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Validation of diagnostic tests to support plant health

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Abstract:

The 5th work package of the VALITEST project aimed at developing guidelines on a horizontal approach allowing the laboratories to undertake proficiency testing (PT) without having to participate in proficiency tests for all the tests they use.
After identifying the needs and expectations of the laboratories, the acceptability of this approach has been discussed with representatives of an accreditation body. According to them, the views of experts collected previously do not constitute a sufficient level of evidence and the suitability of the approach should be demonstrated based on the analysis of proficiency testing results. Based on existing datasets it was possible to draw some conclusions but not to demonstrate the suitability of the approach.
The most appropriate approach identified to limit the PT participation plan is to group the tests and evaluate the proficiency for these groups as described by the European guidance document EA-4/18. However, the identification of the groups of tests can only be done by the laboratory itself taking into account all its specific parameters. As a follow-up to the work carried out within VALITEST, a case study will be developed to accompany the EA-4/18 guidance document and facilitate the implementation of this approach by laboratories.

Partners involved: ANSES, WBF, EPPO, FERA, NIB, ULG, NVWA, CREA
Task 5.3

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TERMS, ABBREVIATIONS AND DEFINITIONS

Terms and definitions used in this document are based on Standards of the European and Mediterranean Plant Protection Organization (PM 7/76: Use of EPPO Diagnostic Standards [1]; PM 7/98: Specific requirements for laboratories preparing accreditation for a plant pest diagnostic activity [2]):

- Field (PM 7/98): Fields include bacteriology, botany, entomology, mycology, nematology, phytoplasmology and virology
- Method (PM 7/76): Methods include: bioassay methods, biochemical methods, fingerprint methods, isolation/extraction methods, molecular methods, morphological and morphometric methods, pathogenicity assessment and serological methods
- Pest (PM 7/76): Any species, strain or biotype of plant, animal or pathogenic agent injurious to plants or plant products
- Test (PM 7/76): The application of a method to a specific pest and a specific matrix

Abbreviations:

- Cofrac: French accreditation body
- EA : European co-operation for accreditation
- ELISA: Enzyme-linked immune sorbent assay
- EPPO: European and Mediterranean Plant Protection Organization
- EPTIS: European PT information system
- EURL: European Union reference laboratories
- GEVES: French variety and seed study and control group
- IEC: International electrotechnical commission
- IF: Immunofluorescence
- ILAC: International laboratory accreditation cooperation
- ISO: International organization for standardization
- ISTA: International seed testing association
- NRL: National reference laboratory
- PCR: Polymerase chain reaction
- PT: Proficiency test

1 Purpose

The VALITEST project mostly aims at validating diagnostic tests available for a selection of relevant plant pests. The goal of using validated tests is to ensure the reliability of the results based on which management measures may be taken. However, the targeted level of performance of a validated test is only ensured if it is performed by a proficient laboratory. This is usually evaluated through a proficiency test (PT) scheme. Participating in a PT enables a laboratory to verify (or confirm) its competence to carry out specific tests. When available and appropriate, it is mandatory for ISO/IEC 17025 accreditation, and serves to demonstrate to stakeholders, including regulatory bodies the competence of the laboratory to perform these tests. At present the approach is based on pest specific PT (including one or several tests for one pest). Considering the difficulties of organizing PT (time, cost, material availability, complexity, number of pests regulated in plant health, number of matrices), all the tests cannot be covered. This approach is therefore neither feasible in the short term nor in the long term.

As presented in the "description of action", the aim of the 5th work package of the VALITEST project was to develop guidelines on an approach based on the establishment of PT programs that would allow a demonstration of competence without the need for a laboratory to participate in PT for all tests performed by the laboratory.

A first deliverable of the work package focused on the needs of the laboratories and on the applicability of the horizontal PT approach.

The present document considers the constraints of laboratories working on plant health. Based on interviews and data analysis, it evaluates the feasibility of undertaking proficiency testing through a horizontal approach.

2 Normative and regulatory framework

Several regulatory and normative documents must be considered in order to understand the requirements with which laboratories must comply.

2.1 Regulation (EU) 2017/625 of the European Parliament and of the Council of 15 March, 2017

In the European Union, laboratories involved in official analysis are working under Regulation 2017/625 [3] 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. According to this regulation: (§50) "*Laboratories designated by the competent authorities to carry out analyses, tests and diagnoses on samples taken in the context of official controls and other official activities should possess the expertise, equipment, infrastructure and staff to carry out such tasks to the highest standards. To ensure sound and reliable results, those laboratories should be accredited for the use of these methods according to standard EN ISO/IEC 17025 on 'General requirements for the competence of testing and calibration laboratories'. The accreditation should be delivered by a national accreditation body operating in accordance with Regulation (EC) No 765/2008 of the European Parliament and of the Council*".

