Optimization of Proficiency Assessment

EPPO Workshop for Heads of laboratories 20-04-2023

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ANSES

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Valitest

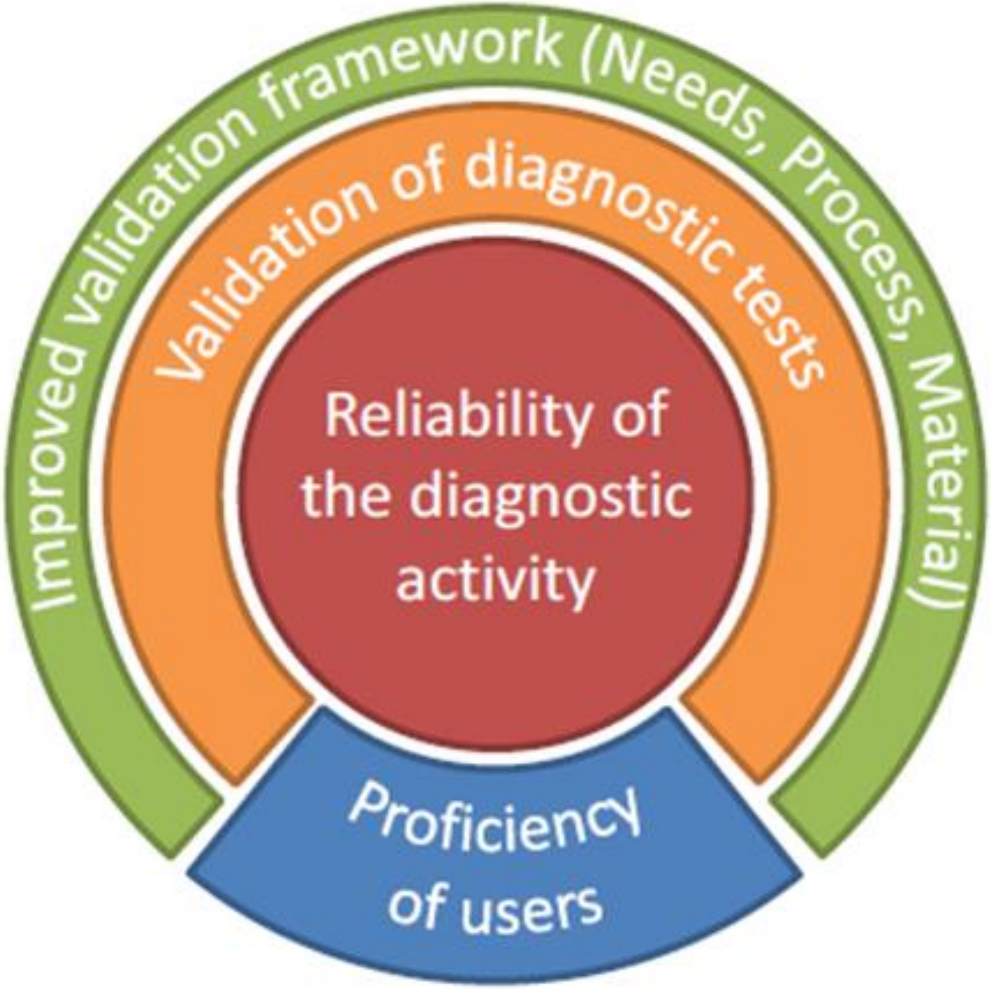
VALITEST project in brief

H2020-VALITEST project n°773139: Validation of diagnostic tests in support of plant health

- *Funded by EU commission: 3 millions €*
- *Duration: 3 years 1/2 (01/05/18 to 30/10/21)*
- *Coordination: ANSES – Plant Health Laboratory*
- *Consortium: 16 partners*

anses
Investigate, evaluate, protect
crea
PIORIN
Netherlands Food and Consumer Product Safety Authority
Ministry of Agriculture, Nature and Food Quality
NIB
NATIONAL INSTITUTE OF BIOLOGY
Clear Detections
revolutionary diagnostics
fera
YSEDIAG
Anticorps et Diagnostic
Ipa&d Lab
INTERNATIONAL PLANT ANALYSIS & DIAGNOSTICS
BIOREBA
Your Partner in Agro-Diagnostics
LOEWE®
Schweizerische Eidgenossenschaft
Confédération suisse
Confederazione Svizzera
Confederaziun svizra
ug
Gembloux Agro-Bio Tech
Université de Liège
AGRINNOVA
WAGENINGEN
UNIVERSITY & RESEARCH

VALITEST project in brief



WP 5: Optimization of proficiency assessment

Context

Current approach of proficiency assessment is based on regular proficiency tests for each combination of method/matrix/pest

Accredited testing activities performed by the laboratory in Bacteriology

- Detection of *Clavibacter insidiosus* in seeds by IF
- Detection of *Clavibacter michiganensis* subsp. *michiganensis* in symptomatic plant material by isolation, IF, real-time PCR, PCR sequencing, pathogenicity test or MALDI-TOF MS
- Detection of *Clavibacter michiganensis* subsp. *michiganensis* in seeds by IF
- Detection of *Clavibacter sepedonicus* in symptomatic plant material by isolation or MALDI-TOF MS
- Detection of *Clavibacter sepedonicus* in tubers by IF or PCR
- Detection of *Erwinia amylovora* in symptomatic or asymptomatic plant material by isolation
- Detection of *Pantoea stewartii* subsp. *stewartii* in seeds by isolation or real-time PCR
- Detection of *Pseudomonas savastanoi* pv. *phaseolicola* in seeds by isolation
- Detection of *Pseudomonas syringae* pv. *actinidiae* in symptomatic plant material by isolation
- Detection of *Pseudomonas syringae* pv. *actinidiae* in asymptomatic plant material by PCR
- Detection of *Ralstonia solanacearum* in symptomatic plant material or tubers by isolation, IF, real-time PCR, PCR, PCR sequencing or pathogenicity test
- Detection of *Ralstonia solanacearum* in water, soil or other substrate by isolation
- Detection of *Xanthomonas campestris* pv. *vesicatoria, X. euvesicatoria, X. gardneri, X. perforans, X. vesicatoria* in symptomatic plant material by isolation, IF or PCR
- Detection of *Xanthomonas fragariae* in symptomatic plant material by isolation, IF or real-time PCR
- Detection of *Xanthomonas axonopodis* pv. *phaseoli, X. fuscans* subsp. *fuscans* in symptomatic plant material by isolation or PCR
- Detection of *Xanthomonas axonopodis* pv. *phaseoli, X. fuscans* subsp. *fuscans* in seeds by isolation
- Detection of *Xanthomonas axonopodis* pv. *citri* in symptomatic plant material by isolation
- Detection of *Xanthomonas axonopodis* pv. *dieffenbachiae* in symptomatic plant material by isolation, IF or PCR
- Detection of *Xanthomonas arboricola* pv. *pruni* in symptomatic plant material by isolation, IF or real-time PCR
- Detection of *Xylella fastidiosa* in symptomatic plant material by isolation, real-time PCR or PCR sequencing
- Detection of *Xylella fastidiosa* in asymptomatic plant material by real-time PCR
- Detection of *Xylophilus ampelinus* in symptomatic plant material by PCR

