

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 (3 sessions: 2x ToBRFV, 1x TSWV)
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 training activities
Test Performance Studies organisation

How to organise Test Performance Study?

TSWV example

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Support: Dr. Ana Vučurović (NIB)

On-line training, 16th March 2021



NACIONALNI INŠTITUT ZA **BIOLOGIJO**
NATIONAL INSTITUTE OF **BIOLOGY**



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Outline of training session

Timing*	Timeline
9:00	Introduction
	Introduction to the pest
	Diagnosis of the pest
	Scope of the TPS
	Test selection for preliminary study
10:30	break
11:00	Preliminary study and test selection for TPS
	In-house validation
	Selection of TPS participants, contract
	Technical instructions
12:30	break
13:30	Test panel composition
	Descriptors of reference material/ samples included in TPS
	Randomization, distribution of samples, consumables provided by the organizer of TPS
	Collection of participants results
	TPS results analysis
finish: 15:00	Conclusion

*Time zone: CET

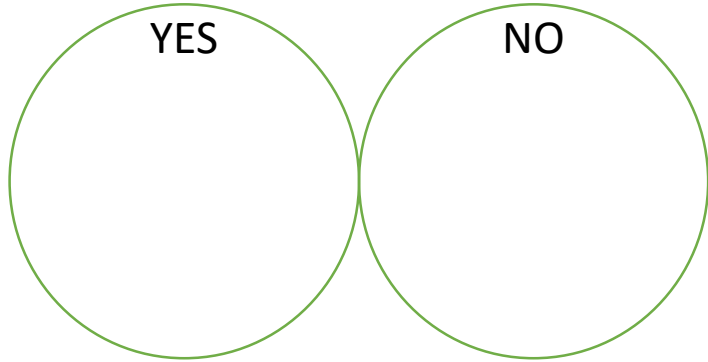
How to use whiteboard in Zoom?

1. Go to View Options at the very top of your screen
2. Click on the ^ for the drop-down menu
3. Choose Annotate
4. Pick Text, Draw or stamp

Wish list for today (one or two words)

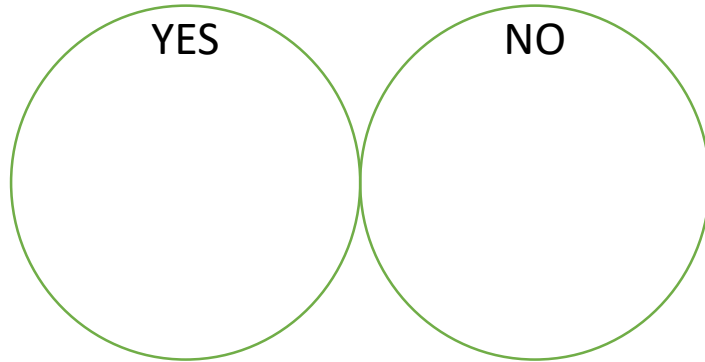
Did you organized any Test Performance Study (TPS)?

YES NO

Two overlapping circles with green outlines. The left circle is labeled 'YES' and the right circle is labeled 'NO'. The circles overlap in the middle.

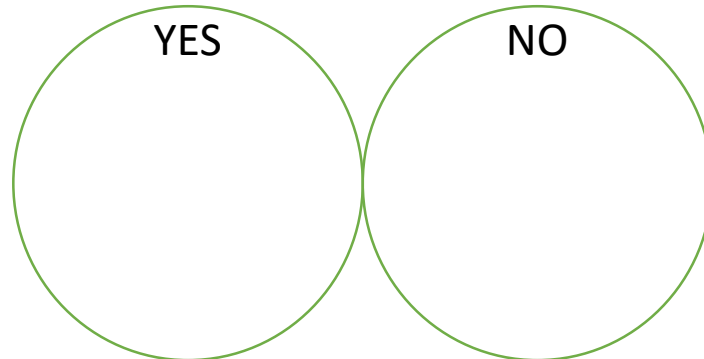
Are you planning to organize TPS in near future?

YES NO

Two overlapping circles with green outlines. The left circle is labeled 'YES' and the right circle is labeled 'NO'. The circles overlap in the middle.

Did you ever take part in TPS as TPS participant?

YES NO

Two overlapping circles with green outlines. The left circle is labeled 'YES' and the right circle is labeled 'NO'. The circles overlap in the middle.

Tomato spotted wilt tospovirus (TSWV)

It is not my area of expertise

I am virologist, but I never worked with TSWV

I have some/many experiences with diagnosis/ research of TSWV and/or other tospoviruses

Tomato spotted wilt tospovirus (TSWV)

- One of the most important plant viruses
- Host range **>1000** plant species (tomato, pepper, a variety of ornamentals,...)

Symptoms in tomato



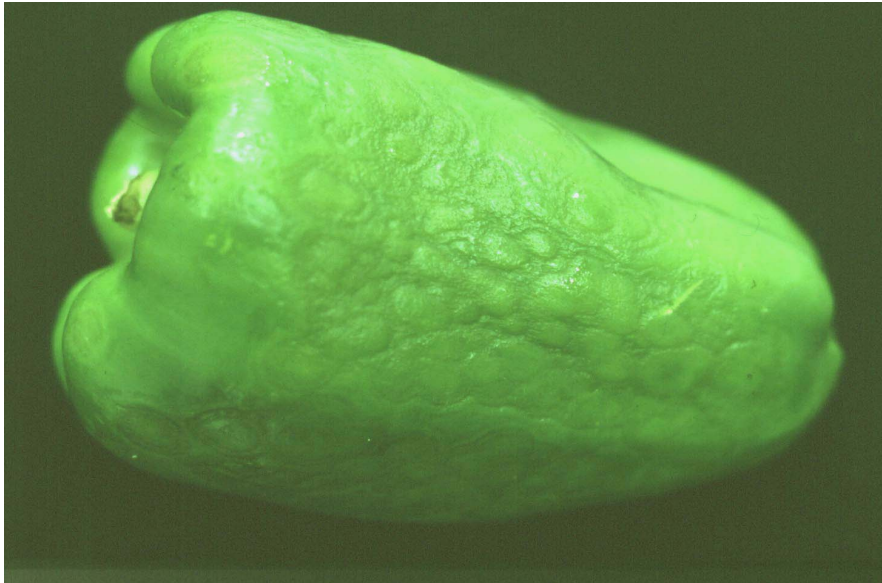
Images: Nejc Jakoš, NIB



Image: Magda Rak Cizej, IHP Žalec

Tomato spotted wilt tospovirus (TSWV)

