What did we achieve through VALITEST an EU project on validation in plant pest diagnostics?

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Abstract

Ensuring the reliability of diagnostic activities is an essential cornerstone of Plant Health strategies to reduce the risk of entry and spread of plant pests in a region and ultimately their impacts. Diagnostic tests should be validated to ensure that they are fit for purpose. Validation is usually done by diagnostic laboratories although companies commercializing diagnostic kits also produce validation data for their products. Due to the high number of pest, matrix and method combinations and given the significant resources required to validate tests, it is essential that validation data are shared with the entire diagnostic community and produced in a harmonized way to facilitate their use by different stakeholders. Indeed, the selection of tests to be used in specific contexts is not the sole responsibility of diagnostic laboratories and also involve National Plant Protection Organizations. The VALITEST EU project (2018-2021) was established to tackle all these issues. New validation data for tests targeting important pests for the EPPO region were produced. Guidelines to improve and harmonize the validation framework were developed. Sharing of validation data and experience was ensured through the development of new or existing databases, the organization of training courses and the dissemination of the project outputs in scientific publications and Standards. Finally, the involvement of researchers, diagnosticians, policy makers, inspectors, industries etc. and the establishment of the European Plant Diagnostic Industry Association were important actions to strengthen the interactions between Plant Health stakeholders.

Keywords: plant pest diagnostics, validation, test performance study, high-throughput sequencing, reference material, training

INTRODUCTION

The Food and Agriculture Organization (FAO) estimates that annually between 20 to 40 percent of global crop production is lost due to pests. Each year, plant diseases cost the global economy around 220 billion USD, and invasive insects around 70 billion USD (FAO, 2019a). Protecting crops against these losses from farm to fork is critical to ensure global food security, achieve sustainable and competitive agriculture as well as for the protection of biodiversity and ecosystems. Efficient surveillance mechanisms are key for the fulfilment of these important goals, as they enable effective monitoring and control of introduction and spread of plant pests (Carvajal-Yepes *et al.,* 2019). Early diagnosis and a rapid response are crucial to reduce the risk of entry and spread of plant pests and ultimately their impacts. Plant pests can be managed most effectively when detected in time and when control measures are implemented at an early stage of infestation (Koch *et al.* 2020).

National Plant Protection Organizations (NPPOs) routinely conduct inspections, supported by testing for export certification, import, pest surveillance and eradication programs. Accurate identification of a pest is a prerequisite for taking phytosanitary action. In addition, to enable safe trade, testing must be completed quickly and to a high level of confidence.

In the European Union, the need to validate (including through test performance studies; TPS) existing and new tests for the detection and identification of pests using harmonized approaches was recognized for both Animal and Plant Health fields and a specific topic on "*Validation of diagnostic tools for animal and plant health*" was included in the EU's research and innovation funding programme 2014-2020 (called Horizon 2020). A contribution from the EU of around 3 million EUR was granted for this Plant Health topic. One of the requirements of the EU Commission was that projects should involve different stakeholders in Plant Health and that cooperation with international and standardization bodies should also be ensured. A consortium of 16 partners composed of research institutions, private companies (such as diagnostic kit providers) and National Plant Protection Organizations was formed. The European and Mediterranean Plant Protection Organization (EPPO), an intergovernmental organization responsible for international cooperation in plant protection for the European, Mediterranean and Central Asian region was also part of the consortium. Since 1998, EPPO has established a work programme in pest diagnostics to harmonize procedures across its region. This involves the preparation of pest-specific diagnostic protocols, as well as horizontal Standards providing for example guidance on the validation of tests or on inter-laboratory comparisons.

The VALITEST (for Validation of diagnostic tests to support plant health) project started on 2018-05-01 and finished on 2021-10-31 (Trontin *et al.*, 2021). The main achievements and lessons learned from the project are presented here. All deliverables and outputs of the project are (or will soon be) available on the VALITEST website (https://www.valitest.eu/index) and on the zenodo repository of the project (https://zenodo.org/communities/valitest/?page=1&size=20).

It is noted that validation terminology varies between different international and national organizations. The terminology used in this article is according to EPPO terminology defined in the EPPO Standard PM 7/76 *Use of EPPO Diagnostic protocols* (EPPO, 2018).