→ Not sustainable with the resources available

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Objectives

- Identification of possible horizontal proficiency tests.
- Consultation of accreditation bodies.
- Preparation of guidelines

Applicability: Identification of critical points

Evaluate the applicability of the horizontal proficiency testing approach:

Discussion during an EPPO workshop (2019/02 –Paris*): half a day with diagnostics and QA experts to brainstorm by groups of expertise

* https://www.eppo.int/MEETINGS/2019_meetings/w_pm7_98

Applicability: Identification of critical points

Factors determining the correctness and reliability of a test

Factors	ISO 17025 (2017)	EPPO PM7/84(2) *
Human factors	X	X
Accommodation and environmental conditions	X	X
Test methods and method validation	X	X
Equipment	X	X
Measurement traceability	X	
Sampling	X	X
Handling of test items	X	X
Reference material		X

* Basic requirements for quality management in plant pest diagnostic laboratories

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Applicability: Identification of critical points

Extent to which these factors contribute to the uncertainty ?

For each method/test:

⇒ is a proficiency result valid for a different test
(e.g. a result valid for different ELISA tests ?)

⇒ is it valid for a different method (serology, molecular) ?

⇒ is it valid for all methods in the field (bacteriology) ?

⇒ Is it valid for all methods across fields ?

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Applicability: Identification of critical points

Factors	Views of experts
Human factors	Isolation/morphology/Bioassay: Only valid for the test
	Molecular/serology: Valid across fields
Accommodation and environmental conditions	Valid across fields
Test methods and method validation	Not under the scope of PT
Equipment	Valid across fields (except dedicated equipment)
Measurement traceability	Valid across fields
Sampling	Not under the scope of PT
Handling of test items	Valid across fields
Reference material	Not under the scope of PT

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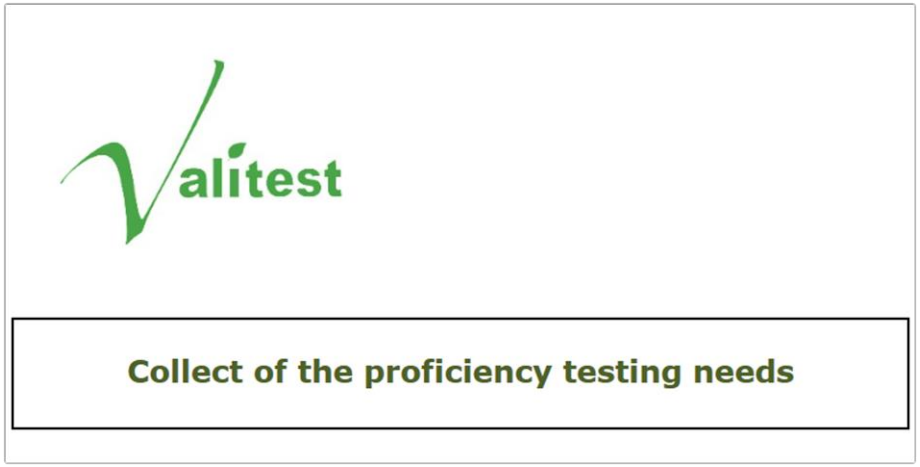
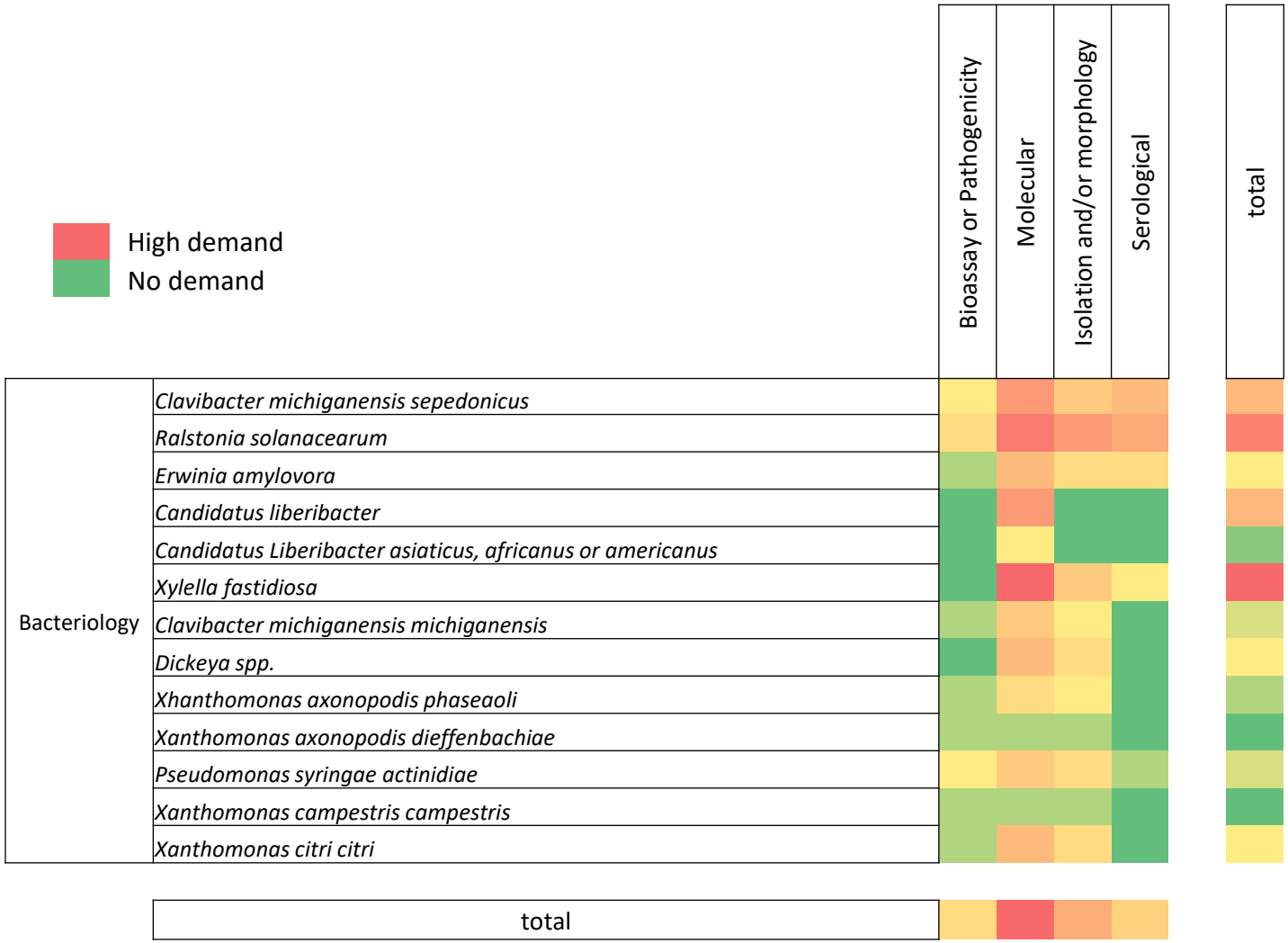
Needs of laboratories

