Applying a systems approach to validation and proficiency testing in plant virology to meet ISO-17025 accreditation requirements

Annelien Roenhorst[1], Denise Altenbach[2], Marleen Botermans[1], Michele Della Bartola[3], Francesco Faggioli[4], Adrian Fox[5], Doris Gambin[6], Anne Giesbers[1*], Sabine Grausgruber-Gröger[7], Miroslava Hejlová[8], Colin Jeffries[9], Christel de Krom[1], Irena Mavrič Pleško[10], Natasa Mehle[11,12], Jasna Milanović[13], Antonio Olmos[14], Carla Oplaat[1], Hana Orságová[8], Sebastjan Radišek[15], Matthieu Rolland[16], Anna Rosińska[17], Ana Belén Ruiz-García[14], Christina Varveri[18], Marcel Westenberg[1]

1. Netherlands Institute for Vectors, Invasive plants and Plant health (NIVIP), National Plant Protection Organization (NPPO), Netherlands Food and Consumer Product Safety Authority (NVWA), Geertjesweg 15, 6706 EA Wageningen, The Netherlands
2. Research group Molecular Diagnostics of Regulated Plant Pests, Division of Methods Development and Analytics, Agroscope, Federal Department of Economic Affairs, Education and Research EAER, Route de Duillier 60, 1260 Nyon, Switzerland
3. Plant Health Laboratories, Plant Science Division, Department of Agriculture, Food and the Marine, Backweston Laboratory Campus, W23X3PH Celbridge, Ireland
4. National Reference Plant Protection Institute, Research Center for Plant Pathology and Certification (CREA), Council for Agricultural Research and Economics, Via Carlo Giuseppe Bertero 22, 141 Rome, Italy
5. Plant Virology, Fera Sceince Ltd, York Biotech Campus, YO41 1LZ, York, United Kingdom
6. Plant Health Diagnostic Laboratory, Rural Affairs Department, Plant Protection Directorate, 110 Annibale Preca Street, LJA 1915, Hal-Lija, Malta
7. Plant Health and Reference Laboratories,Institute for Sustainable Plant Production, Austrian Agency for Health and Food Safety (AGES), Spargelfeldstraße 191, 1220 Vienna, Austria
8. National reference laboratory, Department of Plant Pest Diagnostics, Central Institute for Supervising and Testing in Agriculture, Šlechtitelů 773/23, 77900 Olomouc, Czech Republic
9. Plant Health Sciences, National Plant Protection Organization, Science and Advice for Scottish Agriculture (SASA), Roddinglaw Road, EH12 9FJ Edinburgh, United Kingdom

10. Plant Protection Department, Agricultural Institute of Slovenia, Hacquetova ulica 17, 1000 Ljubljana, Slovenia
11. Department of Biotechnology and Systems Biology, National Institute of Biology (NIB), Večna pot 121, 1000 Ljubljana, Slovenia
12. School for Viticulture and Enology, University of Nova Gorica, Dvorec Lanthieri Glavni trg 8, 5271 Vipava, Slovenia
13. Center for Plant Protection, Croatian Agency for Agriculture and Food, Gorice 68b, 10000 Zagreb, Croatia
14. Plant Protection and Biotechnology, Instituto Valenciano de Investigaciones Agrarias (IVIA), CV-315, Km 10.7, 46113 Moncada, Spain
15. Plant Protection Diagnostics Laboratory, Slovenian Institute of Hop Research and Brewing, Cesta Žalskega tabora 2, 3310 Žalec, Slovenia
16. Plant Health Laboratory, Bacteriology Virology GMO Unit, French Agency for Food, Environmental and Occupational Health & Safety (ANSES), 7 Rue Jean Dixméras, 49044 Angers, France
17. Phytosanitary Reference Laboratory, Central Laboratory, Main Inspectorate of Plant Health and Seed Inspection, Żwirki i Wigury 73, 87-100 Toruń, Poland
18. Department of Phytopathology, Benaki Phytopathological Institute, St. Delta 8, 14561 Kifissia, Greece

*Corresponding author: A.K.J. Giesbers; E-mail: a.k.j.giesbers@nvwa.nl

Abstract

Recent changes in European Union plant health regulations require diagnostic laboratories to obtain ISO/IEC 17025 accreditation for all tests on regulated pests conducted under official controls. However, meeting all requirements of this standard is challenging due to the vast number of pest, host, and matrix combinations that require test validation and proficiency testing. In plant virology, adopting a risk-based systems approach to validation can help to meet ISO/IEC 17025 accreditation requirements. This approach acknowledges that workflows, which may consist of single or multiple tests, are used for the detection and identification of specific viruses, viroids, and

phytoplasmas. For each test within a workflow, validation is determined through a risk analysis based on its specific purpose, while the entire diagnostic process is validated through the combination of tests. This approach could fit within the definition of a "flexible scope" as defined by the European co-operation for Accreditation. When combined with diagnostic expertise and contextual understanding in plant health diagnostics as well as proficiency testing at the method level, this approach improves overall diagnostic proficiency in a cost-effective way and may facilitate accreditation and compliance with plant-health regulations in the European Union.

Keywords: diagnostic expertise, diagnostic process, plant-pest diagnostics, performance criteria, phytoplasmas, purpose of the test, reference material, risk analysis, viruses, viroids, workflow

Introduction

In 2016 and 2017 plant health regulations in the European-Union (EU) underwent significant changes. Regulation (EU) 2016/2031 on protective measures against pests of plants came into effect in 2016. This regulation aimed to enhance more effective measures to protect the Union's territory and its plants and ensure safe trade (Plant Health Regulation (PHR); European Parliament and Council, 2016). It includes Annexes specifying regulated pests, commodities and plant health requirements. In addition, Regulation (EU) 2017/625 on official controls and other official activities performed (Official Controls Regulation (OCR); European Parliament and Council, 2017), was imposed on plant health, to ensure a harmonised application of official controls in the plant health domain, which had previously been largely outside the scope of these rules. Official controls are conducted by the competent authorities in EU Member States to verify business compliance with agri-food chain legislation and may involve inspections and/or laboratory testing. For plant-health diagnostic laboratories this meant that all tests targeting pests listed in the Annexes of the PHR and performed within the framework of official controls must comply with the requirements of the OCR,

Firstly, the OCR introduced the requirement that laboratories performing tests under official controls must be designated as official laboratories, comprising both European Union and National Reference Laboratories, and other Official Laboratories. Secondly, this regulation requires all official

laboratories to be accredited in accordance with ISO/IEC 17025 *General requirements for the competence of testing and calibration laboratories* (ISO/IEC, 2017). This accreditation requirement applies to all tests under official controls conducted on any regulated pest as listed in the Annexes of the Plant Health Regulation ((EU) 2019/2072; European Commission, 2019). These regulatory changes had a significant impact on the activities of official plant-pest diagnostic laboratories. The large number of regulated pests (Table 1), combined with their wide range of hosts and matrices, makes full accreditation across all pest-matrix-method combinations difficult, if not impossible.

In recognition of these challenges, Article 41 of the OCR introduced an exemption from the accreditation requirement, which was later formalized by Delegated Regulation (EU) 2021/1353 (European Commission, 2021a). This regulation allowed official laboratories that do not fulfil accreditation requirements to conduct official testing for regulated pests under specific conditions. However, despite this exemption, all tests still require validation, and since the exemption currently applies only to Official Laboratories, it does not provide a solution for European Union and National Reference Laboratories. Therefore, the European Commission is considering possibilities for extending the scope of this regulation.