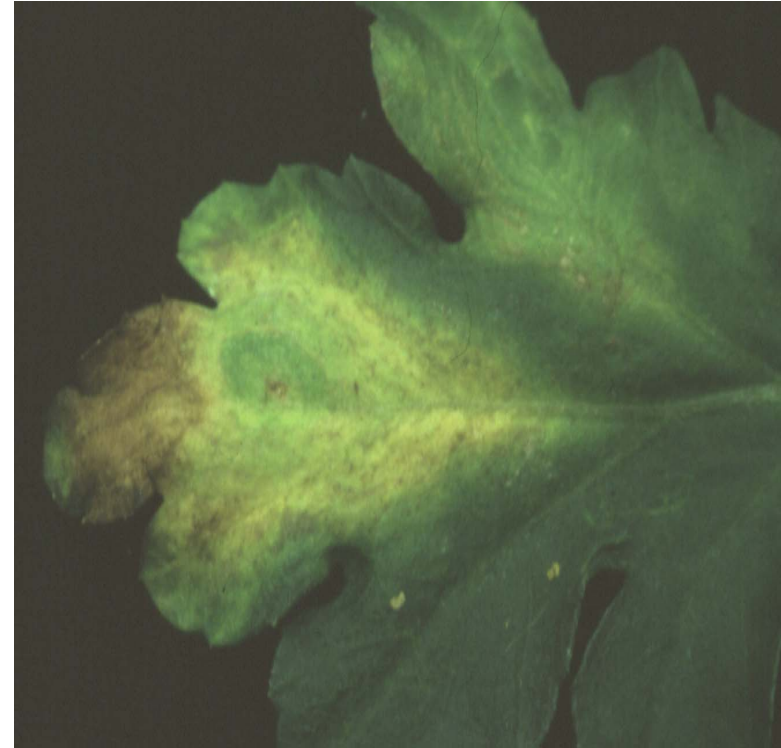
pepper:



Images: NIB archive

Tomato spotted wilt tospovirus (TSWV)

Chrysanthemum:



Images: NIB archive

Tomato spotted wilt tospovirus (TSWV)

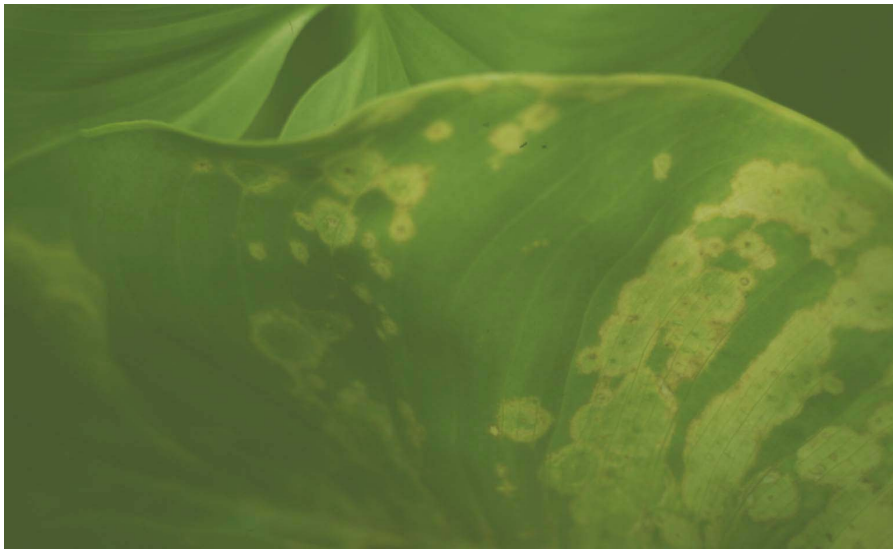
Cyclamen:



Image: NIB archive

Tomato spotted wilt tospovirus (TSWV)

Zantedeschia:



Images: NIB archive

Tomato spotted wilt tospovirus (TSWV)

Nerium oleander:

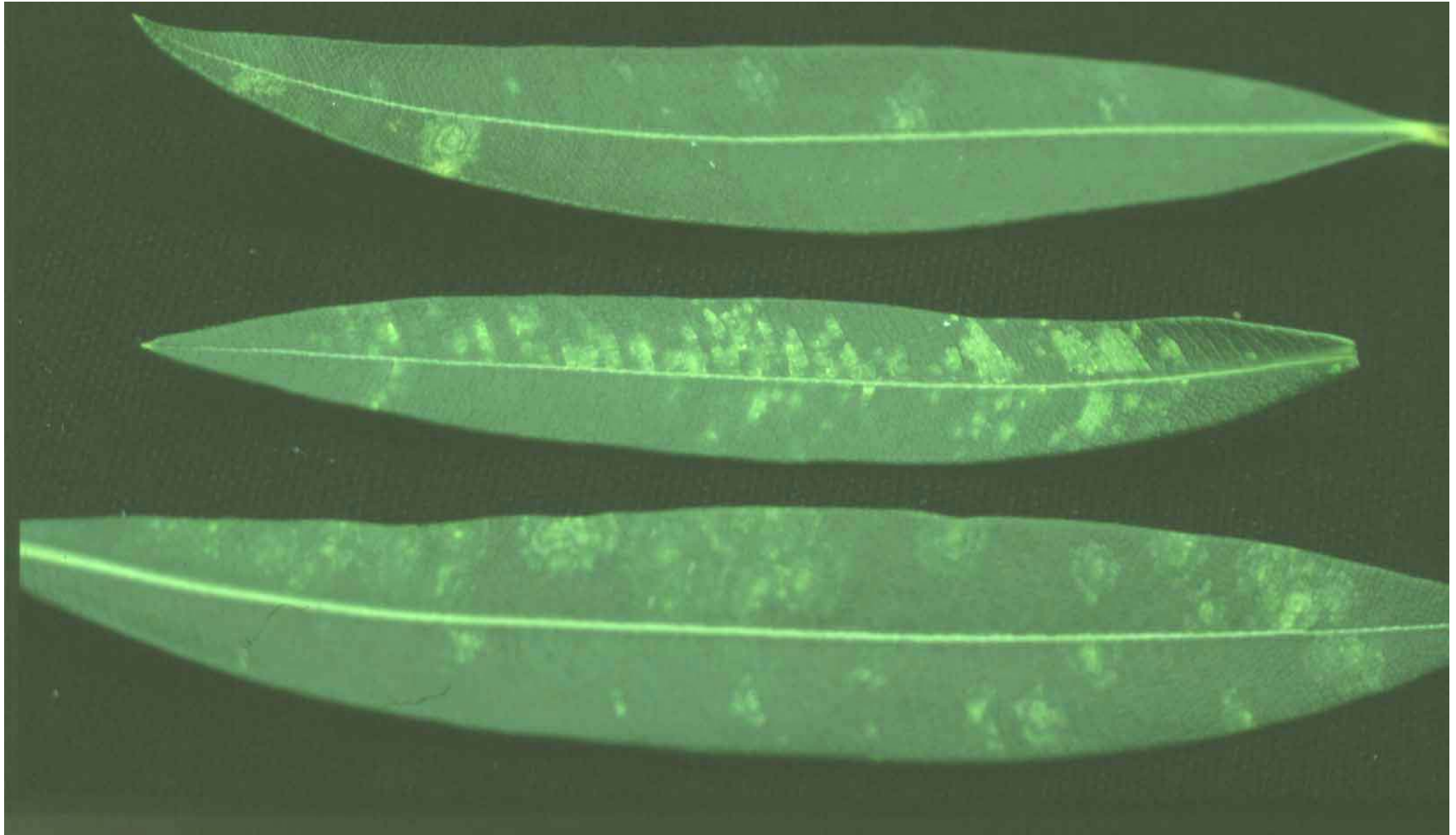
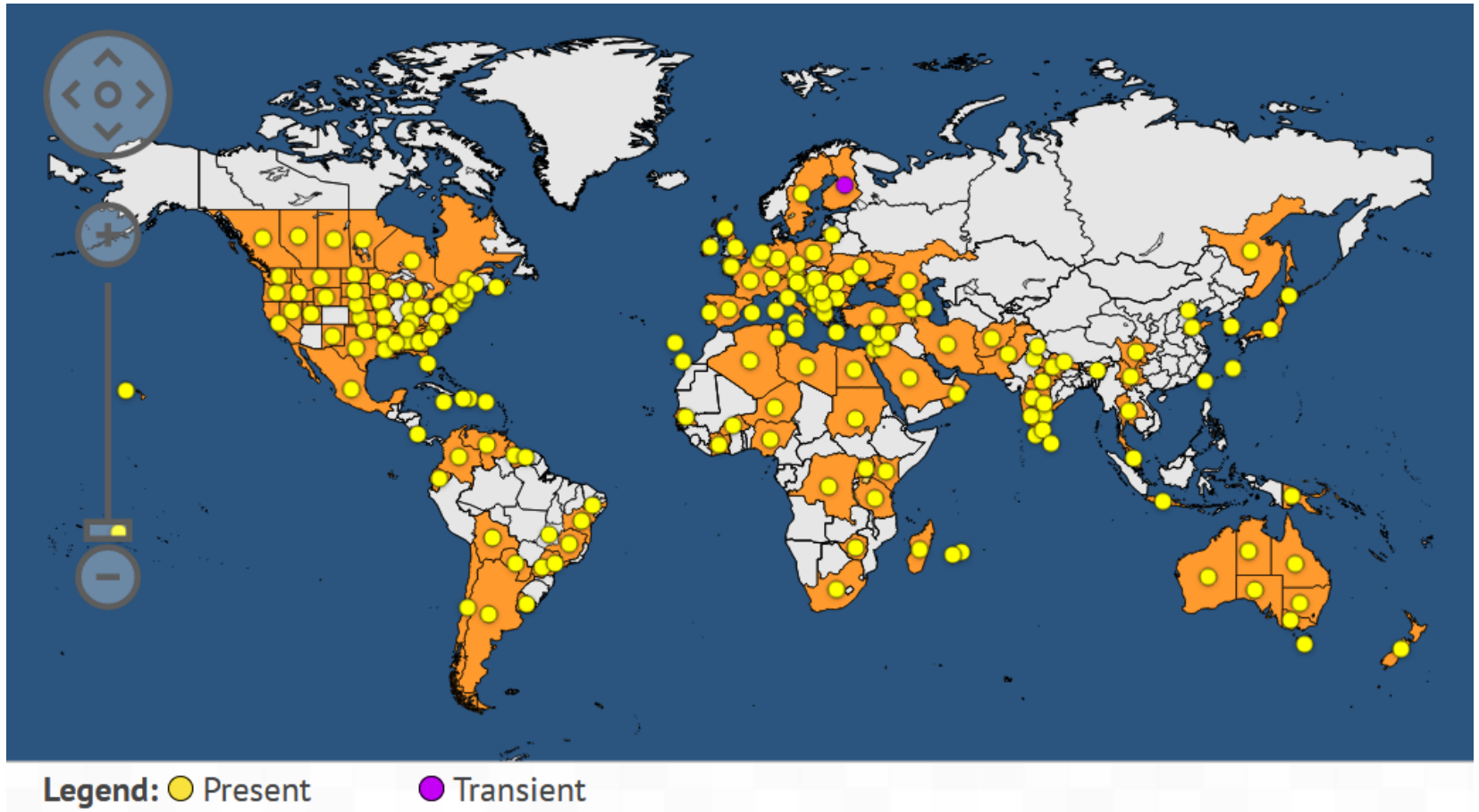