II - Survey addressed to all the laboratories of the EPPO database on diagnostic expertise:



Needs of laboratories: Data collected



Needs of laboratories: Data collected

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.

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…).

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

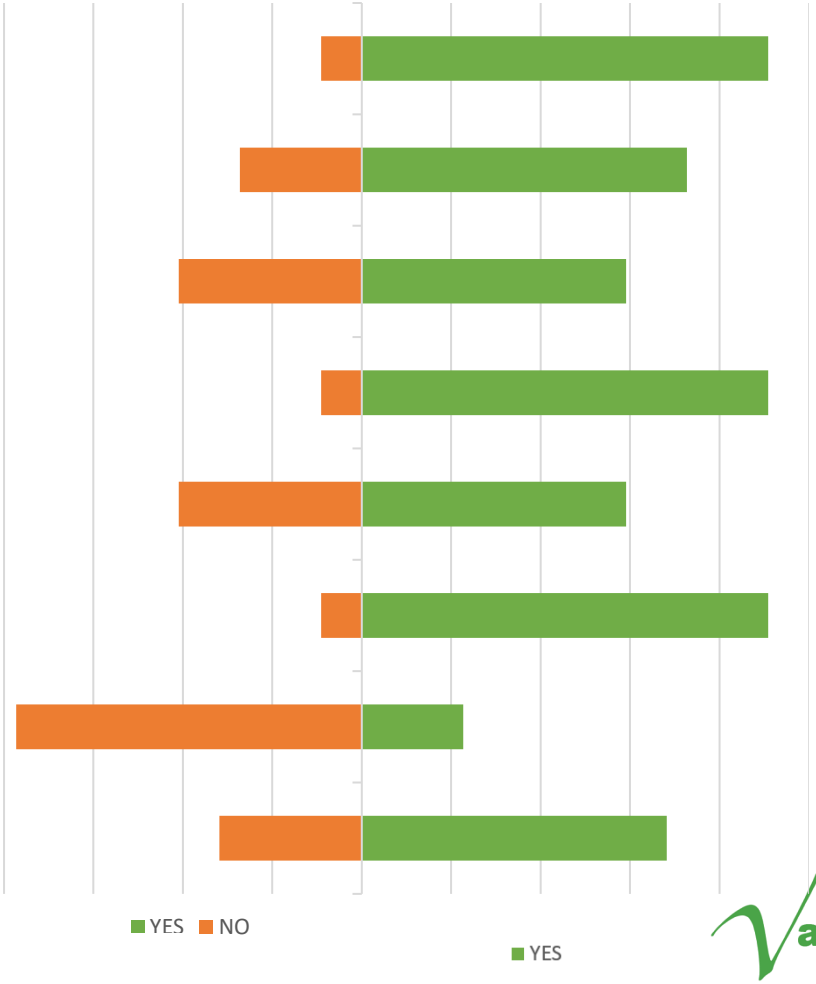
The proficiency test plan should include emerging pests as soon as validated tests are available.

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

Samples should be as similar as possible to routine samples?

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

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



Outputs: Deliverable 1

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Grant agreement N. 773139

DELIVERABLE N° 5.1

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

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Validation of diagnostic tests to support plant health

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AB consultation

Question raised:

Is it possible to assess the proficiency of laboratories horizontally ?

Is a PT result robust from one PT to another, using different methods, on different pests ?

AB consultation

- European accreditation recommendations: EA-4/18 INF2010 - Guidance on the level and frequency of proficiency testing participation
  - Risk based approach
  - The laboratory should identify sub-disciplines: “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”

AB consultation

- Consultation of Cofrac (French accreditation body)

  - Use of the collected data to identify analysis groups defined in such a way that a PT result is valid for all the group ?

