This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N° 773139

Grant agreement N. 773139

DELIVERABLE N° 5.1 – V2

Title: Analysis of the needs of the laboratories and applicability of the horizontal proficiency testing approach

Valitest

Validation of diagnostic tests to support plant health

Due date	Month 18
Actual submission data	15-11-2019 (Month 19)
Start date of the project	01-05-2018
Deliverable lead contractor (organization name)	ANSES
Participants (Partners short names)	ANSES, WBF, EPPO, FERA, NIB, ULG, NVWA, CREA
Author(s) in alphabetical order	M. Rolland
Contact for queries	Mathieu.rolland@anses.fr
Level of dissemination	Public
Type of deliverable	Report

Abstract:
The 5th work package of the VALITEST project aims 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.
A range of data has been collected using three different approaches, i) the study of accreditation scopes of some laboratories involved in diagnostics, ii) a workshop with diagnostic experts, iii) a survey sent to laboratories listed in the EPPO database on diagnostic expertise. The data collected allow a better understanding of the expectations of laboratories, of what they would consider as acceptable and of the applicability of the horizontal proficiency testing approach.
According to the data collected, a PT plan should be conducted over 3 to 4 years. Each year a single PT should be organized per field. It could contain several pests, however, to limit the workload no more than 3 tests should have to be performed on a maximum of 10 samples. The PT should include whenever possible the different pests included in the scope of accreditation of the laboratories, but also emerging pests. The pests to consider as a priority have been identified. PT samples should be as similar as possible to routine samples, ideally different matrices should be included in each PT. Among the different factors determining the reliability of a test, the design of the PT plan should especially focus on human factors (i.e. the proficiency of the staff to use the different techniques). The laboratories do not wish to spend more than 3 500 € per year for a service providing proficiency tests covering all their needs.

Partners involved: ANSES, WBF, EPPO, FERA, NIB, ULG, NVWA, CREA
Task 5.1

HISTORY OF CHANGES			
Version	Publication date	Authors	Change
1.0	15 Nov. 2019	Rolland Mathieu	Initial version
2.0	10 Feb. 2020	Rolland Mathieu	Following the review of the project, tables 2 and 3 have been modified, *Leptinotarsa decemlineata* has been added to the entomology section.

TABLE OF CONTENTS

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:

- ELISA: enzyme linked immune sorbent assay
- EPPO: European and Mediterranean Plant Protection Organization
- ISO: International Organization for Standardization
- LAMP: loop mediated isothermal amplification
- 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 achieved through a proficiency test (PT) scheme. Participating in a PT enables a laboratory to check whether it is (or confirm that it remains) competent 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 its competence for these tests. The current approach is based on pest specific PTs. Considering the difficulties of organizing PTs (time, cost, material availability, complexity, number of pests regulated in plant health), all the pests and tests cannot be covered. This approach is not adequate nor sustainable.

The 5th work package of the VALITEST project aims at developing guidelines on a horizontal approach allowing the laboratories to undertake proficiency testing without having to participate in PTs for all the tests performed routinely.

This first deliverable of the work package focuses on the needs of the laboratories and on the applicability of the horizontal PT approach. This work is based on data collected using three different methods, i) the study of accreditation scopes of some laboratories involved in diagnostic, ii) a workshop organized by EPPO 'Workshop on the revision of PM 7/98 Specific requirements for laboratories preparing accreditation for a plant pest diagnostic activity' (called hereafter EPPO Workshop on quality assurance) during which a practical session was dedicated to this work, iii) a survey sent to laboratories listed in the EPPO database on diagnostic expertise [3].

Based on this work, guidelines on an approach to undertake horizontal proficiency testing will be delivered by the group at month 24 of the project.

2 Proficiency testing needs of the laboratories

Within quality management systems, proficiency testing aims at ensuring the quality of the results and the trust of all the stakeholders in these results. Proficiency testing is a highly recommended activity for laboratories willing to

maintain their accreditation, however, PT can be used more broadly by any laboratory for any pest or test to ensure the ability of the laboratory to provide valid results.

Two strategies have been employed in order to identify the needs of laboratories in terms of proficiency testing, i) the methods and pests under accreditation have been listed, ii) experts of laboratories have been asked through a survey which pests should be prioritized for the organization of PTs.

2.1 Needs associated with the accreditation of laboratories

To identify the needs associated with the accreditation of laboratories, the accreditation scopes of 24 laboratories have been analyzed. These accreditation scopes are from laboratories listed on the EPPO database on diagnostic expertise [3] that have an accredited ISO quality assurance system and for which the accreditation scope could be retrieved (out of the 70 laboratories listed in the database, 24 were listed as working under accreditation). For each field, the methods used under accreditation have been listed in table 1, the pests detected or identified have been listed in table 2.

Table 1: Number of laboratories accredited per field and method (out of the 24 accreditation scopes retrieved from the EPPO database on diagnostic expertise [3]). Combinations (field and method) accredited in at least one laboratory are highlighted in grey.

	Bacteriology	Botany	Entomology	Nematology	Mycology	Virology and Phytoplasmology
Bioassay/Pathogenicity	7	0	0	1	1	1
Biochemistry	0	0	0	0	0	0
Fingerprint	0	0	0	0	0	0
Molecular	17	1	2	8	11	18
Isolation and morphology	11	2	4	9	9	3
Serology	13	0	0	0	1	13

Table 2: Number of laboratories accredited per field and pest (out of the 24 accreditation scopes retrieved from the EPPO database on diagnostic expertise [3]).

Bacteriology	
Any bacteria	3
Clavibacter michiganensis subsp. *sepedonicus*	11
Ralstonia solanacearum	10
Erwinia amylovora	4
Candidatus liberibacter	4
Xylella fastidiosa	3
Clavibacter michiganensis subsp. *michiganensis*	3
Dickeya spp.	2
Xanthomonas axonopodis pv. *phaseaoli*	1
Xanthomonas axonopodis pv.*dieffenbachiae*	1
Pseudomonas syringae pv.*actinidiae*	1
Xanthomonas campestris pv. *campestris*	1
Xanthomonas citri pv.*citri*	1

Mycology	
Any fungi or oomycete	5
Phytophthora ramorum	5
Monilinia fructicola	2
Synchytrium endobioticum	1
Tilletia spp.	1
Venturia inaequalis	1
Guignardia citricarpa	1
Fusarium circinatum	1
Ascochyta spp.	1
Drechslera spp.	1
Leptosphaeria nodorum	1
Microdochium spp.	1
Ustilago nuda	1

Virology and Phytoplasmology	
Any virus, viroid or phytoplasma	6
Pospiviroids	7
PPV	6
PVY, PVA, PVM, PVS, PVX	4
ArMV	4
GFLV,GVA,GLRaV-1,GLRaV-3	3
PLRV	2
CTV	2
Phytoplasma mali	2
Flavescence dorée	2
Phytoplasma pyri	1
BNYVV, CMV, BBTV, BBrMV	1
BCMNV	1
PepMV	1
TBRV, ACLSV, ApMV, CLRV, PDV, PNRSV, ASGV, RpRSV, SMYEV, TNV, ToRSV	1
Other potato viruses	1

Botany	
Any plant	1
Ambrosia artemisiifolia	1
Acroptilon repens	1
Cenchrus pauciflorus	1

Nematology	
Any nematode	4
Potato cyst nematodes	8
Bursaphelenchus xylophilus	2
Meloidogyne fallax/chitwoodi	1
Ditylenchus dipsaci/destructor	1

Entomology	
Any arthropod	2
Anoplophora glabripennis	1
Bemisia tabaci	1
Leptinotarsa decemlineata	1
Phthorimaea operculella	1
Tuta absoluta	1