The ISO/IEC 17025 standard establishes specific technical requirements for testing laboratories. It serves as a framework for implementing quality management systems designed to improve a laboratory's ability to consistently produce valid and reliable results and forms the basis for accreditation. Following its initial release in 1999, the European and Mediterranean Plant Protection Organization (EPPO) has published two complementary standards to support plant-health diagnostic laboratories in quality assurance and accreditation.PM7/84 *Basic requirements for quality management in plant pest diagnostic laboratories* (EPPO, 2021a), first published in 2007, outlines general quality management requirements for diagnostic laboratories. PM7/98 *Specific requirements for laboratories preparing accreditation for a plant pest diagnostic activity* (EPPO, 2021b), first published in 2010, details the specific requirements for laboratories preparing accreditation, including guidance on the validation of tests across different methods and disciplines. These validation and accreditation activities are generally considered at the "test" level. However, in plant virology tests are often used in combination, as part of a workflow (Roenhorst et al., 2018). Furthermore, within the EPPO region there is an additional requirement to perform a

confirmatory test in critical cases (EPPO, 2024a), preferably using a test based on a different biological principle, effectively requiring a sequence of tests.

Within the European Union, the need for "Validation of diagnostic tools for animal and plant health", prompted the inclusion of this topic in the EU Horizon 2020 funding programme. This resulted in the VALITEST project *Validation of diagnostic tests to support plant health* (Trontin et al., 2021 and 2023). The project objectives were to improve and further harmonise validation procedures, as well as to strengthen the infrastructure for sharing validation data and expertise. Several specific outcomes from VALITEST have been incorporated in EPPO standards and databases. These include PM7/98 (EPPO, 2021b), PM7/122 *Guidelines for the organization of interlaboratory comparisons by plant pest diagnostic laboratories* (EPPO, 2022a), PM7/147 *Guidelines for the production of biological reference material* (EPPO, 2021c), the EPPO Database on Diagnostic Expertise (https://dc.eppo.int/validation_data/validationlist), and the commercial EPDIA database on diagnostic kits (https://www.epdia.eu/).

Many authors have highlighted the lack of standardisation in plant health and emphasised the need to improve the generation, use, and exchange of validation data (Cardwell et al., 2018a; Harrison et al., 2023; Groth-Helms et al., 2023; Sharma and Luster 2023). The EPPO database on Diagnostic Expertise (https://dc.eppo.int/validation_data/validationlist) as well as the National Plant Diagnostic Network National Data Repository in the United States (https://www.npdn.org/public/intro_to_ndr), provide platforms for sharing validation data among plant pest diagnostic laboratories, although the latter is not publicly accessible. Sharing of validation data may reduce validation efforts as it allows laboratories to verify their competence to perform a test according to the relevant performance characteristics (EPPO, 2021b). In addition, Harmon et al. (2023) further outlined recommendations for development of reference collections to support test development and validation. These could be implemented in databases such as EPPO-Q-bank, a database to support plant pest diagnostic activities (https://qbank.eppo.int/; Bertaccini et al., 2024).

While existing standards and publications (EPPO, 2021b; Growth-Helms et al., 2023) provide guidance on the validation of diagnostic tests, they do not solve the challenges regarding the large

number of tests to be validated, where a test is defined as the application of a method to a specific pest and a specific matrix (EPPO 2024a). This is due to a lack of availability of reference material for many pests and a lack of resources to cover the costs of development and validation of the vast number of tests required. In plant virology, for example, National Reference Laboratories must deal with over 500 quarantine and approximately 100 regulated non-quarantine pests (Table 1), which have host ranges varying from a few to over a hundred plant species. To address these challenges, new strategies in validation and quality assurance are being explored to increase efficiency without compromising confidence in the final result from a diagnostic process.

In addition to validation, ISO/IEC 17025 accreditation requires laboratories to demonstrate their competence by participating in interlaboratory comparisons, such as proficiency testing. In plant health, however, diagnostic tests are often derived from research, meaning that there is no "official laboratory" for comparison. Moreover, fulfilling this requirement is frequently unfeasible for many of the regulated pests as proficiency testing is not conducted due to a shortage of reference materials and/or resources.

In order to address the challenges described a systems approach to validation in plant virology is proposed. This approach focuses on the purpose of tests within diagnostic workflows, which may consist of a single test or combinations of tests. Such approach when combined with proficiency testing at the method level improves overall diagnostic proficiency in a cost-effective way and contributes to meeting the ISO/IEC accreditation requirements.

Challenges of test validation in plant virology

The ISO/IEC 17025 accreditation requirement applies to all pests listed in the Annexes of Implementing Regulation (EU) 2019/2072, as amended by (EU) 2021/2285 and further amendments (European Commission, 2019, 2021b, 2024). Annex II lists the Union quarantine pests and includes approximately 57 viruses, two viroids, 44 phytoplasmas, hereafter referred to as viruses, and additionally more than 450 begomoviruses that are listed as a group at genus level (Table 1). Additional Annexes of this regulation list around 100 regulated non-quarantine pests. Quarantine viruses are considered either absent from the EU or have a limited distribution and are

regulated regardless of the plant species in which they occur. Consequently, diagnostic tests for these viruses must be validated for each relevant matrix, defined by plant species, plant part, and/or developmental stage. This requirement makes test validation extensive and challenging, not least because reference materials of quarantine pests are often unavailable or difficult to obtain. In contrast, other disciplines, such as veterinary medicine, benefit from commercially available "gold standard" reference materials, which significantly facilitate the validation processes and the organisation of proficiency testing. Additionally, host plants to be tested may not be known in advance of their arrival in the laboratory. For regulated non-quarantine pests, validation tends to be more straightforward because reference materials generally have greater availability and relevant host plants and matrices are specified in the regulation.

Furthermore, accreditation overlooks the importance of context and the approaches to the diagnostic process in the laboratory that can be divided into "detection" and "diagnosis". Workflows for detection aim to screen pre-determined sample types by a fixed range of tests for selected viruses, where further tests may be carried out for confirmation of a positive result. Workflows for diagnosis include an assessment of symptoms that led to the submission of the sample, and selection of (an) appropriate test(s) dependent upon the likelihood of a symptom being associated with a specific virus in that host. In the latter workflows, each test, including the visual assessment, contributes to the final result. Therefore, the differing applications of tests as well as the importance of expert judgement in the design of workflows should be considered within the requirements for validation and subsequent accreditation.

Challenges in obtaining accreditation

While EPPO standards and publications provide guidance on the validation of diagnostic tests in preparation for accreditation, they do not address the challenge posed by the large number and different applications of tests required in plant virology. The ISO/IEC 17025 standard accreditation is primarily based on fixed scopes, where laboratories are accredited for detecting and/or identifying a specific organism (X) within a defined matrix (Y) using a specified method (Z). Alternatively, a flexible scope allows laboratories to extend their accreditation by adding new tests under an already accredited method and report results as accredited without prior approval from the accreditation body. For instance, a laboratory accredited for detecting potato virus Y (PVY) in

potato leaf material by ELISA, could add the detection of potato virus S in the same matrix using the same method, or add an additional matrix for PVY, provided that the new test is validated accordingly. However, even a flexible scope requires extensive validation of each new test, meaning that the overall workload and burden of validation remain substantial.