Factors	Views of experts
Human factors	Isolation/morphology/Bioassay: Only valid for the test
	Molecular/serology: Valid across fields
Accommodation and environmental conditions	Valid across fields
Test methods and method validation	Not under the scope of PT
Equipment	Valid across fields (except dedicated equipment)
Measurement traceability	Valid across fields
Sampling	Not under the scope of PT
Handling of test items	Valid across fields
Reference material	Not under the scope of PT

  - Example:

✓ PT on a serological test → ? → ✓ Serological tests on other pests/matrices

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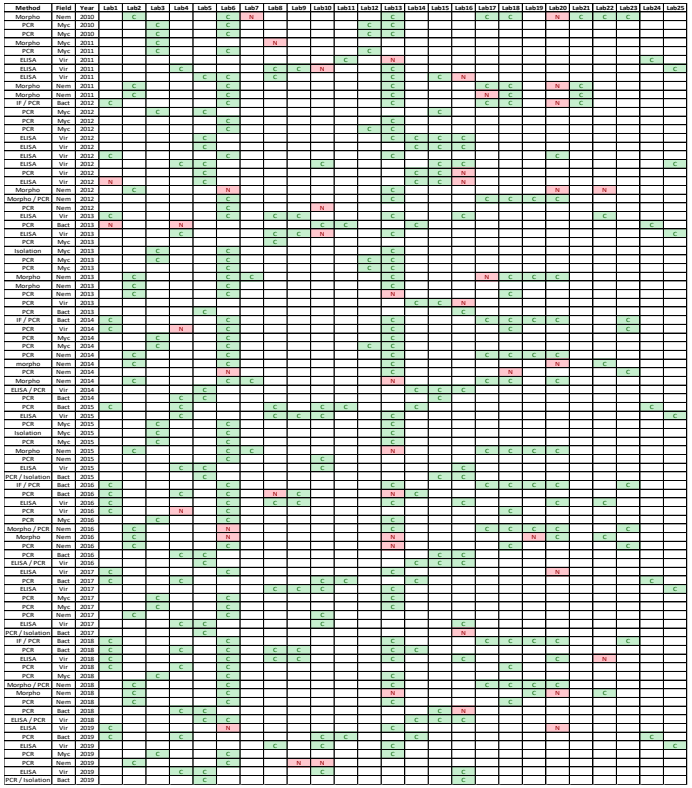
AB consultation

- Consultation of Cofrac (French accreditation body)
  - A PT is required for each combination of method/matrix/pest
    - Before accreditation
    - At least once per accreditation cycle
  - Each laboratory has to conduct its own risk analysis to design its own PT participation plan
  - Limited PT participation requires an enforceable document demonstrating the feasibility of this approach on the basis of objective data

AB consultation

- Demonstrate the feasibility of approach on the basis of objective data ?



PT data of a network of laboratories using the French official methods from 2010 until 2019

Considering the available data and the identified biais, it is not possible to conclude concerning the robustness of a PT result

Outputs : Deliverable 2

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Grant agreement N. 773139

DELIVERABLE N° 5.2

Title: Guidelines on an approach to undertake horizontal proficiency testing

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Validation of diagnostic tests to support plant health

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AB consultation

- Consultation of EA (European Accreditation): EA-4/18 INF2010 - Guidance on the level and frequency of proficiency testing participation

  The laboratory should identify sub-disciplines: “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”

AB consultation

- Consultation of EA (European Accreditation): EA-4/18 INF2010 - Guidance on the level and frequency of proficiency testing participation

  - Case Study 1 – Environmental Chemistry Testing Laboratory
  - Case Study 2 – Microbiology Testing Laboratory
  - Case Study 3 – Clinical Testing Laboratory
  - Case Study 4 – Physical Testing Laboratory
  - Case Study 5 – Matrix Approach (Clinical Chemistry)

  => Plant health ?

AB consultation

- Consultation of EA:
  - Considering the number of disciplines, it is not possible to include a specific case study for each
  - EA encouraged us to build our own case study

Case Study 1 – Environmental Chemistry Testing Laboratory

Accredited testing activities performed by the laboratory:

- Polychlorinated Biphenyls (PCB) by GC-MS in Soils and Sewage Sludge
- Polyaromatic Hydrocarbons (PAH) by GC-MS in Soils and Sewage Sludge
- Volatile Organic Compounds (VOC) by Purge and Trap GC-MS in Waters
- Metals by ICP-MS in Soils, Sewage Sludge and Waters
- pH in Soils, Sewage Sludge and Waters

Considerations for determinations of sub-disciplines:

For pH the laboratory identifies that it utilises the same standard ISO Method for all three matrices (Soils, Waters and Sewage Sludges). This ISO Method has been validated against all three matrices and therefore the laboratory identifies this as one sub-discipline

For the analysis of metals the laboratory identifies that it uses the same measurement technique (ICP-MS) for all three matrices (Soils, Waters and Sewage Sludge). However, the preparation of Water samples compared to Soils and Sewage Sludges is significantly different. As such the laboratory identifies that it cannot declare this as one sub-discipline, but as the methodologies for soils and sewage sludge are demonstrably comparable they can be. Therefore the laboratory identifies two more sub-disciplines

For PAH and PCB analysis the laboratory identifies that it uses the same measurement technique (GC-MS) and the extraction of the matrices (Soils and Sewage Sludge) is identical for both matrices. However, via its initial validation of the methods it is apparent that PCB and PAH are effected in different ways by variations in the methodology and therefore acceptable performance or problematic performance on PCB would not necessarily mean the same for PAH (and vice versa). Therefore the laboratory identifies two more sub-disciplines

For its VOC method the laboratory only has one matrix (water) to consider. However the laboratory is aware that the method analyses several different properties that could potentially react in different ways to problems with the method. Through its method validation data the laboratory has demonstrated that the differing properties react in comparable ways to variations in the method. Therefore the laboratory identifies one more sub-discipline.