Article 38 of the regulation lists the obligations of official laboratories; among these, "*upon request by the European Union reference laboratory or national reference laboratory, official laboratories shall take part in inter-laboratory comparative tests or proficiency tests that are organised for the analyses, tests or diagnoses they perform as official laboratories*".

2.2 Normative framework

2.2.1 ISO/IEC 17025 (2017) - General requirements for the competence of testing and calibration laboratories

As presented above, official laboratories have to be accredited according to the standard ISO/IEC 17025:2017 [4]. Furthermore, for laboratories that are not required by national regulations to have this accreditation, having this accreditation provides an important competitive advantage and many laboratories comply with it.

According to this standard (§7.7.2), laboratories have to monitor their performance by comparing their results with other laboratories, including, but not limited to participation in proficiency testing and participation in inter-laboratory comparisons.

2.2.2 ILAC P9:06/2014 - ILAC Policy for Participation in Proficiency Testing Activities

To clarify the position of accreditation bodies concerning the requirements to participate in proficiency tests, a guidance document has been published by the International laboratory accreditation cooperation (ILAC) [5].

According to this document, (§4.2), the "*minimum PT activity according to a laboratory's or inspection body's (where relevant) scope is:*

- *evidence of satisfactory participation prior to gaining accreditation where PT is available and appropriate;*
- *further and ongoing activity that is appropriate to the scope of accreditation and consistent with the PT participation plan*."

This document also states that (§4.3) accreditation bodies shall have a policy on the use of PT activities in the assessment and accreditation, including "*requirements regarding the minimum level and frequency of participation in PT by accredited laboratories*".

2.2.3 EA-4/18:2010 - Guidance on the level and frequency of proficiency testing participation

The European co-operation for accreditation (EA) had a similar approach as ILAC and published its own guidance document [6].

In this document, an original approach is developed concerning the level and frequency of participation in PT:

Extract from *EA-4/18 INF:2010 Guidance on the level and frequency of proficiency testing participation*, section 4

The first step for the laboratories should be to identify the sub-disciplines that apply to them for the tests/calibrations for which they are accredited. Ideally a laboratory would participate in a specific PT for every measurement technique it uses and for every property measured in every product. However, it is acknowledged that this is unlikely to be feasible, both logistically and economically. Therefore accreditation bodies should expect laboratories to identify groups of sets of measurement techniques, properties and products on which the outcome of a PT for one of these sets can be directly correlated to the others sets of measurement techniques, properties and products contained within the group. These groups of sets of measurement techniques, properties and products are termed a sub-discipline.

A sub-discipline, as defined above, may contain more than one measurement technique, property or product as long as equivalence and comparability can be demonstrated. The first consideration for a laboratory, when determining a sub-discipline, is that it should generally not contain different technical competences. Different technical competences can usually be identified by the need for different qualifications, training, and use of different equipment, knowledge or experience.

When determining a sub-discipline it may be helpful to consider a stepwise approach working up from measurement technique through properties to products. This is because it is more likely that there will be several products and/or properties associated with one measurement technique within a given sub-discipline than vice versa:

(i) With reference to the measurement technique: It is possible but not common to include different measurement techniques in the same sub-discipline.

(ii) With reference to the property to be measured, determined or identified: It may be possible to include more than one property (parameter) in the same sub-discipline.

(iii) With reference to products to be tested: It may be possible to include different products in the same sub-discipline provided that the matrices, objects or materials included, are of equivalent nature.

When a laboratory determines that more than one measurement technique, property or product is classified under the same sub-discipline, accreditation bodies should evaluate whether a laboratory can justify and demonstrate equivalence. This can usually be done by e.g.:

- The method validation data, or,

- Use of the same standard method

Once the laboratory has defined its sub-disciplines the "level of participation" can be deemed to have been defined. Accreditation bodies will also need to evaluate the suitability of a laboratories "frequency" of participation, based on level of risk and should expect a minimum frequency of participation for each sub-discipline to be set by the laboratory.

