Introduction of the webinar and training activities

Test Performance Studies organisation

Videos	What is a TPS?	On the week 02/15
Videos	VALITEST TPS: selection of the pests and of the TPS organizers	On the week 02/15
Webinar 1	Preparing the TPS plan	Friday 19/02, 11am
Webinar 2	**Selection of the tests and associated documents**	**Wednesday 24/02, 2pm**
Webinar 3	Selection of participants and contract	Monday 1/03, 2pm
Webinar 4	Preparation and dispatch of samples	Friday 5/03, 11am
Webinar 5	Production of reference material for TPS	Wednesday 10/03, 2pm
Practical training sessions	How to organise Test Performance Studies?	15-18/03 (4 sessions)
Webinar 6	How to tackle the analysis of TPS results?	Monday 22/03, 2pm
Videos	Calculate performance characteristics of a test and get useful information from your validation data by statistical analysis.	On the week 22/03
Webinar 7	Q&A session: the statistical analysis of TPS results	Monday 29/03, 2pm
Practical training sessions	How to analyse the results of Test Performance Studies?	30/03-1/04 (3 sessions)
Webinar 8	From TPS organisation to analysis of the results: example of the TPS on ToBRFV	Wednesday 7/04, 2pm
Videos	Reporting TPS results	To be confirmed/announced

VALITEST webinar series and training activities
Test Performance Studies organisation

Selection of the tests and associated documents

24 February 2021

Hanna Mouaziz (ANSES)

Q&A session with the support of TPS organizers
AM. Chappé (ANSES), M. Luigi (CREA), T. Dreo (NIB)

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

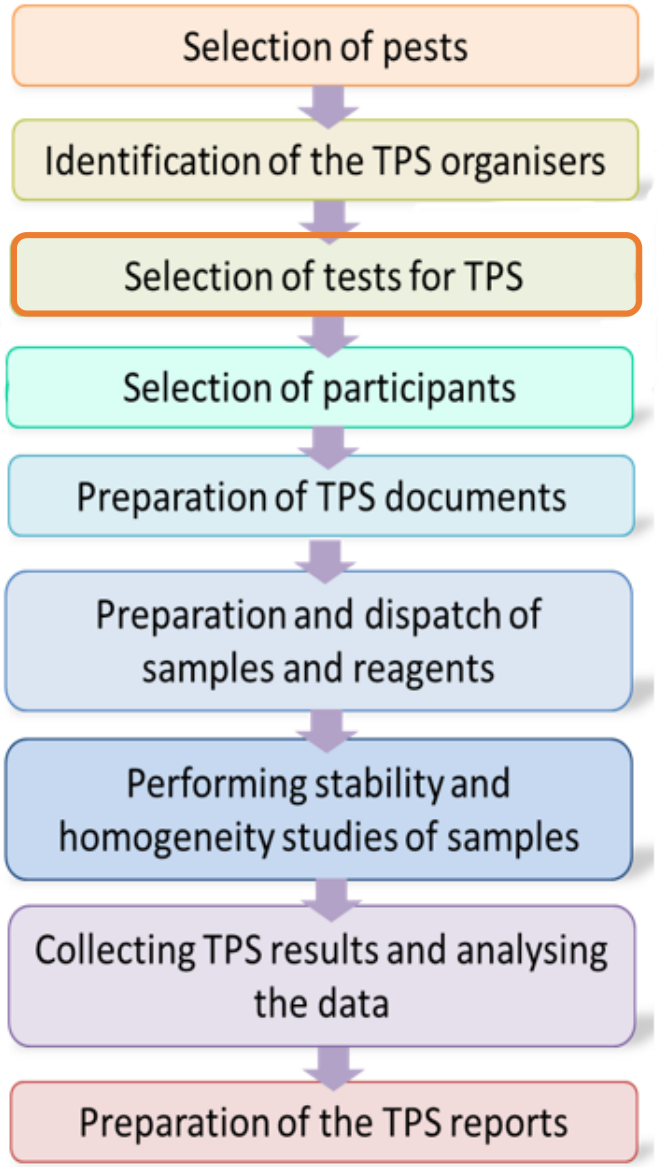
Outline of the presentation

- Introduction: TPS workflow and definitions

- Common rules defined in the framework of VALITEST project
  → Data from TPS organizers
  → Example: TPS organized on *Bursaphelenchus xylophilus* (AM. Chappé - ANSES)
  → Interviews of TPS organizers

- Q&A session with TPS organizers:
  AM. Chappé (ANSES), M. Luigi (CREA) and T. Dreo (NIB)

TPS workflow



Webinar 2	Selection of the tests and associated documents

Valitest

Definitions

Test (EPPO, PM 7/76 (5)): Application of a **method** to a specific **pest** and a specific **matrix**
EPPO (2018), PM 7/76 (5) Use of EPPO Diagnostic Standards, Bulletin OEPP/EPPO Bulletin 48 (3), 373–377

Method (EPPO, PM 7/76 (5)): includes **bioassay** methods, **biochemical** methods, **fingerprint** methods, **isolation/extraction** methods, **molecular** methods, **morphological** and **morphometrical** methods, **pathogenicity assessments**, and **serological** methods.