Resulting sub-disciplines from this exercise:

- Polychlorinated Biphenyls (PCB) by GC-MS in Soils and Sewage Sludge
- Polyaromatic Hydrocarbons (PAH) by GC-MS in Soils and Sewage Sludge
- Volatile Organic Compounds (VOC) by Purge and Trap GC-MS in Waters
- Metals by ICP-MS in Soils and Sewage Sludge
- Metals by ICP-MS in Waters
- pH in Soils, Sewage Sludge and Waters

Prepare a similar document based on the accreditation scopes and analysis offers of ANSES and NVWA on bacteriology

(ANSES/EPPO/NVWA)

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Case study – Plant pathology

Accredited testing activities performed by the laboratory in Bacteriology

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- Detection of *Clavibacter michiganensis* subsp. *michiganensis* in seeds by IF
- Detection of *Clavibacter sepedonicus* in symptomatic plant material by isolation or MALDI-TOF MS
- Detection of *Clavibacter sepedonicus* in tubers by IF or PCR
- Detection of *Erwinia amylovora* in symptomatic or asymptomatic plant material by isolation
- Detection of *Pantoea stewartii* subsp. *stewartii* in seeds by isolation or real-time PCR
- Detection of *Pseudomonas savastanoï* pv. *phaseolicola* in seeds by isolation
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- Detection of *Pseudomonas syringae* pv. *actinidiae* in asymptomatic plant material by PCR
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- Detection of *Xanthomonas arboricola* pv. *pruni* in symptomatic plant material by isolation, IF or real-time PCR
- Detection of *Xylella fastidiosa* in symptomatic plant material by isolation, real-time PCR or PCR sequencing
- Detection of *Xylella fastidiosa* in asymptomatic plant material by real-time PCR
- Detection of *Xylophilus ampelinus* in symptomatic plant material by PCR

<u>7 measurement technique:</u>

- Isolation
- IF
- Real-time PCR
- PCR
- PCR sequencing
- Pathogenicity test
- MALDI-TOF MS

<u>6 products:</u>

- Symptomatic plant material
- Asymptomatic plant material
- Seeds
- Tubers
- Water
- Soil/substrate

Valitest

Case study – Plant pathology

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- Detection of *Xylophilus ampelinus* in symptomatic plant material by PCR

	Product					
Measurement technique	**SP**	**AP**	**Se**	**T**	**W**	**So**
Isolation						
IF						
Real-time PCR						
PCR						
PCR sequencing						
Pathogenicity test						
MALDI-TOF MS						

SP: Symptomatic plant material
AP: Asymptomatic plant material
Se: Seeds
T: Tubers
W: Water
So: Soil/substrate

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Case study – Plant pathology

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Characteristic / Measurement technique	*Clavibacter insidiosus*						*Clavibacter michiganensis* subsp. *michiganensis*						*Clavibacter sepedonicus*						*Erwinia amylovora*					
Product	SP	AP	Se	T	W	So	SP	AP	Se	T	W	So	SP	AP	Se	T	W	So	SP	AP	Se	T	W	So
Isolation							■						■						■	■				
IF			■				■		■							■								
Real-time PCR							■																	
PCR																■								
PCR sequencing							■																	
Pathogenicity test							■																	
MALDI-TOF MS							■						■											

Characteristic / Measurement technique	*Pantoea stewartii* subsp. *stewartii*						*Pseudomonas savastanoï* pv. *phaseolicola*						*Pseudomonas syringae* pv. *actinidiae*						*Ralstonia solanacearum*					
Product	SP	AP	Se	T	W	So	SP	AP	Se	T	W	So	SP	AP	Se	T	W	So	SP	AP	Se	T	W	So
Isolation			■						■				■						■			■	■	■
IF																			■			■		
Real-time PCR			■																■			■		
PCR														■					■			■		
PCR sequencing																			■			■		
Pathogenicity test																								
MALDI-TOF MS																								

Characteristic / Measurement technique	*Xanthomonas campestris* pv *vesicatoria, X. euvesicatoria, X. gardneri, X. perforans, X. vesicatoria*						*Xanthomonas fragariae*						*Xanthomonas axonopodis* pv. *phaseoli, X. fuscans* subsp. *fuscans (X. a. pv. phas var. fuscans)*						*Xanthomonas axonopodis* pv. *citri*					
Product	SP	AP	Se	T	W	So	SP	AP	Se	T	W	So	SP	AP	Se	T	W	So	SP	AP	Se	T	W	So
Isolation	■						■						■		■				■					
IF	■						■																	
Real-time PCR							■																	
PCR	■												■											
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Characteristic / Measurement technique	*Xanthomonas axonopodis* pv. *dieffenbachiae*						*Xanthomonas arboricola* pv. *pruni*						*Xylella fastidiosa*						*Xylophilus ampelinus*					
Product	SP	AP	Se	T	W	So	SP	AP	Se	T	W	So	SP	AP	Se	T	W	So	SP	AP	Se	T	W	So
Isolation	■						■						■											
IF	■						■																	
Real-time PCR							■						■	■										
PCR	■																		■					
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Pathogenicity test																								
MALDI-TOF MS																								

SP: Symptomatic plant material
AP: Asymptomatic plant material
■ Activity performed under accreditation

Se: Seeds
T: Tubers

W: Water
So: Soil/substrate

Case study – Plant pathology

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- Detection of *Xylophilus ampelinus* in symptomatic plant material by PCR

Characteristic / Measurement technique	*Clavibacter insidiosus*						*Clavibacter michiganensis* subsp. *michiganensis*						*Clavibacter sepedonicus*						*Erwinia amylovora*					
Product	SP	AP	Se	T	W	So	SP	AP	Se	T	W	So	SP	AP	Se	T	W	So	SP	AP	Se	T	W	So
Isolation							■						■						■	■				
IF							■		■							■								
Real-time PCR							■																	
PCR							■									■								
PCR sequencing							■																	
Pathogenicity test							■																	
MALDI-TOF MS							■						■											

Characteristic / Measurement technique	*Pantoea stewartii* subsp. *stewartii*						*Pseudomonas savastanoï* pv. *phaseolicola*						*Pseudomonas syringae* pv. *actinidiae*						*Ralstonia solanacearum*					
Product	SP	AP	Se	T	W	So	SP	AP	Se	T	W	So	SP	AP	Se	T	W	So	SP	AP	Se	T	W	So
Isolation			■				■		■				■						■			■	■	
IF																			■					
Real-time PCR			■																■			■		
PCR													■						■			■		
PCR sequencing																			■			■		
Pathogenicity test																			■					
MALDI-TOF MS																								