Vision statement

This concept note presents the outcomes of VALITEST, an EU funded project on diagnostic test validation. Beyond the evaluation of the performance of specific tests used in plant pest diagnostics, this project improved diagnostic procedure by tackling areas such as the organisation of test performance studies, the statistical analysis of data generated during validation studies and by developing guidelines for the use of Reference Material and High-Throughput Sequencing technologies in plant pest diagnostic laboratories. Additionally, it strengthened interactions between stakeholders in Plant Health including companies producing diagnostic kits to reach better diagnostics.

EVALUATION OF TESTS

By providing information on the performance of the tests that are used in diagnostics, validation is essential to ensure the reliability of a diagnostic activity. Validation studies can be performed both in one laboratory or involve several laboratories. It consists of the evaluation of different performance criteria such as analytical sensitivity, analytical specificity, selectivity, repeatability, and reproducibility (EPPO, 2021a). Diagnostic sensitivity and diagnostic specificity (also called rate of true positives and rate of true negatives) can also be evaluated during validation studies. A test can be considered as validated when its performance characteristics meet the level required for a specific intended use. Tests are currently mostly validated on an intralaboratory basis or through limited interlaboratory comparisons (i.e. test performance studies (TPS), sometimes referred to as ring tests). In addition, sharing validation data in publicly available resources remains limited. Thus, the first goal of VALITEST was to produce new or additional validation data for the detection and identification of plant pests through the organization of two rounds of TPS. In total, 12 TPS, targeting 11 pests of interest for various stakeholders in the EPPO region, were organized in the framework of VALITEST and produced validation data (e.g. diagnostic sensitivity, diagnostic specificity, reproducibility…) for 83 tests (Table 1). The two rounds of TPS included laboratories from 31 countries spread over four continents. Between 11 and 34 participants from 8 to 20 different countries were selected for each TPS (Trontin *et al.* 2021). Prior to each TPS, preliminary studies were carried out by TPS organizers to support the selection of the tests to be included in each TPS. These also contributed to the production of a substantial amount of validation data (e.g. analytical sensitivity, analytical specificity, repeatability…) for a total of 131 tests.

HARMONIZING AND IMPROVING THE VALIDATION FRAMEWORK

In addition to the production of new validation data, one objective of VALITEST was to further harmonize and improve the validation framework and to adapt it to new technologies used in diagnostics. Based on the expertise of the partners and on the experience gained through the organization of several TPS, different guidelines were produced.

Substantial knowledge gained on the organization of test performance studies

TPS are the ultimate approach to evaluate and compare the performance of tests. However, the organization of a TPS is a complex process which requires time and resources. In addition, TPS organizers need a high level of expertise to ensure a smooth process and reliable results.

Substantial knowledge and experience were gained in the two rounds of VALITEST TPS. The organization of TPS was shown to be easier if timelines, rules and criteria, which need to be followed, are defined and formalized in advance. In the first round of TPS, a framework and associated documentation for the preparation (including definition of the scope, the selection of tests and the selection of participating laboratories) and the organization of TPS have been created. For the analysis of TPS results, harmonized documents for the calculation and graphical representation of performance criteria such as diagnostic sensitivity and diagnostic specificity were also developed. Those documents were used and further improved and finalized in the second round of TPS.

One of the major outputs of VALITEST is a book written by the TPS organizers *Critical points for the organisation of test performance studies in microbiology: plant pathogens as a case study* (Vučurović *et al.*, 2022). This book provides further details on each step of the process and examples of TPS documents and forms. Those general recommendations for the organization of TPS are applicable to any TPS organization and can help diagnostic laboratories in the field of plant health.

Better insight into statistical analysis of data

An appropriate and harmonized approach for the statistical analysis of validation data is important to facilitate the interpretation of performance characteristics, the comparison of tests and to increase the confidence in the conclusions drawn from the validation data. However, up to now, there was limited guidance on the use of statistical analysis in the context of plant health diagnostics. During VALITEST, a framework, proposing new statistical tools to be used for the analysis of validation data, has been prepared by a group composed of diagnosticians and statisticians. This framework was evaluated using 10 datasets obtained from the TPS, which allowed the recommendations to be refined. The choice of the statistical methods for the determination of the performance characteristics was based on the applicability of the method in the context of plant health diagnostic laboratories, the minimum number of samples and replicates required for a statistical method to perform correctly, the ease of application and interpretation of the results. These guidelines also provide information on how to establish the panel of samples, how to deal with inconclusive and missing results and how to identify and deal with outliers. The proposed statistical tools will facilitate the comparison of the performance characteristics between tests. A paper will be published in the EPPO bulletin (Massart *et al.*, in press).