EPPO (2018), PM 7/76 (5) Use of EPPO Diagnostic Standards, Bulletin OEPP/EPPO Bulletin 48 (3), 373–377

Test performance study (TPS): Evaluation of the **performance** of one or more **tests** by two or more **laboratories** using defined samples (evaluation of a test). A TPS is also referred as **ring tests or collaborative trials** (EPPO PM 7/76 (5)).
Test performance study is part of **validation studies** and usually follows **preliminary studies conducted in-house.**
EPPO (2018), PM 7/76 (5) Use of EPPO Diagnostic Standards, Bulletin OEPP/EPPO Bulletin 48 (3), 373–377

Valitest

Common rules – VALITEST project

Selection of tests is critical to obtain relevant results in TPS

- **A set of common rules was prepared in the framework of VALITEST project to ensure transparent process for the selection of tests for TPS**

VALITEST project

VALITEST aims at producing validation data for the detection and identification of plant pests. Two rounds of tests performance studies (TPS) have been organized:

- The first round of TPS organized in 2019 targeted six pests identified as important by the consortium before the beginning of the project

Erwinia amylovora

Pantoea stewartii subsp. *stewartii*

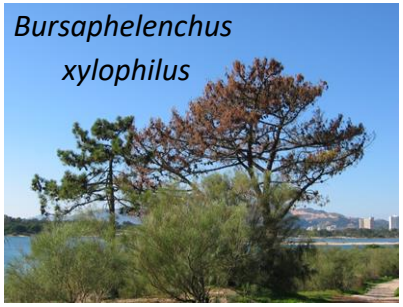
NIB NATIONAL INSTITUTE OF BIOLOGY

Fusarium circinatum

fera

citrus tristeza virus

Bursaphelenchus xylophilus



anses

plum pox virus

Netherlands Food and Consumer Product Safety Authority
Ministry of Agriculture, Nature and Food Quality

Valitest

VALITEST project

- For the second round of TPS organized in 2020, the pests were selected based on the needs expressed by various stakeholders in order to better align validation priorities with stakeholders needs and with the market

Xanthomonas citri pv. *citri*

plum pox virus
(on-site detection)

tomato brown rugose fruit virus

Xylophilus ampelinus

tomato spotted wilt tospovirus

Cryphonectria parasitica

Common rules – VALITEST project

1. **Definition of TPS scope and Collection of available data**
2. **Criteria to be reached by a test**
3. **Analysis of available data and pre-selection of tests**
4. **Preliminary studies according to the criteria and selection of the final tests**

Common rules – VALITEST project

1. **Definition of TPS scope and Collection of available data**
2. **Criteria to be reached by a test**
3. **Analysis of available data and pre-selection of tests**
4. **Preliminary studies according to the criteria and selection of the final tests**

1. Definition of TPS scope and Collection of available data

- **Definition of TPS scope**

  Detection and/or Identification of **pest x** in **matrix y** by **method z**

  → Preparation of a list of known diagnostic methods

  - **Applicability for detection and/or identification**
  - **Use on symptomatic and/or asymptomatic material**
  - **Possibility of cross-reactions**
  - **EU pest status (regulated quarantine, non-quarantine)**
  - **Availability of plant material**
  - **Expertise of TPS organizer**

Valitest

1. Definition of TPS scope and Collection of available data

➢ Collection of available data

→ Preparation of a list of known diagnostic methods (TPS Round 1 and 2)

Pest	TPS organiser	Selected methods (TPS scope)
1st round of TPS		
Erwinia amylovora	NIB	real-time PCR, LFDs and LAMP
Pantoea stewartii* subsp. *stewartii	NIB	real-time PCR, PCR and LAMP
Citrus tristeza virus	ANSES	ELISA, Conventional RT-PCR, Real-time RT-PCR, RT-LAMP, TPIA and ImmunoStrip
Bursaphelenchus xylophilus	ANSES	conventional PCR, real-time PCR and LAMP
Plum pox virus	NVWA	DAS-ELISA, RT-PCR and real-time RT-PCR
Fusarium circinatum	FERA	plating, PCR and real-time PCR
2nd round of TPS		
Tomato spotted wilt tospovirus	NIB	DAS-ELISA, on-site tests, conventional and real-time RT-PCR
Xylophilus ampelinus	FERA	DAS-ELISA, IF, conventional and real-time PCR
Cryphonectria parasitica	UNITO	conventional and real-time PCR
Plum pox virus (on-site testing)	ANSES	on-site tests: LFD (serologic), LFD RPA (molecular) and LAMP (molecular)
Tomato brown rugose fruit virus	CREA	DAS-ELISA, on-site tests, conventional and real-time RT-PCR
Xanthomonas citri* pv. *citri	ANSES	conventional, real-time PCR, LAMP and direct molecular tests performed from Immunostrips or Whatman™ FTA cards

→ **A WIDE DIVERSITY**

Molecular methods:
Conventional PCR and RT-PCR
Real-time PCR and RT-PCR
LAMP, LFD RPA

Serological methods:
LFD
ELISA
Immunofluorescence

Other methods:
Tissue Print Immunoassay
Plating

Valitest

1. Definition of TPS scope and Collection of available data

- **The scope needs to be clearly defined for each pest and for each method**

Data from TPS Round 1&2 → A WIDE DIVERSITY

SAMPLE TYPE → Plant extract (wood, leave, seed, fruit), freeze-dried extracts
Infected/non-infected plant material
Plant material with deactivated pathogen
DNA and RNA extract, culture, tissue-print

MATRIX → Leaves/fruits, freeze-dried leaves/fruits
Wood, seed, shoots, stem material, reference culture

SUITABLE FOR → Symptomatic and/or Asymptomatic

PURPOSE → Detection and/or Identification

CONTROLS → NIC, NAC, PAC, PIC, NC, PC

NIC: Negative isolation control, NAC: Negative amplification control, PAC: Positive amplification control, PIC: Positive isolation control, NC: Negative control, PC: Positive control

Scope definition - TPS Round 1 → D1.1. "Minimum performance parameters to select tests for validation & selection of laboratories for TPS"
Scope definition - TPS Round 2 → D1.3. "List of tests for validation - Round 2" → Valitest - Publications

1. Definition of TPS scope and Collection of available data

Example of TPS Round 1 – Scope definition for *Bursaphelenchus xylophilus*

Disease: Pine wilt disease

Wilting symptoms in hot and dry conditions

May remain **asymptomatic** in colder conditions

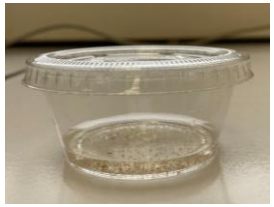
EU pest status

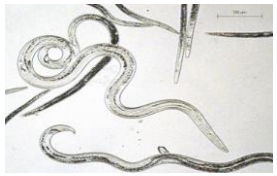
Bursaphelenchus xylophilus is **regulated (quarantine pest)**

