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° 1.1 – V2

Title: Minimum performance parameters to select tests for validation and selection of laboratories for TPS

Validation of diagnostic tests to support plant health

Due date:	Month 3
Actual submission date	04-03-2019 (Month 11)
Start date of the project	01-05-2018
Deliverable lead contractor (organization name)	NIB, NVWA
Participants (Partners short names)	NIB, NVWA, FERA, ANSES
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Level of dissemination	Public
Type of Deliverable	Report

Abstract:
The aim of deliverable 1.1. is to prepare criteria to select tests for validation and to select laboratories for TPS (test performance study). Criteria for selection of tests for the TPS for each pest have been set (see **Tables 7-12**). These criteria have been divided in five groups: 1) validation data, 2) applicability, 3) protocols, 4) chemicals and 5) equipment. For selection of participants for the TPS selection criteria have also been set (see **Table 13**). Amongst the most important criteria for selection for participants of TPS are technical expertise for the pest group and the method, authorization to work with the specific pest and that the participating laboratory has quality assurance in place. These criteria enable evaluation of whether participants are proficient to perform the tests, have the necessary equipment and a permit to work with viable regulated organism. The scope of the testing for specific pests was set and common rules for each selection process was defined.

Partners involved Task NIB, NVWA, FERA, ANSES

HISTORY OF CHANGES		
Version	Publication date	Change
1.0	04 March 2019	Initial version
2.0	11 February 2020	Following the review of the project, harmonization of the vocabulary between “in-house”, “prevalidation” and “preliminary study”. Consistency of the use of the term “preliminary study”. Clarification of the definition of “test”.

Contents

1 Purpose

The aim of deliverable 1.1. is to prepare criteria to select tests for validation and to select laboratories for TPS (test performance study) in the frame of WP1 of VALITEST project, in which the aim is to coordinate (prepare and organize) test validations and running of TPSs for prioritized pests in a range of matrices and for a range of diagnostic technology related platforms (both laboratory and on site-based). Test is defined in EPPO Standard PM 7/76 as the application of a method to a specific pest and a specific matrix. TPS Round 1 (in year 1) is focused on six preselected pests (*Bursaphelenchus xylophilus*, *Erwinia amylovora*, *Pantoea stewartii* subsp. *stewartii*, citrus tristeza virus, plum pox virus and *Fusarium circinatum*) for which the test/participant selection criteria are listed here and weighted according to the scope of each TPS, also defined in this deliverable. Furthermore, to ensure a transparent process for selection of tests for validation and selection of laboratories for TPS, a detailed set of common rules for each selection process was defined and described, also included as a part of this deliverable.

2 Scope

The criteria prepared in this deliverable will be directly used to select tests for validation and to select laboratories for TPS in Round 1 (in year 1 and 2). During the TPS process the results will be evaluated and the criteria adapted accordingly in Round 2 if needed. In addition, during the first year of the project, analysis in the frame of WP4 of VALITEST project will conclude with the selection of further pests where tests validation is a priority and which will be the focus of TPS in Round 2 (in year 2 and 3). The criteria prepared in this deliverable will be used as a guidance for selection of tests for validation and selection of laboratories for TPS in Round 2. Furthermore, the outcome of this deliverable is applicable to any TPS organization and could help new EU reference laboratories (in the field of plant health) in the future.

3 Reference documents

EPPO (2009) PM7/91 (1) *Gibberella circinata.* EPPO Bulletin 39, 298–309

EPPO (2013) PM 7/20 (2) *Erwinia amylovora*. EPPO Bulletin, 43, 21–45

EPPO (2018), PM 7/76 (5) Use of EPPO Diagnostic Standards. EPPO Bulletin 48, 373–377

EPPO (2018) PM 7/98 (3) Specific requirements for laboratories preparing accreditation for a plant pest diagnostic activity. EPPO Bulletin, 48, 387–404.

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Lee RF, Bar-Joseph M (2000) Tristeza. In: Timmer, L.W., Garnsey, S.M., Graham, J.H. (Eds.), Compendium of Citrus Diseases. APS Press, St. Paul, MN, 61–63.

Martelli GP, Agranovsky, AA, Bar-Joseph M, Boscia D, Candresse T, Coutts RHA, Dolja VV, Falk BW, Gonsalves D, Hu J, Jelkmann, Karasev AV, Minafra A, Namba S, Vetten, HJ, Wisler CG, Yoshikawa N (2005) Virus taxonomy. In: Fauquet, C.M., Mayo, M.A., Maniloff, J., Desselberger, U., Ball, L.A. (Eds.), Eighth Report of the International Committee on Taxonomy of Viruses. Elsevier Academic Press, San Diego, CA.

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Saponari M, Manjunath K & Yokomi RK (2008) Quantitative detection of Citrus tristeza virus in citrus and aphids by real-time reverse transcription-PCR (TaqMan). Journal of Virological Methods 147, 43–53.

4 Terms, abbreviations and definitions

CTV – Citus tristeza virus

EPPO – European and Mediterranean Plant Protection Organization

LAMP - Loop-mediated isothermal amplification

LFD - Lateral flow device

NAC – Negative amplification control

NC – Negative control

NIC – Negative isolation control

PAC – Positive amplification control

PIC – Positive isolation control

PC – Positive control

PPV – Plum pox virus

TPS – test performance study

5 Methodology

The process of criteria selection for tests included in TPS started in the beginning of the project where the criteria listed in EPPO protocol PM 7/98 (Specific requirements for laboratories preparing accreditation for a plant pest diagnostic activity) and additional criteria coming from the WP1 partners expertise were discussed in detail during several WP1 teleconferences and meetings. For the purpose of setting the weight for each criterion for a specific pest, the scope of the testing for a specific pest was needed, therefore the tables with specific data for each pest were prepared. Similar procedure was used for criteria which are used for selection of laboratories, participating in test performance study. The outcome of WP1 group, including the rules for how to execute the selection was presented and discussed in Steering Committee meetings. The final list of criteria and their corresponding weights were finalized in project month 10 (February 2019).

6 Definition of scope of testing for selected pests

To define weighted criteria for TPS tests selection it is necessary to precisely define the scope for a specific pest included in the TPS. The definition of the scope include selection of methods and for every method identification of: sample type (DNA, sample spiked with pest,…), matrix (seeds, leaves,…), purpose (detection or identification), controls, number of samples and number of laboratories. The selection of different methods for different pests relies on differences between the current diagnostic needs for each of the six pests listed above. In some cases, methods are needed for fast detection on-site, while in other cases ability to detect the pest in very low concentrations is more important. Methods differ in their applicability for detection or screening, and between the uses on symptomatic or asymptomatic material. In the frame of the scope definition, methods selection could depend also on plant material available and expertise of test performance study organizer.