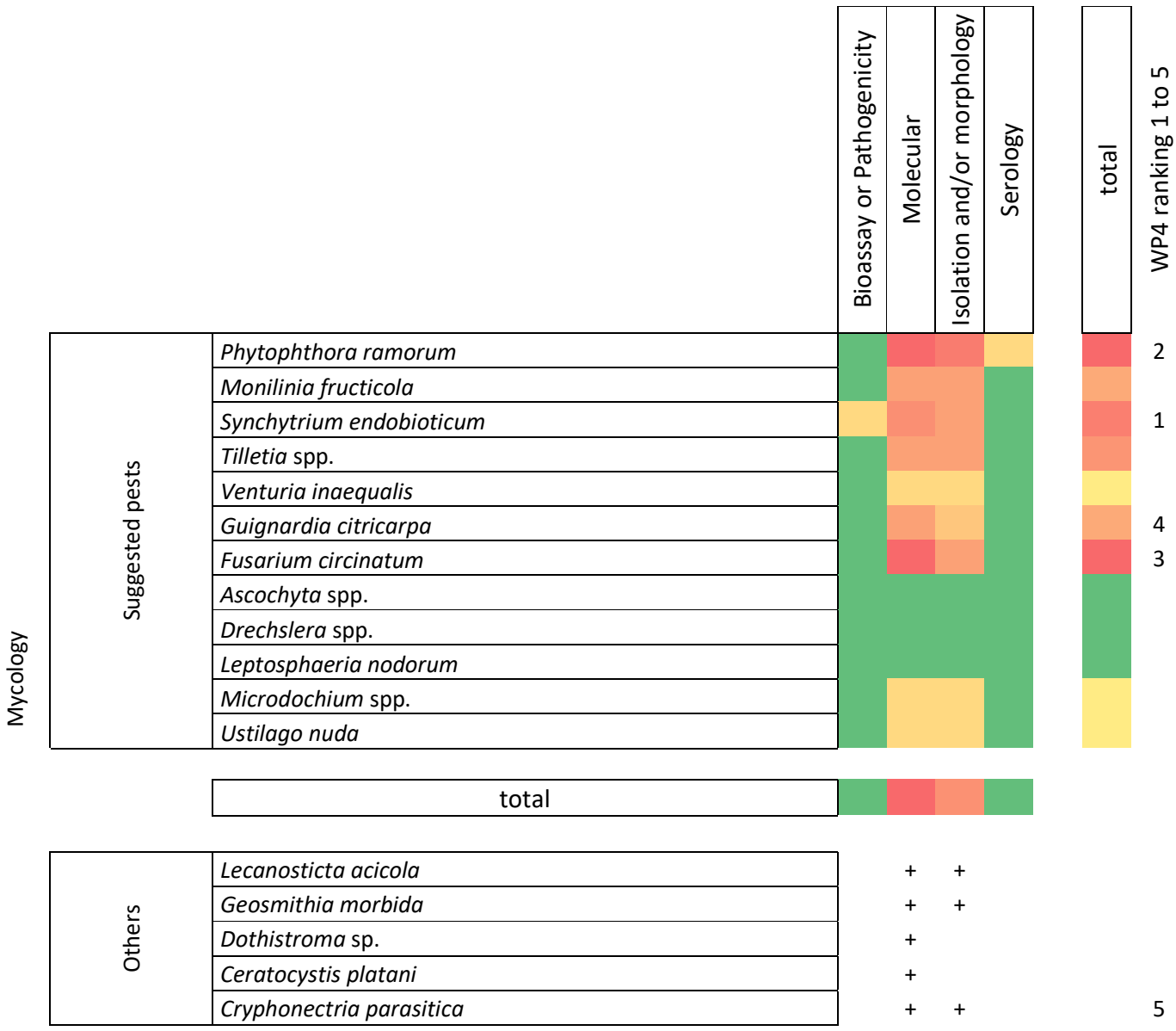
For all the considered fields, accreditation mainly focuses on bioassay, molecular, isolation, morphological and serological methods. The list of pests for which the 24 laboratories have tests under accreditation is important but does not necessarily reflect the needs in terms of proficiency testing, as tests for other pests may be as important or more important for proficiency testing. Furthermore it is important to keep in mind that an accreditation scope is defined strategically to answer needs of clients or regulators. To obtain a more precise list of the proficiency testing needs, experts have been asked to specify the need of their laboratory based on the lists of pests in table 2 and methods used in table 1.