It should also be considered that according to ISO/IEC 17025:2005 (5.9.1) the laboratory should have quality control procedures (of which PT is one) and that these should be planned. Therefore, once the "level" and "frequency" of participation is established, laboratories should be expected to develop a proficiency testing strategy which takes into account those factors highlighted in "General Aspects" points 1-5. The extent and content of this strategy will depend upon the circumstances and scope of the individual laboratory. This should form part of the laboratory's overall quality control strategy. It is recommended that the strategy covers, at least, one accreditation cycle (period between full reassessments), and that this strategy is reviewed by the laboratory for its suitability on an annual basis, usually during the formal management review. The classification of sub-disciplines may be different for every laboratory. For this reason, accreditation bodies should expect laboratories to be able to justify the technical arguments that have led to the laboratories decision on the "level" and "frequency" of participation in PT. It is recommended that laboratories document this justification.

3 Specificity of plant health testing

The main challenge faced in plant health is the diversity of pests and pest/host matrices and possible combinations, and consequences for validation. More than 300 pests (bacteria, fungi and chromista, insects and mites, nematodes, phytoplasmas, viruses and viroids, invasive plants) are recommended for regulation as quarantine pests by EPPO[1]. These recommendations are largely implemented in EPPO member countries. Official plant pest diagnostic laboratories also perform analyses on exported plants and plant products for pests that are regulated by importing countries, in order to fulfil their international obligations under the International Plant Protection Convention (FAO, 1987). The laboratories potentially need to test hundreds of pests on thousands of pest/host matrices under accreditation.

One pest may affect several hosts, and the number of hosts may increase over time, in particular when pests invade new areas and encounter (potential) new host plants. In addition, depending on their biology, pests may be found in different parts of the plants or plants products e.g. in the roots, leaves, fruits, woody parts, packaging material. Pests may also be present in substrates such as soil and water. For example, the stem nematode *D. dipsaci sensu lato (s.l.),* attacks more than 1 200 species of wild and cultivated plants, it can attack aerial parts of plants but also bulbs tubers and seeds and may be present in soil.

As a consequence, the number of possible matrices to be tested and that would need to be validated for a single pest can be huge.

[1] For more information see:
https://www.eppo.int/media/uploaded_images/RESOURCES/eppo_standards/pm1/pm1-002-29-en.pdf

Another challenge is the lack of reference material (including for regulated pests and their 'look-alikes') Reference material is needed for development of tests, validation of tests and as controls to be included in the routine use of tests. Access to reference material can be limited (in particular for phytoplasmas, live nematodes, infected plant material, invasive alien plants). Consequently, the organization of PTs is not always possible.

4 Main PT offer

In plant health, the limited existing PT offer is one of the difficulties laboratories have to face. During the preparation of this document, discussions have been initiated with several structures organizing PT in order to identify how they work and the main difficulties they meet. One of the objectives of this work was also to stimulate the development of the PT organization market. The data collected are provided for information.

4.1.1 Fapas:

Fapas (Fera Science Ltd, Proficiency Testing Group, Great Britain) has previously run some plant health diagnostic PT under the PhytoPAS scheme name. This scheme stopped after six distributions for two reasons; economic viability and licencing issues of shipping infested plant material internationally. The last PT was organized in 2012.

The most recent report (July 2012), contained six individual PT organized simultaneously. Each PT involved between 7 and 13 participants. The six PT were as follows:

- Detection of *Ralstonia solanacearum* (DNA test materials)
- Detection of *Clavibacter sepedonicus* (DNA test materials)
- Enumeration of *Clavibacter sepedonicus* (immuno-fluorescence slides test material)
- Detection of potato spindle tuber viroid (freeze dried leaf test materials)
- Detection of pepino mosaic virus (freeze dried leaf test materials)
- Identification of *Phytophthora ramorum* (culture test materials)

For the detection tests, 4 or 6 samples were supplied to each participant, of which some were positive. Instructions were provided to participants for the reconstitution / handling of all samples, but participants could use any test to analyze the samples.

In 2012, the price for a single laboratory participation in one PT was 250 £. Apart from the sample shipping difficulties at that time, this was not an economically viable price for the PT to continue. Based on its experience, Fapas draws the attention of potential future organizers to the following points:

- The method being targeted
- The priority organisms / plant diseases being targeted
- Whether the presence of matrix is important or just DNA
- Whether images or slides will be part of the PT
- Whether samples need to be challenging with the presence of competing organisms / vectors / viruses

Based on the experience of Fapas, licencing for shipping the samples was another major issue. The receiving laboratories must indeed apply for the necessary licence or import permit to receive the test materials. Once the licences or import permits are in place and evidence is sent to the PT organizer that samples can be shipped. The licence application by the participant laboratories is likely to be the most time consuming and bureaucratic part of the entire operation, so sufficiently advanced application is advised. Procedures for providing licences differ between countries as do requirements for obtaining the licence.