Characteristic / Measurement technique	*Xanthomonas campestris* pv *vesicatoria, X. euvesicatoria, X. gardneri, X. perforans, X. vesicatoria*						*Xanthomonas fragariae*						*Xanthomonas axonopodis* pv. *phaseoli, X. fuscans* subsp. *fuscans (X. a. pv. phasvar. fuscans)*						*Xanthomonas axonopodis* pv. *citri*					
Product	SP	AP	Se	T	W	So	SP	AP	Se	T	W	So	SP	AP	Se	T	W	So	SP	AP	Se	T	W	So
Isolation	■						■						■		■				■					
IF																								
Real-time PCR							■																	
PCR	■												■											
PCR sequencing																								
Pathogenicity test																								
MALDI-TOF MS																								

Characteristic / Measurement technique	*Xanthomonas axonopodis* pv. *dieffenbachiae*						*Xanthomonas arboricola* pv. *pruni*						*Xylella fastidiosa*						*Xylophilus ampelinus*					
Product	SP	AP	Se	T	W	So	SP	AP	Se	T	W	So	SP	AP	Se	T	W	So	SP	AP	Se	T	W	So
Isolation	■						■						■											
IF																								
Real-time PCR							■						■	■										
PCR	■																		■					
PCR sequencing													■											
Pathogenicity test																								
MALDI-TOF MS																								

SP: Symptomatic plant material
AP: Asymptomatic plant material
■ Activity performed under accreditation

Se: Seeds
T: Tubers

W: Water
So: Soil/substrate

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7 sub-disciplines

- Detection of bacteria by isolation
- Detection of bacteria by IF
- Detection of bacteria by real-time PCR
- Detection of bacteria by PCR
- Detection of bacteria by PCR sequencing
- Detection of bacteria by pathogenicity test
- Detection of bacteria by MALDI-TOF MS

Measurement technique

Product	SP	AP	Se	T	W	So
Isolation	■	■	■	■	■	■
IF	■		■	■		
Real-time PCR	■	■	■	■		
PCR	■	■		■		
PCR sequencing	■			■		
Pathogenicity test	■					
MALDI-TOF MS	■					

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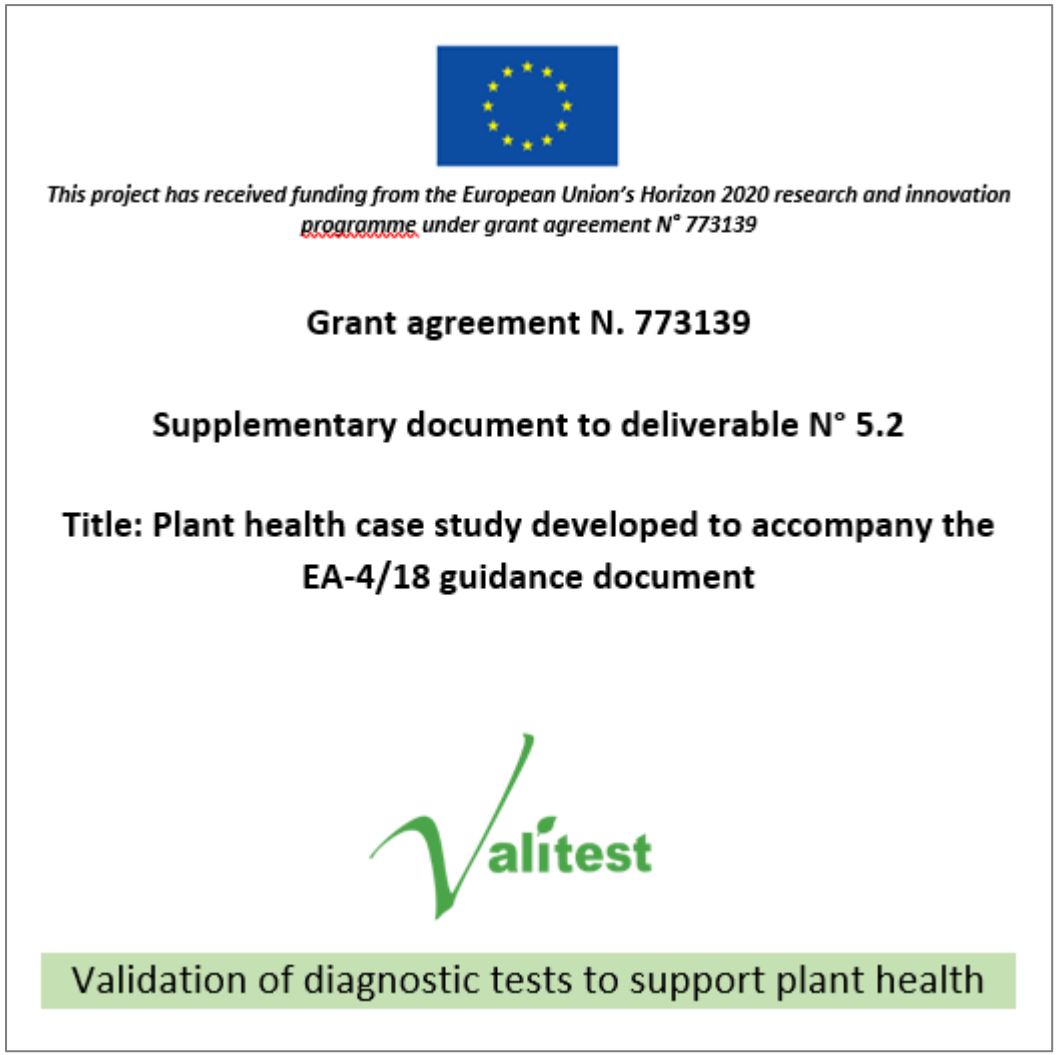
Valitest

- Detection of bacteria by isolation
- Detection of bacteria by IF
- Detection of bacteria by real-time PCR
- Detection of bacteria by PCR
- Detection of bacteria by PCR sequencing
- Detection of bacteria by pathogenicity test
- Detection of bacteria by MALDI-TOF MS

Proficiency testing strategy

- One proficiency test by sub-discipline (i.e. one PT per measurement technique: Isolation, IF, real-time PCR…) and by accreditation cycle.
- Evaluate the necessity to undertake PTs specifically covering all the products in the scope on a periodic basis.
- Participate in PTs covering as many pest species/taxa as possible during each accreditation cycle.
- Prioritize the participation in the different PTs (testing activities, number of samples, analyses identified as technically challenging, higher phytosanitary risk).
- Elaborate a detailed and duly justified proficiency testing strategy. It is recommended that this strategy includes a multi-annual PT participation plan covering an accreditation cycle.