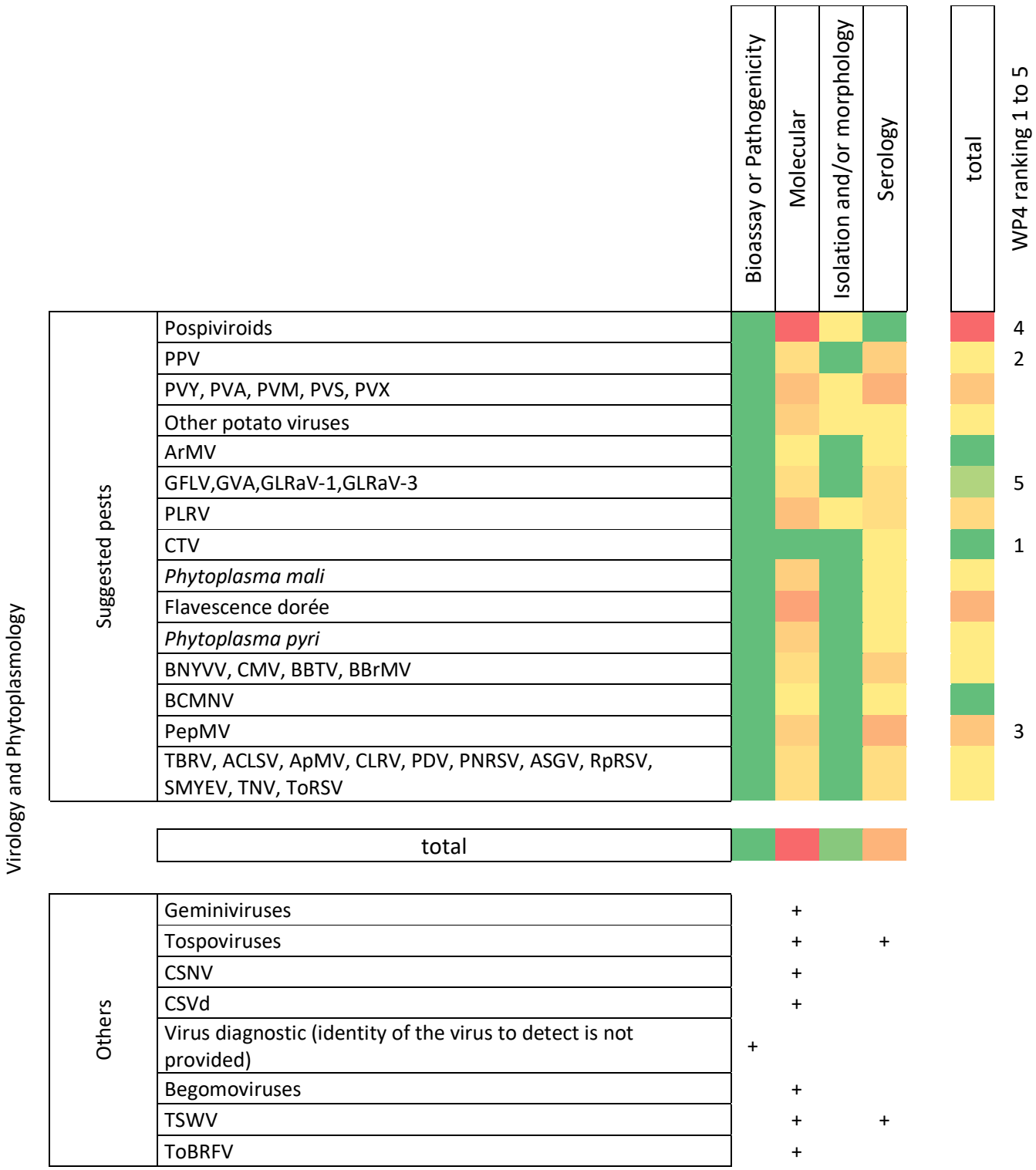
2.2 Results of the survey

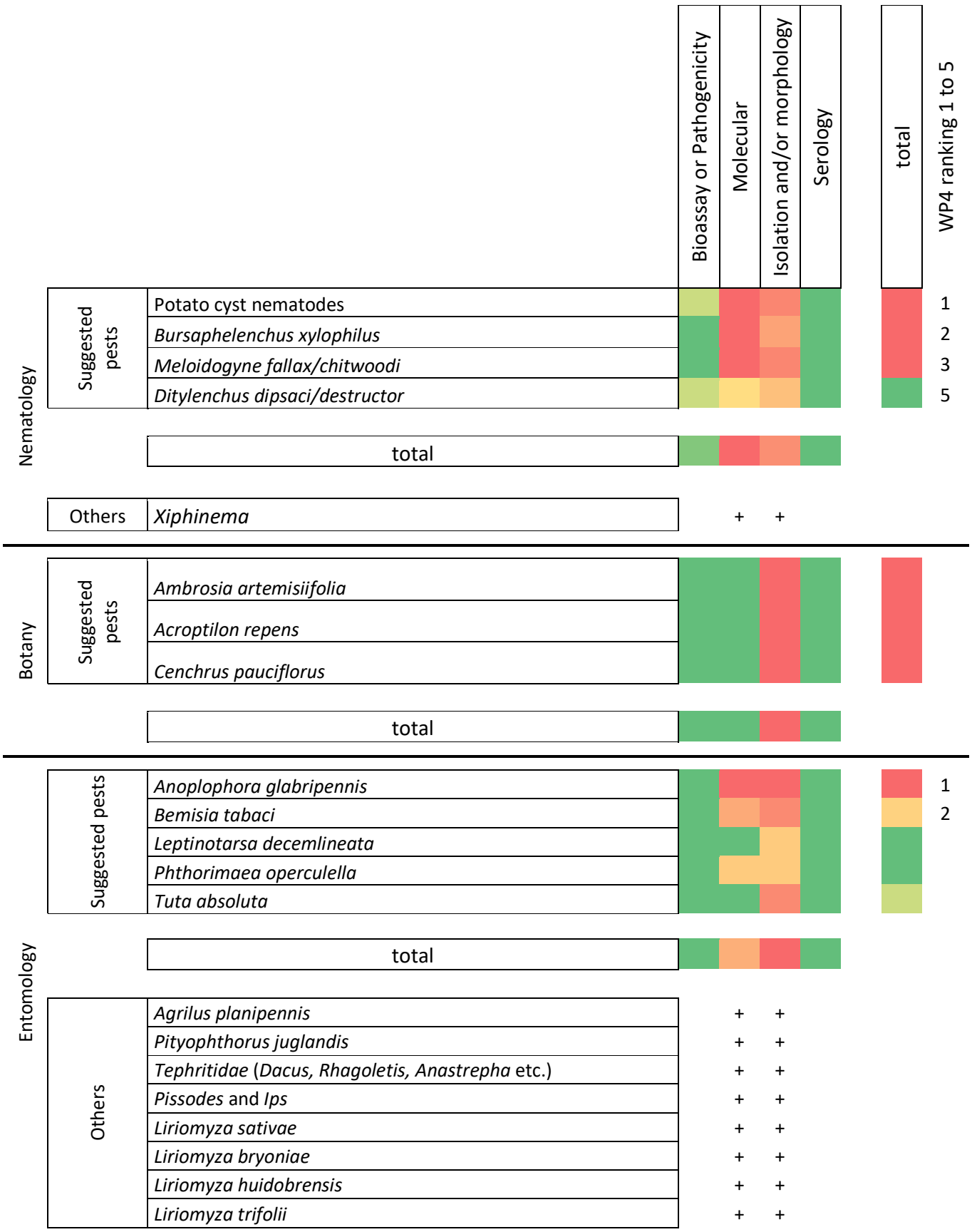
A survey has been sent to experts of laboratories listed in the EPPO database on diagnostic expertise [3] (content of the survey provided in annex 1). The survey allowed views to be collected from 22 experts working in 12 countries (Australia, Belgium, the Czech Republic, France, Germany, Ireland, Italy, the Netherlands, Slovenia, Spain, Switzerland and the United Kingdom). The list of pests previously identified (table 2) was provided and experts were asked to identify for which pest they need a PT and if so for which method. Participants also had the possibility to add pests not listed in table 2. Table 3 presents the results obtained during the survey but also the pests ranked by WP4 as representing the highest demand for testing and impact.

Table 3: Identified PT needs (data from the survey). For the suggested pests, the demand is illustrated using a colour scale ranging from dark green (no demand), to red (highest demand in the field). Added pests are listed below, for these pests, each method listed by at least one laboratory is notified (+), WP4 ranking is represented on the right of the table.

		Bioassay or Pathogenicity	Molecular	Isolation and/or morphology	Serology	total	WP4 ranking 1 to 5
Bacteriology	Suggested pests	*Clavibacter michiganensis* subsp. *sepedonicus*					2
		Ralstonia solanacearum					3
		Erwinia amylovora					4
		Candidatus liberibacter					5
		Candidatus liberibacter asiaticus, africanus or *americanus*					
		Xylella fastidiosa					1
		Clavibacter michiganensis subsp. *michiganensis*					
		Dickeya spp.					
		Xanthomonas axonopodis pv. *phaseoli*					
		Xanthomonas axonopodis pv. *dieffenbachiae*					
		Pseudomonas syringae pv. *actinidiae*					
		Xanthomonas campestris pv. *campestris*					
		Xanthomonas citri pv. *citri*					
	total						
Others		*Pantoea stewartii*	+	+			
		Curtobacterium flaccumfaciens subsp. *flaccumfaciens*	+	+			
		Xanthomonas oryzae pv. *oryzae/oryzicola*	+	+			


Mycology
Suggested pests
Phytophthora ramorum
Monilinia fructicola
Synchytrium endobioticum
Tilletia spp.
Venturia inaequalis
Guignardia citricarpa
Fusarium circinatum
Ascochyta spp.
Drechslera spp.
Leptosphaeria nodorum
Microdochium spp.
Ustilago nuda
total
Others
Lecanosticta acicola
Geosmithia morbida
Dothistroma sp.
Ceratocystis platani
Cryphonectria parasitica
Bioassay or Pathogenicity
Molecular
Isolation and/or morphology
Serology
total
WP4 ranking 1 to 5
2
1
4
3
5


Virology and Phytoplasmology
Suggested pests
Pospiviroids
PPV
PVY, PVA, PVM, PVS, PVX
Other potato viruses
ArMV
GFLV,GVA,GLRaV-1,GLRaV-3
PLRV
CTV
Phytoplasma mali
Flavescence dorée
Phytoplasma pyri
BNYVV, CMV, BBTV, BBrMV
BCMNV
PepMV
TBRV, ACLSV, ApMV, CLRV, PDV, PNRSV, ASGV, RpRSV, SMYEV, TNV, ToRSV
total
Others
Geminiviruses
Tospoviruses
CSNV
CSVd
Virus diagnostic (identity of the virus to detect is not provided)
Begomoviruses
TSWV
ToBRFV
Bioassay or Pathogenicity
Molecular
Isolation and/or morphology
Serology
total
WP4 ranking 1 to 5
4
2
5
1
3


Bioassay or Pathogenicity
Molecular
Isolation and/or morphology
Serology
total
WP4 ranking 1 to 5
Nematology
Suggested pests
Potato cyst nematodes
Bursaphelenchus xylophilus
Meloidogyne fallax/chitwoodi
Ditylenchus dipsaci/destructor
1
2
3
5
total
Others
Xiphinema
+ +
Botany
Suggested pests
Ambrosia artemisiifolia
Acroptilon repens
Cenchrus pauciflorus
total
Entomology
Suggested pests
Anoplophora glabripennis
Bemisia tabaci
Leptinotarsa decemlineata
Phthorimaea operculella
Tuta absoluta
1
2
total
Others
Agrilus planipennis
Pityophthorus juglandis
Tephritidae (Dacus, Rhagoletis, Anastrepha etc.)
Pissodes and Ips
Liriomyza sativae
Liriomyza bryoniae
Liriomyza huidobrensis
Liriomyza trifolii
+ +

The data represented above allow identifying some trends in the needs of laboratories. First considering the methods, the accreditation scopes and the interest of the laboratories both show a difference between fields. In bacteriology, proficiency testing is considered as needed for the four categories of methods (Bioassay or Pathogenicity; Molecular; Isolation and/or morphology; Serology), in mycology the demand focusses on molecular and morphological methods, in virology it focusses on molecular and serological methods, in nematology on molecular and morphology, in botany on morphology and in entomology on molecular and morphology. A horizontal assessment meant to demonstrate the proficiency in the different fields will have to take into account all the different methods, while PT plans focused on a single field could be limited to some methods.