4.1.2 ISTA

The international seed testing association (ISTA) is organizing PT on several aspects of seed testing. Seed health PT are organized to monitor the ability of laboratories accredited by ISTA to detect seed borne pathogens using methods published by ISTA.

So far these PT were mainly organized by the French variety and seed study and control group (GEVES). About two PT are organized every year, a pluriannual scheme is designed to cover as many pests, fields and matrices as possible. The price for a single laboratory participation in one PT varies from 600 € to 2 000 € according to the amount of work required to organize the PT. One of the main difficulties is the availability of infested material. The sample dispatched corresponds to naturally or artificially infested seed lots. Isolated pests can also be provided additionally to increase the coverage of the PT.

ISTA has made available its procedure associated with the organization and analysis of results of the seed health PT [7].

4.1.3 Proficiency tests organized by EU Reference Laboratories

Regulation EU 2017/625 [3] lists the responsibilities of European Union Reference Laboratories (EURL, Article 94) and national reference laboratories (NRL, Article 101). These are respectively asked to coordinate the implementation of the reference methods by the NRL and by official laboratories, in particular, by organizing regular inter-laboratory comparative testing or proficiency tests and by ensuring appropriate follow-up of such tests.

In plant health, EURL have been designated very recently (2019) and just started to organize PT. The scope of these PT is set by the EURL on the basis of guidance provided by the European Commission. They are intended to cover the main identified threats. Many countries have had NRL for many years and PT organized in the framework of the official control represent an important part of the PT available for laboratories. However, these PT are organized to check the ability of laboratories to use specific tests used for the official analysis, furthermore, the participation in these PT may be restricted to NRLs.

Such PT are organized in different disciplines and several strategies have been developed to reduce the workload while ensuring that they are able to evaluate the proficiency of laboratories. For example, in the animal health field in France, three NRL in charge of foodborne bacteria (*Salmonella*, *Listeria monocytogenes* and coagulase positive *Staphylococcus*) organize one common annual PT. The preparation of the sample, the contact with participants and the collection of results have been subcontracted to a private company after a public call for tender. In 2018, 71 laboratories participated in this PT.

4.1.4 Information concerning the available PT.

The European PT Information System (EPTIS) was an EU project that aimed at making an inventory of regularly operated PT schemes. The project resulted in a database [8] including 590 PT schemes called the EPTIS database which went online in 2000. At the end of the project, the partners agreed to continue the development of the database on a voluntary and non-profit basis. They laid down the rules of their cooperation and invited other organizations to join. Today, the EPTIS database lists around 4,000 PT schemes from around 40 countries worldwide. However, plant health is almost absent from the database. Currently organizations such as the EPPO or the NRLs can relay information about the organization of PT, however in most cases the laboratories must rely on their knowledge of the available PT. Making the plant health PT offer accessible through a platform such as the EPTIS database could increase its visibility and facilitate the choice of laboratories willing to plan their PT participation.

5 Other means to ensure and demonstrate the proficiency

PT have to be considered by laboratories as a part of their quality system to ensure and demonstrate their proficiency. Furthermore, in some sectors participation in PT may be difficult and other quality assurance measures should be considered.

The standard ISO/IEC 17025:2017 [4] lists (§7.7.1) other measures usable by laboratories to monitor the validity of their results. Based on this list, in version 4 of the EPPO Standard PM7/98 [2], a table has been included on internal and external checks specifically applicable to plant health diagnostic. It specifies for each control whether it should be used to monitor the actual performance of the test, of an operator within a laboratory or of the laboratory.

In the context of its flexible scope accreditation, the Dutch national plant protection organization (NPPO) developed a strategy based on similar controls and identified three levels of controls according to their frequency of use: 1st line controls are integrated in all analysis. 2nd line controls are organized internally (i.e. blind samples). 3rd line controls correspond to PT. 2nd and/or 3rd line controls are performed regularly on the level of the method and of the group of organisms. The selection of 2nd and 3rd line controls is done to ensure the coverage of a wide range of species, tests, matrices and staff.

6 Actions conducted during the project

The table below presents a simplified chronology of the main actions carried out during the project and the associated outputs.