→ **Specific and sensitive methods**

Availability of plant material

Difficult to produce infected wood in large quantity and risky to send such material across EU





wood extracts spiked with nematodes / DNA (extracts from culture)

DNA (extracts from culture)

Detection

Real-time PCR

LAMP

Identification

Conventional PCR

Valitest

Common rules – VALITEST project

1. **Definition of TPS scope and Collection of available data**
2. **Criteria to be reached by a test**
3. **Analysis of available data and pre-selection of tests**
4. **Preliminary studies according to the criteria and selection of the final tests**

Question to the audience

According to you, what are the main sources to get access to relevant information on methods and tests available for the pest studied?

(Select maximum 2 answers)

- **Research articles**
- **International standards**
- **EPPO database (validation data)**
- **EUPHRESCO final reports**
- **Kits providers websites**

Interviews – TPS organisers

“How did you get access to relevant information on methods and tests available for the pest studied?”

1. Definition of TPS scope and Collection of available data

➢ Collection of available data

- Research articles
- International standards (e.g. EPPO, IPPC)
- EPPO database (validation data)
- EUPHRESCO final reports
- Kits providers websites (kits instructions)
- EPPO dedicated survey


Bulletin OEPP/EPPO Bulletin (2013) 43 (1), 105–118 ISSN 0250-8052. DOI: 10.1111/epp.12024

European and Mediterranean Plant Protection Organization
Organisation Européenne et Méditerranéenne pour la Protection des Plantes

PM 7/4 (3)

Diagnostics
Diagnostic

PM 7/4 (3) *Bursaphelenchus xylophilus*

Specific scope

This standard describes a diagnostic protocol for *Bursaphelenchus xylophilus*[1].

Specific approval and amendment

First approved in 2000-09. Revised on 2009-09.
Second revision approved on 2012-09

Introduction

Bursaphelenchus xylophilus, the pine wood nematode, is an EPPO A2 pest. The nematode is native to North America [Canada, US, Mexico (unconfirmed)] and is thought to have been carried to Japan at the beginning of the 20th century on timber exports. In Japan, the nematode has been causing massive mortality of native pine trees (*P. thunbergii*, *P. luchuensis*), where it is the forest pest. It has also spread to China, Korea, Taiwan. More recently, it has also killed pines only exotic species including *P. sylvestris*, southern areas of the country. Regardless of rence of pine wilt disease in North America, such is widely dispersed in native conifer

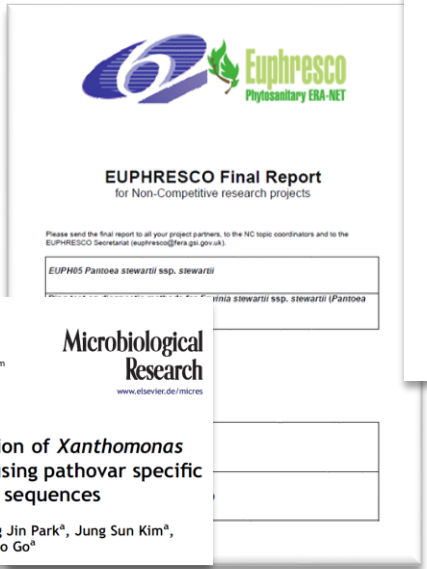
(maturation feeding by either sex), or, depending on *Monochamus* species, to the trunks or larger branches, including wood waste or debris, weakened trees or trees that recently died, during oviposition by the females. Transmission of *B. xylophilus* to the live trees during feeding by the adult beetles can result in the development of wilt disease in the tree, but only in susceptible species of *Pinus* under


Food and Agriculture Organization of the United Nations
International Plant Protection Convention
Protecting the world's plant resources from pests

ISPM 27
ANNEX 15
ENG

STANDARD FOR PHYTOSANITARY MEASURES 27

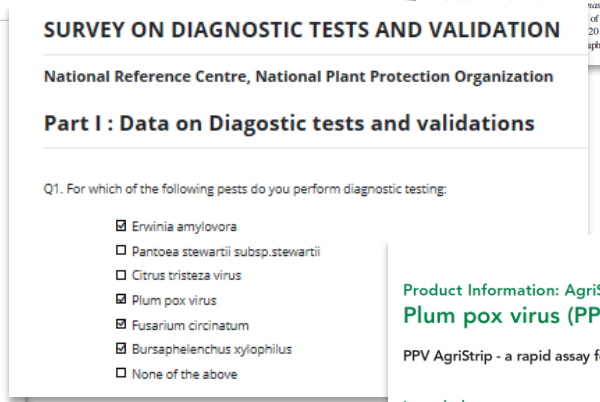
DP 15:
Citrus tristeza virus


Euphresco
Phytosanitary ERA-NET

EUPHRESCO Final Report
for Non-Competitive research projects

Please send the final report to all your project partners, to the NC topic coordinators and to the EUPHRESCO Secretariat (euphresco@fera.gsi.gov.uk).