Understanding and specifying needs for the routine use of HTS in plant pest diagnostics.

High-throughput sequencing (HTS) is one of the most significant advances in molecular diagnostics since the advent of the PCR methods in the 1980s. By having the potential to detect the nucleic acids of any organism present in a sample, HTS provides new possibilities and opportunities in routine plant health diagnostics (Olmos *et al.*, 2018). However, standardized best-practice guidelines to ensure the harmonized and proper implementation of this new technique were lacking up to now. A recommendation on 'Preparing the use of HTS technologies as a diagnostic tool for phytosanitary purposes' was adopted by the Commission on Phytosanitary Measures governing body of the International Plant Protection Convention (IPPC) in 2019 (FAO, 2019b). This recommendation encourages the development of best-practice operational guidelines covering analysis results and quality control measures for HTS that "ensure HTS data outputs are robust and accurate, have biological significance in a phytosanitary context, and are implemented in a harmonized way, including test validation and quality assurance" (FAO, 2019). In addition, the recommendation highlights the need for validating HTS tests. During VALITEST, guidelines were developed for the use of HTS as a routine test in plant diagnostic laboratories. These were reviewed externally by 42 experts from 18 countries (from 5 continents) and 29 Institutes (universities, research centres, diagnostic laboratories, NPPOs, EPPO), with expertise in pest diagnostics. The guidelines provide technical recommendations for each step of the test including laboratory work and bioinformatic analyses. They also include recommendations on test selection, development and optimization, validation and verification, internal and external quality checks, including the use of proper external and internal controls, and interpretation and reporting of test results. The guidelines have been developed, irrespective of the chemistry, equipment and software, and are applicable to any plant pest in any matrix. They have been designed to allow flexibility within this fast-evolving technology. The guidelines target plant health diagnostic laboratories that intend to routinely use HTS technologies for the detection and identification of pest and are applicable for any organism (e.g. arthropods, bacteria, fungi, nematodes, invasive plants, protozoa, viroids, viruses or weeds) and any type of matrix (e.g. pure microbial culture, plant tissue, soil, water), regardless of the type of HTS technology (e.g. amplicon sequencing, shotgun sequencing) and their application (e.g. surveillance programme, phytosanitary certification, crop protection). In addition, their adoption by research laboratories would also improve the overall reliability of generated HTS datasets and of their comparison. Two publications (Lebas *et al.* and Massart *et al.)* have been prepared and should be published in 2022.

Ensuring the production of high-quality reference material:

Reference material is essential to ensure traceability when performing diagnostic activities. In Plant Health, reference material is usually produced by individual diagnostic laboratories due to the limited commercial offer. To help TPS organizers in that task, quality guidelines were developed for the production of reference materials to be used in interlaboratory studies. First, a list of criteria (i.e. the intended use of the reference material, its identity, traceability, commutability, homogeneity, stability, assigned value and purity) to consider for the description of reference material was established. Then, a general standard operating procedure (SOP) for the production of reference material for use in plant health diagnostics was developed. The steps required during the production process (e.g. identification of the material, multiplication, verification of the homogeneity, stability, commutability, purity, quantity and identity of the material) depend on the sources of the reference material (e.g. field material, working collection, reference material or certified reference material) and on the intended use of the material. For each step in the process critical points should be identified, as well as the criteria that reference material have to meet and their minimum required levels. Further details are available in Chappé *et al.* (2020) and Chappé *et al.* (2019). These guidelines were used to develop a new EPPO Standard (see below)

Guidelines are important, however their production alone is not sufficient, and it is essential that access to reference material is enhanced. As recommended in the White Paper Phytosanitary diagnosis and collections developed in the framework of another EU funded project (Q-Collect), it is important that appropriate basic funding is secured for reference material collections and that a common policy towards collection management is established to ensure sharing of reference material (see report of the second Q-Collect workshop https://www.eppo.int/MEETINGS/2015_meetings/wk_q_collect_workshop).