Table 1: Simplified chronology of the main actions carried out in the framework of WP5 and associated outputs

Timeline	Action	Output
February 2019	Dedicated session during the EPPO workshop on the revision of PM 7/98	Experts agreed on the feasibility of the approach and expressed their needs
June 2019	Analysis of the accreditation scopes of laboratories listed in the EPPO database on diagnostic expertise, working under quality assurance	Identification of the needs of laboratories associated with quality assurance
August 2019	Questionnaire sent to the laboratories listed in the EPPO database on diagnostic expertise	Laboratories contacted expressed their needs (results from 22 laboratories in 12 countries)
September to November 2019	Analysis of the collected results and writing of deliverable 5.1	Deliverable 5.1: Analysis of the needs of the laboratories and applicability of the horizontal proficiency testing approach

February 2019 to May 2020	Analysis of the bibliography	EA-4/18 suggests to group sets of measurement techniques, properties and products on which the outcome of a PT for one of these sets can be directly correlated to the others. Groups may contain more than one measurement technique property or product as long as equivalence and compatibility can be demonstrated
May 2020	Meeting with Cofrac	The collected data are not considered sufficient to justify the identification of sub-disciplines as suggested by EA-4/18. Limited PT participation should rely on an enforceable document demonstrating the feasibility of this approach on the basis of objective data
May 2020	Collection of available data	An initial analysis of the data tends to show that no PT can be considered as equivalent to another
May to June 2020	Meetings with PT providers	Identify the main offers of PT organization available on the market
July 2020	Meeting with EA	EA cannot include a case study for each discipline. EA encourages WP5 to build a case study for plant health even if it cannot be included in the revision of EA-4/18.
October 2020	End of the statistical analysis of the available data	Considering the available data, it is not possible to demonstrate the equivalence of PT for different measurement techniques, properties or products
October 2020	Establishment of a working group for the development of a case study in plant health	EPPO will ensure that a case study in plant health is made available (similar to the case studies provided for several disciplines in the document EA-4/18)

6.1 Actions and conclusions reported in deliverable 5.1

A first deliverable of the work package focused on the needs of the laboratories and on the applicability of the horizontal PT approach. This work was based on data collected using three different methods:
i) a workshop organized by EPPO in Paris in February 2019 'Workshop on the revision of PM 7/98 Specific requirements for laboratories preparing accreditation for a plant pest diagnostic activity' during which a practical session was dedicated to this work,
ii) the study of accreditation scopes of some laboratories involved in diagnostics,
iii) a survey sent to laboratories listed in the EPPO database on diagnostic expertise[9].
The collected data allowed a better understanding of the expectations of laboratories, of what laboratories would consider acceptable and of the applicability of the horizontal proficiency testing approach.

6.2 Meeting with Cofrac

In May 2020, EPPO and ANSES WP5 members met representatives of Cofrac, the French accreditation body. During this meeting, the outcomes of the work performed between February 2019 and May 2020 were presented. Considering the concept of sub-disciplines developed in the document EA-4/18 and the data presented in the deliverable 5.1 - table 4 (extent to which a PT demonstrates the proficiency of a laboratory), WP5 members asked if views of the experts present during the EPPO workshop on the revision of PM 7/98 could be used to identify sub-disciplines as defined by EA-4/18.

To facilitate the discussions, several examples were considered. The answer given by Cofrac was that a PT is required for each test, at least before accreditation and once per accreditation cycle. It was also stated that each laboratory has to conduct its own risk analysis to design its own PT participation plan.

According to Cofrac representatives, a limited PT participation should rely on an enforceable document demonstrating the feasibility of this approach through objective data analysis. The views of experts do not constitute a sufficient level of evidence.

6.3 Equivalence of PT for different measurement techniques, properties or products

One possibility investigated during the project was the possibility to evaluate the proficiency of a laboratory for some tests based on the results of PT organized on other tests with similarities in terms of matrix, pest and/or method. In such a case, the appropriate participation in a selection of PT (PT scheme) could cover the scope of analysis of a laboratory while requiring limited time and consumables. However, a horizontal PT scheme must provide the same level of evidence concerning the proficiency of the laboratory. To determine whether this approach is consistent with the normative and regulatory requirements (see §2), the ideal would be to determine whether proficiency of the laboratory for the tests it performs can be predicted based on a participation in a PT programme involving similar methods in the same field (bacteriology, entomology, mycology, nematology or virology) or even in other fields.