EUPH05 Pantoea stewartii ssp. *stewartii*

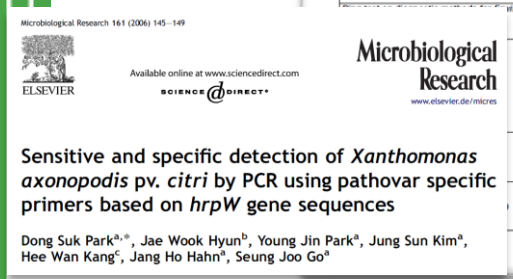

SURVEY ON DIAGNOSTIC TESTS AND VALIDATION

National Reference Centre, National Plant Protection Organization

Part I : Data on Diagostic tests and validations

Q1. For which of the following pests do you perform diagnostic testing:

- ☑ Erwinia amylovora
- ☐ Pantoea stewartii subsp.stewartii
- ☐ Citrus tristeza virus
- ☑ Plum pox virus
- ☑ Fusarium circinatum
- ☑ Bursaphelenchus xylophilus
- ☐ None of the above


Microbiological Research 161 (2006) 145–149

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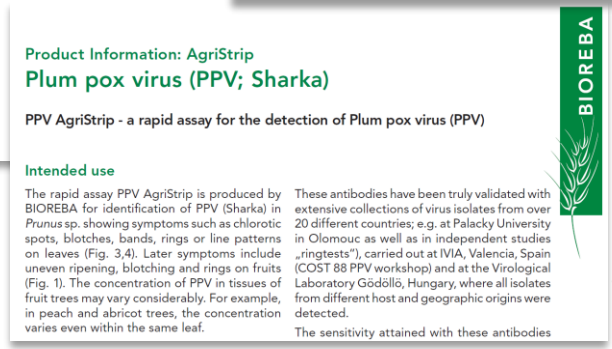
Sensitive and specific detection of *Xanthomonas axonopodis* pv. *citri* by PCR using pathovar specific primers based on *hrpW* gene sequences

Dong Suk Park[a,*], Jae Wook Hyun[b], Young Jin Park[a], Jung Sun Kim[a], Hee Wan Kang[c], Jang Ho Hahn[a], Seung Joo Go[a]


Product Information: AgriStrip

Plum pox virus (PPV; Sharka)

PPV AgriStrip - a rapid assay for the detection of Plum pox virus (PPV)

BIOREBA

Intended use

The rapid assay PPV AgriStrip is produced by BIOREBA for identification of PPV (Sharka) in *Prunus* sp. showing symptoms such as chlorotic spots, blotches, bands, rings or line patterns on leaves (Fig. 3,4). Later symptoms include uneven ripening, blotching and rings on fruits (Fig. 1). The concentration of PPV in tissues of fruit trees may vary considerably. For example, in peach and abricot trees, the concentration varies even within the same leaf.

These antibodies have been truly validated with extensive collections of virus isolates from over 20 different countries; e.g. at Palacky University in Olomouc as well as in independent studies „ringtests"), carried out at IVIA, Valencia, Spain (COST 88 PPV workshop) and at the Virological Laboratory Gödöllö, Hungary, where all isolates from different host and geographic origins were detected.

The sensitivity attained with these antibodies

Valitest

1. Definition of TPS scope and Collection of available data

Data from TPS Round 1&2

Pest	TPS organiser	Selected methods (TPS scope)	Nb of tests identified by collection of data
1st round of TPS			
Erwinia amylovora	NIB	real-time PCR, LFDs and LAMP	**46**
Pantoea stewartii* subsp. *stewartii	NIB	real-time PCR, PCR and LAMP	**30**
Citrus tristeza virus	ANSES	ELISA, Conventional RT-PCR, Real-time RT-PCR, RT-LAMP, TPIA and ImmunoStrip	**22**
Bursaphelenchus xylophilus	ANSES	conventional PCR, real-time PCR and LAMP	**23**
Plum pox virus	NVWA	DAS-ELISA, RT-PCR and real-time RT-PCR	**22**
Fusarium circinatum	FERA	plating, PCR and real-time PCR	**7**
2nd round of TPS			
Tomato spotted wilt tospovirus	NIB	DAS-ELISA, on-site tests, conventional and real-time RT-PCR	**76**
Xylophilus ampelinus	FERA	DAS-ELISA, IF, conventional and real-time PCR	**10**
Cryphonectria parasitica	UNITO	conventional and real-time PCR	**3**
Plum pox virus (on-site testing)	ANSES	on-site tests: LFD (serologic), LFD RPA (molecular) and LAMP (molecular)	**5**
Tomato brown rugose fruit virus	CREA	DAS-ELISA, on-site tests, conventional and real-time RT-PCR	**13**
Xanthomonas citri* pv. *citri	ANSES	conventional, real-time PCR, LAMP and direct molecular tests performed from Immunostrips or Whatman™ FTA cards	**20**