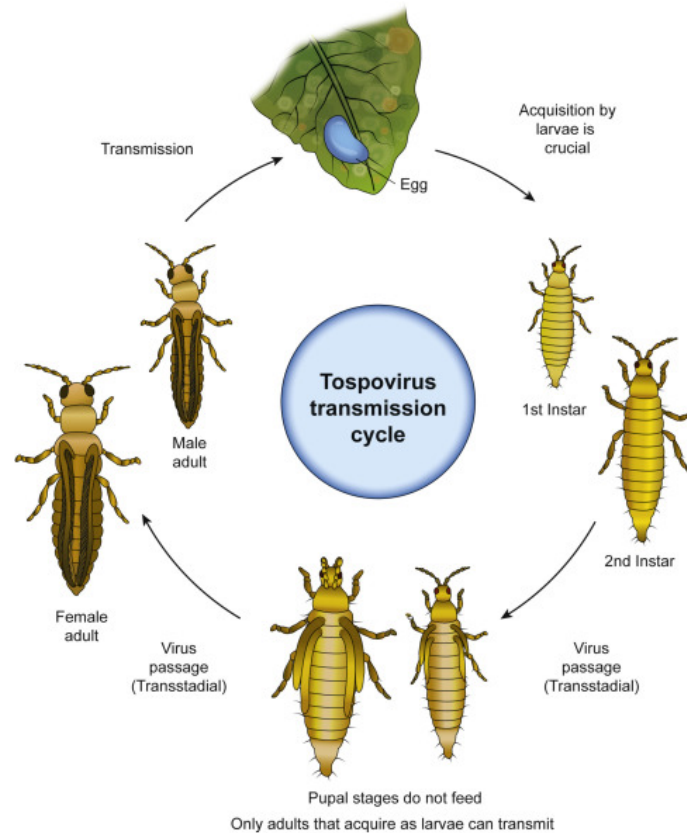
Image: NIB archive

Tomato spotted wilt tospovirus (TSWV)



Source of distribution map: EPPO global database <https://gd.eppo.int/taxon/TSWV00/distribution>

Tomato spotted wilt tospovirus (TSWV)



The most important vector: Western flower thrips

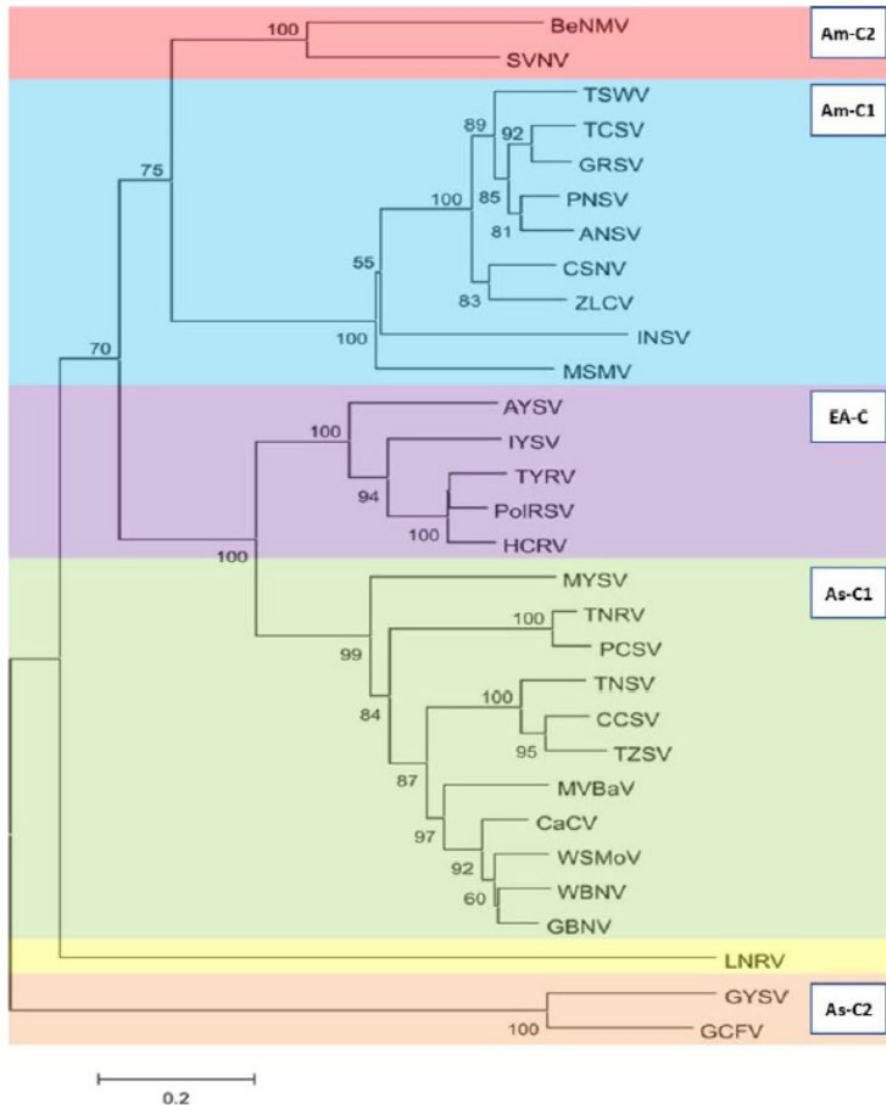
Tomato spotted wilt tospovirus (TSWV)

- Regulated non-quarantine pest (Commission implementing Regulation (EU) 2019/2072 of 28 November 2019)

planting material of *Lactuca sativa* L., *Solanum lycopersicum* L., *Solanum melongena* L., *Begonia x hiemalis* Fotsch, *Capsicum annuum* L., *Chrysanthemum* L., *Gerbera* L., *Impatiens* L. New Guinea Hybrids, *Pelargonium* L. must be free of TSWV

- Emergence of resistance breaking strains!!!

Tomato spotted wilt tospovirus (TSWV)



SIMILAR SYMPTOMS

Source of figure: EPPO PM 7/139

Diagnosis of the pest

Bulletin OEPP/EPPO Bulletin (2020) **50** (2), 217–240

ISSN 0250-8052. DOI: 10.1111/epp.12676

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

PM 7/139 (1)

Diagnostics

PM 7/139 (1) Tospoviruses (Genus *Orthotospovirus*)

Specific scope

This Standard describes a diagnostic protocol for detection and identification of tospoviruses¹.