Concerning the pests, the study of the accreditation scope and the survey allowed to list the pests on which there is some interest in developing a PT. It will not be possible to include all the listed pests in a proficiency plan. However, considering the different levels of interest for the listed pests, it will be possible to prioritize (also taking into account the methods to be used). It is worth noting that at least in bacteriology, mycology and nematology, the pests with the highest PT demand also correspond to the pests presenting, according to WP4, the highest demand for testing and impact. This however is not true in virology. Indeed, citrus tristeza virus for example has been ranked by WP4 as having the highest ranking in terms of demand for testing and impact while there has been no demand for proficiency testing on this virus.

3 Identification of critical points, overlaps

During the EPPO Workshop on quality assurance held in Paris in February 2019, a session was dedicated to the 5th work-package of the VALITEST project. Experts were asked to work in subgroups (i) Bacteriology, (ii) Botany, Entomology & Nematology, (iii) Mycology and (iv) Virology and Phytoplasmology and to brainstorm on the identification of critical points and the possible organization of horizontal proficiency assessment (files used during the workshop are provided in annex 2).

For each factor determining the correctness and reliability of the tests (according to ISO 17025 [4] and PM 7/84 [5]) and each type of method (according to PM 7/76 [1]), the groups were asked to brainstorm on the applicability of a proficiency result to other tests, methods, fields etc. For example, if a laboratory obtains satisfactory results during a PT using a molecular method, should it be considered that the factor « accommodation and environmental conditions » is properly managed for the test only, the method, the field, or across fields. Table 4 below attempts to summarize the answers collected.

Table 4: Extent to which a PT demonstrates the proficiency of a laboratory for factors affecting the reliability of a test (data from the EPPO Workshop on quality assurance)

Factors determining the correctness and reliability of the tests (ISO17025 & PM7/84)	Extent to which the proficiency is demonstrated after a PT
Human factors	- Bioassay, isolation or extraction, morphology or morphometry, pathogenicity: the proficiency is only demonstrated for the test - Fingerprint, molecular (reaction), serology: the proficiency applies to all the fields - Molecular (extraction only): the proficiency only applies to the matrix or target
Accommodation and environmental conditions	If the procedures are the same, then the proficiency applies to all the fields.
Test methods and method validation	Not under the scope of the Proficiency testing
Equipment	Covered by the quality assurance system of the laboratory
Measurement traceability	If the procedures are the same, then the proficiency applies to all the fields.
Sampling	Not horizontal, rarely covered in the PTs (difficult to achieve).
Handling of test items	If the procedures are the same, then the proficiency applies to all the fields.
Reference material	Pest dependent, or at least depends on the material maintenance procedure.

During the workshop, experts commented that the first means to ensure the correctness and reliability of a test is the quality assurance system. Based on the answers collected during this workshop, for most factors determining the correctness and reliability of a test, the demonstrated proficiency can be considered as horizontal. In other words, for most factors, if proficiency is demonstrated with one test, the lab can be considered proficient for other tests, methods or even other fields. This, however, is not true for human factors, sampling and reference material.

Concerning human factors, some methods (bioassay, isolation, morphology or pathogenicity) mainly rely on staff expertise. For these methods, proficiency is associated with the tested genus or species and cannot be horizontal. For serological and molecular tests, the required expertise generally applies to the technique and not to the pest. Proficient staff can therefore apply the method to other tests and fields. Serological methods are considered as diverse, but if each technique is assessed separately (immunofluorescence, ELISA), then proficiency can be considered as demonstrated for all the tests using this technique. For molecular methods a difference was made between the extraction step and the reaction itself. Based on the data collected, the proficiency to extract nucleic acids cannot be generalized as some matrices and/or pests are more difficult to work with. Concerning the reaction, proficiency does not depend on the field but on the technique and a laboratory proficient to perform PCR cannot be considered as proficient to perform isothermal amplification. If each technique is assessed separately (PCR, real-time PCR, LAMP) then proficiency can be considered as demonstrated for all the tests using this technique. Some experts considered barcoding as too specific to one field to be horizontal.

Concerning sampling, the proficiency cannot be generalized. However, integrating sampling in a PT is difficult to achieve and this factor is rarely covered by the PTs. Some experts questioned during the EPPO workshop considered that integrating sampling in the proficiency testing would improve the correctness and reliability of the diagnostic activity.

The reference material is pest dependent but also depends on the procedures and infrastructures developed by the laboratory to maintain its reference materials. As such, the control of this factor by the laboratory is not assessed by the PTs.

All the points above will have to be taken into account when designing a horizontal PT plan.

4 Applicability of the horizontal proficiency testing approach

In the survey sent to experts of laboratories listed in the EPPO database on diagnostic expertise [3], several questions were asked to understand their expectations and to identify the acceptability of a horizontal proficiency testing strategy (survey provided in annex 1). Answers to the questions are represented in Figure 1 to 6. Figure 1 presents answers to eight of the questions. For each question, respondents were allowed to comment their answer; these comments are provided in annex 3.

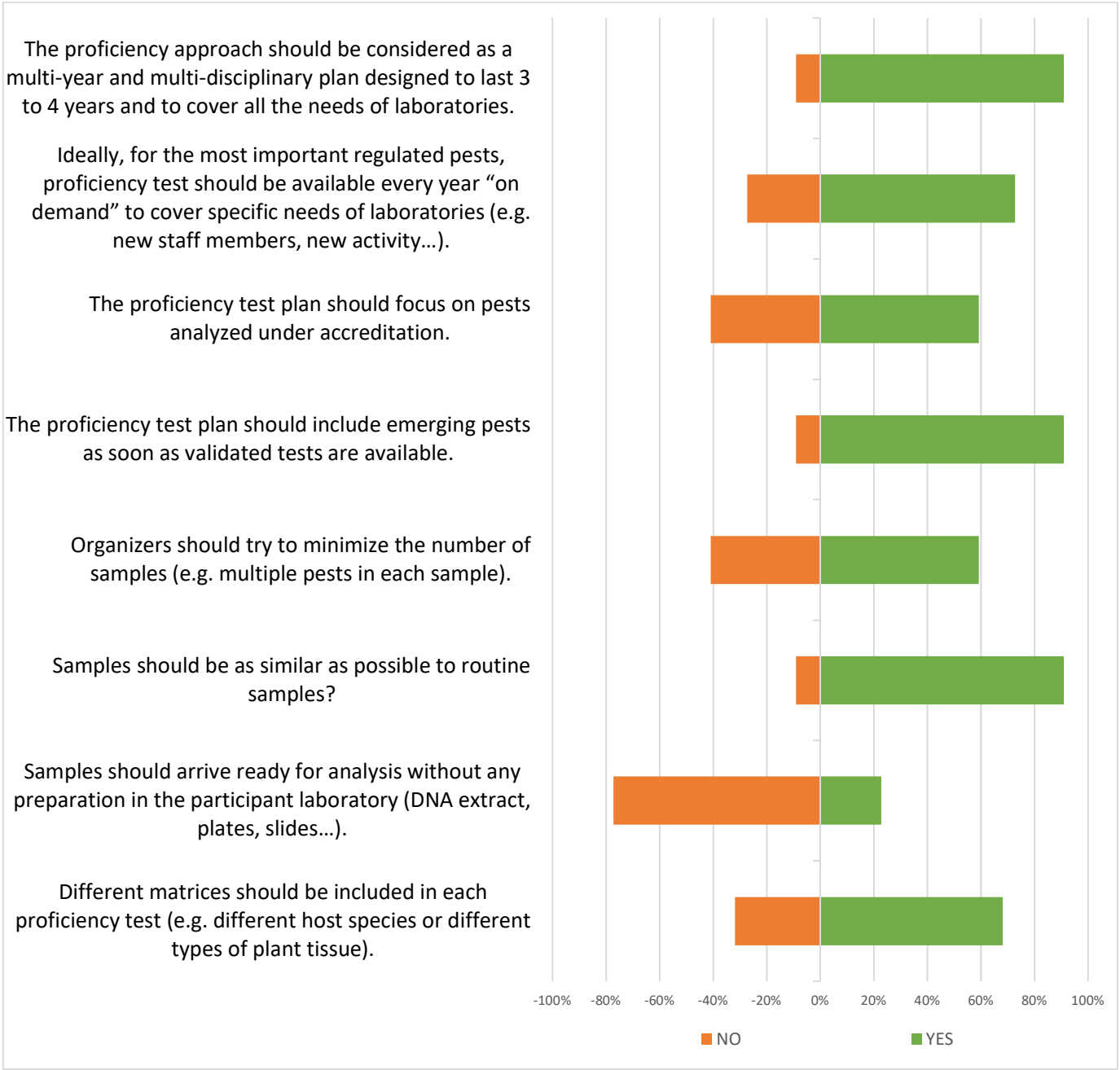


Figure 1: Answers collected during the survey on features of PT sent to laboratories of the EPPO database on diagnostic expertise [3]