Outputs : Supplementary document to Deliverable 2



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Grant agreement N. 773139

Supplementary document to deliverable N° 5.2

Title: Plant health case study developed to accompany the EA-4/18 guidance document

Valitest

Validation of diagnostic tests to support plant health

Valitest

Thank you for your attention!

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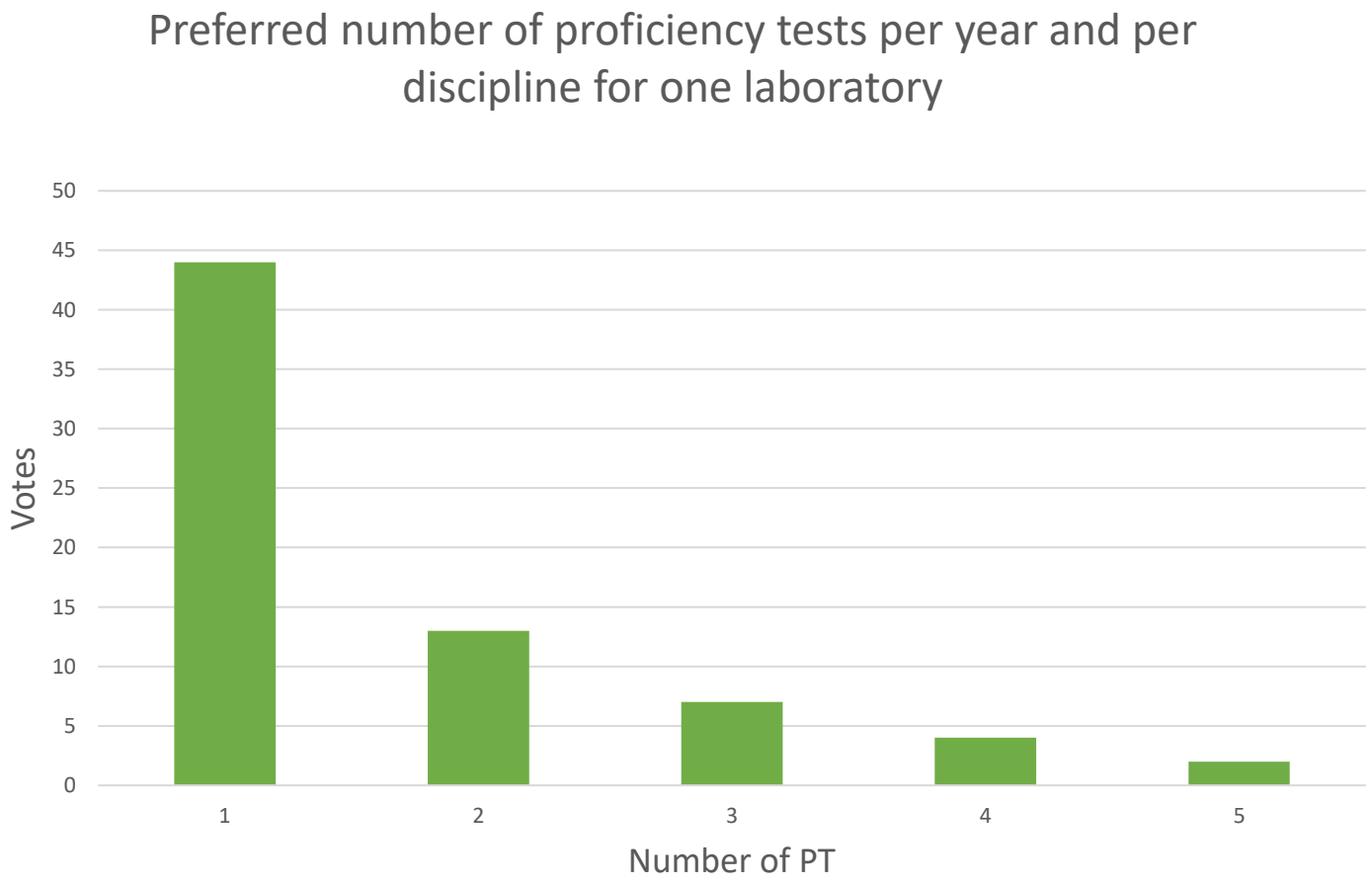
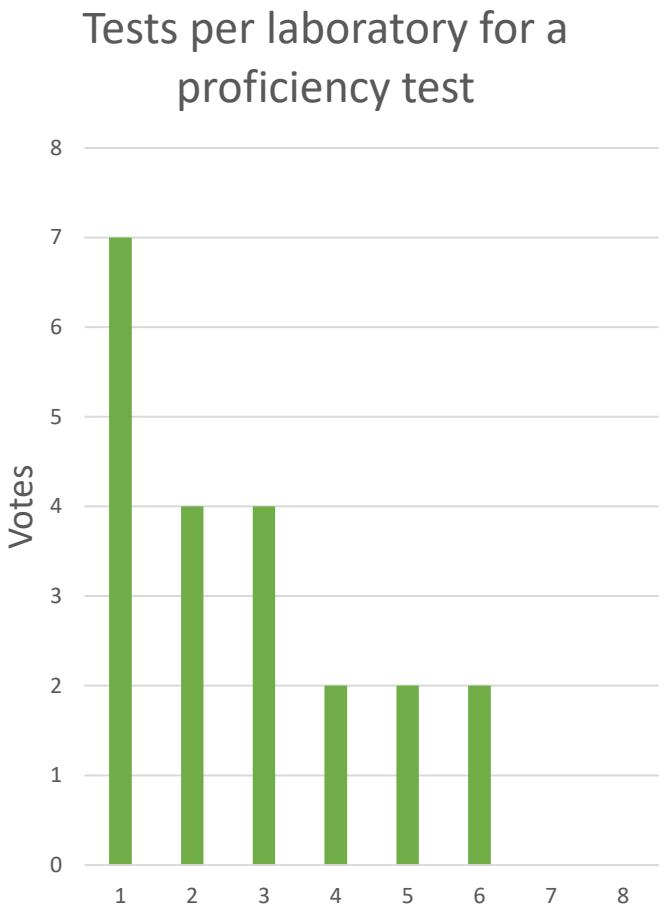
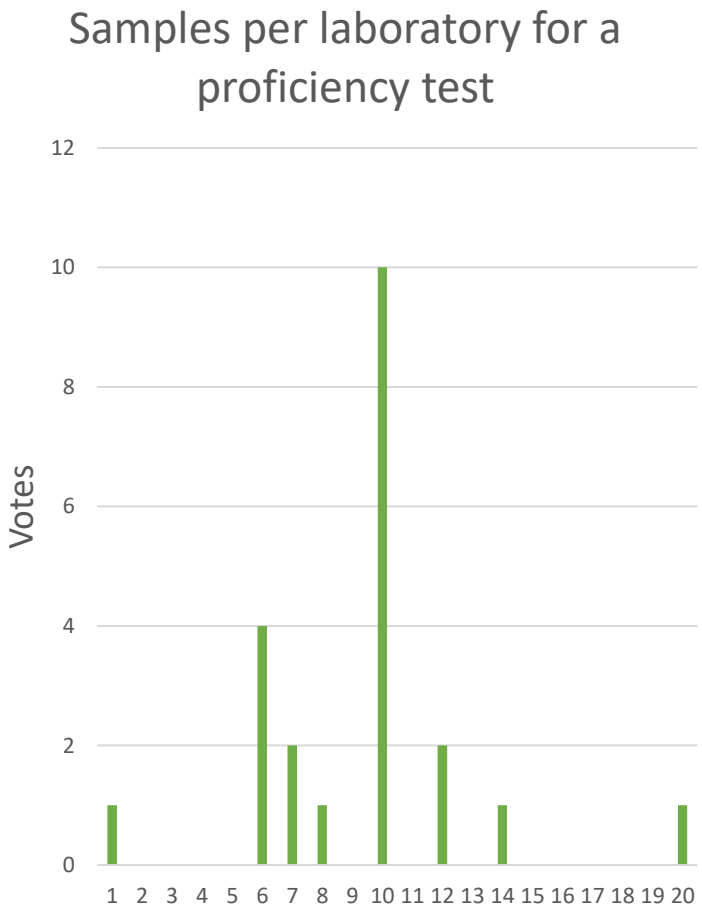
Valitest