Further on, weightings, which are assigned to the criteria, described below (Section 8 - Setting the weighted criteria for selection of tests for TPS), differ between the pests, depending on the scope of the TPS for each pest (Table 1 -

Table 6). For example, in the case of *Pantoea stewartii* subsp. *stewartii*, which is an emerging pest for Europe the aim of the TPS is to identify tests that are able to detect the pest in asymptomatic plant material as it can be transmitted with infected seeds. Therefore, it is important to be able to detect the presence of the pest in very low concentrations (see table 8). On the contrary, in the case of testing for *Erwinia amylovora* in symptomatic material, detection of the pest in low concentrations is not critical.

6.1 *Erwinia amylovora (see Table 1)*

The scope of the test performance study is: detection of *Erwinia amylovora* in symptomatic plant material.

Fire blight, a disease caused by *Erwinia amylovora (E. amylovora)*, is a quarantine disease in most countries. The pest was found up till now in the majority of EU countries, excluding so-called 'protected zones' in which fire blight is considered as absent. Therefore, most commonly, detection of the causative bacteria is performed from symptomatic samples. Based on that, the TPS starting material will include extracts of tree shoots with fire blight symptoms and extracts from healthy shoots with or without added target and/or other bacteria. The plant material of *Malus domestica*, *Pyrus communis*, *Amelanchier* and *Pyracantha* was collected in the season of 2018 and is available for preparation of samples for TPS. The purpose of the TPS will be detection of the pest in symptomatic shoots. The TPS will incorporate 15-20 samples. The number of participants is approx. 30 laboratories. The purpose of the TPS will be detection of the pest using both serological and molecular methods, as recommended by the EPPO standard PM 7/20 *Erwinia amylovora*. The methods of choice for laboratory detection of *E. amylovora* are based on the fact that molecular methods real-time PCR and LAMP have high analytical sensitivity, high analytical specificity, can show the presence of the pest even in the case when fire blight may be masked by the presence of other pathogens, senescence of plant material or pesticides used. Serological LFD methods were selected because of their practicality for on-site use. The selected methods were previously validated in ERA NET projects and validation data are available for some of them; however, direct comparison of validation results are hindered by the differences in sample preparation and/or modification of tests. Therefore, preliminary study will be done by TPS organizer and will allow direct comparison of the tests on the same material. The methods are well established in the laboratory of TPS organizer.

Table 1: Scope definition for *Erwinia amylovora*

	Methods					
	plating	**IF**	**PCR**	**real-time PCR**	**LAMP**	**other methods applicable for on-site**
sample type (DNA, plant material with deactiv. pests, etc.)	-	-	-	Plant extract/DNA	Plant extract/DNA	Plant extract
matrix (type of plant material: seed, leaves, etc.)	-	-	-	shoots	shoots	shoots
suitable for: symptomatic / asymptomatic sample	-	-	-	symptomatic	symptomatic	symptomatic
purpose: detection / identification	-	-	-	detection	detection	detection
type of controls needed (NIC, NAC, PAC, PIC, IC, etc)	-	-	-	PAC NAC NIC	PAC NAC NIC	PAC NAC NIC
no. of samples	-	-	-	15-20	15-20	15-20
max no. of participants	-	-	-	30	30	30

6.2 *Pantoea stewartii* subsp. *stewartii (see Table 2)*

The scope of the test performance study is: (molecular) detection of *Pantoea stewartii* subsp. *stewartii* in asymptomatic plant material (maize seeds).

Pantoea stewartii subsp. *stewartii* is endemic to America and has been introduced to other parts of the world with maize seeds. It causes a disease called Stewart's wilt. The principal host of the pest is *Zea mays* (maize). Asymptomatic infection of maize plants with *P. stewartii* subsp. *stewartii* have not been reported, however the bacterium can be found on or in maize seeds which can serve as mode of transmission. Infected seeds do not show any characteristic symptoms, therefore testing of the seeds is the only possibility to prevent spread of the pest with planting material. Based on that, the TPS starting material will include extracts of commercially produced maize seeds with or without added target or other bacteria. The purpose of the TPS will be detection of the pest in asymptomatic seeds. The TPS will incorporate 15-20 samples. The number of participants is approx. 20 laboratories. The purpose of the TPS will be detection of the pest using molecular methods, as they exhibit high analytical sensitivity which is essential for testing latently infected plant material. We chose PCR and real-time PCR methods for laboratory detection of *P. stewartii* subsp. *stewartii* as they have shown higher analytical sensitivity than LAMP method. Some of them are also able to distinguish *P. stewartii* subsp. *stewartii* from highly similar and non-pathogenic *P. stewartii* subsp. *indologenes*. Selection of the methods and tests was based on the publications and experience of diagnostic laboratories. Both methods are well established in the laboratory of the TPS organizer. Detection of the pest in the field is one of the future perspectives, therefore LAMP was selected as a potential on-site method. Only a few LAMP tests are available with minimal validation data, consequently all identified LAMP tests will be included in the preliminary study in order to select the best performing test for TPS, if they are found to have suitable performance characteristics.

Table 2: Scope definition for *Pantoea stewartii* subsp. *stewartii*

	Methods					
	plating	**IF**	**PCR**	**real-time PCR**	**LAMP**	**other methods applicable for on-site**
sample type (DNA, plant material with deactiv. pests, etc.)	-	-	Plant extract from maize seeds / DNA	Plant extract from maize seeds / DNA	Plant extract from maize seeds / DNA	-
matrix (type of plant material: seed, leaves, etc.)	-	-	seed	seed	Seed	-
suitable for: symptomatic / asymptomatic sample	-	-	asymptomatic	asymptomatic	asymptomatic	-
purpose: detection / identification	-	-	detection	detection	detection	-
type of controls needed (NIC, NAC, PAC, PIC, IC, etc)	-	-	PAC NAC NIC	PAC NAC NIC	PAC NAC NIC	-
no. of samples	-	-	15-20	15-20	15-20	-
max no. of participants	-	-	20	20	20	-

6.3 Citrus tristeza virus *(see Table 3)*

The scope of the test performance study is: detection of citrus tristeza virus in symptomatic plant material and in plant material spiked with the virus in order to mimic asymptomatic samples.