DISSEMINATION OF VALITEST OUTPUTS

EPPO Database on Diagnostic Expertise

The Standard ISO/IEC 17025:2017 (ISO, 2017) requires that all the tests for which a laboratory is preparing accreditation should be validated. Validation data should be generated by the laboratory or should be publicly available in which case, the laboratory should provide objective evidence that it can perform the test according to the established performance characteristics. Therefore, it is important that validation data is made available to the diagnostic community in an easily accessible way.

The EPPO Database on Diagnostic Expertise was created in 2007 (Roy *et al.*, 2010). Its first aim was to allow identification of experts who can provide diagnosis of regulated pests and those who can help in the identification of new or unusual species.

A section 'validation data for diagnostic tests' was created in 2012 at the request of laboratories, which were engaging in an accreditation process. It was considered that sharing validation data will save resources and promote collaboration within the EPPO region. The section on validation data includes data for diagnostic tests for regulated pests. Validation data can be deposited by any registered diagnostic laboratory and can be retrieved by the users of the database in the form of a harmonized validation sheet in PDF format including the description of the test evaluated (pest x matrix x method) and the associated performance data.

During the VALITEST project, a survey was organized to identify the needs for improvement of the database, which resulted in the following upgrades to the database:

- The database can now be searched using key words (searchable descriptors are pest, method, plant species, test, matrix, EPPO-IPPC test).
- Combined and flexible queries (e.g. multiple pest queries) are now possible.
- Sorting of information within different methods has been improved.
- Searches for tests used for detection, identification or both can now be made.
- Searches for kits can now be made.

As a result of the project, the format and content of the whole database was further improved and made more user friendly and more searchable, in particular for the section on validation data for diagnostic tests. All validation data generated during the VALITEST project is (or will soon be) available via the database.

Diagnostic kit database

A wide range of kits for serological or molecular diagnostics are available from commercial suppliers worldwide. Each may differ in performance characteristics, intended use and validation data available.

During the project, a European Plant Diagnostic Industry Association (EPDIA) was formed by the commercial partners of the project. The partners of EPDIA have created a database that helps potential users to find the diagnostic tool they need. It includes information from different companies on test kits for various pests, suppliers, purpose of the test, performance criteria, manuals and more for different techniques such as ELISA or PCR (https://www.epdia.eu/diagnostic-kits-european-plant-diagnostic-industry-association.php?lang=en).

Training courses

Training activities were organized in the framework of the VALITEST project. Due to the Covid-19 pandemic, the physical workshops planned for diagnostic laboratories could not be organized. All training activities were held online in the format of webinars, practical training sessions and videos. Three series of activities were organized on the following topics:

- Concept of test validation in plant health.
- TPS organization.
- Use and validation of High-Throughput Sequencing (HTS) tests for diagnostics of plant pests.

All webinars were recorded, and videos are available on the VALITEST website to ensure the maximal dissemination of the results of the project (https://www.valitest.eu/training/activities_and_webinars).

In addition to the webinars, several videos were also prepared by the partners to:

- Illustrate and describe the whole project.
- Illustrate specific steps in the process of TPS organization and share experience from the TPS organizers via interviews (such as the selection of pests and TPS organizers, tests, and participants).
- Explain specific notions related to the statistical analysis of TPS data.

These videos provide very valuable feedback from TPS organizers who explain the difficulties they were faced with during the organization of the TPS but also provide tips that are useful for laboratories envisaging to organize TPS.

Videos can be seen on the EPPO YouTube account in a playlist specific to VALITEST.