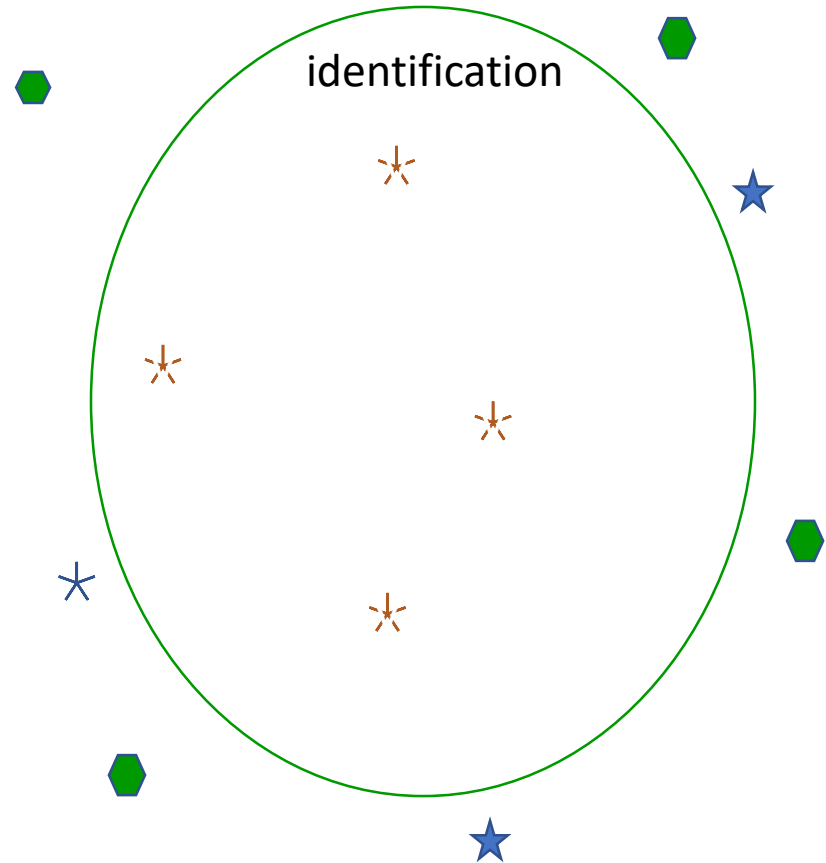
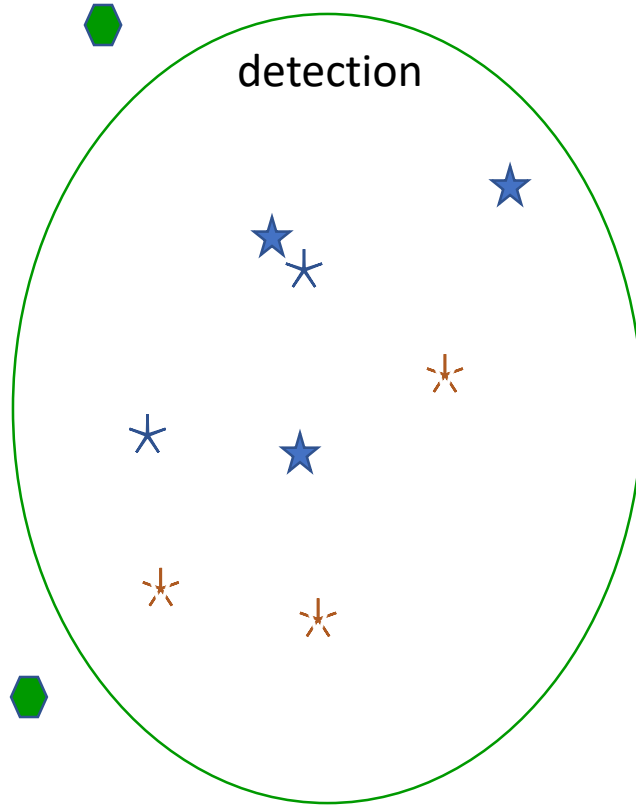
This protocol replaces the EPPO Standard PM 7/34 on *Tomato spotted wilt*, *Impatiens necrotic spot* and *Watermelon silver mottle* tospoviruses.

This Standard should be used in conjunction with PM 7/76 *Use of EPPO diagnostic protocols*.

Specific approval and amendment

Approved in 2020-04.

Detection vs. Identification ? *



Detection vs. Identification ?