Most laboratories agree to consider the proficiency approach as a multi-year and multi-disciplinary plan. However, this plan should be able to be adapted to new emerging pests (see comments in the annex 3). Laboratories would appreciate to have access to PT on the most important pests every year but they realize that it might be too difficult to organize

(see comments in the annex 3). Laboratories do not want to see the proficiency testing limited to tests performed under accreditation and strongly agree on the need to include emerging pests as soon as possible and maybe even before a validated test is available (see comments in the annex 3). Results are balanced concerning the possibility to minimize the number of samples. Minimizing the number of samples should therefore not be considered as a priority. When it comes to the nature of the samples, most laboratories supported that "samples should be as similar as possible to routine samples". Prepared samples (i.e. DNA extracts) should be avoided as much as possible, although it was recognized that they are convenient to work with. Finally, when relevant, different matrices should be included in each PT, although it does not seem to be a priority for laboratories.

During the survey, participants were also asked how many PT, samples and tests would seem appropriate every year. Obtained results are presented in figures 2 to 4.

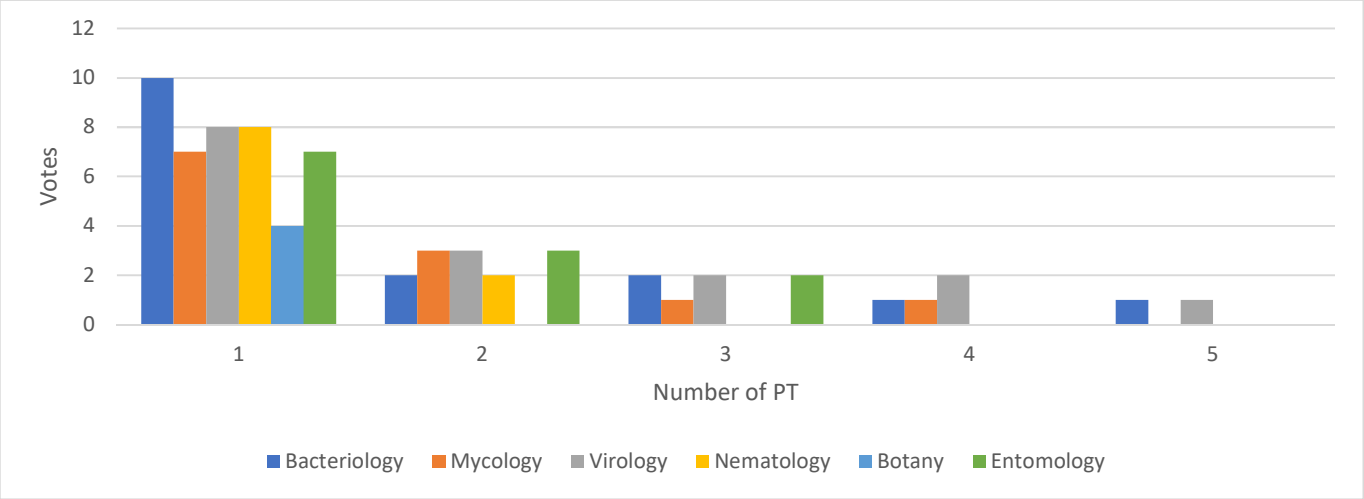


Figure 2: Number of proficiency tests considered as appropriate per year and per discipline for one laboratory (results of the survey).

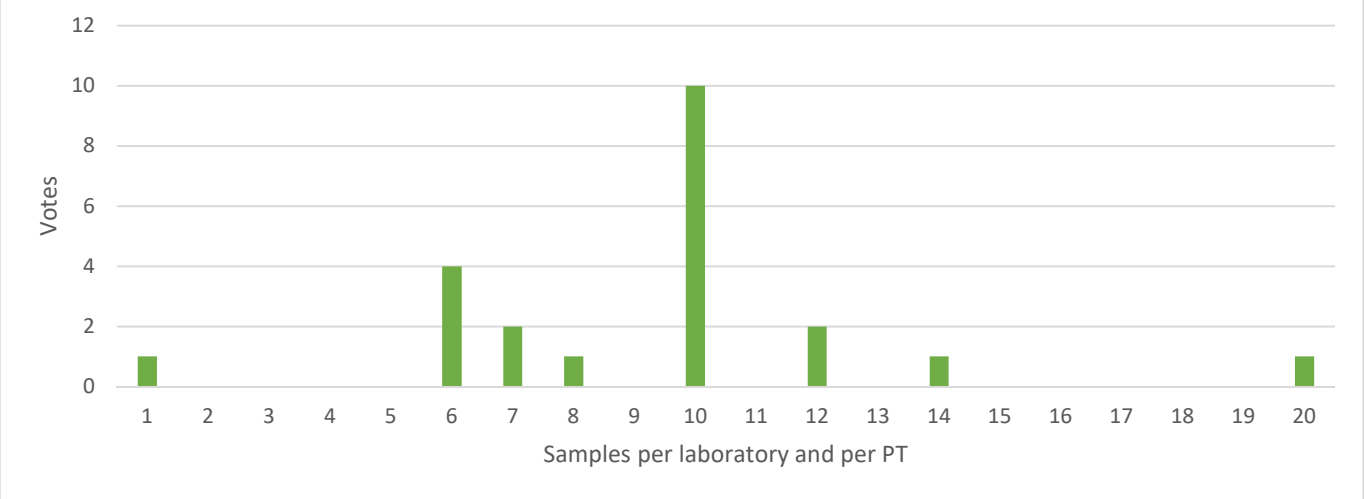


Figure 3: Samples per laboratory considered as appropriate for a proficiency test (results of the survey).

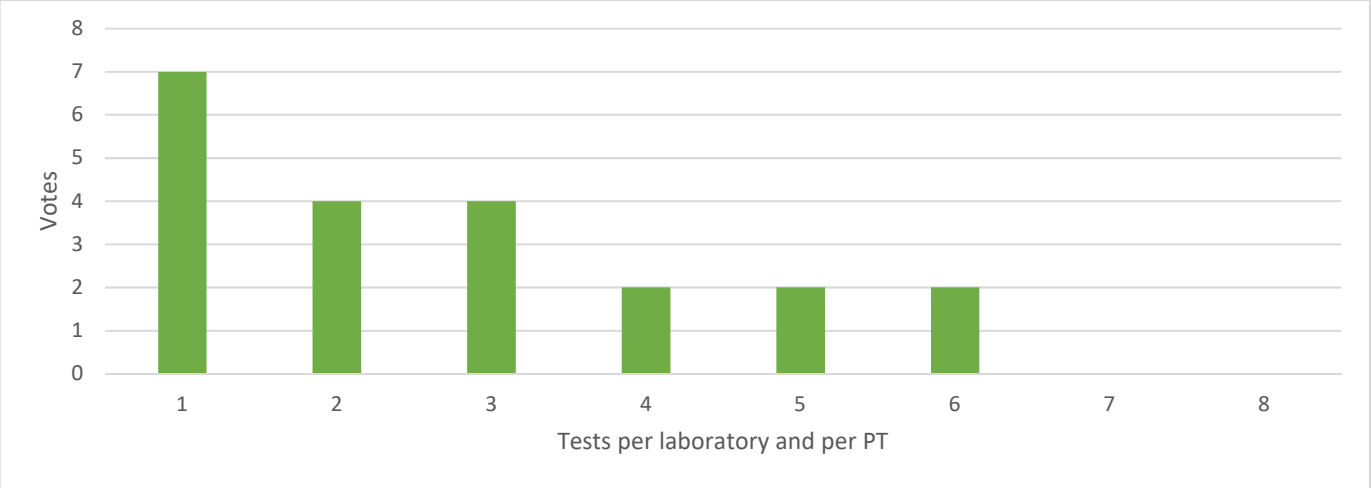


Figure 4: Tests per proficiency test considered as appropriate by laboratory (results of the survey).