Systems approach to validation and proficiency testing in plant virology

Rationale for a systems approach to validation in plant virology

A systems approach to validation could reduce the workload associated with test validation by combining tests within diagnostic workflows. This approach mirrors how diagnostic workflows work in plant virology, where viruses, being too small to be seen with the naked eye, are detected and identified through inferences from indirect evidence. Over time, various diagnostic methods have been developed to detect and identify plant viruses. These include visual assessment of symptoms, bioassay, electron microscopy, and a range of serological and molecular methods. Each method has limitations in terms of reliability and the potential for results to be misinterpreted, potentially leading to false positives or false negatives being inferred. To mitigate these risks, it is often standard practice to use multiple tests for analysing a single sample, either sequential or in parallel. While sequential tests are based on previous results and would also indicate false results, tests run in parallel can complement one another and/or mutually substantiate results. Examples of workflows consisting of sequential testing of both asymptomatic samples and symptomatic samples are described in Roenhorst et al (2018). In addition, Fox et al. (2018) show how different tests are used in a diagnostic process that involves both sequential and parallel testing for the detection and identification of known and novel viruses in *Ullucus tuberosus*. For workflows consisting of a single test, test validation can be supported by using controls that allow monitoring of its critical steps. Both strategies, therefore, will contribute to confidence and accuracy of the final result of a diagnostic process.

The multi-test approach is also reflected in EPPO diagnostic standards, which use flow diagrams to outline different workflows. These diagrams focus on the detection and identification of specific target pests and specify which tests can be used at different stages of the diagnostic process. In

this way, these standards provide alternative workflows allowing to adapt to sample matrix, host species, and presence of symptoms. For example, EPPO PM7/146 *Tomato brown rugose fruit virus*, outlines different workflows regarding the sample matrix (EPPO, 2022b). Additionally, they allow for adjustments based on the capabilities of individual laboratories. These flow diagrams also show that a single test can serve multiple purposes within different workflows. This flexibility enables laboratories to optimise their diagnostic processes by using the same tests across various workflows.

Design of a systems approach to validation

Figure 1 illustrates the building blocks of the systems approach to validation for workflows in plant virology. The left side lists the different diagnostic methods used for virus detection and identification, while the right side indicates the performance criteria for validation. The core principle of the systems approach is that all relevant performance criteria are considered, but that not all need to be assessed for each individual test within a workflow. Instead, the scope of validation for each test is determined through a risk analysis based on its specific purpose within the workflow, which as such allows the overall diagnostic process to be validated (EPPO, 2021b). It is important to note that if a test is used across different workflows, its validation should meet the requirements of its purpose in each workflow.

Tests used in diagnostic workflows can range from highly specific to practically generic. Generally, specific tests are based on Enzyme-Linked Immunosorbent Assay (ELISA), Loop-mediated Isothermal Amplification (LAMP) or Real-time Polymerase Chain Reaction (Real-time PCR), whereas tests based on Bioassay and High-throughput Sequencing (HTS) are the most generic. Bioassay, in particular mechanical inoculation of test plants, enables the detection, but rarely the identification, of mechanically transmissible viruses based on a selection of suitable test plants (EPPO, 2022c). HTS tests allow the detection and identification of multiple viruses based on the choice of the platform and reference database used (EPPO, 2022d). Moreover, both bioassay and HTS can detect and identify viruses without prior knowledge of the target, an important capability in diagnosing symptomatic samples lacking further diagnostic indicators. However, the interpretation of results of tests based on these methods highly rely on the expertise of staff. Tests based on conventional Polymerase Chain Reaction (PCR) and Real-time PCR can be designed either as generic tests that

target all or several species within a genus or as species-specific tests. For PCR subsequent sequencing and analysis of the amplicon can be used for identification or confirmation. To illustrate, the EPPO diagnostic standard on '*Candidatus* Phytoplasma phoenicium' provides generic and specific PCR and real-time PCR tests for detection and identification in accordance with the outlined workflows (EPPO, 2021d). Therefore, when designing diagnostic workflows for specific purposes, it is important to consider the characteristics of the available tests. For example, employing a single generic test to screen for multiple viruses can reduce the number of individual tests required when the number of positives is expected to be low, since species-specific tests for identification only need to be performed on samples that test positive in the initial generic test.

Test performance at method level

While analytical sensitivity and analytical specificity are directly linked to a test, the performance criteria selectivity, repeatability, and reproducibility, also include general features. For selectivity, these may involve specific plant species or matrices that contain components interfering with test performance regardless of the target organism. In such cases, experience with one test can possibly be extrapolated to another test based on the same method. Where relevant, "worst case" matrices can be included in assessing the selectivity, such as rosaceous crops which are known for inhibition in PCR tests (Menzel et al., 2002). If no inhibition is observed in these crops, specifically at low virus concentrations, it is generally not expected in other crops. Furthermore, repeatability and reproducibility rely, at least partially, on the overall competence of a laboratory, including both infrastructure and staff expertise. As a result, the efforts required to evaluate these performance criteria may be adjusted based on a risk analysis.

The systems approach in practice

Use of workflows

In practice, validating a test workflow should focus on minimising the risk of false negative or false positive results. If a workflow involves only a single test, all relevant performance criteria must be evaluated. However, when a workflow consists of multiple tests, it is sufficient to assess only the

performance criteria relevant to the specific role of each test in the workflow following a risk analysis as highlighted in EPPO PM7/98 (EPPO, 2021b). Species-specific tests typically involve a single test that both detects and identifies the target virus, and as such, all performance criteria must be evaluated (see Figure 2A).

For workflows consisting of more than one test, validation efforts for individual tests may possibly be reduced. When the first test in a workflow is a generic test that detects, but does not identify the target virus, the main risk lies in a false negative result because no further testing will be performed if the result is negative. If this initial test yields a positive result, the subsequent test(s) must ensure accurate identification, with the primary concern now being the risk of a false positive result. Table 2, adapted from Roenhorst et al. (2018), and Figure 2B outline the focus of validation and associated performance criteria for both detection and identification when these steps are separated across different tests. Since repeatability and reproducibility also depend on a laboratory's overall competence, including its infrastructure and expertise of staff, the assessment of these criteria can be adapted accordingly. For these reasons, repeatability and reproducibility are not included in Table 2. Moreover, accurate interpretation of test results at each step of the workflow, particularly in relation to the specific purpose of testing, is crucial to ensuring confidence in the overall result.

The possible advantage of such a workflow is illustrated by EPPO standard PM7/152 *Begomoviruses*, where three generic PCR tests can be used to screen symptomatic tomato plants for the presence of regulated begomoviruses (EPPO,2022d). If all three tests yield a negative result, it can be concluded that no begomoviruses are present. When the screening for regulated begomoviruses was the only reason of testing, the diagnostic process stops here. When a positive result is obtained for at least one of the tests, sequencing and analysis of the amplicon(s) should be performed for identification but also allows to exclude a false positive result. However, when it is important to identify the cause of the observed symptoms, negative results of the begomovirus tests require further testing by other tests to finalise the diagnostic process.

For generic tests based on PCR-sequencing or HTS that can combine detection and identification of a specific target, also all performance criteria are relevant and must be assessed (Figure 2C).

However, due to the inherent characteristics of HTS tests, analytical specificity may be assured by default when using appropriate (and validated) reference databases (EPPO, 2022d; 2024b), and selectivity data may allow for extrapolation based on the experience with another test based on the same method in the same matrix.

Regardless of whether a workflow consists of a single test or multiple tests, additional confirmatory testing may be needed. This is particularly relevant when there is uncertainty about test results, e.g., when results are contradictory or inconsistent with expectations. Furthermore, in the context of detecting quarantine pests, the precautionary principle may necessitate additional testing to verify their absence. Confirmation is also advisable when a virus is detected for the first time in a particular plant species or geographic region, or other so-called 'critical cases' (EPPO, 2024a). Furthermore, the need for confirmatory testing will depend on the risks associated with the individual case and in some instances may be required by legislation.