To test the predictability of PT results, the first step was to gather data corresponding to PT results of a set of laboratories using several tests and methods in different fields, over time. Among the consortium some partners organize PT regularly. However, in most cases, participants are free to use the test of their choice and do not necessarily inform the organizer of their choice. It was nevertheless possible to gather a dataset corresponding to the need: the results of PT organized by the Plant Health Laboratory of ANSES, France, since 2010. ANSES organizes these PT in the framework of its responsibilities as NRL. These inter-laboratory comparisons are organized for laboratories performing analysis in the framework of official controls. The dataset is available in annex 1, it includes the results of 25 laboratories participating in some of the 89 PT organized by ANSES from 2010 to 2019. The proficiency tests include different fields of Plant Health (bacteriology, nematology, mycology, virology) and the tests used comprise serological, molecular and morphological methods.

The question raised concerning the possible horizontality of a proficiency assessment, is to know whether the results of a laboratory are reproducible using different tests based on the same method (in one single field or in different fields).

The data described above provide an insight into the proficiency of some laboratories while carrying out a series of given tests over a long period of time. However, they have not been generated to answer this specific question and they present a major bias. Indeed, after a non-conforming PT result, a participant is asked by the NRL to propose and implement corrective actions. A non-conforming result is therefore not supposed to be repeated in the ANSES dataset while it could have been repeated in the absence of corrective action. Considering this bias it is not possible to conduct an analysis concerning the reproducibility or the predictability of the results.

Two alternative statistical analyses can be considered. The first analysis performed on our dataset is a logistic regression to determine if the result of the laboratories (conforming or non-conforming) can be explained by the available variables (method, field, year). The results of this analysis (presented in annex 2) shows the absence of correlation between the variables and the PT results ($p<0,0001$).

The second approach considered to analyze these data is based on the scoring methods developed to follow the recommendations of the standard ISO 13528:2015 [10] and currently available in the literature for qualitative testing. Uhlig *et al.* [11] have developed the L-score, based on the logistic model, which takes into account both the level of competence of the laboratories and the level of difficulty of the tasks asked in the PT. With the a-score, Beavis *et al.* [12] use the binomial distribution and also take into account the success rate of the participants and the PT failure rate. The s-score, (to be published) has been more recently developed to limit the constraints on the number of participants or the number of samples provided during each PT. However, applied to our data, the scoring methods were not able to establish a prediction. This was due to missing data (not all laboratories participate in each PT).

During the various PT campaigns, in case of non-conforming results, the participants and organizers systematically tried to identify the causes in order to implement the appropriate corrective actions. The identified causes of such non conformity are listed in the table below.

Table 2: Causes identified following a non-conforming result to a PT.
The grey shading is used to highlight the positive values. Since the tests can be based on different methods, the total number of non-conformities may be lower than the row totals.

	Field				Method			
	Bacteriology	Mycology	Nematology	Virology	Morphology	PCR	Serology	Total
Human factors	1	0	7	1	7	2	1	9
Accommodation and environmental conditions	0	0	0	0	0	0	0	0
Test methods and method validation	1	0	3	2	0	4	3	6
Equipment	0	0	5	0	4	2	0	5
Measurement traceability	0	0	0	0	0	0	0	0
Sampling	0	0	0	0	0	0	0	0
Handling of test items	0	0	0	0	0	0	0	0
Reference material	0	0	0	0	0	0	0	0
Unidentified cause	5	1	9	11	8	11	7	26
Total	7	1	24	14	19	19	11	46

Data: ANSES, FR

The causes of non-conforming results could be identified in about 40% of cases and these were mostly in nematology. The differences between disciplines are related to the number of PTs and success rates, but also to the way the causes of failure are determined. In these results, the human factor is the main factor explaining non-conforming results. It is especially true for tests based on morphology requiring a high expertise and an appropriate training. This result is in line with the statements of the experts presented in deliverable 5.1 who considered the human factor as the main factor on which restrictions had to be made, especially for morphological methods. The test methods and their validation are the second factor explaining non-conforming results. Indeed, in the identified cases, laboratories did not confirm appropriately their ability to reproduce the level of performance of the method using their equipment or did not use the correct reagents. This was the main cause on non-conformity in PCR and serology. Finally, the equipment was identified as a cause of non-conforming results both in morphology and PCR. This was mainly associated with the cleaning of the equipment used in nematology to isolate the individuals from the analyzed matrix, regardless of the method used subsequently (morphology or PCR). These results highlight the need for a PT scheme to cover the skills of the individuals ensuring the analysis, and the appropriate verification of the test by laboratories. Special attention must be paid to the decontamination of the equipment used in nematology to prepare the samples, meaning that sample preparation in nematology should be included in the PT scheme.