Dissemination through Standards

In addition to being published in international scientific journals and books, most of the VALITEST results and outputs were used to develop EPPO Standards to be used by stakeholders or to revise existing ones. Most of the validation data generated in the TPS and preliminary studies were or will be used to revise EPPO Diagnostic Standards on specific pests (Table 1). In addition, the experience gained on TPS organization and analysis of validation, data was used to improve the EPPO Diagnostic Standard on the organization of interlaboratory comparisons (PM 7/122, 2014 - under revision). Finally, two new Standards were developed: PM 7/147 *Guidelines for the production of biological reference material* (EPPO, 2021b) and another one on considerations for the use of High Throughput Sequencing in plant health diagnostics (PM 7/NEW, in preparation). These Standards are (or will be when approved) published in the EPPO Bulletin with free access, and via the EPPO Website (https://www.eppo.int/RESOURCES/eppo_standards/pm7_diagnostics) and the EPPO Global Database (https://gd.eppo.int/standards/PM7/)

STRENGTHENING LINKS WITH STAKEHOLDERS

The last objective of VALITEST was to better understand the need of different stakeholders (e.g. researchers, diagnosticians, policy makers, inspection services, industries, seed companies, growers' associations, etc.) at national and EU levels and to further strengthen their collaboration for better diagnostics.

Identification of the needs of different stakeholders

VALITEST has integrated a strong stakeholder focus across all work-packages to ensure the delivery of practical and relevant outputs throughout the project's lifetime. One example is the organization of two online surveys targeting laboratories and NPPOs to identify testing needs.

The survey for laboratories covered different topics: (1) current testing priorities, (2) requirements for new or improved tests, (3) validation data available, (4) the use of on-site testing kits, and (5) the use of HTS. A survey for National Plant Protection Organizations was also conducted and asked representatives to rank their top 10 priority pests. Results from these surveys were combined and a pest ranking (supplemented with additional information on their national and international status) was the basis for the selection of priorities for the organization of the second round of TPS. To support this selection, a framework was created to aggregate the ranked results from the two surveys according to the priorities given by respondents.

A mathematical framework has also been developed to support, inter alia, resource allocation for and design of sampling and test programmes in different plant health contexts see Harrison *et al.* (this issue).

Establishment of links with accreditation bodies regarding proficiency testing

Ensuring that laboratories are proficient is essential for a reliable diagnostic service. However, laboratories in plant health cannot undertake proficiency testing (PT) for all the tests they use. The VALITEST partners aimed to develop a horizontal approach that would ensure the proficiency of laboratories through their participation to a limited but specific number of PT.

The needs and expectations of the laboratories were identified and possible solutions were discussed with representatives of an accreditation body. The most appropriate approach identified to limit the PT participation plan was that a laboratory should identify sets of tests (grouped by methods) for which the outcome of a PT using one test can be directly correlated to the proficiency of the laboratory in the use of other tests. Such approach is described by the European Cooperation for Accreditation (EA) in the EA-4/18 guidance document on the level and frequency of proficiency testing participation (EA, 2021). A case study was developed and will be discussed with EA in the coming months.

Establishment of the European Plant Diagnostic Industry Association

At the start of the project, the diagnostic industry was not structured as an entity that can be solicited by other stakeholders. The project provided the opportunity to establish the foundations for a structure to improve communication concerning offers and demands for plant health diagnostic tests in a sustainable manner. The European Plant Diagnostic Industry Association (EPDIA, www.epdia.eu) has been created during the VALITEST project.

EPDIA's mission is to engage on behalf of its members, with all relevant European decision makers in order to represent their interests and to contribute to:

- The promotion of a Quality Charter for the production and the development of tools by the plant diagnostics industry.

- The promotion and the disclosure of information to the market on phytodiagnostic technologies and their validation.
- The representation of the plant diagnostics Industry within European and international institutions.

CONCLUSION, RECOMMENDATIONS AND BEYOND VALITEST

The Strategic Framework for the International Plant Protection Convention (IPPC) 2020-2030 adopted in 2021 recognizes the importance of diagnostics. It highlights the need for internationally accepted standards for accurate diagnostics, but also for networks to help countries identify pests in a more reliable and timely manner. It also underlines the fact that developments in molecular biology and genetic sequences will not only deliver new tools but also new challenges for plant health diagnostics (FAO, 2021). Activities conducted in the VALITEST project have contributed to this strategic objective. The guidelines developed to improve the validation framework and the validation data generated throughout the organisation of TPS, are the result of international collaborations not only within the EPPO region but also with diagnosticians, researchers and companies from other parts of the world and are being used to revise major EPPO Standards on diagnostics. The preparation of guidelines for HTS is a nice example of successful international collaboration and is an important step toward the development of standardized HTS tests for pest detection and identification. Lessons learnt from VALITEST include:

- the need to find compromises between what is ideal and what is practical e.g. when designing panel of samples for optimal statistical analysis or when producing reference material;
- the need for anticipation and the importance of logistics for the good progression of test performance studies;
- the need of a thorough knowledge of the biological constraints associated with the pest and the plant material for the production of reference material (seasonal availability, survival/stability, delay to produce samples).