The effectiveness of using workflows composed of multiple tests depends on the specific context. If a generic test is used to detect a particular virus but frequently yields positive results due to the presence of other viruses, extensive follow-up testing may be required. In such a case a virus-specific test is more practical. Conversely, a generic test may be well-suited for screening when target viruses are unlikely to be present. It also implies that when a positive result from the initial test is followed by a negative result in a subsequent test, particularly when unexpected, it is essential to investigate the discrepancy. Possible explanations for discrepant results include lower analytical sensitivity of the second test, which for instance may happen when a pospiviroid is detected by Real-time PCR and PCR-sequencing is required for identification (EPPO, 2021e). Other reasons may include the presence of a deviating isolate of the target virus, the presence of another virus, or human error. Dullemans et al (2020) demonstrate the challenges in interpreting test results for strawberry latent ringspot virus when encountering deviating isolates, different strains or even distinct species. However, if a generic conventional PCR test is used for detection of a particular virus and sequencing of the amplicon identifies a non-target virus, obviously no further testing is needed.

Quality control at sample level

While diagnostic workflows that separate detection from identification may improve efficiency, they do not validate the overall diagnostic process when applied to new virus-host-matrix combinations. In such cases alternative approaches should be considered to minimise the risk of false negative and false positive results. The use of extra controls in addition to the regular negative and positive controls can provide supplementary data to substantiate the validity of a test result. Including a non-infected ("healthy") sample of the same matrix as the test sample in ELISA can help to monitor background reactions. Similarly, in PCR tests, the use of a host internal control, such as nad5 mRNA, can reveal potential inhibition (Menzel et al., 2002; Botermans et al., 2013). Martin et al. (2025) provide a comprehensive overview of controls used to monitor test performance and errors across different diagnostic methods. Although the use of additional controls does not replace test validation, they are crucial for supporting the reliability of results by identifying procedural issues or matrix effects that might otherwise go unnoticed.

Importance of competence and expertise

Adopting a flexible scope based on a systems approach of validation requires competence of the laboratory and diagnosticians (Roenhorst et al., 2018; Van Leeuwen et al., 2020). This competence includes both infrastructure and expertise of staff. Beyond meeting the general requirements of the ISO/IEC 17025 standard, the laboratory must have the necessary infrastructure to compile and maintain databases enabling access to data from previous analyses, such as photos of symptoms, test plant reactions, and sequence data. Diagnosticians should also have access to external databases, scientific literature, and other information regarding viruses occurring in specific crops, their symptoms and impact, distribution, regulatory status, etcetera. It is also essential for them to be part of the research community, especially with access to applied and extension experts. Additionally, validation reports and supporting data should be publicly accessible or available on request to facilitate the verification of the validation status of each test. Furthermore, diagnosticians need to maintain a thorough understanding of the entire diagnostic process and the objectives of testing, particularly when workflows are flexible and tests may be performed in different sequences and combinations. This expertise is particularly important when workflows and tests are not predetermined and described in international standards, leaving it up to the diagnostician to determine the diagnostic process. The challenge becomes even greater when the diagnostician must also decide which viruses to test for, based on the plant species, observed

symptoms, and the sample's origin. They should be aware of whether tests are validated for detection, identification, or both, and apply this understanding when interpreting test results and deciding if further testing is necessary. Gill et al. (2005) highlighted the crucial role of expertise and contextual understanding in diagnostic work within health clinics. They explained that interpreting test results from a Bayesian perspective adds significant value, as it considers the probability of having a certain disease versus not having that disease. Finally, given the dynamic nature of plant health, diagnostic laboratories should continuously update and refine their workflows and validations to keep pace with emerging pests, new diagnostic methods, and regulatory changes.

Selecting workflows and tests

Most plant-health diagnostic laboratories manage a diverse range of samples and diagnosticians must take multiple factors into account when choosing the most appropriate workflow, such as the target (virus, viroid, phytoplasma), host plant, symptoms, sample matrix, objective of testing (certification, surveillance, import, export), and the availability of tests. Furthermore, choosing the most appropriate workflow may also depend on efficiency and cost-effectiveness, required accuracy, and regulatory compliance.

Routine testing and certification

For routine testing of standard samples for a limited number of specific viruses in designated crops, workflows are generally predefined. Such workflows typically involve a single test that combines detection and identification. Such a test requires validation through assessment of all relevant performance criteria. Additionally, certification programmes aimed to detect multiple viruses simultaneously will benefit from validated tests based on a single method. For instance, laboratories conducting seed-potato certification, will gain efficiency advantages by employing either ELISA or Real-time RT-PCR tests to screen for all common potato viruses. Using tests based on the same method allows for combined sample preparation and multiplex tests enable simultaneous detection and/or identification of multiple viruses. Furthermore, pooling of samples may enhance efficiency and cost-effectiveness of testing.

Surveillance

EU or national surveillance programmes require screening for many different viruses, including less prevalent (regulated) viruses. For several of these viruses, specific tests may not be available. Moreover, changes in the regulation may require surveillance programmes to be adapted and "new" tests to be developed and/or validated. In such cases an HTS test for generic detection and identification of viruses could be the ideal tool as it can screen for all known viruses and, depending on the test, even detect viruses new to science. For validation, relevant performance criteria should be assessed at least for the target viruses.

HTS tests may be particularly beneficial when samples are composed of symptomatic plant material collected during surveys based on visual inspection. In this case HTS will potentially be able to detect all viruses present in the sample. However, if applicable, determining the cause of the symptoms may be more challenging (Fox, 2020). Furthermore, since not all laboratories will have the option of using HTS for diagnostic purposes, other tests must be considered. Additionally, HTS tests might lack the analytical sensitivity for testing symptomless field samples composed of multiple plants. In such cases, tests detecting groups of viruses can provide useful alternatives, e.g., generic conventional PCR tests capable of detecting all known or relevant viruses within a genus. In these cases, diagnostic expertise is essential for determining which test should initiate the workflow. The advantages of using 'generic' PCR tests are shown in EPPO PM7/139 *Tospoviruses*, which describes generic tests allowing the detection of viruses belonging to different clades (EPPO, 2020), and EPPO PM7/152 *Begomoviruses*, describing three tests allowing detection and identification of over 200 begomoviruses infecting tomato and cucurbits (EPPO, 2022e). These generic tests are primarily suited for detection, where only samples that test positive then require sequence analysis or a specific test for identification.

Symptomatic samples

Apart from the often symptomless, samples from certification and surveillance programmes, laboratories receive symptomatic samples from visual inspections or surveys. For such symptomatic samples, predefined workflows are typically unavailable, leaving the design of the diagnostic process to identify the causal agent of the observed symptoms up to the diagnostician. Having a set of validated generic detection tests along with additional tests for identification will

enable the design of efficient tailored workflows, even for individual samples. The availability of such tests will help the laboratory to address the challenge of not being able to prepare for every possible virus-host-matrix combination, which often arises from a lack of reference material for certain viruses and/or insufficient knowledge of their host ranges. Moreover, most laboratories lack the resources to implement and maintain validated and accredited tests for the wide diversity of samples they may encounter. In this regard, a systems approach to test validation, allied with staff expertise, supports the flexibility needed to adapt workflows for specific cases.

Demonstrating proficiency

In addition to test validation, demonstrating proficiency is of key importance for obtaining and maintaining accreditation. Therefore, laboratories aim to participate in proficiency testing relevant to their scope of accreditation. However, given the diversity of samples regarding organisms and matrices, plant health laboratories often perform tests for numerous viruses for which no proficiency testing is organised. In such cases, laboratories may consider addressing this requirement at the method level, as factors such as equipment, procedures, and staff expertise, are generally consistent across tests based on the same method. As a practical approach, laboratories could strive to participate in at least one proficiency test per method. In this regard, the EPPO database on Diagnostic Expertise serves as a hub for exchanging details on proficiency tests being organised (https://dc.eppo.int/pt/pt_list). If no proficiency test is offered for a particular test or method, laboratories may organise an interlaboratory comparison with at least one other laboratory. Over time, the inclusion of different virus species and genera, prioritisation of the most frequently used tests, and staff rotation, can help ensure a thorough evaluation at the method level. This method-based approach enables plant health diagnostic laboratories to demonstrate the validity of their results, even when proficiency tests for individual tests are not available. Moreover, this approach is risk based and cost effective, as overall proficiency is still demonstrated while reducing the number of proficiency tests. Further guidance on the organisation of interlaboratory comparisons is provided in EPPO PM7/122 (EPPO, 2022a).