WP5
Data collected



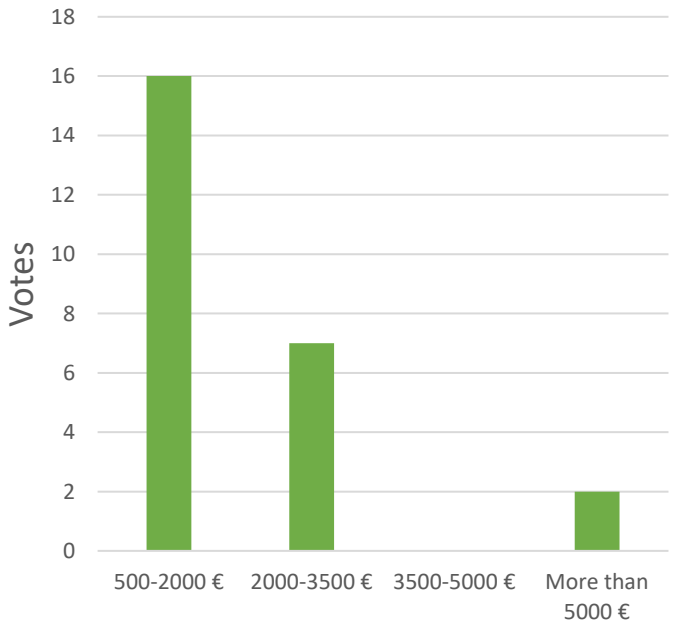
WP5
Data collected



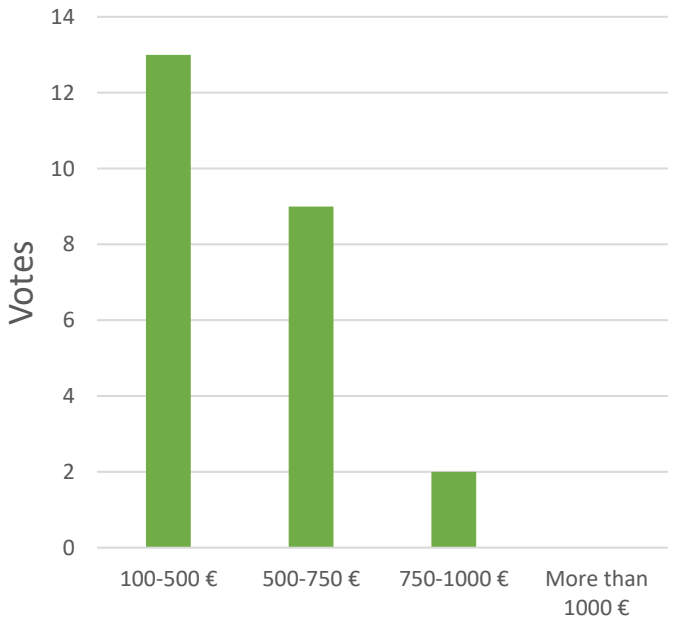


WP5
Data collected

Annual fee for a PT plan covering the different disciplines



Annual fee for a PT plan covering one single discipline



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