures
Performing the method – impact of laboratory materials and ware?	Impacts on the fluorescence measurements: dust on the test plate/ foil, talc on the gloves, touching of the foil/bottom of the test plate Increased possibility of contamination: pipeting tips without a filter.	Inclusion of internal controls: NTC 1, NTC 2. Using laboratory gloves without talc, using pipeting tips with filter. Dust-free conditions for storage of test plates and foil.	02D-Pos48
Performing the method – impact of the laboratory equipments?	Non- accurate pipeting of small volumes.	Using only calibrated pipettes. Inclusion of PK 1 and PK 2 with known C_t value (verifying suitability of C_t values).	02D-Obr30, 02D-Pos43
Performing the method – impact of the apparatus?	Theoretically possible	Verifying suitability of the apparatus by comparing with another device/ apparatus before using it for diagnostic purposes.	Example as in Report of suitability testing D0002/12
Performing the method – impact of the chemicals?	Old chemicals, improper storage of chemicals, improper choice of chemicals, poor quality of a lot of chemicals, contaminated chemicals – impact on amplification, false negative/positive results.	Inclusion of internal controls NTC 1 and NTC 2. Inclusion of PK 1 and PK 2 with known C_t values (verifying suitability of C_t values). Recording the expiration dates of chemicals, lot/inventory number or of a PPS mix preparation date.	02D-Obr30, 02D-Nav24
Performing the method – impact of the environment?	Impact of the temperature when pipeting the small volumes.	Verifying the temperature in the room where PCR mixes are being prepared, and in the room where DNA is being added.	02D-Obr30, 02D-Pos48
Performing the method – analysis of the results	Wrong Threshold setting – false negative results; signal that is not a consequence of amplification – false positive results.	Verification of the amplification curves (for example Multicomponent Plot)	02D-Pos48, 02D-Pos43
Determination and entry of the test results	Wrong interpretation of the results obtained by the method.	Clearly defined interpretation of the results (for example, detection limit, parallel tests, and controls). The responsible authorized person for survey and interpretation of the results must verify the accuracy of entered data.	02D-Pos43, 02D-Pos48 02D-Nav01, 02R-Sez07
End result of testing – a result that provides the final confirmation of presence/ absence	End result depends on the partial results for various individual amplicons – not clearly set rules for conveying of the end result may lead to the wrong conclusions. Wrong formulation of the end results may confuse the client.	Clearly stated criteria for finalizing the samples. Only the persons responsible and authorized for performing the finalizing step can finalize the sample. Formulation of the test results is specified.	02D-Pos10, 02D-Pos43, 02R-Sez07 02D-Pos18
Informing	End results not sent to all parties that are eligible according to the contract for receiving the end result. Delay in informing about the results of the analyses.	Clearly stated guidelines for informing about the end results. All correspondence with the client is being archived (traceability of the correspondence about the individual sample is also assured). BiaLims system prompts us automatically about the proximity of the due dates/ deadlines for the submission of the results of analyses.	02D-Nav01, 02D-Nav16

(continued)

Table *(continued)*

A step in the process	Possible impact on the result	Measures for reducing the uncertainty of measurement	Document that defines the measures
Issuing the test reports	Test report not sent to all parties that are eligible according to the contract for receiving the end result.	Clearly stated guidelines for issuing and sending the test reports.	02D-Nav01
Archiving	A complaint from a client, orderer or owner, about the execution of the analyses.	Organized sorted archive of test reports for the samples from previous years. Assured traceability from receiving the sample to issuing the test report. Samples kept in storage at least until issuing the test report. All important DNA extracts are being kept in the collection of DNA isolates.	02D-Vzo01, 02D-Pos18, 02D-Nav01, 02R-Nav02, 02R-Nav08
Other/remarks	/		

Report 2 – Detection of Flavescence dorée (FD) and Bois noir (BN) by real-time PCR

Validated method:

- French Method MOA006 parts A et B version 1b – Detection of phytoplasmas from 16SrV group(flavescence dorée) and 16SrXII group (bois noir)/Matrix: *Vitis sp.*
- Erratum MOA006 part A on 07 March 2012.