In conclusion, it is important to take into account that the data presented above were not generated specifically to answer whether or not the assessment of the proficiency can be done horizontally. The analysis was based on datasets generated prior to the project for a different purpose. Based on the available data it was not possible to answer the question of the reproducibility of the result of a laboratory using different tests based on one method. To answer this specific question, a dedicated experimental set up should be designed. According to the available data, non-conforming results correspond to isolated events, a laboratory may have had many successful results to PT in different fields using different methods, it is not possible to predict the results of subsequent tests. Furthermore, since non conformities are not reproducible in the dataset, identified non-conformities could not have been detected by participating in a different PT, even using a similar method in the same field.

6.4 Application of EA-4/18 in the case of plant health laboratories: building a case study

EA has established specific policies regarding the level and frequency of proficiency testing participation [6]. It recognizes that participating in a specific PT for every test is unlikely to be feasible. It suggests to laboratories that they identify sets of tests for which the outcome of a PT using one test can be directly correlated to the proficiency using other tests. This approach limits the number of PT participation (although not as significant as a fully horizontal approach), and the efforts of the laboratory, while ensuring the validity of the results.

EA-4/18 [6] recommends that a quality control strategy is developed for at least one accreditation cycle (period between full reassessments) and that this strategy is reviewed annually. The strategy should be developed taking into account PT and other quality control procedures, but also the level of risk presented by the laboratory.

In order to illustrate how a laboratory might identify the sets of tests adequately covering its scope, the guidance document presents case studies in environmental, clinical, physical testing and in microbiology. The guidance does not provide any case study taking into account the specificities of plant health (§3). The members of WP5 have met with the EA members in charge of the revision of this document and have proposed to prepare a case study to be added in the next revision of the document. Considering the number of disciplines, EA cannot add a case study for each and can therefore not make an exception for plant health. However, the group in charge of the revision of the guidance document highly encouraged WP5 to prepare such a document taking into account the specificity of plant health and to make it public.

During the revision process of the EA-4/18 guidance document, a consultation of Plant pests diagnostic laboratories was organized and comments were provided by EPPO. One laboratory had already expressed the willingness to participate in the drafting of a case study specific to plant health. In the framework of the project, contacts have been made with the laboratories and an Expert Working Group will be established to develop a plant health case study. This follow-up of the work initiated during the project will benefit to all the plant health laboratories including official control laboratories.

7 Conclusion

According to the available data, a fully horizontal approach may not be currently suitable to evaluate the proficiency. In order to draw finalized conclusions, it would be necessary to analyze data generated specifically to address this question.

Taking into account the restrictions associated with the limited existing PT organization offer, and using other available quality assurance measures, laboratories can build their own PT participation plan in order to ensure the validity of their results. One suitable approach to limit the PT participation plan is to group the tests and evaluate the proficiency for these groups. However, this analysis has to be done by each laboratory taking into account all the parameters associated with its offer and activity. As a follow-up to the work carried out within VALITEST, the development of a specific case study for plant health in the framework of the EA-4/18 guidance document will be initiated. It is expected that it will facilitate the implementation of this approach by laboratories.