According to the collected data, laboratories seem to consider that one single PT per field and per year is enough. Concerning the number of samples per PT, 10 is the median value and also the most commonly cited number. Ten samples per PT could be the number of samples to target for the development of a horizontal PT even if it will not be appropriate for all laboratories. Finally, for the number of tests per PT, collected data range from 1 to 8 (an outlier value has been suppressed for the analysis) with a mean value of 2.7. For each horizontal PT, no more than 3 different tests should therefore be included.

5 Financial participation for a proficiency testing plan

During the survey, respondents were asked what would be the most important drivers for them to participate or not in a horizontal PT plan (i.e. to an offer of several PTs allowing the laboratory to demonstrate its proficiency on the needed diagnostic tests without having to do it test by test, pest by pest). The question of the annual participation fee they would accept to pay for this service was also raised. Collected answers are presented in figures 5 and 6.

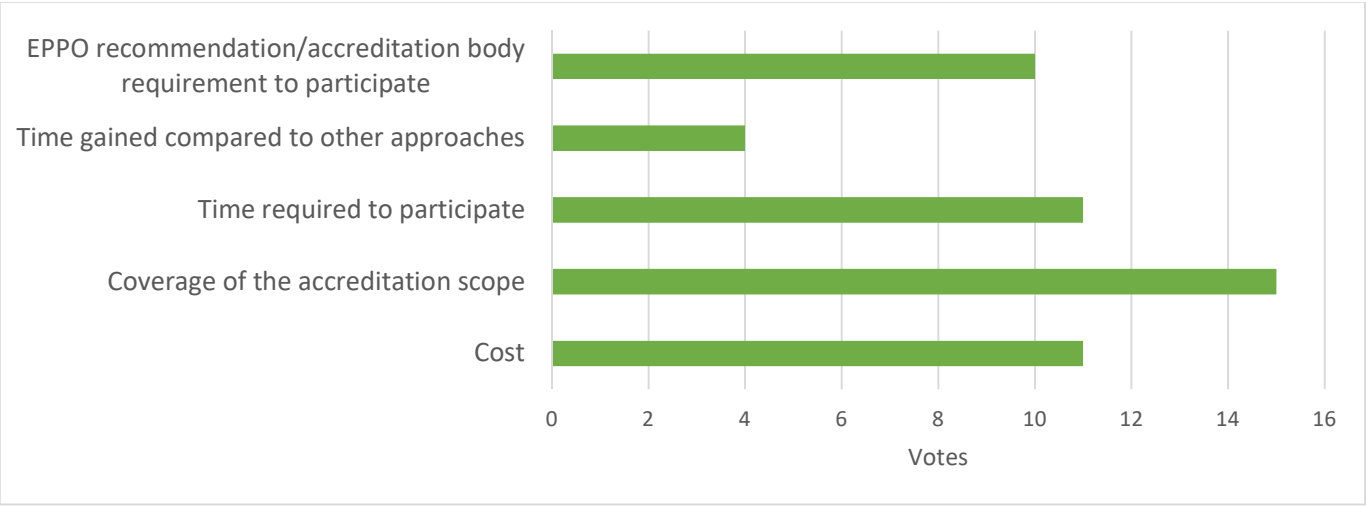


Figure 5: Drivers to participate to a horizontal proficiency (results of the survey).

Although laboratories consider that a PT plan should not be limited to tests they are performing under accreditation, the coverage of their scope of accreditation by the PT plan remains the main driver. The cost of the plan comes in second position and will also have a great impact on the success of a proposed service. Results obtained concerning the time aspect seem contradictory. Saving time does not seem to be important while the time required to participate gathered as many votes as the cost. Despite this surprising result, the time required to proceed with the PT plan should

not be too long to avoid discouraging potential participants. Finally, the EPPO recommendation or the accreditation bodies requirement would of course increase the participation of laboratories to PT plans.

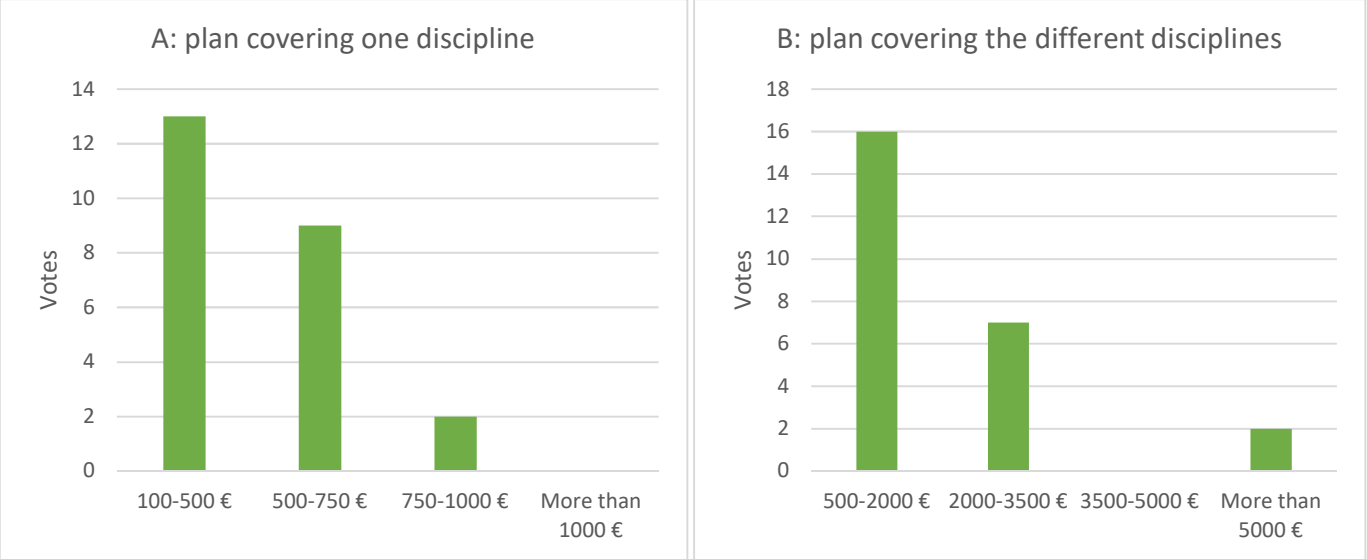


Figure 6: Annual participation fee for a proficiency testing plan (results of the survey, several answers possible).

For a plan covering one single discipline, the fee laboratories would accept to pay to participate ranges from 100 € to 1 000 € and most of the collected answers are between 100 € and 500 €. For a plan covering the different disciplines the fee could be superior to 5 000€ for 2 respondents out of the 22. Most respondents were in favour of a fee between 500 and 2 000 € for a multidisciplinary PT.

6 Conclusions

Different data have been collected using three different methodologies, i) the study of accreditation scopes of some laboratories involved in diagnostics, ii) a workshop with experts, iii) a survey sent to laboratories listed in the EPPO database on diagnostic expertise [3]. The collected data allow a better understanding of the expectations of laboratories, of what laboratories would consider acceptable and of the applicability of the horizontal proficiency testing approach.

From the collected data, the following conclusions have been reached:

- A PT plan should last 3 to 4 years.
- Each year a single PT should be organized per field, it could contain several pests as long as no more than 3 tests have to be performed on a maximum of 10 samples.
- The PT should include whenever possible the different pests analyzed under accreditation, but also emerging pests. The pests to consider as a priority have been listed.
- Samples should mimic as much as possible the routine samples, ideally different matrices should be included in each PT.
- Among the different factors determining the reliability of a test, the design of the PT plan should especially focus on the human factor (i.e. the proficiency of the staff to use the different techniques).
- The laboratories are unlikely to spend more than 3 500 € per year for a service providing PTs covering all their needs.