Citrus tristeza virus (CTV), genus Closterovirus (Martelli *et al*., 2005), is the causal agent of tristeza, a major disease on Citrus causing decline of trees and impacting fruits production. The virus has a host range restricted to most species of the family *Rutaceae* (Roistacher, 1991) and can be disseminated long distances by movement of virus-infected plant material and locally by several aphid species in a semi-persistent mode (Lee and Bar-Joseph, 2000).
CTV probably originated in Malaysia and other countries of Southeast Asia, the putative area of origin of citrus, and it has been disseminated to almost all citrus-growing countries through the movement of infected plant material (IPPC, 2016).
Types and severity of symptoms induced by CTV are associated with different viral strains. The most virulent isolates (aggressive isolates) cause stem pits in wood of twigs, small and large lateral branches and the main trunk. They also reduce growth of the tree accompanied by a decline in fruit yield, fruit size and quality in severe cases (Saponari *et al*., 2008).
Detection and identification of CTV can be achieved using biological, serological or molecular amplification tests.
The purpose of the TPS is to compare the performance of different tests (including rapid detection tests like LAMP and on-site tests) and to generate robust validation data (as they will be obtained by a selection of competent laboratories using standard operating procedures) in order to help laboratories and decision makers to choose the best detection strategy for their purpose. For this a first selection of tests will be performed based both on a bibliographic review and on experimental investigations conducted by the TPS organiser. Only the most sensitive and specific tests will be selected to be included in the TPS.

TPS will be composed of 20/30 samples including CTV infected samples (diversity of isolates, and in particular aggressive isolates, different infection levels) and samples not infected by CTV. The number of participants is limited to a maximum of 16 laboratories

Table 3: Scope definition for citrus tristeza virus

	Methods					
	ELISA	**RT-PCR**	**real-time PCR**	**LAMP**	**other methods applicable for on-site: Immunostrip**	**Other : TPIA (Tissue Print Immunoassay)**
sample type (DNA, plant material with deactiv. pests, etc.)	Freeze dried leaves / Freeze dried ground leave extracts	Freeze dried leaves / Freeze dried ground leave extracts	Tissue-print / Freeze dried leaves / Freeze dried ground leave extracts	Freeze dried leaves / Freeze dried ground leave extracts	Freeze dried leaves	Tissue-print
matrix (type of plant material: seed, leaves, etc.)	Leaves	Leaves	Woody cuttings/ Leaves	Leaves	Leaves	Woody cuttings
suitable for: symptomatic / asymptomatic sample	symptomatic/ asymptomatic	symptomatic/ asymptomatic	symptomatic/ asymptomatic	symptomatic/ asymptomatic	symptomatic/ asymptomatic	symptomatic/ asymptomatic
purpose: detection / identification	detection	detection	detection	detection	detection	detection
type of controls needed (NIC, NAC, PAC, PIC, IC, etc)	NC, PC	NC, PC NAC,PAC	NC, PC NAC,PAC	NC, PC NAC,PAC	NC, PC	NC, PC
no. of samples	20	20	20 (30 for TP-rt-PCR)	20	20	30
max no. of participants	16	16	16	16	16	16

6.4 Plum pox virus *(see Table 4)*

The scope of the test performance study is: Detection of plum pox virus in symptomatic and asymptomatic leaf material of *Prunus* spp.

Plum pox, also known as sharka, is caused by plum pox virus (PPV). PPV may infect a wide variety of *Prunus* species, including, almond, apricot, cherry, nectarine, peach, plum, as well as wild and ornamental species (e.g *Prunus besseyi, Prunus insititia, Prunus tomentosa, Prunus triloba* and *Prunus spinose*). In fruit trees, infection may eventually result in deformation of fruits and severe yield reduction. At present more than ten different strains and recombinants are distinguished, based on biological, serological and molecular characteristics. PPV is present in many European countries, and is regulated for plants for planting to control the disease (EU II/AII). Therefore, the availability of reliable tests is required to guarantee the absence of PPV in this material. For detection of the virus testing can be performed on symptomatic leaves, flowers, and/or fruits. In plant material without symptoms, both shoots and leaves can be tested.

The scope of the TPS is 'detection of PPV in symptomatic and asymptomatic leaves of *Prunus* spp.', with a focus on a broad detection of 'all' variants. The TPS will include approx. 15 samples and concerns both serological (DAS-ELISA) and molecular methods (real-time RT-PCR and RT-PCR). The method LAMP will not be included due to limited amount of starting material and minimal experience by the TPS organizer. It will be evaluated if the on-site method LFD will be

incorporated when it is possible to use the same sample set as used for DAS-ELISA. The number of participants is limited to a maximum of 20 laboratories.

Table 4: Scope definition for Plum pox virus

	Methods				
	DAS-ELISA	**RT-PCR**	**real-time RT-PCR**	**LAMP**	**other methods applicable for on-site: LFD**
sample type (DNA, plant material with deactiv. pests, etc.)	Freeze dried ground leaf extracts	Freeze dried ground leaf extracts or RNA extracts	Freeze dried ground leaf extracts or RNA extracts	-	Freeze dried leaves or Freeze dried ground leaf extracts
matrix (type of plant material: seed, leaves, etc.)	leaves	leaves	leaves	-	leaves
suitable for: symptomatic / asymptomatic sample	symptomatic/ asymptomatic	symptomatic/ asymptomatic	symptomatic/ asymptomatic	-	symptomatic/ asymptomatic
purpose: detection / identification	detection	detection	detection	-	detection
type of controls needed (NIC, NAC, PAC, PIC, IC, etc)	PC, NC (plant), NC (buffer)	NIC, NAC, PAC, PIC	NIC, NAC, PAC, PIC	-	PC, NC (plant), NC (buffer)
no. of samples	15	15	15	-	15
max no. of participants	20	20	20	-	20

6.5 Fusarium circinatum (see Table 5)

The scope of the test performance study is: Identification of *Fusarium circinatum* from culture material using plating methods and detection from culture material and DNA extracts using molecular methods.

Fusarium circinatum is the causal agent of pitch canker disease which primarily affects *Pinus sp.* Whilst the pest has been reported in some European countries, the serious threat to the pine forest industry means this pest is seen as of high importance. There is a wide range of host materials that can be tested for the presence of *Fusarium circinatum*, including infected tree material, seeds and potential insect vectors. Many laboratories also deploy multiple methods to confirm positives findings, which often include plating methods for identification. The ability to obtain sufficient volumes of infected material which can be easily homogenised for distribution is challenging therefore the TPS organisers have chosen to provide viable reference cultures to allow more standardised TPS material. The ability to supply viable cultures within the TPS will also allow laboratory who solely undertake plating methods to also partake. Laboratories have been given the choice as to which methods they would like to undertake (Table 5) to capture the variety of methods and combinations being deployed. The TPS will consist of a maximum of 6 cultures for plating and molecular methods along with additional DNA extracts for molecular methods. For the identification of *Fusarium circinatum* both mating types will be included and plated on media as described in the EPPO standard PM7/91 (1) *Gibberella circinata*. For molecular methods both conventional PCR and real-time PCR are established methods so these will be included in the TPS. Molecular methods from both the EPPO protocol and other published assays will be evaluated for inclusion in the TPS.