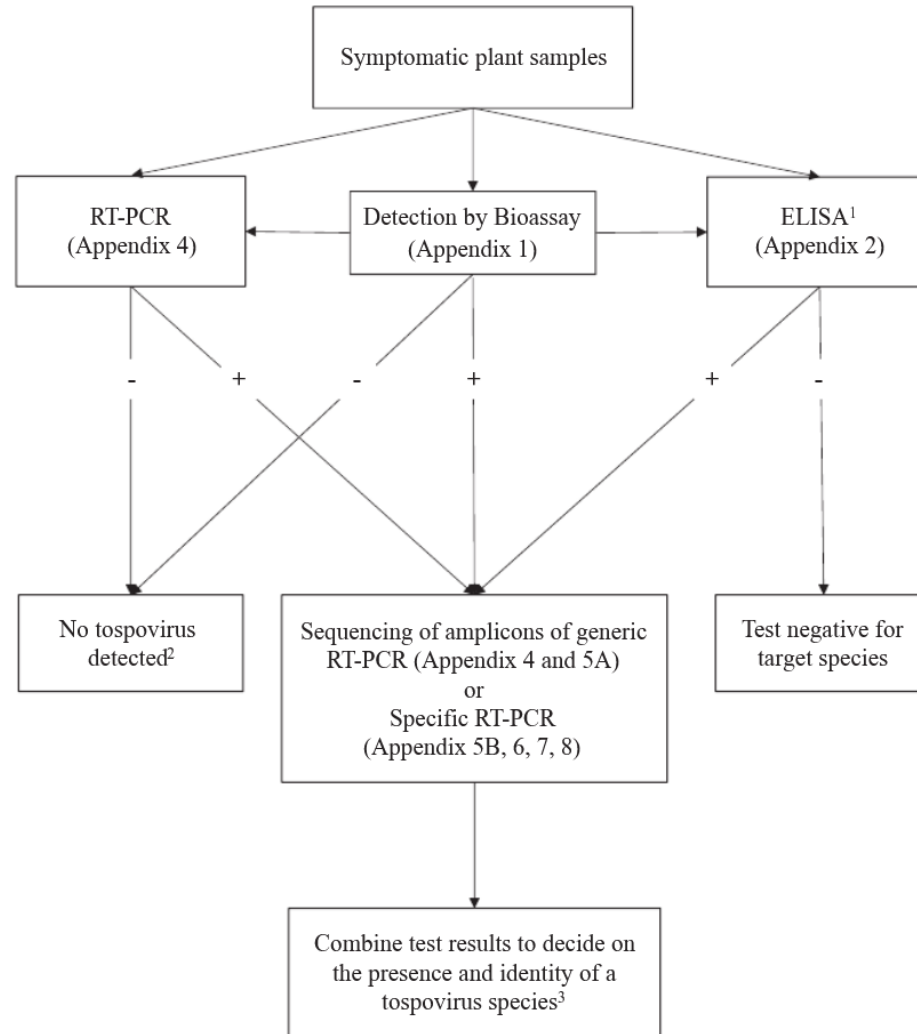
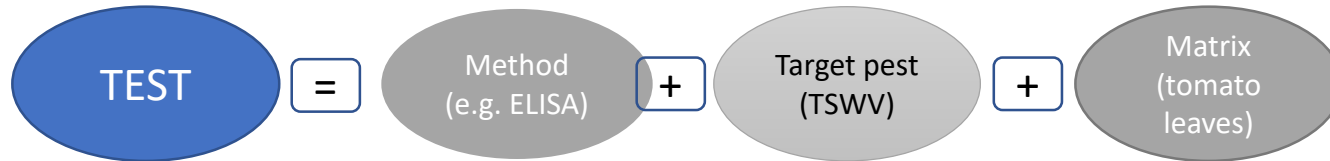


Fig. 3 Flow diagram for detection and identification of tospoviruses in symptomatic plant samples.
EPPO PM7/139

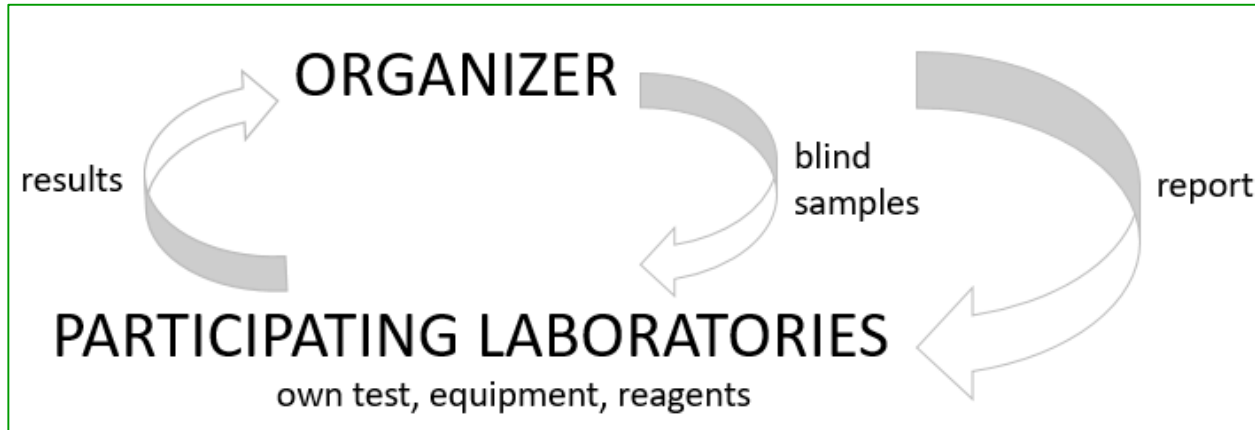
Method vs. Test ?



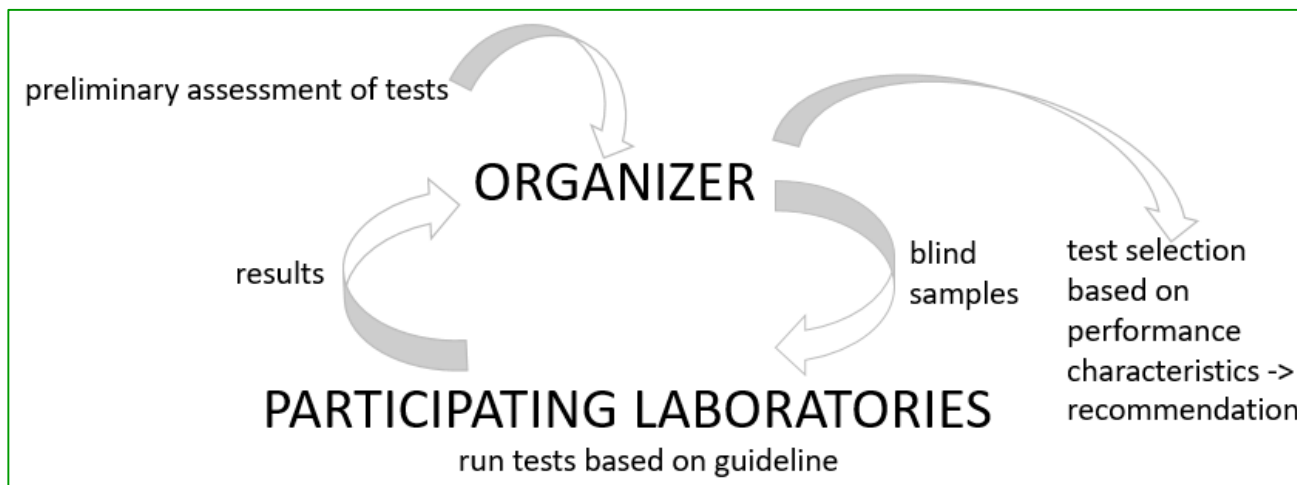
Proficiency test (PT) vs. Test Performance study (TPS)?