Number of tests identified depends on the pest

TPS Round 1
7 → 46

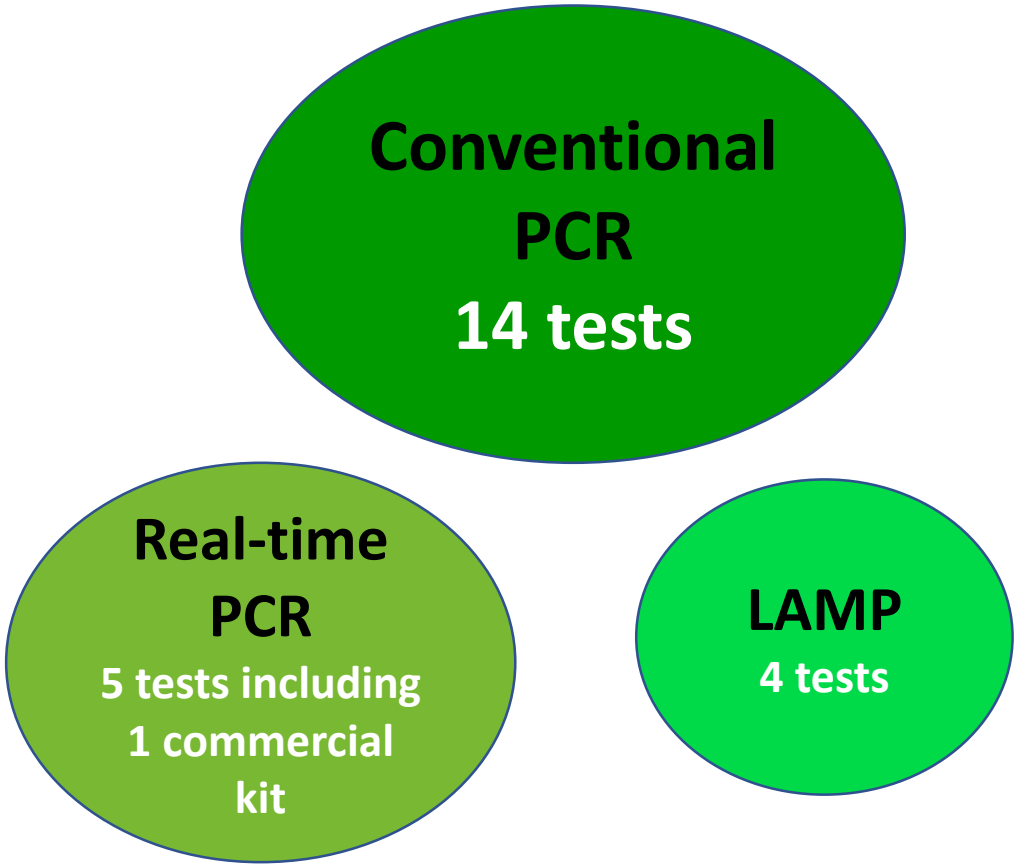
TPS Round 2
3 → 76

Valitest

1. Collection of available data and definition of TPS scope

Example of TPS Round 1 – *Bursaphelenchus xylophilus*

LITERATURE REVIEW → **23 TESTS**



Valitest

Common rules – VALITEST project

1. Definition of TPS scope and Collection of available data
2. Criteria to be reached by a test
3. Analysis of available data and pre-selection of tests
4. Preliminary studies according to the criteria and selection of the final tests

2. Criteria to be reached by a test

- **Criteria were set to objectively select appropriate tests for the defined scope for a specific pest according to:**
  - the use of the **EPPO standard PM 7/98** *(Specific requirements for laboratories preparing accreditation for a plant pest diagnostic activity)*
  - the **experience of TPS organizers**

- **For each criterion, there is a:**
  - **Descriptor** (quantitative or qualitative)
  - **Target** to be reached by a test
  - **Relative weight** different for the different use of the test (laboratory use or on-site use)
  - **Conclusion** whether the criterion is met by the test

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2. Criteria to be reached by a test

- **Performance criteria taken into account:**
  - **Analytical sensitivity** (how much)
  - **Analytical specificity** (what)
    - exclusivity (non-target organism): false positives
    - inclusivity (target organisms): false negatives
  - **Selectivity** (matrix variation) depending on the scope of the test
  - **Repeatability** (consistent results between replicates)
  - **Reproducibility** (effect of operator, time of analysis, equipment)

Note: if a criterion is not relevant for a specific method/pest combination it can be ignored

Valitest

2. Criteria to be reached by a test

Criteria	Descriptor (%, number, text)	Target	Relative Weight (lab)	Relative Weight (on-site)
VALIDATION DATA (prior preliminary studies)				
Available validation data	Yes/No			
→ Validation data available for selected matrix	Yes/No			
Analytical sensitivity (LOD) (pure culture or DNA diluted in water)	Conc. (absolute value if possible or relative conc. or low/medium/high)			
Sensitivity in plant material (selected matrix)	Conc. (absolute value if possible or relative conc. or low/medium/high)			
Diagnostic sensitivity (comparison of different tests)	%			
Analytical specificity	Level			
a) Exclusivity (Non-target organism): False positives	Level			
b) Inclusivity (Target organisms): False negatives	Level			
Selectivity	Presence of cross reactions with matrix			
Repeatability (near LOD)	Level	**100% at LOD**	**medium**	**medium**
Reproducibility/ robustness	%			
Results of interlaboratory comparisons available	Yes/No			
Additional information (not a criterion!)				
Type of matrix				
Extraction method				
Use on sympt./asympt.				
Other				

Valitest

2. Criteria to be reached by a test

- **If some of the tests show similar value and performance, then also less important criteria are taken into account:**
  - **Applicability**
  - **Protocols**
  - **Chemicals**
  - **Equipment**

Note: if a criterion is not relevant for a specific method/pest combination it can be ignored

2. Criteria to be reached by a test

Criteria	Descriptor (%, number, text)	Target	Relative Weight (lab)	Relative Weight (on-site)
APPLICABILITY				
Applicability in different matrixes	level	**high**	**medium**	**medium**
Amount of material which is included in one sample	Amount of plant units tested			
Standardized preparation of the reaction (e.g., ready to use reagents)	Yes/No			
Availability and relevance of controls (in the case of kits)	Yes/No			
PROTOCOLS				
Available detailed protocols	Yes/No			
Simple test procedure	Yes/No			
Simplicity of data analysis	Yes/No			
User friendly test	Yes/No			
Time needed to complete analysis (less than one hour/ one day/ several days)	Duration in time unit			
CHEMICALS				
risks associated with chemicals and consumables	description of the risk (harmful, toxic, …)			
feasibility to transport the chemicals	Yes/No			
shipment of chemicals and samples (safety and transport regulations)?	Possible/Not possible and Easy/Not easy			
Stability of chemicals at ambient temperature	Yes/No			
EQUIPMENT				
No equipment/ instrument needed (relevant only for on-site tests)	Yes/No			
Test not exclusively developed for a specific instrument	Yes/No			
Cost of obligatory equipment/ instruments (up to 10.000 EUR/ 10.000-50.000 EUR/ more than 50.000 EUR?)	Cost in euro			