Table 5: Scope definition for *Fusarium circinatum*

	Methods				
	Plating	**PCR**	**real-time PCR**	**LAMP**	**other methods applicable for on-site**
sample type (DNA, plant material with deactiv. pests, etc.)	Culture	Culture/DNA	Culture/DNA	-	-
matrix (type of plant material: seed, leaves, etc.)	Reference Cultures	Reference Cultures & Extracts from Cultures	Reference Cultures & Extracts from Cultures	-	-
suitable for: symptomatic / asymptomatic sample	symptomatic	symptomatic	symptomatic	-	-
purpose: detection / identification	Identification	Detection	detection	-	-
type of controls needed (NIC, NAC, PAC, PIC, IC, etc)	PC, NC	PAC, NAC, NIC	PAC, NAC, NIC	-	-
no. of samples	6	6 Cultures / 4 Extracts	6 Cultures / 4 Extracts	-	-
max no. of participants	15	15	15	-	-

6.6 Bursaphelenchus xylophilus (see Table 6)

The scope of the test performance study is: detection of *Bursaphelenchus xylophilus* in asymptomatic plant material and its identification.

Bursaphelenchus xylophilus is the causal agent of the pine wilt disease, which may express wilting symptoms in hot and dry conditions, but may remain asymptomatic in colder conditions.

The tests shall be applied to symptomatic and asymptomatic material (wood samples). Nevertheless it is difficult to produce infected wood in large quantity and is risky to send such material across EU. Consequently, the biological material will be composed either of wood extracts spiked with nematodes or of DNA extracts. These two types of biological material will allow the validation of the different steps of the process (extraction, amplification) but also comparison between tests (through DNA extracts). Tests based on RNA detection were not retained as they are too sensitive to environmental conditions with risk of possible contaminations.

TPS will include 15/20 samples per participants. The number of participants is limited to a maximum of 20 laboratories to get enough data and allow reliable statistical analysis of the data.

Table 6: Scope definition for *Bursaphelenchus xylophilus*

	methods				
	PCR	**real-time PCR**	**LAMP**	**other methods applicable for on-site**	**Other**
sample type (DNA, plant material with deactiv. pests, etc.)	DNA	wood extract/ DNA	wood extract/ DNA	-	-
matrix (type of plant material: seed, leaves, etc.)	extracts from Cultures	wood, extracts from Cultures	wood, extracts from Cultures	-	-
suitable for: symptomatic / asymptomatic sample	symptomatic/ asymptomatic	symptomatic/ asymptomatic	symptomatic/ asymptomatic	-	-
purpose: detection / identification	identification	detection	detection	-	-
type of controls needed (NIC, NAC, PAC, PIC, IC, etc)	NAC, PAC	NAC, PAC, PC, NC	NAC, PAC	-	-
no. of samples	max 15	20	20	-	-
max no. of participants	20	20	20	-	-

7 Common rules for selection of tests for TPS

Common rules for selection of tests for validation are described, ensuring a transparent process for selection of test for TPS.

7.1 Definition of the scope of testing

- The scope needs to be clearly defined for each pest (e.g. use for detection or screening, symptomatic material, selection of methods...)

7.2 Weighting and targeted values for each criterion to be reached by a test

- Targeted values for each criterion to be reached by a test are defined. It is necessary to explain how the targeted values have been defined (e.g. value associated with the best performance whatever the use of the test).
- Criteria are weighted to allow selection of appropriate tests for the defined scope for a specific organism. First the most important criteria (high weight) are considered and if some of the tests show similar value and performance, then also less important criteria are considered. Criteria can also have different weight depending on test's use: on-site versus laboratory use.

7.3 Collection of available data

- Preparation of a list of known diagnostic methods for the specific pest.
- Collection of validation data available for different tests: in research articles, EPPO database (validation data), EUPHRESCO final reports, from EPPO/dedicated questionnaires, through internet search, emails sent to commercial kits providers.

7.4 Analysis of available data

- Analysis of performance values from the available validation data for each test [see reference: WP1 Summary table of validation data; internal document to WP1].
- Objective comparison of performance among the tests identified.

7.5 First selection of tests

- Validation data from different sources are not always necessarily presented homogeneously. Experienced and critical judgement of TPS organizers is needed to make a pre-selection of tests for validation. Results of previous preliminary studies can be used to characterise a test.

7.6 Preliminary studies

- Preliminary study is conducted in-house by the TPS organizers to provide missing validation data to help select the final tests for TPS.

7.7 Selection of the final tests

- Criteria are documented to select tests for TPS among pre-selected tests for validation. If a criterion is not relevant for a specific method/pest combination it can be ignored.

8 Setting the weighted criteria for selection of tests for TPS

Weighted criteria were set (Tables 7-12) to objectively select tests from a list of tests for a specific pest, each having advantages and disadvantages under specific circumstances or needs, depending on the scope of the TPS. To be able to establish such criteria, the use of the EPPO standard PM 7/98 (Specific requirements for laboratories preparing accreditation for a plant pest diagnostic activity) as well as the professional experience of TPS organizers was required.

Apart from criteria, the tables 7-12 contain also criteria descriptors (quantitative or qualitative), targets to be reached by a test, relative weight of a criterion, different for the different use of the test (laboratory use or on-site) and the conclusion whether the criterion is met by the test. Criteria descriptors can be quantitative, as for example

concentration of pest to be detected (not all pest can be easily counted e.g. viruses). Descriptors can also be simple Yes/No answers or even relative. As already emphasized above, criteria could be differently weighted to allow selection of appropriate tests for the defined scope for a specific pest. First the most important criteria (high weight) are considered and if some of the tests show similar value and performance, then also less important criteria are used (medium or low weight). The most critical criteria of each test used in diagnostic purposes are analytical sensitivity, analytical specificity (exclusivity and inclusivity), selectivity, repeatability and reproducibility. Less important criteria dealing with applicability, chemicals, and equipment help to evaluate other properties of specific tests. The applicability of the test is evaluated based on sample throughput and complexity of the test procedures. However it is important also to evaluate how accessible or stable are the required chemicals and/or equipment which is needed to perform a specific test for a specific scope. However, if a criterion is not relevant for a specific method/pest combination it can be ignored (no weight).

Criteria can also have different weight depending on test's use: on-site versus laboratory use. Conclusions about how each test met the criteria are combined and a decision is made whether the test is appropriate for the scope for a specific pest.

Table 7 - 12 contains criteria which are described and weighted for each pest.