EPPO PM7/122 (Guidelines for the organization of interlaboratory comparisons by plant pest diagnostic laboratories)

PT:



TPS:

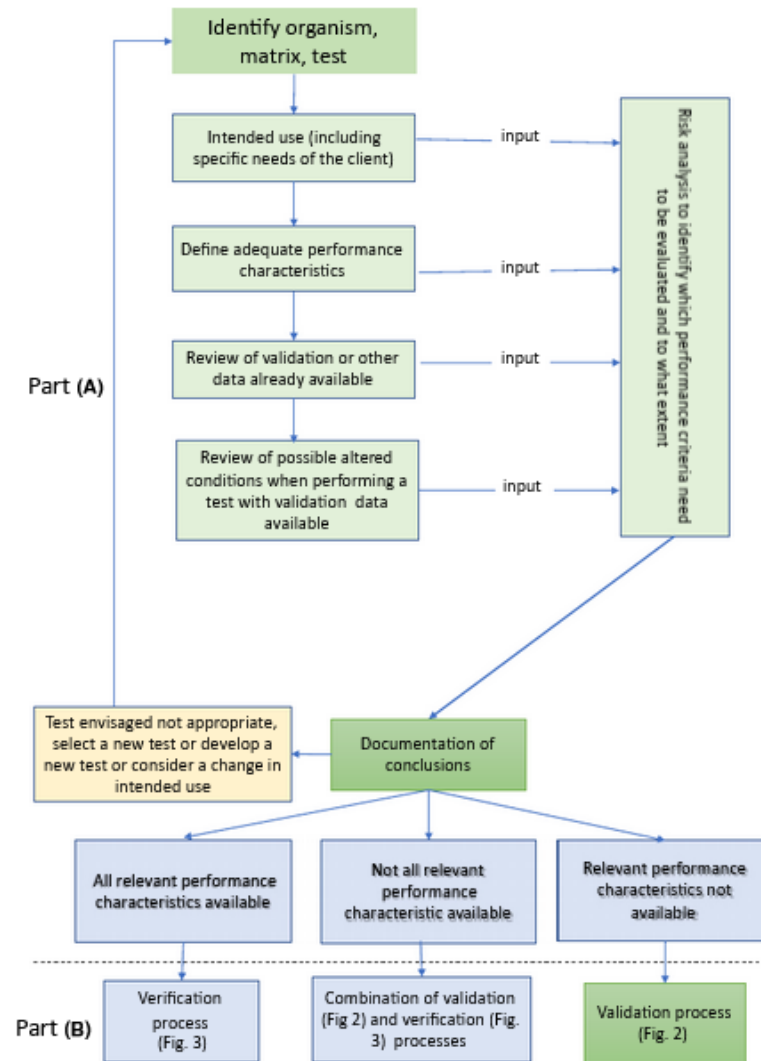


Validation vs. Verification?



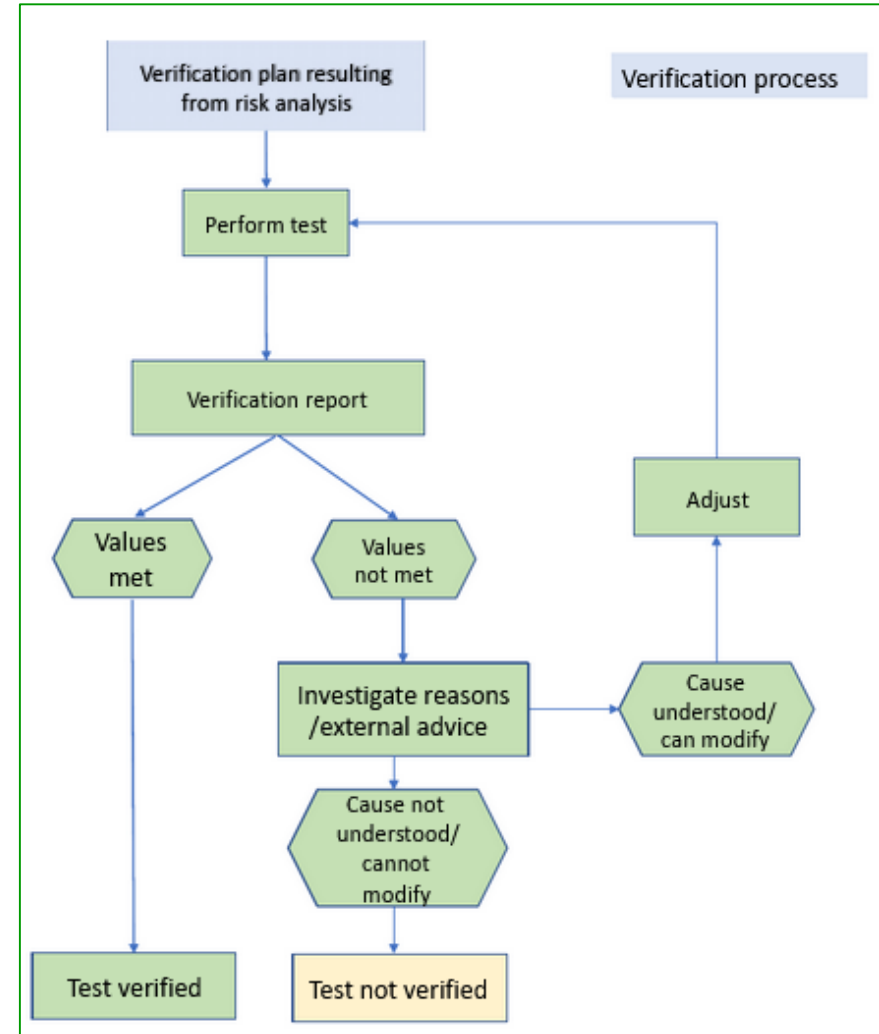
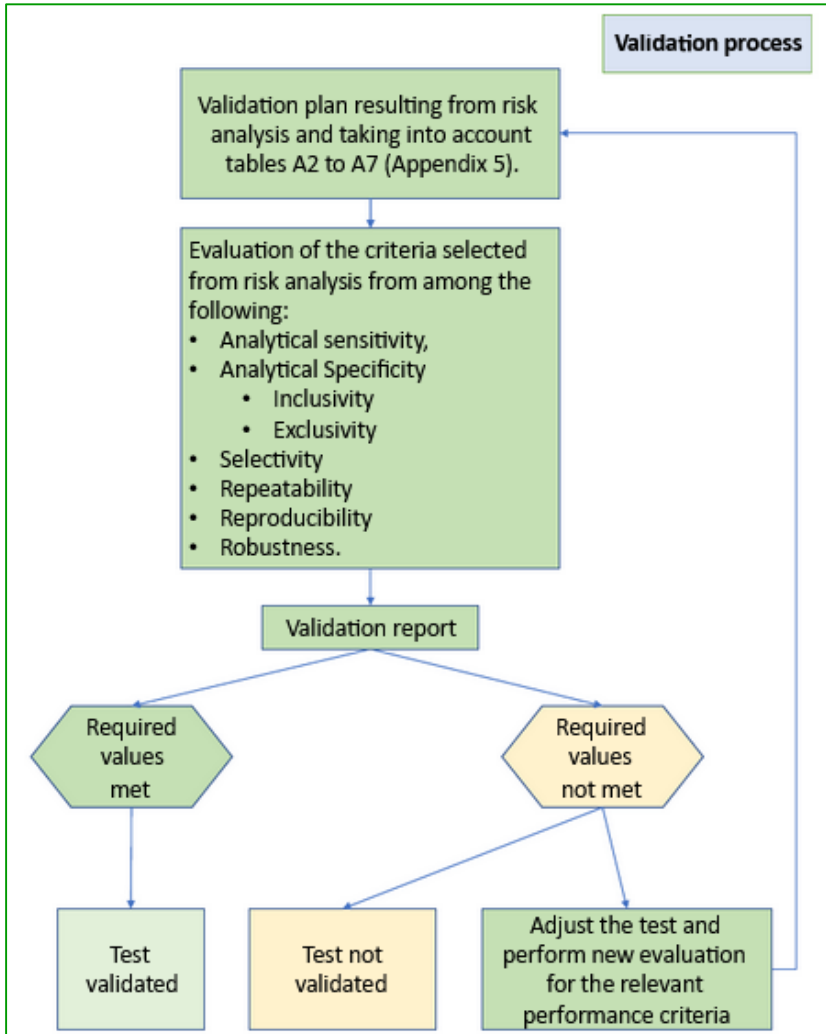
Validation vs. Verification?