2. Criteria to be reached by a test

Criteria	Descriptor (%, number, text)	Target	Relative Weight (lab)	Relative Weight (on-site)
APPLICABILITY				
Applicability in different matrixes	level	**high**	**medium**	**medium**
Amount of material which is included in one sample	Amount of plant units tested			
Standardized preparation of the reaction (e.g., ready to use reagents)	Yes/No			
Availability and relevance of controls (in the case of kits)	Yes/No			
PROTOCOLS				
Available detailed protocols	Yes/No			
Simple test procedure	Yes/No			
Simplicity of data analysis	Yes/No			
User friendly test				
Time needed to complete analysis (less than o				
CHEMICALS				
	egulations)?	Possible/Not possible and Easy/Not easy		
	ient temperature	Yes/No		
EQUIPMENT				
No equipment/ instrument needed (relevant only for on-site tests)	Yes/No			
Test not exclusively developed for a specific instrument	Yes/No			
Cost of obligatory equipment/ instruments (up to 10.000 EUR/ 10.000-50.000 EUR/ more than 50.000 EUR?)	Cost in euro			

Tables "criteria for selection of tests" TPS Round 1 → D1.1. "Minimum performance parameters to select tests for validation & selection of laboratories for TPS"

Tables "criteria for selection of tests" TPS Round 2 → D1.3. "List of tests for validation - Round 2"

Valitest - Publications

Common rules – VALITEST project

1. Definition of TPS scope and Collection of available data
2. Criteria to be reached by a test
3. Analysis of available data and pre-selection of tests
4. Preliminary studies according to the criteria and selection of the final tests

3. Analysis of available data and pre-selection of tests

- **Analysis of available data :**
  - **Analysis of performance** values from the available validation data for each test
  - **Objective comparison of performance** among the tests identified

Interviews – TPS organisers

What was the major difficulty you encountered to select the tests? How did you circumvent this difficulty?

3. Analysis of available data and pre-selection of tests

➢ **Analysis of available data :**

- **Analysis of performance** values from the available validation data for each test
- **Objective comparison of performance** among the tests identified

Difficulties

Extensive validation data
Lack of validation data or no validation data → **Comparison is very difficult**

Specific equipment required for some tests

Availability of commercial kits and/or reagents

Test not widely used in diagnostic laboratories in EU
(no EU research publications and not included in EPPO and IPPC diagnostic protocols)

Valitest

3. Analysis of available data and selection of first tests

Example of TPS Round 1 – *Bursaphelenchus xylophilus*

→ Availability of validation data from literature sources

→ Tests reported in EPPO and IPPC diagnostic protocols

→ Tests reported in EPPO survey results

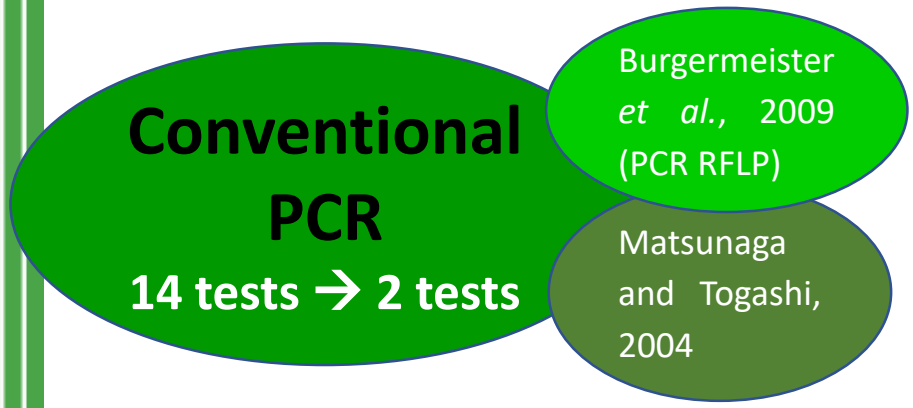
→ Available results of interlaboratory studies or intralaboratory validation data

→ Experience from TPS organizer

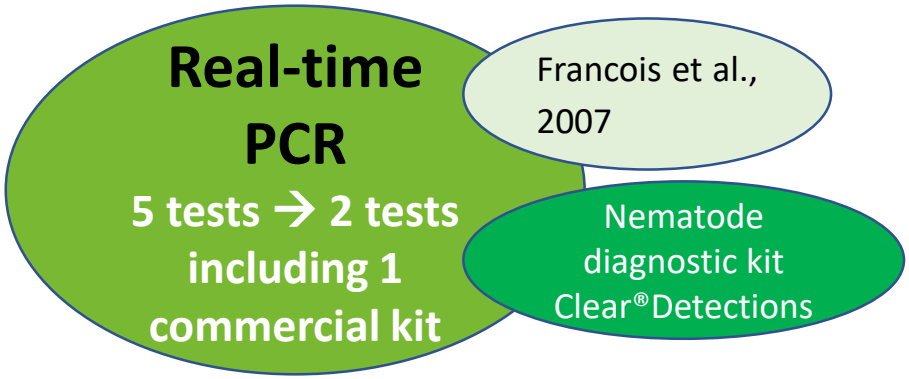
6 PRE-SELECTED TESTS

3. Analysis of available data and pre-selection of tests

Example of TPS Round 1 – ***Bursaphelenchus xylophilus***

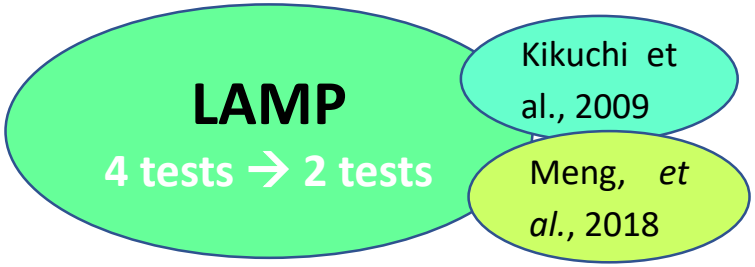


- Test Burgermeister **widely used in the EU region** and considered as a **gold standard**



- **Large offer of tests** in literature → **only tests corresponding to the TPS scope (focused on high analytical sensitivity and high analytical specificity)** selected for preliminary studies

- **Only 1 commercial kit** available and **not previously submitted to an interlaboratory study**



- **on-site testing** crucial for **early detection**
  → tests selected to **document their performance**
  → **pre-selection** based on the **results of EPPO survey**