8.1 *Erwinia amylovora*

Table 7: Criteria for selection of tests for TPS for *Erwinia amylovora*

Criteria	Descriptor (%, number, text)	Target	Relative Weight (lab)	Relative Weight (on-site)	Conclusion for the test (OK/Not OK)
Validation data (prior preliminary studies) available validation data					
available validation data	Yes/No	Yes	medium	medium	
→ validation data available for selected matrix	Yes/No	Yes	low	low	
analytical sensitivity (LOD)	conc.	medium	medium	high	
analytical specificity	level	medium	high	high	
a) exclusivity (Non-target organism): False positives	level	medium or low	high	high	
b) Inclusivity (Target organisms): False negatives	level	low (zero tolerance)	high	high	
selectivity	presence of cross reactions with matrix	No	high	high	
repeatability	level	high at medium target conc.	high	high	
reproducibility	%	100% at medium target conc.	high	high	
results of interlaboratory comparisons available	Yes/No	Yes	low		
Validation data (after preliminary studies)					
analytical sensitivity (LOD)	conc.	medium	medium	high	
analytical specificity	level	medium	high	high	
a) exclusivity (Non-target organism): False positives	level	medium or low	high	high	
b) inclusivity (Target organisms): False negatives	level	low (zero tolerance)	high	high	
selectivity	presence of cross reactions with matrix	No	high	high	
repeatability	level	high at medium target conc.	high	high	
reproducibility	%	100% at medium target conc.	high	high	
APPLICABILITY					
applicability in different matrices	level	high	medium	medium	
sample throughput	level	low (on-site) to high (lab)	medium	medium	

amount of material which is included in one sample	amount of plant units tested	medium	medium	medium	
standardized preparation of the reaction (e.g., ready to use reagents)	Yes/No	Yes	medium	high	
availability and relevance of controls (in the case of kits)	Yes/No	Yes	medium	high	
PROTOCOLS					
available detailed protocols	Yes/No	Yes	high	high	
simple test procedure	Yes/No	Yes	low	medium	
simplicity of data analysis	Yes/No	Yes	low	medium	
user friendly test	Yes/No	Yes	low	medium	
time needed to complete analysis (less than one hour/ one day/ several days)	Duration in time unit	the fastest	low	medium	
easy to multiplex?	Yes/No	Yes	low	NA	
database/library dependent (yes/ no) (for example fatty acids profiling, sequencing,...)	NA	NA	NA	NA	
CHEMICALS					
availability of chemicals/ reagents/ kits					
a) in all EU countries	Yes/No	Yes	low	low	
b) in all EPPO countries	Yes/No	Yes	low	low	
cost of consumables and chemicals	Cost in euro per test	NA	low	low	
stability of chemicals at ambient temperature	Yes/No	Yes	NA	high	
risks associated with chemicals and consumables	description of the risk (harmful, toxic, …)	lowest risk for use	medium	medium	
duration of validity of chemicals/reagents	Duration in time unit	the longest	low	low	
feasibility to transport the chemicals	Yes/No	NA	NA	NA	
shipment of chemicals and samples (safety and transport regulations)?	Possible/Not possible and Easy/Not easy	NA	NA	NA	
EQUIPMENT					
no equipment/ instrument needed (relevant only for on-site tests)	Yes/No	Yes	NA	medium	
test not exclusively developed for a specific instrument	Yes/No	Yes	medium	medium	
cost of obligatory equipment/ instruments (up to 10,000 EUR/ 10,000-50,000 EUR/ more than 50,000 EUR?)	cost in euro	NA	low	high	

8.2 *Pantoea stewartii* subsp. *stewartii*

Table 8: Criteria for selection of tests for TPS for *Pantoea stewartii* subsp. *stewartii*

Criteria	Descriptor (%, number, text)	Target	Relative Weight (lab)	Relative Weight (on-site)	Conclusion for the test (OK/Not OK)
Validation data (prior preliminary studies) available validation data					
available validation data	Yes/No	Yes	medium	medium	
→ validation data available for selected matrix	Yes/No	Yes	low	low	
analytical sensitivity (LOD)	conc.	low	high	high	
analytical specificity	level	high	high	high	
a) exclusivity (Non-target organism): False positives	level	low	high	high	
b) Inclusivity (Target organisms): False negatives	level	low (zero tolerance)	high	high	
selectivity	presence of cross reactions with matrix	No	high	high	
repeatability	level	high at low target conc.	high	high	
reproducibility	%	100% at low target conc.	high	high	
results of interlaboratory comparisons available	Yes/No	Yes	low		
Validation data (after preliminary studies)					
analytical sensitivity (LOD)	conc.	low	high	high	
analytical specificity	level	high	high	high	
a) exclusivity (Non-target organism): False positives	level	low	high	high	
b) inclusivity (Target organisms): False negatives	level	low (zero tolerance)	high	high	
selectivity	presence of cross reactions with matrix	No	high	high	
repeatability	level	high at low target conc.	high	high	
reproducibility	%	100% at low target conc.	high	high	
APPLICABILITY					
applicability in different matrices	level	low	medium	medium	
sample throughput	level	medium	medium	medium	
amount of material which is included in one sample	amount of plant units tested	high amount preferable	medium	medium	

standardized preparation of the reaction (e.g., ready to use reagents)	Yes/No	Yes	medium	high	
availability and relevance of controls (in the case of kits)	Yes/No	Yes	medium	high	
PROTOCOLS					
available detailed protocols	Yes/No	Yes	high	high	
simple test procedure	Yes/No	Yes	low	medium	
simplicity of data analysis	Yes/No	Yes	low	medium	
user friendly test	Yes/No	Yes	low	medium	
time needed to complete analysis (less than one hour/ one day/ several days)	Duration in time unit	the fastest	low	medium	
easy to multiplex?	Yes/No	Yes	low	NA	
database/library dependent (yes/ no) (for example fatty acids profiling, sequencing,...)	NA	NA	NA	NA	
CHEMICALS					
availability of chemicals/ reagents/ kits					
a) in all EU countries	Yes/No	Yes	low	low	
b) in all EPPO countries	Yes/No	Yes	low	low	
cost of consumables and chemicals	Cost in euro per test	NA	low	low	
stability of chemicals at ambient temperature	Yes/No	Yes	NA	high	
risks associated with chemicals and consumables	description of the risk (harmful, toxic, …)	lowest risk for use	medium	medium	
duration of validity of chemicals/reagents	Duration in time unit	the longest	low	low	
feasibility to transport the chemicals	Yes/No	NA	NA	NA	
shipment of chemicals and samples (safety and transport regulations)?	Possible/Not possible and Easy/Not easy	NA	NA	NA	
EQUIPMENT					
no equipment/ instrument needed (relevant only for on-site tests)	Yes/No	Yes	NA	medium	
test not exclusively developed for a specific instrument	Yes/No	Yes	medium	medium	
cost of obligatory equipment/ instruments (up to 10,000 EUR/ 10,000-50,000 EUR/ more than 50,000 EUR?)	cost in euro	NA	low	high	