Based on this feedback and on their knowledge of PT organization, partners of the VALITEST project involved in the 5th work-package will now develop guidelines on an approach to undertake horizontal proficiency testing (Deliverable D5.2 of the project).

REFERENCES

1. *PM 7/76 (5) Use of EPPO Diagnostic Standards.* EPPO Bulletin, 2018. **48**(3): p. 373-377.
2. *PM 7/98 (3) Specific requirements for laboratories preparing accreditation for a plant pest diagnostic activity.* EPPO Bulletin, 2018. **48**(3): p. 387-404.
3. *EPPO database on diagnostic expertise*. Available from: http://dc.eppo.int/.
4. *ISO/IEC 17025:2017 General requirements for the competence of testing and calibration laboratories.*
5. *PM 7/84 (2) Basic requirements for quality management in plant pest diagnostic laboratories.* EPPO Bulletin, 2018. **48**(3): p. 378-386.

ANNEX 1 – Survey sent to experts of laboratories listed in the EPPO database on diagnostic expertise

The text in orange presents the type of answer expected.

VALITEST: collect of the proficiency testing needs

Introduction:

The following survey is performed in the framework of the 5th work package of the VALITEST project.

This project aims at improving the diagnostic in plant health by:

- producing validation data for existing tests,
- adapting the validation process to new technologies,
- developing recommendations for the quality of reference materials,
- optimizing proficiency evaluation,
- stimulating the interactions between stakeholders in Plant Health.

If you want to know more about the project, the consortium, the outputs, please visit our website: https://www.valitest.eu

In Work package 5, our goal is to optimize proficiency evaluation through a horizontal assessment. In other words, we try to identify strategies allowing laboratories to demonstrate their proficiency on a selection of tests corresponding to their need without having to do it test by test.

At this stage we need to identify the needs of laboratories to build a coherent proficiency testing offer, this is why we need as many experts as possible to answer to following questions.

Thank you very much.

Instructions:

You cannot save your answers but you can come back on the webpage several times.

We recommend not using the Firefox web-browser (some issues have been reported).

Contact

Contact data will not be analysed. All the collected data can be accessed or modified upon request sent at valitest@anses.fr.

Contact mail (text, 100 Char)

Laboratory (text, 100 Char)

Country (text, 100 Char)

Proficiency testing needs

You will find below a list of pests analyzed under accreditation in different laboratories.

Indicate for each pest if you need a proficiency test and if so for which category of method. (with proposed methods: Bioassay or Pathogenicity; Molecular; Isolation and/or morphology; Serological; Participants can select one or more methods)

Bacteriology	Bioassay or Pathogenicity	Molecular	Isolation and/or morphology	Serological
Clavibacter michiganensis subsp. *sepedonicus*				
Ralstonia solanacearum				
Erwinia amylovora				
Candidatus Liberibacter solanacearum				
Candidatus Liberibacter asiaticus, africanus or americanus				
Xylella fastidiosa				
Clavibacter michiganensis subsp. *michiganensis*				
Dickeya spp.				
Xhanthomonas axonopodis pv. *phaseoli*				
Xanthomonas axonopodis pv.*dieffenbachiae*				
Pseudomonas syringae pv.*actinidiae*				
Xanthomonas campestris pv. *campestris*				
Xanthomonas citri pv. *citri*				

Mycology	Bioassay or Pathogenicity	Molecular	Isolation and/or morphology	Serological
Phytophthora ramorum				
Monilinia fructicola				
Synchytrium endobioticum				
Tilletia spp.				
Venturia inaequalis				
Guignardia citricarpa				
Fusarium circinatum				
Ascochyta spp.				
Drechslera spp.				
Leptosphaeria nodorum				
Microdochium spp.				
Ustilago nuda				

Virology and Phytoplasmology				
Pospiviroids				
PPV				
PVY, PVA, PVM, PVS, PVX				
ArMV				
GFLV,GVA,GLRaV-1,GLRaV-3				
PLRV				
CTV				
Phytoplasma mali				
Flavescence dorée				
Phytoplasma pyri				
BNYVV, CMV, BBTV, BBrMV				
BCMNV				
PepMV				
TBRV, ACLSV, ApMV, CLRV, PDV, PNRSV, ASGV, RpRSV, SMYEV, TNV, ToRSV				
Other potato viruses				

Nematology				
Potato cyst nematodes				
Bursaphelenchus xylophilus				
Meloidogyne fallax/chitwoodi				
Ditylenchus dipsaci/destructor				

Botany				
Ambrosia artemisiifolia				
Acroptilon repens				
Cenchrus pauciflorus				

Entomology				
Anoplophora glabripennis				
Bemisia tabaci				
Leptinotarsa decemlineata				
Phthorimaea operculella				
Tuta absoluta				

Can you identify other needs in terms of proficiency tests to be offered? (With proposals of pests and of methods: Bioassay or Pathogenicity; Molecular; Isolation and/or morphology; Serological; Participants can select one or more methods)

Pest	Bioassay or Pathogenicity	Molecular	Isolation and/or morphology	Serological

Which horizontal proficiency testing approach?

Based on discussions held during a quality assurance EPPO workshop (Paris, 2019-02-11/13), the following proposals were made, please agree or disagree (comment optional if in agreement and required if in disagreement; Comment limited to 200 characters):

- The proficiency approach should be considered as a multi-year and multi-disciplinary plan designed to last 3 to 4 years and to cover all the needs of laboratories. (Yes; No; comments)

- Ideally, for the most important regulated pests, PT should be available every year "on demand" to cover specific needs of laboratories (e.g. new staff members, new activity…). (Yes; No; comments)

- The PT plan should focus on pests analyzed under accreditation. (Yes; No; comments)

- The PT plan should include emerging pests as soon as validated tests are available. (Yes; No; comments)

- Organizers should try to minimize the number of samples (e.g. multiple pests in each sample). (Yes; No; comments).

- According to you, to how many proficiency tests can a laboratory participate per year and per discipline (Table with all disciplines)

- According to you, how many samples per laboratory seems appropriate for a proficiency test (required number)

- According to you, how many test per laboratory seems appropriate for a PT (required number)

- Samples should be as similar as possible to routine samples. (Yes; No; comments)

- Samples should arrive ready for analysis without any prior preparation (DNA extract, plates, slides…). (Yes; No; comments)

- Different matrices should be included in each PT (e.g. different host species or different plant tissues). (Yes; No; comments)

Participation to proficiency tests

- What would be the most important drivers for you to participate or not to a horizontal proficiency test plan (i.e. an offer of several proficiency tests allowing your laboratory to demonstrate its proficiency on the needed diagnostic tests without having to do it test by test)

Cost	
Coverage of the accreditation scope	
Time required to participate	
Time gained compared to other approaches	
EPPO/accreditation body requirement to participate	

  Others: (free text, 200 char)
- What annual participation fee would seem acceptable to participate in a proficiency testing plan covering the different disciplines
  - 500-2000 €
  - 2000-3500 €
  - 3500-5000 €
  - More than 5000 €
- What annual participation fee would seem acceptable to participate in a proficiency testing plan covering one single discipline
  - 100-500 €
  - 500-750 €
  - 750-1000 €
  - More than 1000 €

Thank you very much for participating.

The outputs of this work will be made public on the website of the VALITEST project (https://www.valitest.eu/).

ANNEX 2 – Documents used during the EPPO Workshop on quality assurance held in Paris in February 2019

Answers from the group:

Bacteriology

Extent of the demonstrated proficiency

To which extent is the proficiency of a laboratory demonstrated following a successfull proficiency test

	Factors determining the correctness and reliability of the tests (ISO17025 & PM7/84)								
Types of methods (PM7/76-5)	Human factors	Accommodation & environmental conditions	Test methods & method validation	Equipment	Measurement traceability	Sampling	Handling of test items	Reference material	Biological material (Matrix)
Bioassay									
Biochemical									
Fingerprint									
Isolation/extraction									
Molecular									
Morphological and morphobiometric									
Pathogenicity assessment									
Serological									

For each type of method and factor, the group shall decide if he consideres a successful proficiency test to demonstrate the proficiency of the laboratory on the:
Test / Method / Field (Mycology, nematology…) / across fields; additional comments are welcome

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N° 773139

Horizontal proficiency assessment: initial questions

Answers from the group:

Bacteriology

Should different pests be included in each round of the Proficiency test?