Reference of internal SOP: AMO07S23

Identification of uncertainty sources	Type of uncertainty	Frequency	Actions to reduce/avoid the uncertainty and associated documentation	Contribution to the overall uncertainty
Staff				
Identification of batches and samples	Error when allocating results to a sample	Rare	Transfer of simplified sample number on the parcel and on the registration documents **APRO6002**	Negligible
Labelling of stands and containers/tubes (PCR)	Error when allocating results to a sample	Rare	Use of simplified sample number on the containers during the analytical process (bags, tubes, plan of PCR plates…) **APQA**	Negligible
Staff training	Human error during the analytical process	Rare	Training and authorization of operators **APR02003/APR02004**	Negligible
Reading and interpretation of PCR results	Risk of false positive and false negative results	Rare	Decision rules clearly defined in the quality management system **AMO07T42** Training and authorization of operators **APR02003/APR02004**	Negligible
Report editing: transfer of information	Transcript error	Not frequent	Overall review by the technical leader. Check of correct record of the results on the report by the technical leader and the administrative leader. Report signed by the administrative leader: **APR07001**	Negligible

(continued)

Table *(continued)*

Identification of uncertainty sources	Type of uncertainty	Frequency	Actions to reduce/avoid the uncertainty and associated documentation	Contribution to the overall uncertainty
Execution of the analysis and preparation of buffers, solutions and reaction mixes	Error during the preparation process (quantity, . . .)		Complete and detailed standard operating procedure which enables reliable traceability **AMO07P32**, **AMO05T28** et **AMO07T42**	Negligible
Materials				
Distribution of the target phytoplasmas in plant tissues	Random distribution of the phytoplasmas in the plant tissues	Frequent	Sampling of the necessary amount/quantity of petiole and/or main veins on symptomatic vine leaves **AMO07T42**	Not negligible
Sample degraded when it arrived at the laboratory	Development of saprophytic organisms and/or degradation of plant material leading to the reduction of the level of contamination	Rare	Sample refused, information sent to the client: **AMMQSE06**–**APR06002**	Negligible
Degradation of samples during storage	Development of saprophytic organisms and/or degradation of plant material leading to the reduction of the level of contamination	Rare	The requirements related to storage conditions are defined and applied **APQA**, **APRO6002**, **AMO07T42**.	Negligible
Sample with a lot of plant material ('rich' sample)	Risk for the presence of inhibitors	Occasional	The correct result of the vine positive control allows confirmation that amplification is not inhibited **AMO07T42**.	Negligible
Quality of reagents, solutions, buffers and media	Non-respect of requirements for storage conditions, expiration date, contaminations	Not likely	Definition of areas and conditions for storage according to the suppliers' recommendations and those defined by the laboratory. Temperature control of climatic chambers (e.g. fridge) for storage of reagents: **APQM** Storage areas separated from the zones dedicated for samples reception and storage of positive controls: **APQA** Use of relevant controls in each test performed **AMO07T42** Regular change of the BET staining bath according to **APQT01**	Negligible
Preparation of solutions, buffers and media	Error in measuring weight, volumes and pH	Not likely	Metrological controls of balances, pipettes, pH meter to ensure the compliance with the requirements and the maximum error defined: **APQM**	Negligible

(continued)

Table *(continued)*

Identification of uncertainty sources	Type of uncertainty	Frequency	Actions to reduce/avoid the uncertainty and associated documentation	Contribution to the overall uncertainty
Equipment/Measurements				
Equipment under metrological survey or not	Non respect of metrological and/or technical requirements	Not likely	Definition and enforcement of a metrological quality plan **APQM**. Procedures and calibration/ verification programmes: **APQM/ APR03032** Pipette: **APR03009/APR03010** Balances: **APR03007/APR03008** pH-meter: **APR03006** temperatures: **APR03031** PCR machines qualifying: **APR03036** Establishment of a plan for maintenance and cleaning **APRO3005**, **APQN**. Technical confirmation by metrology leader and technical leader before putting into service **APRO3001**.	Negligible
Environment				
Cross-contamination: general	Risk of false positive results	Rare	Plan for equipment and benches disinfection Respect of 'one way traffic' during the analysis process: **APQA**	Negligible
Cross-contamination: PCR	Risk of false positive results	Rare	Separated rooms for «reaction mix preparation », « addition of DNA extracts », « amplification »and « electrophoresis » and use of appropriate controls **APQT01**	Negligible
Samples Storage	Development of saprophytic organisms and/or degradation of plant material leading to the reduction of the level of contamination	Rare	The requirements related to storage conditions are defined and applied **APQA**, **APRO6002**, **AMO07T42**.	Negligible
Method/Process				
Positive control for the whole process	Control not detected	Occasional	Defined rule: If the positive extraction control doesn't react correctly, either another sample from the same extraction series is positive and then the run is validated or no sample gave a positive result in the run (rare case) and the run is not validated. The experiment should be repeated. Use of « vine » internal positive control for each sample enabling validation of the quality of each extract **AMO07T42**.	Negligible
Sensitivity/ limit of detection	Presence of the target below the limit of detection	Possible Frequency not determined	Risk associated with the method used. Use of controls at the limit of detection **MO07T42**. Performance criteria of the test defined in the internal validation report FD BN 2009	Not negligible Linked to the limits of the methods used