8.3 Citrus tristeza virus

Table 9: Criteria for selection of tests for TPS for citrus tristeza virus

Criteria	Descriptor (%, number, text)	Target	Relative Weight (lab)	Relative Weight (on-site)	Conclusion for the test (OK/Not OK)
Validation data (prior preliminary studies) available validation data					
available validation data	Yes/No	Yes	medium	medium	
→ validation data available for selected matrix	Yes/No	Yes	low	low	
analytical sensitivity (LOD)	dilutions	None	medium	medium	
analytical specificity	%	None	high	high	
a) exclusivity (Non-target organism): False positives	% of non target strains / samples detected	0%	high	high	
b) Inclusivity (Target organisms): False negatives	% of target strains / samples not detected	0%	high	high	
selectivity	presence of cross reactions with matrix	No	high	high	
repeatability	% of agreement between repetitions	100% at LOD	medium	medium	
reproducibility	% of agreement between repetitions in different conditions	100% at LOD	medium	medium	
results of interlaboratory comparisons available	Yes/No	Yes	low	low	
Validation data (after preliminary studies)					
analytical sensitivity (LOD)	dilutions	Lowest level	high	high	
analytical specificity	% of true positive detected and true negative not detected	Highest level	high	high	
a) exclusivity (Non-target organism): False positives	% of non target strains / samples detected	0%	high	high	
b) inclusivity (Target organisms): False negatives	% of target strains / samples not detected	0%	high	high	
selectivity	presence of cross reactions with matrix	No	high	high	
repeatability	% of agreement between repetitions	100% at LOD	medium	medium	

reproducibility	% of agreement between repetitions in different conditions	100% at LOD	medium	medium	
APPLICABILITY					
applicability in different matrices	Description + Yes/No	NA	medium	medium	
sample throughput	Yes/No	Yes	high	high	
amount of material which is included in one sample		NA	low	low	
standardized preparation of the reaction (e.g., ready to use reagents)	Yes/No	Yes	low	high	
availability and relevance of controls (in the case of kits)	Yes/No	NA	medium	high	
PROTOCOLS					
available detailed protocols	Yes/No	Yes	medium	high	
simple test procedure	Yes/No	Yes	medium	high	
simplicity of data analysis	Yes/No	Yes	medium	high	
user friendly test	Yes/No	Yes	medium	high	
time needed to complete analysis (less than one hour/ one day/ several days)	Duration in time unit	the fastest	medium	high	
easy to multiplex?	NA	NA	NA	NA	
database/library dependent (yes/ no) (for example fatty acids profiling, sequencing,...)	NA	NA	NA	NA	
CHEMICALS					
availability of chemicals/ reagents/ kits					
a) in all EU countries	Yes/No	NA	low	low	
b) in all EPPO countries	Yes/No	NA	low	low	
cost of consumables and chemicals	Cost in euro per test	NA	low	medium	
stability of chemicals at ambient temperature	NA	NA	NA	medium	
risks associated with chemicals and consumables	description of the risk (harmful, toxic, …)	lowest risk for use	high	high	
duration of validity of chemicals/reagents	Duration in time unit	the longest	low	medium	
feasibility to transport the chemicals	Yes/No	NA	very low	high	
shipment of chemicals and samples (safety and transport regulations)?	Possible/Not possible and Easy/Not easy	NA	very low	high	
EQUIPMENT					
no equipment/ instrument needed (relevant only for on-site tests)	Yes/No	Yes	NA	high	
test not exclusively developed for a specific instrument	Yes/No	Yes	medium	medium	
cost of obligatory equipment/ instruments (up to 10,000 EUR/ 10,000-50,000 EUR/ more than 50,000 EUR?)	cost in euro	NA	high	high	

8.4 Plum pox virus

Table 10: Criteria for selection of tests for TPS for plum pox virus

Criteria	Descriptor (%, number, text)	Target	Relative Weight (lab)	Relative Weight (on-site)	Conclusion for the test (OK/Not OK)
Validation data (prior preliminary studies) available validation data					
available validation data	Yes/No	Yes	medium	medium	
→ validation data available for selected matrix	Yes/No	Yes	low	low	
analytical sensitivity (LOD)	conc.	low	high	high	
analytical specificity	level	high	high	high	
a) exclusivity (Non-target organism): False positives	level	low	high	high	
b) Inclusivity (Target organisms): False negatives	level	Low	high	high	
selectivity	presence of cross reactions with matrix	No	high	high	
repeatability	%	100% at low target conc.	medium	medium	
reproducibility	%	100% at low target conc.	medium	medium	
results of interlaboratory comparisons available	Yes/No	Yes	low	low	
Validation data (after preliminary studies)					
analytical sensitivity (LOD)	conc.	low	high	high	
analytical specificity	level	high	high	high	
a) exclusivity (Non-target organism): False positives	level	low	high	high	
b) inclusivity (Target organisms): False negatives	level	Low	high	high	
selectivity	presence of cross reactions with matrix	No	high	high	
repeatability	%	100% at low target conc.	medium	medium	
reproducibility	%	100% at low target conc.	medium	medium	
APPLICABILITY					
applicability in different matrices	level	high	high	high	
sample throughput	level	medium	medium	medium	

amount of material which is included in one sample	NA	NA	NA	NA	
standardized preparation of the reaction (e.g., ready to use reagents)	Yes/No	Yes	medium	high	
availability and relevance of controls (in the case of kits)	Yes/No	Yes	medium	high	
PROTOCOLS					
available detailed protocols	Yes/No	Yes	high	high	
simple test procedure	Yes/No	Yes	medium	medium	
simplicity of data analysis	Yes/No	Yes	medium	medium	
user friendly test	Yes/No	Yes	medium	medium	
time needed to complete analysis (less than one hour/ one day/ several days)	Duration in time unit	NA	NA	Na	
easy to multiplex?	Yes/No	NA	NA	NA	
database/library dependent (yes/ no) (for example fatty acids profiling, sequencing,...)	NA	NA	NA	NA	
CHEMICALS					
availability of chemicals/ reagents/ kits					
a) in all EU countries	Yes/No	Yes	high	high	
b) in all EPPO countries	Yes/No	Yes	high	high	
cost of consumables and chemicals	Cost in euro per test	low	high	high	
stability of chemicals at ambient temperature	Yes/No	Yes	NA	high	
risks associated with chemicals and consumables	description of the risk (harmful, toxic, …)	lowest risk for use	high	high	
duration of validity of chemicals/reagents	Duration in time unit	the longest	low	low	
feasibility to transport the chemicals	Yes/No	NA	NA	NA	
shipment of chemicals and samples (safety and transport regulations)?	Possible/Not possible and Easy/Not easy	NA	NA	NA	
EQUIPMENT					
no equipment/ instrument needed (relevant only for on-site tests)	Yes/No	Yes	NA	High	
test not exclusively developed for a specific instrument	Yes/No	Yes	High	High	
cost of obligatory equipment/ instruments (up to 10,000 EUR/ 10,000-50,000 EUR/ more than 50,000 EUR?)	cost in euro	NA	low	high	