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3. Analysis of available data and pre-selection of tests

➢ From literature

Conventional PCR | Burgermeister *et al.*, 2009 (PCR RFLP) | Leal, 2005 | Li, 2008

Criteria	Descriptor (%, number, text)	Target	Relative Weight (lab)	Value	Conclusion for the test (OK/NOK)	Value	Conclusion for the test (OK/NOK)	Value	Conclusion for the test (OK/NOK)
Validation data (prior preliminary studies)									
available validation data	Yes/No	Yes	**medium**	**Yes**	**OK**	Yes	OK	NO	NOK
→ validation data available for selected matrix	Yes/No	Yes	**low**	**yes**	**OK**	Yes			
analytical sensitivity (LOD)	nb individuals	<10	**medium**	**5**	**OK**	5			
analytical specificity	%	None	**high**	**100**	**OK**				
a) false positives	% of non target populations detected	0%	**high**	**0**	**OK**	5%			
b) false negatives	% of target populations not detected	0%	**high**	**0**	**OK**	0%			
selectivity	presence of cross reactions with matrix	No	**high**	**no**	**OK**				
repeatability	% of agreement between repetitions	100% at LOD	**medium**	**100%**	**OK**	100%			
reproducibility/ robustness	% of agreement between repetitions in different conditions	100% at LOD	**medium**	**100%**	**OK**	100%			
Rationale				**Validation data OK. Test widely used in EU laboratories**		The test is less efficient regarding specificity		No validation data for performance criteria	

3. Analysis of available data and pre-selection of tests

Data from TPS Round 1&2

Pest	TPS organiser	Selected methods (TPS scope)	Nb of tests identified by collection of data	Nb of pre-selected tests
1st round of TPS				
Erwinia amylovora	NIB	real-time PCR, LFDs and LAMP	**46**	**9**
Pantoea stewartii* subsp. *stewartii	NIB	real-time PCR, PCR and LAMP	**30**	**8**
Citrus tristeza virus	ANSES	ELISA, Conventional RT-PCR, Real-time RT-PCR, RT-LAMP, TPIA and ImmunoStrip	**22**	**16**
Bursaphelenchus xylophilus	ANSES	conventional PCR, real-time PCR and LAMP	**23**	**6**
Plum pox virus	NVWA	DAS-ELISA, RT-PCR and real-time RT-PCR	**22**	**18**
Fusarium circinatum	FERA	plating, PCR and real-time PCR	**7**	**7**
2nd round of TPS				
Tomato spotted wilt tospovirus	NIB	DAS-ELISA, on-site tests, conventional and real-time RT-PCR	**76**	**19**
Xylophilus ampelinus	FERA	DAS-ELISA, IF, conventional and real-time PCR	**10**	**10**
Cryphonectria parasitica	UNITO	conventional and real-time PCR	**3**	**3**
Plum pox virus (on-site testing)	ANSES	on-site tests: LFD (serologic), LFD RPA (molecular) and LAMP (molecular)	**5**	**4**
Tomato brown rugose fruit virus	CREA	DAS-ELISA, on-site tests, conventional and real-time RT-PCR	**13**	**8**
Xanthomonas citri* pv. *citri	ANSES	conventional, real-time PCR, LAMP and direct molecular tests performed from Immunostrips or Whatman™ FTA cards	**20**	**20**

Number of pre-selected tests for preliminary studies

TIME AND BUDGET CONSTRAINTS

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Common rules – VALITEST project

1. Definition of TPS scope and Collection of available data
2. Criteria to be reached by a test
3. Analysis of available data and pre-selection of tests
4. Preliminary studies according to the criteria and selection of the final tests

4. Preliminary studies and selection of the final tests

Example of TPS Round 1 – *Bursaphelenchus xylophilus*

6 PRE-SELECTED TESTS for preliminary studies

Method	**Tests for validation:**
Real-time PCR	Real-Time PCR Nematode diagnostic kits Clear®Detections Francois *et al.*, 2007
Conventional PCR	Matsunaga and Togashi, 2004 Burgermeister *et al.*, 2009
On-site methods	**Molecular (LAMP):** Kikuchi *et al.*, 2009 Meng, *et al*., 2018

Tables "tests selected" TPS Round 1 → D1.2. "List of tests for validation - Round 1"
Tables "tests selected" TPS Round 2 → D1.3. "List of tests for validation - Round 2"
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4. Preliminary studies according to the criteria

Example of TPS Round 1 – *Bursaphelenchus xylophilus*

Conventional PCR

Burgermeister *et al.*, 2009 (PCR RFLP)

Criteria	Descriptor (%, number, text)	Target	Relative Weight (lab)	Value	Conclusion for the test (OK/NOK)
Validation data (after preliminary studies)					
analytical sensitivity (LOD)	nb individuals	Lowest level	high	**1**	**ok**
analytical specificity	% of true positive detected and true negative not detected	Highest level	high	**100%**	**ok**
a) false positives	% of non target populations detected	0%	high	**0%**	**ok**
b) false negatives	% of target populations not detected	0%	high	**0%**	**ok**
selectivity	presence of cross reactions with matrix	No	high	**no**	**ok**
repeatability	% of agreement between repetitions	100% at LOD	medium	**100%**	**ok**
reproducibility	% of agreement between repetitions in different conditions	100% at LOD	medium	**100%**	**ok**

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4. Preliminary studies and selection of the final tests

Example of TPS Round 1 – *Bursaphelenchus xylophilus*

Methods/tests / Criteria	Conventional PCR		Real-time PCR		LAMP PCR	
	Burgermeister *et al.*, 2009	Matsunaga and Togashi, 2004	François *et al.*, 2007	Diagnostic kit Clear®Detections	Kikuchi *et al.*, 2009	Meng *et al.*, 2018
Limit of detection	**1 nematode**	1 nematode	1 nematode	10 nematodes**	1 nematode	The test did not perform at all
Sensitivity *	**100%**	100%	100%	100%	100%	
Repetability *	**100%**	100%	100%	100%	100%	
Reproductibility*	**100%**	100%	100%	100%	100%	
Analytical specificity (inclusivity and exclusivity)	**100%**	100%	100%	100%	100%	