Concluding remarks

The implementation of the OCR in plant health has revealed the challenges diagnostic laboratories face in meeting the requirement to be ISO/IEC 17025 accredited for the testing of official samples. The vast number of regulated pests and their associated host plant species makes it difficult, if not impossible, for laboratories to obtain accreditation for every possible combination, because of a lack of reference material, incomplete knowledge of host ranges, as well as limited financial resources. Nevertheless, laboratories recognise the need to substantiate the validity of results. A recent literature review by Nascimento et al. (2025) revealed an increased emphasis on analytical sensitivity and specificity, but at the same time indicated the need for more uniform validation practices meeting the diverse diagnostic applications. Here we highlight that a systems approach to validation in plant virology, focusing on workflows rather than single tests, allows for increasing the effectiveness of validation without compromising on the overall reliability of the results. Adjusting validation according to a risk analysis tailored to the purpose of a test within a diagnostic process, may reduce the number of performance criteria to be assessed when used in combination with other tests. Moreover, integrating multiple complementary tests, whether based on the same or different methods, aligns with established diagnostic practices in plant virology. Furthermore, considering the ISO/IEC 17025 requirement of demonstrating proficiency in operating the accredited tests, participation in proficiency tests at method level provides a risk-based alternative given the limited availability of proficiency testing. Nevertheless, a systems approach of validation will still not provide solutions for all official samples that may be encountered, simply because reference material for certain (regulated) viruses may be unavailable and such viruses may occur in plant species not previously recognised as hosts. In such cases the use of additional external or internal controls at sample level as well as additional testing may increase the confidence in test results. Furthermore, it is important to focus on strategies to further exploit the benefits of the use and validation of workflows, since plant pest diagnostic laboratories continue to face novel pests and changing regulatory demands.

At the same time, plant pest diagnostic laboratories vary greatly in their capacity to implement such a systems approach. Significant differences in infrastructure, expertise, and resources, emphasise the need for clear guidance, training, and adequate support, to ensure a consistent application across EU Member States and throughout Europe. Furthermore, sharing of validation

data by companies providing diagnostic kits, would reduce the burden of validation for individual laboratories, which then only need to verify the performance criteria (EPPO, 2021b). In addition, broader and more systematic use of shared repositories for validation data, such as the EPPO Database on Diagnostic Expertise, would help to reduce duplication, and make better use of the collective expertise available.

Furthermore, regarding the accreditation requirements imposed on laboratories performing tests within the framework of official controls, it is important to acknowledge that sample testing comprises only part of the process of official controls. These may include the planning and design of inspections, ideally based on statistically supported sampling strategies to achieve the required accuracy, assessment of plants and symptoms, and collection, storage and transport of samples. This implies that several activities preceding the actual testing can affect sample quality and consequently the meaning of the test results within the broader context, which emphasises the crucial role of expertise and contextual understanding in a plant health diagnostic laboratory.

In conclusion, given the practical constraints and often limited financial resources, a systems approach to validation in combination with demonstrating proficiency at the method level, allows for a risk-based reduction of the burden of validation and proficiency tests. Such an approach could fit within the definition of a "flexible scope" as defined by the European co-operation for Accreditation (see Glossary; EA, 2023), which states: "It (a flexible scope) is also relevant to scopes that include a combination of fixed and flexible activities, or even for primarily fixed scopes that, for example include one or two flexible elements". The adoption of such a flexible scope of accreditation could make it easier for laboratories to expand their capabilities under ISO/IEC 17025. Moreover, further harmonisation of the interpretation of the standard in the field of plant health among national accreditation bodies across EU Member States and throughout Europe, would strengthen the overall framework of plant health diagnostics.

Acknowledgements

The authors thank Géraldine Antoine, Saskia Bosman, Alexia Bottino, Richard O'Hanlon, Justyna Pięcińska, Helga Reisenzein, and Charlotte Trontin, for stimulating discussions and critical reading of the manuscript.

References

Bertaccini A, et al, 2024. EPPO-Q-bank: a curated database to support plant pest diagnostic activities. EPPO Bulletin 54:361-365. https://onlinelibrary.wiley.com/doi/epdf/10.1111/epp.13063

Botermans M, van de Vossenberg BTLH, Verhoeven JThJ, Roenhorst JW, Hooftman M, Dekter R, Meekes ETM, 2013. Development and validation of a real-time RT-PCR test for generic detection of pospiviroids. Journal of Virological Methods 187:43–50. https://www.sciencedirect.com/science/article/pii/S0166093412003072?via%3Dihub

Cardwell K, Dennis G, Flannery AR, Fletcher J, Luster D, Nakhla M, Rice A, Shiel P, Stack J, Walsh C, Levy L. 2018a. Principles of diagnostic assay validation for plant pathogens: a basic review of concepts. Plant Health Progress 19:272-278. https://apsjournals.apsnet.org/doi/full/10.1094/PHP-06-18-0036-RV

Cardwell K, Dennis G, Flannery A, Fletcher J, Luster D, Nakhla M, Rice A, Shiel P, Stack J, Walsh C, Levy L, 2018b. Diagnostic Assay Validation Terminology. The Plant Health Instructor. DOI:10.1094/PHI-I-2018-0709-01. https://www.apsnet.org/edcenter/Pages/AssayValidationGlossary.aspx

Dullemans AM, Botermans M, de Kock MJD, de Krom CE, van der Lee TAJ, Roenhorst JW, Stulemeijer IJE, Verbeek M, Westenberg M, van der Vlugt RAA, 2020. Creation of a new genus in the family Secoviridae stubstantiated by sequence variation of newly identified strawberry latent ringspot virus isolates. Archives of Virology 165:21-31. https://link.springer.com/article/10.1007/s00705-019-04437-0

EA, 2023. European co-operation for Accreditation, EA-2/15 M: 2023 EA requirements for the accreditation of flexible scopes. https://european-accreditation.org/wp-content/uploads/2018/10/ea-2-15-m.pdf

EPPO, 2020. PM7/139 (1) Tospoviruses. EPPO Bulletin 50:217-240. https://onlinelibrary.wiley.com/doi/epdf/10.1111/epp.12676

EPPO, 2021a. PM7/84 (3) Basic requirements for quality management in plant pest diagnosis laboratories. EPPO Bulletin 51:457-467. https://onlinelibrary.wiley.com/doi/epdf/10.1111/epp.12779

EPPO, 2021b. PM7/98 (5) Specific requirements for laboratories preparing accreditation for a plant pest diagnostic activity. EPPO Bulletin 51:468-498. https://onlinelibrary.wiley.com/doi/epdf/10.1111/epp.12780

EPPO, 2021c. PM7/147 (1) Guidelines for the production of biological reference material. EPPO Bulletin 51:499-506. https://onlinelibrary.wiley.com/doi/epdf/10.1111/epp.12781

EPPO, 2021d. PM7/150 (1) '*Candidatus* Phytoplasma phoenicium'. EPPO Bulletin 51:587-599. https://onlinelibrary.wiley.com/doi/epdf/10.1111/epp.12799

EPPO, 2021e. PM7/138 (1) Pospiviroids. Eppo Bulletin 51:144-177. https://onlinelibrary.wiley.com/doi/epdf/10.1111/epp.12717