8.5 *Fusarium circinatum*

Table 11: Criteria for selection of tests for TPS for *Fusarium circinatum*

Criteria	Descriptor (%, number, text)	Target	Relative Weight (lab)	Relative Weight (on-site)	Conclusion for the test (OK/NOK)
Validation data (prior preliminary studies) available validation data					
available validation data	Yes/No	Yes	medium		
→ validation data available for selected matrix	Yes/No	Yes	low		
analytical sensitivity (LOD)	conc.	low	high		
analytical specificity	level	medium	high		
a) exclusivity (Non-target organism): False positives	level	low	high		
b) Inclusivity (Target organisms): False negatives	level	low	high		
selectivity	presence of cross reactions with matrix	No	high		
repeatability	level	high at medium target conc.	high		
reproducibility	%	high at medium target conc.	high		
results of interlaboratory comparisons available	Yes/No	Yes	low		
Validation data (after preliminary studies)					
analytical sensitivity (LOD)	conc.	medium	medium		
analytical specificity	level	medium	high		
a) exclusivity (Non-target organism): False positives	level	low	high		
b) inclusivity (Target organisms): False negatives	level	low	high		
selectivity	presence of cross reactions with matrix	No	high		
repeatability	level	high at medium target conc.	high		
reproducibility	%	high at medium target conc.	high		
APPLICABILITY					
applicability in different matrices	level	high	low		
sample throughput	level	medium/high	low		

amount of material which is included in one sample	plug from culture	low	low		
standardized preparation of the reaction (e.g., ready to use reagents)	Yes/No	Yes	low		
availability and relevance of controls (in the case of kits)	Yes/No	Yes	low		
PROTOCOLS					
available detailed protocols	Yes/No	Yes	medium		
simple test procedure	Yes/No	Yes	low		
simplicity of data analysis	Yes/No	Yes	low		
user friendly test	Yes/No	Yes	low		
time needed to complete analysis (less than one hour/ one day/ several days)	Duration in time unit	fastest (for each method)	low		
easy to multiplex?	Yes/No	Yes	low		
database/library dependent (yes/ no) (for example fatty acids profiling, sequencing,...)	NA	NA	NA		
CHEMICALS					
availability of chemicals/ reagents/ kits					
a) in all EU countries	Yes/No	Yes	low		
b) in all EPPO countries	Yes/No	Yes	low		
cost of consumables and chemicals	Cost in euro per test	lowest available (for each method)	low		
stability of chemicals at ambient temperature	Yes/No	Yes	low		
risks associated with chemicals and consumables	description of the risk (harmful, toxic, …)	lowest risk for use	medium		
duration of validity of chemicals/reagents	Duration in time unit	the longest	low		
feasibility to transport the chemicals	Yes/No	Yes	low		
shipment of chemicals and samples (safety and transport regulations)?	Possible/Not possible and Easy/Not easy	as easy as possible	low		
EQUIPMENT					
no equipment/ instrument needed (relevant only for on-site tests)	NA	NA	NA		
test not exclusively developed for a specific instrument	Yes/No	Yes	medium		
cost of obligatory equipment/ instruments (up to 10,000 EUR/ 10,000-50,000 EUR/ more than 50,000 EUR?)	cost in euro	as low as possible	low		

8.6 *Bursaphelenchus xylophilus*

Table 12: Criteria for selection of tests for TPS for *Bursaphelenchus xylophilus*

Criteria	Descriptor (%, number, text)	Target	Relative Weight (lab)	Relative Weight (on-site)	Conclusion for the test (OK/NOK)
Validation data (prior preliminary studies) available validation data					
available validation data	Yes/No	Yes	medium	low	
→ validation data available for selected matrix	Yes/No	Yes	low	low	
analytical sensitivity (LOD)	nb individuals	<10	medium	medium	
analytical specificity	%	None	high	high	
a) exclusivity (Non-target organism): False positives	% of non target populations detected	0%	high	high	
b) Inclusivity (Target organisms): False negatives	% of target populations not detected	0%	high	high	
selectivity	presence of cross reactions with matrix	No	high	high	
repeatability	% of agreement between repetitions	100% at LOD	medium	medium	
reproducibility	% of agreement between repetitions in different conditions	100% at LOD	medium	medium	
results of interlaboratory comparisons available	Yes/No	Yes	low	low	
Validation data (after preliminary studies)					
analytical sensitivity (LOD)	nb individuals	Lowest level	high	high	
analytical specificity	% of true positive detected and true negative not detected	Highest level	high	high	
a) exclusivity (Non-target organism): False positives	% of non target populations detected	0%	high	high	
b) inclusivity (Target organisms): False negatives	% of target populations not detected	0%	high	high	
selectivity	presence of cross reactions with matrix	No	high	high	
repeatability	% of agreement between repetitions	100% at LOD	medium	medium	

reproducibility	% of agreement between repetitions in different conditions	100% at LOD	medium	medium	
APPLICABILITY					
applicability in different matrices	Description + Yes/No	NA	low	low	
sample throughput	Yes/No	Yes	high	low	
amount of material which is included in one sample		NA	low	medium	
standardized preparation of the reaction (e.g., ready to use reagents)	Yes/No	Yes	low	high	
availability and relevance of controls (in the case of kits)	Yes/No	NA	very low	high	
PROTOCOLS					
available detailed protocols	Yes/No	Yes	medium	medium	
simple test procedure	Yes/No	Yes	medium	high	
simplicity of data analysis	Yes/No	Yes	medium	high	
user friendly test	Yes/No	Yes	medium	medium	
time needed to complete analysis (less than one hour/ one day/ several days)	Duration in time unit	NA	medium	high	
easy to multiplex?	NA	NA	NA	NA	
database/library dependent (yes/ no) (for example fatty acids profiling, sequencing,...)	NA	NA	NA	NA	
CHEMICALS					
availability of chemicals/ reagents/ kits					
a) in all EU countries	Yes/No	NA	low	low	
b) in all EPPO countries	Yes/No	NA	low	low	
cost of consumables and chemicals	Cost in euro per test	NA	low	low	
stability of chemicals at ambient temperature	NA	NA	NA	high	
risks associated with chemicals and consumables	description of the risk (harmful, toxic, …)	lowest risk for use	high	high	
duration of validity of chemicals/reagents	Duration in time unit	the longest	low	low	
feasibility to transport the chemicals	Yes/No	NA	very low	medium	
shipment of chemicals and samples (safety and transport regulations)?	Possible/Not possible and Easy/Not easy	NA	very low	medium	
EQUIPMENT					
no equipment/ instrument needed (relevant only for on-site tests)	Yes/No	Yes	NA	medium	
test not exclusively developed for a specific instrument	Yes/No	Yes	medium	medium	
cost of obligatory equipment/ instruments (up to 10,000 EUR/ 10,000-50,000 EUR/ more than 50,000 EUR?)	cost in euro	NA	high	medium	

9 Common rules for selection of participants for TPS

Selection of competent laboratories is critical to obtain relevant results in TPS. Below a set of common rules for the selection of participants for TPS was prepared to ensure transparent process for selection of participating laboratories.