* : at the limit of detection;
** :with strict respect of the kit provider 's recommandation, i.e by diluting DNA solution to 1/20

Good validation results

only 1 commercial kit available + correct validation results

only 1 LAMP test with correct validation results

5 FINAL TESTS FOR TPS

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4. Preliminary studies and selection of the final tests

Common rules → Final tests for TPS

Example of TPS Round 1 – *Bursaphelenchus xylophilus*

1. **Definition of TPS scope and Collection of available data**

   3 METHODS
   23 tests from literature

2. **Analysis of available data and pre-selection of tests**
3. **Criteria to be reached by a test**

   6 PRE-SELECTED TESTS
   subjected to preliminary studies

4. **Preliminary studies according to the criteria and selection of the final tests**

   5 FINAL TESTS FOR TPS

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Question to the audience

According to you, how long does it take to perform test selection (including preliminary study)?

- **Less than 3 months**
- **Between 3 and 6 months**
- **More than 6 months**

Interviews – TPS organisers

“How long did you need to perform the selection of the tests (including bibliographical searches, defining the scope and the weighted criteria, preliminary studies, and final selection of the tests)?”

Selection of tests

1. **Definition of TPS scope and Collection of available data**
2. **Analysis of available data and pre-selection of tests**
3. **Criteria to be reached by a test**
4. **Preliminary studies according to the criteria and selection of the final tests**

TIMELINE

5 TPS organizers: 2 - 3 MONTHS

1 TPS organizer: 4 - 5 MONTHS

3 TPS organizers: 5 - 8 MONTHS

→ **Nb of staff involved in the TPS organization team**

→ **Nb of selected tests for preliminary studies**

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Selection of tests

Pest	TPS organiser	Selected methods (TPS scope)	① Nb of tests identified by collection of data	② Nb of tests selected for preliminary studies	③ Nb of tests selected for TPS
1st round of TPS					
Erwinia amylovora	NIB	real-time PCR, LFDs and LAMP	**46**	**9**	**6**
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2nd round of TPS					
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Cryphonectria parasitica	UNITO	conventional and real-time PCR	**3**	**3**	**3**
Plum pox virus (on-site testing)	ANSES	on-site tests: LFD (serologic), LFD RPA (molecular) and LAMP (molecular)	**5**	**4**	**3**
Tomato brown rugose fruit virus	CREA	DAS-ELISA, on-site tests, conventional and real-time RT-PCR	**13**	**8**	**5**
Xanthomonas citri* pv. *citri	ANSES	conventional, real-time PCR, LAMP and direct molecular tests performed from Immunostrips or Whatman™ FTA cards	**20**	**20**	**13**

① Identification of methods and tests by collection of data

② Pre-selection of tests according to the defined criteria

③ Selection of final tests after preliminary studies

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Interviews – TPS organisers

Did you encounter unexpected difficulties?

Lessons learnt from test selection

TIME AND BUDGET CONSTRAINTS

→ **not possible to include all available methods and tests/kits (in particular where the number of tests available was very high)**

ABSENCE of CRITICAL INFORMATION

→ **limit the inclusion of the test into the TPS validation procedure**

LACK OF OFFER FOR KITS

→ **In some cases, no commercial kits available on the market or TPS organizer is not aware of its availability**

OFFER OF KITS TOO LARGE

→ **Difficult to validate kits in their original description**

- **TPS Round 1 preselection:** expertise of TPS organizers, previous experience, assessment of feasibility
- **TPS Round 2 preselection:** more input from kits providers especially with regards to modifications

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Lessons learnt from test selection

LACK OF METHOD EXPERTISE OF TPS ORGANIZER

→ **Methods are not included in the TPS**

ADAPTATION OF PROTOCOLS

→ **Most common reasons:**

- **adaptation of sample preparation** including the extraction buffer to a particular matrix
- **replacement of reagents** specified in the original source but no longer available
- **use of different instruments**
- **optimization of processes** (e.g. minimizing the number of different buffers, polymerases, instruments, DNA extraction kits in a laboratory)

COMMERCIAL KIT vs PUBLICATION

→ **Depend on validation data available for commercial kit compared to the (same) test from publication**

more transparent information on validation data and performance required for commercial kits inclusion in TPS

Discussions and **Harmonization** between **TPS organizers** and commercial **kits providers**

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Thank you for your attention!

Any question?

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Introduction of the webinar and training activities

Test Performance Studies organisation

Videos	What is a TPS?	On the week 02/15
Videos	VALITEST TPS: selection of the pests and of the TPS organizers	On the week 02/15
Webinar 1	Preparing the TPS plan	Friday 19/02, 11am
Webinar 2	Selection of the tests and associated documents	Wednesday 24/02, 2pm
Webinar 3	**Selection of participants and contract**	**Monday 1/03, 2pm**
Webinar 4	Preparation and dispatch of samples	Friday 5/03, 11am
Webinar 5	Production of reference material for TPS	Wednesday 10/03, 2pm
Practical training sessions	How to organise Test Performance Studies?	15-18/03 (4 sessions)
Webinar 6	How to tackle the analysis of TPS results?	Monday 22/03, 2pm
Videos	Calculate performance characteristics of a test and get useful information from your validation data by statistical analysis.	On the week 22/03
Webinar 7	Q&A session: the statistical analysis of TPS results	Monday 29/03, 2pm
Practical training sessions	How to analyse the results of Test Performance Studies?	30/03-1/04 (3 sessions)
Webinar 8	From TPS organisation to analysis of the results: example of the TPS on ToBRFV	Wednesday 7/04, 2pm
Videos	Reporting TPS results	To be confirmed/announced