EPPO, 2022a. PM7/122 (2) Guidance for the organization of interlaboratory comparisons by plant pest diagnostic laboratories. EPPO Bulletin 52:604-618. https://onlinelibrary.wiley.com/doi/epdf/10.1111/epp.12889

EPPO, 2022b. PM7/146 (2) Tomato brown rugose fruit virus. EPPO Bulletin 52:665-692. https://onlinelibrary.wiley.com/doi/epdf/10.1111/epp.12891

EPPO, 2022c. PM7/153 (1) Mechanical inoculation of test plants. EPPO Bulletin 52:693-703. https://onlinelibrary.wiley.com/doi/epdf/10.1111/epp.12901

EPPO, 2022d. PM7/151 (1) Considerations for the use of high throughput sequencing in plant health diagnostics. EPPO Bulletin 52619-642. https://onlinelibrary.wiley.com/doi/epdf/10.1111/epp.12884

EPPO, 2022e. PM7/152 (1) Begomoviruses. EPPO Bulletin 52:643-664. https://onlinelibrary.wiley.com/doi/epdf/10.1111/epp.12887

EPPO, 2023. PM4/28 (2) Introduction to PM 4 standards on schemes for the production of healthy plants for planting. EPPO Bulletin 53: 458-459. https://onlinelibrary.wiley.com/doi/epdf/10.1111/epp.12978

EPPO, 2024a. PM7/76 (6) Use of EPPO Diagnostic standards. EPPO Bulletin 54:312-316. https://onlinelibrary.wiley.com/doi/10.1111/epp.13046

EPPO, 2024b. Addendum – New supporting information for PM7/151 Considerations for the use of high throughput sequencing in plant health diagnostics. EPPO Bulletin 54:253. https://onlinelibrary.wiley.com/doi/epdf/10.1111/epp.13026

European Parliament and Council, 2016. Regulation (EU) 2016/2031 on protective measures against pests of plants. Official Journal of the European Union, OJ L 317:4–104. https://eur-lex.europa.eu/eli/reg/2016/2031/oj

European Parliament and Council, 2017. Regulation (EU) 2017/625 on official controls and other official activities performed to ensure the application of food and feed law, rules on animal health and welfare, plant health and plant protection products. Official Journal of the European Union, OJ L 95:1–142. https://eur-lex.europa.eu/eli/reg/2017/625/oj

European Commission, 2019. Commission Implementing Regulation (EU) 2019/2072 establishing uniform conditions for the implementation of Regulation (EU) 2016/2031 as regards protective measures against pests of plants. Official Journal of the European Union, OJ L 319:1–279. https://eur-lex.europa.eu/eli/reg_impl/2019/2072/oj

European Commission, 2021a. Commission Delegated Regulation (EU) 2021/1353 supplementing Regulation (EU) 2017/625 with regard to the cases and conditions under which competent authorities may designate official laboratories which do not fulfil the conditions in relation to all the methods they use for official controls or other official activities. Official Journal of the European Union, OJ L 291:20-23. https://eur-lex.europa.eu/eli/reg_del/2021/1353/oj

European Commission, 2021b. Commission Implementing Regulation (EU) 2021/2285 amending Implementing Regulation (EU) 2019/2072 as regards the listing of pests, prohibitions and requirements for the introduction into, and movement within, the Union of plants, plant products and other objects. Official Journal of the European Union, OJ L 458:173-282. https://eur-lex.europa.eu/eli/reg_impl/2021/2285/oj

European Commission, 2024. Commission Implementing Regulation (EU) 2024/2004 amending Implementing Regulation (EU) 2019/2072 as regards the listing of pests and rules on the introduction into, and movement within, the Union territory of plants, plant products and other objects. Official Journal of the European Union, OJ L 2024/2004. https://eur-lex.europa.eu/eli/reg_impl/2024/2004/oj

Fox A, 2020. Reconsidering causal association in plant virology. Plant Pathology 69:956-961. https://bsppjournals.onlinelibrary.wiley.com/doi/10.1111/ppa.13199

Fox A, Fowkes AR, Skelton A, Harju V, Buxton-Kirk A, Kelly M, Forde SMD, Pufal H, Conyers C, Ward R, Weekes R, Boonham N, Adams IP, 2018. Using high-throughput sequencing in support of a plant health outbreak reveals novel viruses in Ullucus tuberosus (Basellaceae).

Plant Pathology 68:576-587. https://bsppjournals.onlinelibrary.wiley.com/doi/full/10.1111/ppa.12962

Gill CJ, Sabin L, Schmid CH (2005) Why clinicians are natural bayesians. BMJ 330:10801083. https://www.bmj.com/content/330/7499/1080

Groth-Helms D, Rivera Y, Martin FN, Arif M, Harma P, Castlebury LA, 2023. Terminology and guidelines for diagnostic assay development and validation: best practices for molecular tests. PhytoFrontiers 3:23-35. https://apsjournals.apsnet.org/doi/full/10.1094/PHYTOFR-05-22-0059-FI

Harmon CL, Castlebury LA, Boundy-Mills K, Broders KD, Hyten AM, Jacobs, JL, Knight-Connoni VK, Mollov D, Riojas MA, Sharma P, 2023. Standards of diagnostic validation: Recommendations for reference collections. PhytoFrontiers 3:43-50. https://apsjournals.apsnet.org/doi/10.1094/PHYTOFR-05-22-0050-FI

Harrison C, Agstner B, Tomlinson J, Macarthur R, van den Berg F, 2023. Understanding and improving the collection and use of diagnostic test validation data: reflections and next steps. PhytoFrontiers 3:64-70. https://apsjournals.apsnet.org/doi/full/10.1094/PHYTOFR-03-22-0027-FI

IPPC, 2024. Glossary of Phytosanitary Terms. ISPM no. 5. IPPC Secretariat. FAO, Rome (IT). https://www.ippc.int/en/publications/622/ [accessed on 12-06-2025]

ISO/IEC, 2017. ISO/IEC 17025:2017 General requirements for the competence of testing and calibration laboratories. https://www.iso.org/obp/ui/#iso:std:iso-iec:17025:ed-3:v1:en

Martin FN, Batuman O, Luster DG, Miles TD, Rivera Y, Sharma P, Geiser D, Cardwell K, 2025. How controls improve diagnostic assay performance: hitchhiker's guide to diagnostic assay controls. PhytoFrontiers 5:127-137. https://apsjournals.apsnet.org/doi/10.1094/PHYTOFR-10-24-0118-FI

Menzel W, Jelkmann W, Maiss E, 2002. Detection of four apple viruses by multiplex RT-PCR assays with coamplification of plant mRNA as internal control. J. Virol. Methods 99:81-92. https://www.sciencedirect.com/science/article/pii/S0166093401003810?via%3Dihub

Nascimento DM, Sharma P, Luster DG, 2025. A 2023-2024 literature review of validation standards and diagnostic accuracy. PhytoFrontiers. https://doi.org/10.1094/PHYTOFR-12-24-0132-FI

Roenhorst JW, de Krom C, Fox A, Mehle N, Ravnikar M & Werkman AW, 2018. Ensuring validation in diagnostic testing is fit for purpose: a view from the plant virology laboratory. EPPO Bulletin 48:105–115. https://onlinelibrary.wiley.com/doi/10.1111/epp.12445

Sharma P, Luster DG, 2023. State of the field of plant pathogen diagnostic assay development and validation. PhytoFrontiers 3:18-22. https://doi.org/10.1094/PHYTOFR-05-22-0054-FI

Trontin C et al., 2021. VALITEST: Validation of diagnostic tests to support plant health. EPPO Bulletin 51:198-206. https://onlinelibrary.wiley.com/doi/full/10.1111/epp.12740

Trontin C et al., 2023. What did we achieve through VALITEST, an EU project on validation in plant pest diagnostics? PhytoFrontiers 3:75-81. https://apsjournals.apsnet.org/doi/10.1094/PHYTOFR-03-22-0026-FI

Van Leeuwen GCN, Bosman S, Souer E, Edema MJ, van der Blom M, Werkman AW, 2020. Implementation of an ISO 17025 flexible scope in the phytosanitary field at the National Reference Centre in the Netherlands. EPPO Bulletin 50:549-556. https://onlinelibrary.wiley.com/doi/full/10.1111/epp.12682

Glossary

Accreditation: formal recognition by an independent body, generally known as an accreditation body, that a certification body (here, plant pest diagnostic laboratory) operates according to international standards (ISO, https://www.iso.org/certification.html [assessed 08-05-2025].