9.1 Identification of potential participants for a TPS

- Potential participants are identified through surveys, professional contacts, and previous participation in a TPS or proficiency test (PT). All laboratories inside and outside the consortium, including diagnostic laboratories, private laboratories at commercial companies, and laboratories at public institutions, should have the opportunity to express their interest to take part to the TPS.

9.2 Weight and targeted values for each criterion to be reached by a participant

- Qualification criteria to select TPS participants, described in Table 13 are the same for all applicants. One of the most important criteria is that the participating laboratory has quality assurance in place. Targets for each criterion to be reached by a participant are defined. All criteria which have been designated high importance must be met by the participants in order to make sure that the participants are proficient and are able to correctly perform the selected tests, which enables correct analysis and evaluation of TPS results.
- Criteria are weighted to allow objective selection of qualified participants. First, the most important criteria (high weight) are considered and if some of the participants show similar answers and due to the limited number of participants that can apply, then also less important criteria are considered.

9.3 Sending invitations

- An invitation is sent to potential participants, naming the pests, which will be included in the TPS, describing the scope for each pest and specifying which methods will be evaluated in the TPS as well as informing participants about the timeline and deadlines.
- Participants give some practical details by filling the "TPS Participant Information Form" in order to optimize the organization and the reliability of the TPS.
- If the participants do not return the filled "TPS Participant Information Form" before the defined deadline, it will be considered that they are not interested to take part to the TPS.

9.4 Selection of the participants

- Feedback from the participants is analysed using the qualification criteria. If they meet the required criteria, TPS organizer confirms their participation in the TPS by email before a defined date.

10 Criteria for selection of participants of TPS

Weighted criteria were set to objectively select participants for each TPS, with the emphasis on the importance of technical expertise for the pests group, use of the method, and authorization to work with the specific pest and that the participating laboratory has quality assurance in place. Other criteria may give potential participants advantages in being selected to take part in the TPS, as for example previous participation in test performance studies or proficiency tests, ability to perform all the tests or possibility to perform the test and deliver results in the time frame defined. Qualification criteria to select TPS participants are the same for all applicants and all qualified laboratories inside and outside the consortium, including diagnostic laboratories, private laboratories at commercial companies and laboratories at public institutions, should have the opportunity to express their interest to take part to the TPS. As an example the criteria for selection of participants of the *Pantoea stewartii* subsp. *stewartii* TPS is shown in table 13.

Apart from criteria, the tables contain also criteria descriptors (quantitative or qualitative), target values to be reached, relative weight of a criterion and the conclusion whether the criterion is met by the potential participant. All criteria which have been designated high importance must be met by the participants in order to make sure that the participants are proficient and are able to correctly perform the selected tests, which enables correct analysis and evaluation of TPS results. Criteria are weighted to allow objective selection of qualified participants in case when too many laboratories applied to take part in the TPS. If some of the participants give the same answers to the criteria with high importance, then also less important criteria are considered, which help to decide between potential participants.

Additionally, tables with the criteria for selection of participants of TPS contain also the information required from TPS applicants about their equipment (in red), which will help the TPS organizers to plan the TPS and later interpret the results. The information is collected using a separate form, which was sent together with the TPS invitation letter.

Through organization of TPS Round 1 the TPS organizers are gaining experiences which will be helpful in the organization of the TPS Round 2 and the preparation of improved related documents as invitation letter, contract, instruction sheet, etc. For instance it was discovered after sending out the invitations for the TPS Round 1 for *Pantoea stewartii* subsp. s*tewartii* and *Erwinia amylovora*, that more information is needed from the TPS applicants to better plan the TPS and later interpret the results. For example, information about the choice of DNA extraction method which is used by the TPS participant may have some influence on the results and should be recorded or even better, known in advance.

Table 13: Criteria for selection of participants in TPS (example for *Pantoea stewartii* subsp. *stewartii*))

Criteria	Descriptor	Target	Relative weight	Conclusion for the selection as participant
TPS time schedule compatible participant's availability	Yes/No	Yes	High importance	
Ability/willing to perform all the tests	Yes/No	Yes	Medium importance	
Technical expertise for the pest group (e.g. virology, bacteriology, etc.)	nb of years	>1 year	High importance	
Expertise in the use of the method (e.g. ELISA, real-time PCR, etc)	nb of years	>1 year	High importance	
Authorized to work with the specific pest	Yes/No	Yes	High importance	
Possibility to obtain an import document or Letter of Authority (EU countries)	Yes/No	Yes	High importance	
Possibility to obtain an import document or Letter of Authority (EU countries) within 4 weeks to receive samples containing the specific pest (only necessary when viable pests are sent)	Yes/No	Yes	Medium importance	
Previous participation in TPS or PT	Yes /No	Yes	Medium importance	
Available equipment:			High importance	
- IF: UV-microscope	NA	NA		
- ELISA: Plate reader (company/model of instrument, wavelength of filters)	NA	NA		
- (RT-)PCR: thermal cycler / gel electrophoresis system / gel imaging system (company/model of instrument)	Yes/No	Yes with appropriate characteristics		
- real-time (RT-)PCR: Thermal cycler (company/model of instrument)	Yes/No	Yes (should be compatible with TaqMan Universal PCR Master Mix)		
a) channels available (FAM, VIC,...)	Wavelength filter	FAM/BHQ1		
b) for multiplexing (instrument with at least two channels)	NA	NA		
- LAMP:				
a) Thermal cycler with FAM channel (company/model of instrument)	Yes/No	Yes or Genie		
b) Portable amplification device, e.g. Genie®, bCUBE® (company/model of instrument)	Yes/No	Yes or Thermal cycler with FAM channel		
c) other (to measure turbidity,...) (company/model of instrument if applicable)	NA	NA		
- Plating: laminar flow cabinet and autoclave (media sterilization)	NA	NA		
Constraints for delivery?	Yes/No (if yes explanations)	No	Medium importance	
Any problems or limitations with delivery on dry ice?	Yes/No (if yes explanations)	Preferably No	Medium importance	
Has committed to perform the test and deliver results in the time frame defined	Yes/No	Yes	Medium importance	
Traceability in place / QA in place	Yes/No (if Yes please specify)	Yes, preferably ISO17025	High importance	

the information in red need to be specified separately by applicants