ANNEX 1 – Proficiency testing data

Method	Field	Year	Lab1	Lab2	Lab3	Lab4	Lab5	Lab6	Lab7	Lab8	Lab9	Lab10	Lab11	Lab12	Lab13	Lab14	Lab15	Lab16	Lab17	Lab18	Lab19	Lab20	Lab21	Lab22	Lab23	Lab24	Lab25
Morpho	Nem	2010		C				C	N						C				C	C		N	C	C	C		
PCR	Myc	2010			C			C						C	C												
PCR	Myc	2010			C			C						C	C												
Morpho	Myc	2011			C					N																	
PCR	Myc	2011			C			C						C													
ELISA	Vir	2011											C		N											C	
ELISA	Vir	2011				C				C	C	N			C												C
ELISA	Vir	2011					C	C		C					C		C	N									
Morpho	Nem	2011		C				C							C				C	C		N	C				
Morpho	Nem	2011		C				C							C				N	C			C				
IF / PCR	Bact	2012	C					C							C				C	C		N	C				
PCR	Myc	2012			C		C										C										
PCR	Myc	2012						C							C												
PCR	Myc	2012						C						C	C												
ELISA	Vir	2012					C								C	C	C	C									
ELISA	Vir	2012					C									C	C	C									
ELISA	Vir	2012	C					C							C							C					
ELISA	Vir	2012				C	C					C					C	C									C
PCR	Vir	2012					C									C	C	N									
ELISA	Vir	2012	N				C									C	C	N									
Morpho	Nem	2012		C				N							C							N		N			
Morpho / PCR	Nem	2012						C							C				C	C	C	C					
PCR	Nem	2012						C				N															
ELISA	Vir	2013	C					C		C	C				C			C						C			
PCR	Bact	2013	N			N						C	C			C										C	
ELISA	Vir	2013				C				C	C	N			C												C
PCR	Myc	2013								C																	
Isolation	Myc	2013			C			C							C												
PCR	Myc	2013			C			C						C	C												
PCR	Myc	2013						C						C	C												
Morpho	Nem	2013		C				C	C						C				N	C	C	C					
Morpho	Nem	2013		C				C							C												
PCR	Nem	2013		C				C							N					C							
PCR	Vir	2013														C	C	N									
PCR	Bact	2013					C											C									
IF / PCR	Bact	2014	C					C							C				C	C	C	C			C		
PCR	Vir	2014	C			N		C							C					C					C		
PCR	Myc	2014			C			C							C												
PCR	Myc	2014			C			C						C	C												
PCR	Nem	2014		C				C							C				C	C	C	C					
morpho	Nem	2014		C				C							C							N		C			
PCR	Nem	2014						N							C					N					C		
Morpho	Nem	2014		C				C	C						N				C	C		C					
ELISA / PCR	Vir	2014					C									C	C	C									
PCR	Bact	2014				C	C										C										
PCR	Bact	2015	C			C				C		C	C			C										C	
ELISA	Vir	2015				C				C	C	C			C												C
PCR	Myc	2015			C			C							C												
Isolation	Myc	2015			C			C							C												
PCR	Myc	2015			C			C							C												
Morpho	Nem	2015		C				C	C						N				C	C	C	C					
PCR	Nem	2015						C				C															
ELISA	Vir	2015				C	C					C						C									
PCR / Isolation	Bact	2015					C										C	C									
IF / PCR	Bact	2016	C					C							C				C	C	C	C			C		
PCR	Bact	2016	C			C		C		N	C				N	C											
ELISA	Vir	2016	C					C		C	C				C			C				C		C			
PCR	Vir	2016	C			N		C												C							
PCR	Myc	2016			C			C							C												
Morpho / PCR	Nem	2016		C				N							C				C	C	C	C			C		
Morpho	Nem	2016		C				N							N						N	C		C			
PCR	Nem	2016		C				C							N					C					C		
PCR	Bact	2016				C	C										C	C									
ELISA / PCR	Vir	2016					C									C	C	C									
ELISA	Vir	2017	C					C							C							N					
PCR	Bact	2017	C			C						C	C			C										C	
ELISA	Vir	2017								C	C	C			C												C
PCR	Myc	2017			C			C							C												
PCR	Myc	2017			C			C							C												
PCR	Nem	2017		C				C				C															
ELISA	Vir	2017				C	C					C						C									
PCR / Isolation	Bact	2017					C											N									
IF / PCR	Bact	2018	C					C							C				C	C	C	C			C		
PCR	Bact	2018	C			C		C		C	C				C	C											
ELISA	Vir	2018	C					C		C	C				C			C				C		N			
PCR	Vir	2018	C			C		C												C							
PCR	Myc	2018			C			C							C												
Morpho / PCR	Nem	2018		C				C							C				C	C	C	C					
Morpho	Nem	2018		C				C							N						C	N		C			
PCR	Nem	2018		C				C							C					C							
PCR	Bact	2018				C	C										C	N									
ELISA / PCR	Vir	2018					C	C								C	C	C									
ELISA	Vir	2019	C					N							C							N					
PCR	Bact	2019	C			C						C	C			C										C	
ELISA	Vir	2019								C		C			C												C
PCR	Myc	2019			C			C							C												
PCR	Nem	2019		C				C			N	N															
ELISA	Vir	2019				C	C					C						C									
PCR / Isolation	Bact	2019					C											C									

Data: ANSES, FR

Methods: ELISA, Immunofluorescence (IF), Isolation, Morphology (Morpho), PCR,
Fields: Bacteriology (Bact), Mycology (Myc) Nematology (Nem), Virology (Vir)
Results: conforming (C), non-conforming (N)

ANNEX 2 – Logistic regression analysis results

Type II analysis (Variable Result) :

Variables	DDL	Khi² (Wald)	Pr > Wald	Khi² (LR)	Pr > LR
Method	2	4.090	0.129	138.250	< 0.0001
Field	3	5.404	0.144	141.254	< 0.0001
Lab	24	27.600	0.277	177.332	< 0.0001
Year	9	10.552	0.308	146.270	< 0.0001

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