- the importance that information on performance of commercial kits is easily retrievable and that companies have a platform for exchange which is one of the reasons for the creation of EPDIA.
- The importance of sharing experiences and tips among different stakeholders which is why a book on TPS organisation has been prepared (Vučurović et al., 2022).

However, validation is a continuously evolving story, new tests will be developed and will need to be validated, as will new on-site diagnostic technologies that are coming on the market. In this context important players to ensure the production of validation data for tests in the EPPO region are presented below.

The laboratories

Plant pest diagnostic laboratories (including National Reference Laboratories; NRLs) remain the main source of validation data and the majority of data included in the EPPO Database on diagnostic expertise has been generated by individual laboratories. EPPO will continue to encourage laboratories to share the data produced and to support the validation process by updating the EPPO Standards on validation whenever necessary. In 2017 a new EU Regulation (EU 2017/625) on official controls entered into force, and the European Reference Laboratories (EURL) whose activities enhance diagnostic capability and strengthen diagnostic activities in the EU were established. Five EURLs have been designated in the different disciplines (i.e. bacteriology, fungi and oomycetes, insect and mites, plant parasitic nematodes and virology). One of the EURL activities is the validation of tests to make recommendation to the National Reference Laboratories.

EURLs participate in the six EPPO Panels on diagnostics and validation data generated by these laboratories are also populating the EPPO Database on diagnostic expertise.

Euphresco

In order to increase the collaboration among those organizations involved in Plant Health research activities at national and regional levels, Euphresco (European Phytosanitary Research Coordination, www.euphresco.net) was established in 2006 as an ERA-NET project funded by the European Commission. Euphresco has subsequently evolved into a self-sustaining international network hosted by EPPO. The benefits of such coordination are multiple (Giovani *et al.*, 2015). By fostering collaboration at research level, Euphresco allows researchers to work on common problems. Euphresco goes far beyond Europe as members of the network come from five different continents.

Every year, Euphresco members identify research priorities to be tackled through transnational collaboration. Many research projects have been commissioned with the aim of developing new tests for the detection and identification of pests, validating diagnostic tests or evaluating the proficiency of laboratories (examples of pests for which TPS or PT have been organized include '*Candidatus* Liberibacter solanacearum', *Acidovorax citrulli, Xylella fastidiosa,* potato virus Y*,* andean potato latent virus, *Ralstonia solanacearum* and *Clavibacter sepedonicus* '*Candidatus* Liberibacter' spp. causing the Huanglongbing disease on *Citrus* spp.) (Giovani *et al.*, 2019).

The coordination of national activities improves the use of resources allocated to plant health by avoiding duplication and favouring synergies. Synergies have also been pursued with other international initiatives and projects. Recently, the outbreaks of tomato brown rugose fruit virus in several countries pushed countries to validate the use of diagnostic tests. The VALITEST project organized a TPS to validate several tests on plant material, while an Euphresco project was initiated to validate several tests on seed of tomato and pepper.

International collaboration contributes to knowledge exchange, capacity building and harmonization of best practices (including those with diagnostic aims). Projects have been conducted on DNA barcoding (including training sessions available online https://youtube.com/playlist?list=PLoVf4Pt04Db53pUVTI8qwcWkWgUgg46gm) as well as on HTS.

The outputs of research projects have an impact beyond research activities, as they also support national policy-making and international standard setting and practices (Giovani *et al.*, 2017).

RECOMMENDATIONS

- Research institutes, companies or diagnostics laboratories developing tests are encouraged to use the VALITEST outcomes when performing validation studies.
- Resources needed to produce validation data in terms of both expertise and funds should not be underestimated as producing and sharing useful and reliable validation data can be complex.
- Communication between laboratories and other stakeholders is important. For example, as much relevant information as possible should be provided to the risk managers of a National Plant Protection Organization to help them make an informed decision when selecting tests to be used in e.g. surveillance, import inspection.
- Communication between laboratories performing validations and test providers is important to assure reliable results.
- Reference material is essential for the evaluation of tests, and collections should be sufficiently funded and maintained to provide sufficient diversity regarding the target pests but also the ‘look a likes’ (species with which they could be confused).
- Research institutes, companies or diagnostics laboratories developing tests are encouraged to provide validation data and make them publicly available.