Analytical sensitivity: smallest amount of target that can be detected reliably (EPPO, 2024a). This is sometimes referred to as the 'limit of detection'. Further details on the procedures to determine analytical sensitivity are given in PM 7/98 (5) (EPPO 2021b).

Analytical specificity (inclusivity): performance of a test with a range of target organisms covering genetic diversity, different geographical origin, and hosts (EPPO, 2024a). Further details on the procedures to determine inclusivity are given in PM 7/98 (5) (EPPO 2021b).

Analytical specificity (exclusivity): performance of a test regarding cross-reaction with a range of nontargets (e.g., closely related organisms, other organisms in the host/matrix, contaminants) (EPPO, 2024a). Further details on the procedures to determine exclusivity are given in PM 7/98 (5) (EPPO 2021b).

Certification scheme: system for the production of vegetatively propagated plants for planting, intended for further propagation or for sale, obtained from nuclear stock after several propagation stages under conditions ensuring that stated health standards are met (EPPO, 2023).

Confirmation: showing that the result of the (first) analysis is definitely true, e.g., by performing an additional analysis that shows the same result[1].

Detection: part of the diagnostic process, the initial indication of the presence of a particular target (virus) within a sample, usually followed by further analysis to establish its identity[1].

Diagnosis: refers to the determination of the nature and cause of a disease or the process of detection and identification of a pest (i.e. the interpretation of the result of a diagnostic process)[1].

Fixed scope (EA-2/15 M: 2023): ISO 17011:2017 defines a scope of accreditation as specific conformity assessment activities for which accreditation is sought or has been granted. The term "fixed scope" means a clearly defined description of the specific conformity assessment activities for which the body holds accreditations.' (EA, 2023).

Flexible scope (EA-2/15 M: 2023): ISO/IEC 17011:2017 defines a flexible scope as an expression "to allow conformity assessment bodies to make changes in methodology and other parameters which fall within the competence of the conformity assessment body as confirmed by

the accreditation body". The term "flexible scope" is not restricted solely to scopes that are flexible in their entirety. It is also relevant to scopes that include a combination of fixed and flexible activities, or even for primarily fixed scopes that, for example include one or two flexible elements. In some cases, it may be best to define the scope by defined activities; in other cases, it may be better to use the techniques applies and the (technical) field covered by the body. Sometimes different ways may be combined (EA, 2023).

Identification: determination of certain characteristics of an organism, which makes it possible to unambiguously assign it to a specific taxon / to ascertain its identity[1].

Interlaboratory comparison: organisation, performance, and evaluation of measurements of tests on the same or similar items by two or more laboratories in accordance with predetermined conditions (e.g., proficiency testing or test performance studies) (EPPO, 2024a).

Matrix: all the constituents that collectively form a test sample (Cardwell et al., 2018b).

Method[2] (technique)**:** includes (in plant virology) bioassay, enzyme-linked immunosorbent assay (ELISA) high-throughput sequencing analysis (HTS), [immuno-] electron microscopy, polymerase chain reaction (PCR), PCR+Sanger sequencing, Real-time PCR, visual assessment (EPPO, 2024a).

Performance criteria: 'qualities' of a test to describe its performance, including analytical sensitivity, analytical specificity (inclusivity and exclusivity), selectivity, repeatability, reproducibility (EPPO, 2021b). May also include diagnostic sensitivity and specificity, but these are not used in this paper.

Pest: any species, strain, or biotype of plant, animal, or pathogenic agent injurious to plants or plant products (IPPC, 2024).

Proficiency test(ing) (evaluation of the competence of the laboratory): establishing the competence of a laboratory in analysing defined samples using their established test (EPPO, 2024a).

Quarantine pest (virus): pest of potential economic importance to the area endangered thereby and not yet present there, or present but not widely distributed and being officially controlled (IPPC, 2024).

Regulated non-quarantine pest (virus): non-quarantine pest whose presence in plants for planting affects the intended use of those plants with an economically unacceptable impact and which is therefore regulated within the territory of the importing contracting party (IPPC, 2024).

Reference material: material appropriate to be used for testing and diagnosis, such as live cultures, infected plant material, DNA/RNA preparations, images of a diagnostic quality, or mounted (or unmounted) specimens. The reference material used should be documented. It should be ensured that the reference material used is producing the features for which it was selected, for example, expressing a desired antigen for use in serological testing or displaying specific physical features (e.g., sporulation) if used for morphological diagnosis (EPPO 2024a).

Regulated pest (virus): quarantine pest or regulated non-quarantine pest (IPPC, 2024).

Repeatability: level of agreement between replicates of a same sample tested under the same conditions (EPPO, 2024a).

Reproducibility: ability of a test to provide consistent results when applied to aliquots of the same sample tested under different conditions (e.g., time, persons, equipment, location) (EPPO, 2024a).

Screening: process to detect or identify (establish) the presence or absence of an organism in a sample as a preliminary assessment. Triage to eliminate negative samples and move presumptive actionable samples forward for confirmation (Cardwell et al., 2018b).

Selectivity: extent to which variations in the matrix affect the test performance (matrix effect) (EPPO, 2024a). Further details on the procedures to determine selectivity are given in PM 7/98 (EPPO 2021b).

Surveillance: official process whereby information on pests in an area is obtained through general surveillance, specific surveillance, or a combination of both (IPPC, 2024).

Test[2]: application of a method to a specific pest and a specific matrix (EPPO 2024a).

Validation: process to provide objective evidence that a test is suitable for its intended use (EPPO, 2021b), i.e., by assessment of the relevant performance criteria.

Verification: process to provide objective evidence that the laboratory is competent to perform a validated test according to the relevant performance characteristics (EPPO, 2021b).

Workflow: tests used in a diagnostic process and their order of execution (this paper).

[1]Glossary used internally by the EPPO Panel on Diagnostics in Virology and Phytoplasmology (2025; not published)

[2]The term method is also used in the sense of a test, particularly in EU legislation and in standards of the International Plant Protection Convention.

Table 1. Indicative numbers of plant pests regulated in the European Union

Discipline	Union Quarantine pests	Protected zone Quarantine pests	Regulated Non-Quarantine pests	Remarks
Bacteriology	15	2	27	Pests regulated at the level of species, subspecies and pathovars
Entomology	264 15 higher taxonomic level (>8000 species)	15	17 1 genus (~110 species)	Quarantine pests regulated at higher taxonomic levels include >8000 species
Mycology	38 2 genera (~70 species)	4	51	
Nematology	39	-	19	
Virology	94 1 genus (>450 species)	3	~100	Quarantine pests include 57 viruses plus >400 regulated at genus level, 2 viroids,

and 2 phytoplasmas at species level, and

42 phytoplasmas at strain level

Table 2. Main focus of test validation for different steps in a workflow

Workflow[1]	**Detection**	**Identification**
Primary risk	False negative results	False positive results
Performance criteria[1]		
Analytical sensitivity	Limit of detection	-[2]
Analytical specificity (inclusivity)	Detection of all variants of the target virus	-
Analytical specificity (exclusivity)	-	Distinguishing the target virus from (related) other viruses
Selectivity	Inhibition by matrix components	Non-specific reaction due to matrix components

[1]Workflows aiming to detect and identify a target virus can consist of one or multiple tests. When using specific tests workflows generally consist of one test that combines detection and identification, which means that performance criteria of both detection and identification should be considered. When workflows make use of a generic test for detection and an additional test for identification, not all performance criteria necessarily have to be assessed for each test. Repeatability and reproducibility are not included in the table as they apply to each test irrespective of its purpose. Flexible scopes may allow to assess these performance criteria at method level; [2]Not applicable.