No
Yes If yes how many _____

For each pest, should the samples represent (1) the different plant species, (2) the different plant parts and (3) other matrices that can be analyzed by laboratories in routine testing?

1)
2)
3)

What should be the frequency of the Proficiency test?

Should different pests be included in each round?

Any suggestions?

ACTIONS FOR THE LABORATORY

Which new measures should the laboratory put into place if the laboratory does not regularly participate in Proficiency tests?

ANNEX 3 – Comments to the questions on the applicability of the horizontal proficiency testing approach.

Comments are provided as written by the respondents

Question: The proficiency approach should be considered as a multi-year and multi-disciplinary plan designed to last 3 to 4 years and to cover all the needs of laboratories.

Comments of laboratories answering "No":

- A test plan for 3-4 years might be too static for new emerging pests, which require fast action.
- Very difficult to organize

Comments of laboratories answering "Yes":

- Most feasible approach
- We will prefer the combination of both approaches. Multi-disciplinary and broad tests (covering broader spectrum of species or testing more parameters) designed to last 3 years. However, because of actual needs of laboratories (new staff etc.) provide also some basic offer of PT aimed on the most important regulated pests and most important diagnostic methods available annually.
- for newly emerging organisms
- Accurate resourcing to prepare test panels is critical - reflects a major difference between plant biosecurity and animal biosecurity
- the competency might be evaluated on a regular basis and regarding a certain type of technique

Question: Ideally, for the most important regulated pests, proficiency test should be available every year "on demand" to cover specific needs of laboratories (e.g. new staff members, new activity…).

Comments of laboratories answering "No":

- only if high number of requests
- Proficiency tests should cover a wide range of pests rather than test the same ones each year. The training of new staff members is the responsibility of the individual labs and not of proficiency tests!
- Why "on demand"? to cover specific needs of laboratories? The aim of a proficiency test is to show the ability to give reliable results
- Very difficult or impossible to organize "on demand"
- that probably to much to go for
- The frequency would be too high and not necessary. If a lab is involved in many PT, it would probably be tested each year for a similar competency (PCR, serological,...). Especially in view of the previous proposal.

Comments of laboratories answering "Yes":

- Also to cover 17025 needs
- ideally... But in turn, organization of PT should be organized every year. A dedicated structure (private body ?) should be ready to propose such a frequency.
- bilateral, problem orientated

Question: The proficiency test plan should focus on pests analyzed under accreditation.

Comments of laboratories answering "No":

- We might wish to participate in PT for a pest we do not have accreditation (yet)
- This should be open to all labs, not just those under accreditation.
- The proficiency tests are useful and convenient regardless the accreditation
- The scope of accreditation and needs of laboratories can be different. So the PT should focus primarily on regulated and

Comments of laboratories answering "Yes":

- but not restricted to accredited ones
- In particular in the first years, but then it should be extended.
- But not critical - practice and external, friendly evaluation of diagnosticians and technicians is the real value
- Needed for ISO17025 accreditation and specially for NRL performing a limited amount of routine test

quarantine pests and their relatives than on pests analyzed under accreditation.
- pest analysed under accreditation are not specifically defined by now
- No, because the number of analyzable pests under accreditation is currently very small
- we want uniform quality on all tests
- Our lab is not accredited but we also need proficiency tests as a quality control
- The accreditation is dependant of lab strategy.

Question: The proficiency test plan should include emerging pests as soon as validated tests are available.

Comments of laboratories answering "No":

- We might not decide to monitor a particular pest in our country
- The proficiency test plan should include emerging pests without taking into consideration the existence of validated tests

Comments of laboratories answering "Yes":

- absolutely yes, like in the case of ToBRFV
- This would be very useful, but PTs focusing on accredited and well established methods are more important and fundamental to maintain/achieve accredited status
- To help regulator to be confident on the results and help EC to be confident for homogeneity between MS in case of outbreak

Question: Organizers should try to minimize the number of samples (e.g. multiple pests in each sample).

Comments of laboratories answering "No":

- but sample sets should also contain individual mixed infected samples
- This could be a good idea, but I don't know how this could be done for example for the field of forest pathology - different hosts; different organisms; ...
- It is good to involve broader spectrum of related or similar pests in PT because of specificity of diagnostic methods and also try to designed samples as a real samples. However the approach has to be specify in advance.
- difficult to achieve. Biology, load and distribution of inoculum of the pathogens on organs and plants are different, validation of multiple infected / contaminated samples will be a challenge.
- The number of samples is not a problem for us
- This is already the case
- start easy
- Not essential - it might be more economical to send out a number of batches, but not too fussed either way
- not necessary and should favour the full process (grinding process, extraction, amplification)

Comments of laboratories answering "Yes":

- if possible
- As long as the lab is given the opportunity to select only the pest of interest
- case related, if sensitive and specific enough
- minimum 10 samples per test

Question: Samples should be as similar as possible to routine samples?

Comments of laboratories answering "No":

- generally yes, but homogeneity and stability are of utmost importance, hence (freeze) dried samples are most suitable)
- The samples of insects and mites sent must first be identified, which then does not make it possible to provide samples similar to routine samples

Comments of laboratories answering "Yes":

- Comment for the number of tests etc - this is difficult; it depends on the lab and the capacities. My lab could (ideally) do 2 PT per year (current situation).
- Yes, but some pest are not cultivable and difficult to process to prepare artificially contaminated samples (eg rusts, smuts in mycology).
- without compromising the homogeneity of the samples. Homogenisation of the samples is achieved with different

methods and different labs use different machines/tubes/grinding matrices, this step can be problematic (from a similarity to routine samples point of view) if PT samples are submitted in an incompatible format.
- would be optimal but due to biosafety legislation difficult to handle
- prefer plant samples
- to test for the all process from samples preparation to test

Question: Samples should arrive ready for analysis without any preparation in the participant laboratory (DNA extract, plates, slides…).

Comments of laboratories answering "No":

- Samples ready to analyse would mean quicker analysis of PT samples , but result could be less reliable
- sample preparation and/or extraction are part of the test
- The preparation steps are as important as the "analysis".
- not necessarily
- Yes in general, but it depends on the kind of test and the kind of sample
- It is better to have a chance to go through all the process (extraction/detection/identification) than only run e.g. PCR from DNA).
- The preparation of samples is an integral step for each diagnosis
- samples should be similar to routine samples (roots, leaves, soil…) and treated as routine samples
- Nucleic acid extraction also important factor
- No, if feasible, preparation should be included in the PT, because it may be a very important step. However, in some case, it is not possible, from a technical or biological point of view.
- Not necessarily, but it is possible.
- The preparation is an important step in routine analyses and should also be checked
- sample preparation is highly important but due to biosafety legislation highly demanding in administration
- Either/or - our biggest issue with plant tissue is import permits, but really, that is our problem
- The samples should arrive as they would in a diagnostic case
- more complicated but closer than the true life

Comments of laboratories answering "Yes":

- This would ensure homogeneity of samples, however accreditation bodies may want to see also the prep steps being proficiency tested
- This is often the only possibility.

Question: Different matrices should be included in each proficiency test (e.g. different host species or different types of plant tissue).

Comments of laboratories answering "No":

- The number of samples might rise too high
- depends on the type of test
- It depends on the kind of pathogen and the objective of the test
- Different matrices would be beneficial for some pests but not necessary for all.
- No for different host species but yes if the analysis is different according to the matrices
- Depends on the material analyzed in the laboratory.
- Not applicable for entomology and acarology since samples are provided without support matrix

Comments of laboratories answering "Yes":

- only if relevant for the virus species to be tested for (e.g. PSTVD in Tomato and other host), otherwise part of validation
- This is one of the advantage of multi-disciplinary tests which can be big, broad and cover more pests, more matrices and be done by more methods.
- The samples should arrive as they would in a diagnostic case
- if needed regarding the material routinely tested