ACKNOWLEDGMENTS

The authors would like to thank the members of the project advisory board for their support and critical assessment of the VALITEST project progress throughout its duration as well as for bringing external points of view on the activities conducted. The members of the advisory board were Neil Boonham (Newcastle University, GB), Adriana Moreira (FAO IPPC Secretariat, IT), Mark Nakhla, (USDA-APHIS-CPHST, US), Maaike Bruinsma, Moti Sabban and Roland Willmann (ISHI). The VALITEST project was supported by the European Union's Horizon 2020 research and innovation programme under grant agreement n°773139. The content of this publication represents the views of the authors only and is their sole responsibility; it cannot be considered to reflect the views of the European Commission and/or the Research Executive Agency or any other body of the European Union. The European Commission and the Agency do not accept any responsibility for use that may be made of the information it contains.

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Table 1. Summary of the TPS organized in the framework of VALITEST (adapted from Trontin *et al.* 2021) NIB: National Institute of Biology (SI), ANSES: French Agency for Food, Environmental and Occupational Health & Safety (FR), NVWA: Netherland Food and Consumer Product Safety Authority (NL), Fera: Fera Science Limited (UK), UNITO: University of Turin (IT), CREA: Council for Agricultural Research and Economics (IT). PCR: Polymerase Chain Reaction, LFD: Lateral Flow Device, LAMP: Loop-Mediated Isothermal Amplification, RT: Reverse Transcriptase, TPIA: Tissue Print Immunoassay, DAS-ELISA: Double Antibody Sandwich ELISA, IF: Immunofluorescence, RPA: Recombinase Polymerase Amplification

Pest	**TPS organizer**	**Number of tests evaluated in preliminary studies**	**Number of tests selected for TPS**	**Publication of the results** (PM 7= EPPO Standards on Diagnostics)
Bursaphelenchus xylophilus	ANSES	6	5 tests (conventional PCR, real-time PCR, LAMP)	PM 7/004 (under revision)
Citrus tristeza virus (CTV)	ANSES	16	11 tests (ELISA, TPIA, conventional RT-PCR, real-time RT-PCR, RT-LAMP and ImmunoStrip)	PM 7/031 (under revision)
Cryphonectria parasitica	UNITO	3	3 tests (conventional and real-time PCR)	PM 7/045 (under revision)
Erwinia amylovora	NIB	9	6 tests (real-time PCR, LFDs and LAMP)	PM 7/020 (under revision)
Fusarium circinatum	Fera	7	6 tests (plating, PCR, real-time PCR)	PM 7/091 (revision to be started)
Pantoea stewartii subsp. *stewartii*	NIB	8	6 tests (real-time PCR, conventional PCR)	PM 7/060 (revision to be started)

Plum pox virus (PPV)	NVWA	20	8 tests selected (conventional RT-PCR, real-time RT-PCR, DAS-ELISA)	PM 7/032 (under revision)
Plum pox virus (PPV) onsite tests	ANSES	4	3 tests (LFD RPA, LFD)	PM 7/032 (under revision)
Tomato brown rugose fruit virus (ToBRFV)	CREA	9	5 tests (conventional and real-time RT-PCR)	Luigi *et al.*, 2022, PM 7/146 (under revision)
Tomato spotted wilt orthotospovirus (TSWV)	NIB	19	8 tests (DAS-ELISA, on-site tests, conventional and real-time RT-PCR)	Vučurović *et al.*, 2022
Xanthomonas citri pv. c*itri*	ANSES	20	13 tests (conventional and real-time PCR, LAMP and direct molecular tests performed from Immunostrips or WhatmanTM FTA cards)	PM 7/044 (under revision)
Xylophilus ampelinus	Fera	10	9 tests (ELISA, IF, conventional and real-time PCR)	PM 7/096 (revision to be started)
Total of 11 pests	**Total of 6 institutions**	**Total of 131 tests**	**Total of 83 tests**	

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