Captions for figures

Figure 1. Schematic representation of the building blocks of the systems approach to validation for workflows in plant virology. Left side lists diagnostic methods (not exhaustive) used in plant virology, right side indicates performance criteria considered for validation. ELISA: Enzyme-Linked Immunosorbent Assay; HTS: High-throughput Sequencing; LAMP: Loop-mediated Isothermal Amplification; RPA: Recombinase Polymerase Amplification; PCR: Polymerase Chain Reaction. Note that methods are listed alphabetically rather than by importance.

Figure 2. Examples of different workflows for detection and identification in plant virology. Methods (tests) for detection and/or identification are indicated as well as the most relevant performance criteria for these steps in the entire diagnostic process. Note that the degree of specificity, particularly of an HTS test, is depending on the platform and design of the test as well as the reference database used. A. Workflow using a species-specific test that combines detection and identification. B. Workflow using a generic test for detection combined with an additional species-specific test for identification. C. Workflow using a generic test combining detection and identification of one or multiple viruses. Based on a risk analysis, flexible scopes may allow to assess repeatability and reproducibility at method level.

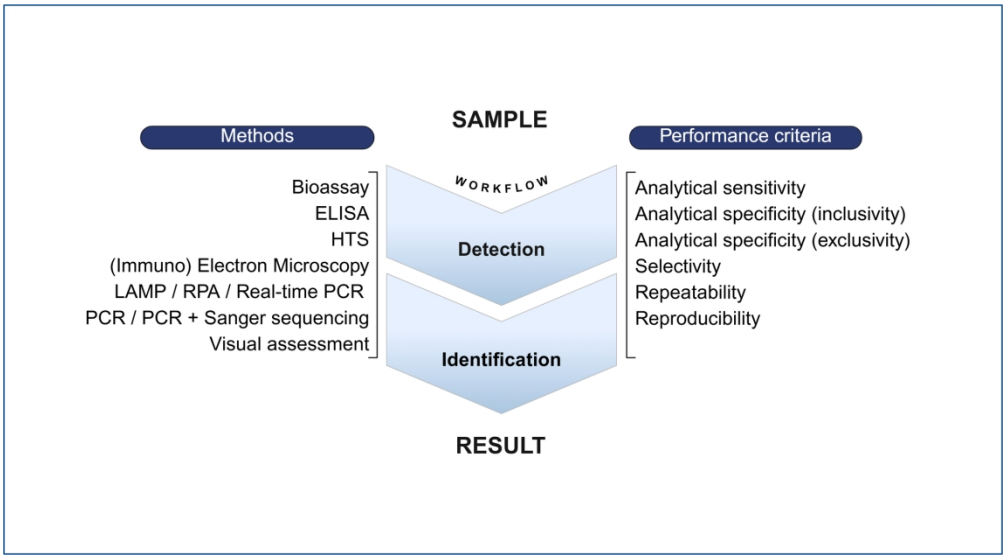

Figure 1. Schematic representation of the building blocks of the systems approach to validation for workflows in plant virology. Left side lists diagnostic methods (not exhaustive) used in plant virology, right side indicates performance criteria considered for validation. ELISA: Enzyme-Linked Immunosorbent Assay; HTS: High-throughput Sequencing; LAMP: Loop-mediated Isothermal Amplification; RPA: Recombinase Polymerase Amplification; PCR: Polymerase Chain Reaction. Note that methods are listed alphabetically rather than by importance.

183x101mm (300 x 300 DPI)

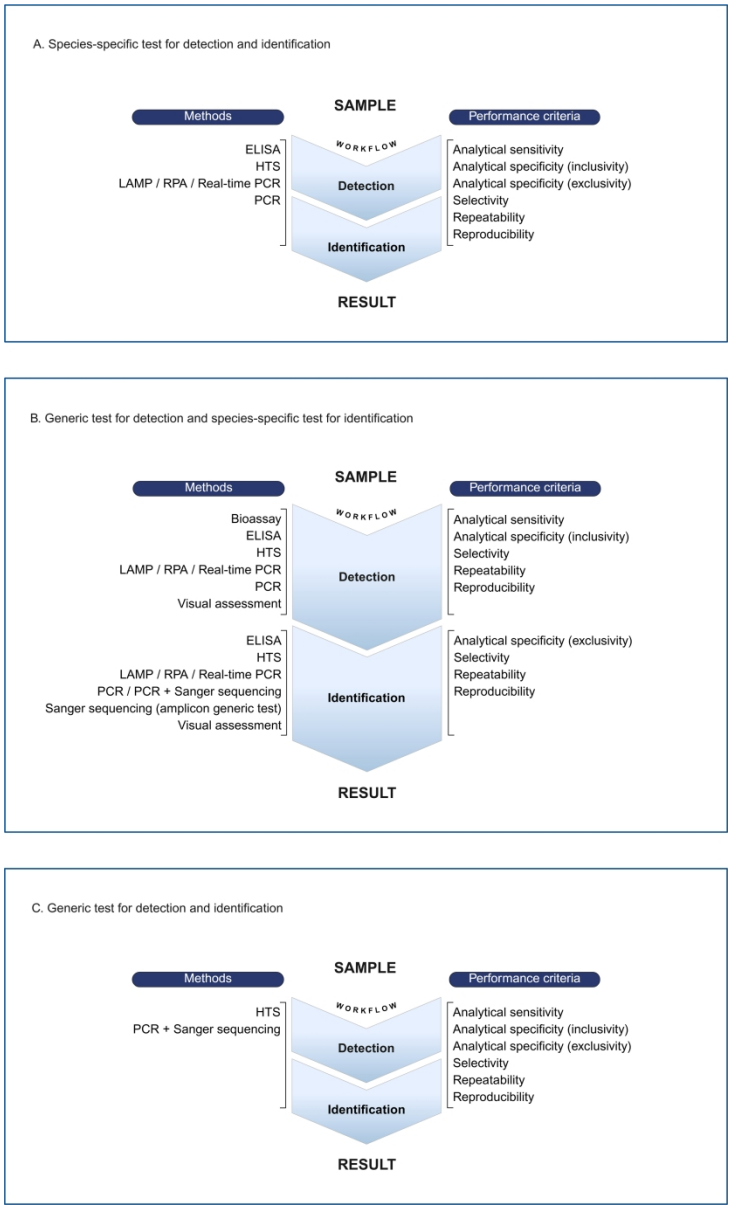

Figure 2. Examples of different workflows for detection and identification in plant virology. Methods (tests) for detection and/or identification are indicated as well as the most relevant performance criteria for these steps in the entire diagnostic process. Note that the degree of specificity, particularly of an HTS test, is depending on the platform and design of the test as well as the reference database used. A. Workflow using a species-specific test that combines detection and identification. B. Workflow using a generic test for detection combined with an additional species-specific test for identification. C. Workflow using a generic test combining detection and identification of one or multiple viruses. Based on a risk analysis, flexible scopes may allow to assess repeatability and reproducibility at method level.

205x339mm (300 x 300 DPI)