EPPO PM7/98(4) (Specific requirements for laboratories preparing accreditation for a plant pest diagnostic activity)



Validation vs. Verification?

EPPO PM7/98(4) (Specific requirements for laboratories preparing accreditation for a plant pest diagnostic activity)



Validation of a test = Evaluation of its performance characteristics

PERFORMANCE CHARACTERISTICS	description
ANALYTICAL SENSITIVITY	How much?
ANALYTICAL SPECIFICITY	What? (inclusivity, exclusivity)
REPRODUCIBILITY	Effect of operator, time of analysis, equipment
REPETABILITY	Consistent results between replicates
SELECTIVITY	Matrix variation

Scope of TPS

- What? (target, matrix, symptomatic/asymptomatic...)
- Why? (plants for planting, certification, national survey,...)
- ...

Scope of TPS

Detection and identification of TSWV in symptomatic tomato leaves

Method	Mark 4 methods you think should be included in TPS
ELISA	
Tissue(dot)-blot immunoassay	
Electron microscopy	
Bioassay (test plants)	
lateral flow devices	
microarray	
LAMP	
RT-PCR	
Immunocapture RT-PCR	
Real-time RT-PCR	
High throughput sequencing	

Scope of TPS

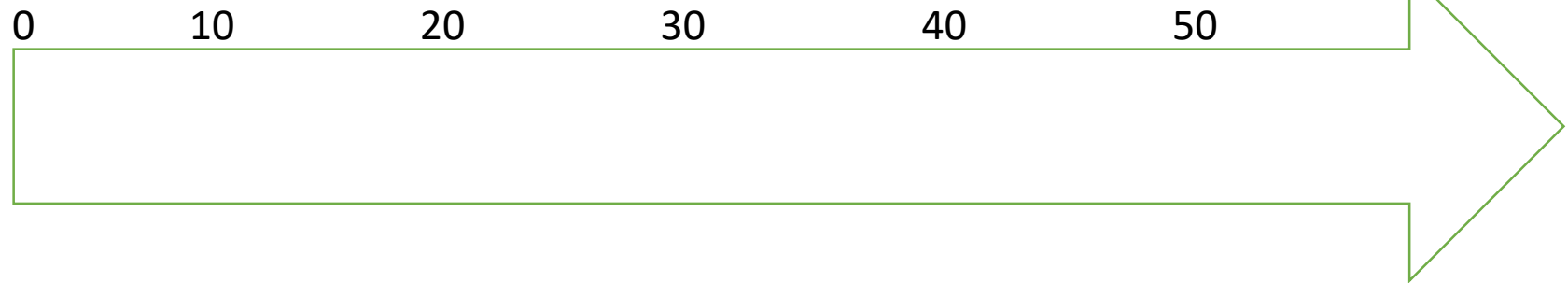
Detection and identification of TSWV in symptomatic tomato leaves

	Methods							
	ELISA		RT-PCR		real time RT-PCR (RT-qPCR)		methods applicable for on-site	
sample type (DNA, plant material with deactiv. pests, etc.)	Infected/ non-infected material	non-plant	Infected/ non-infected material	non-plant	Infected/ non-infected material	non-plant	Infected/ non-infected material	non-plant
matrix (type of plant material: seed, leaves, etc.)	Leaves of tomato		Leaves of tomato		Leaves of tomato		Leaves of tomato	
suitable for: symptomatic / asymptomatic sample	symptomatic		symptomatic		symptomatic		symptomatic	
purpose: detection / identification	detection and identification		detection and identification		detection and identification		detection and identification	

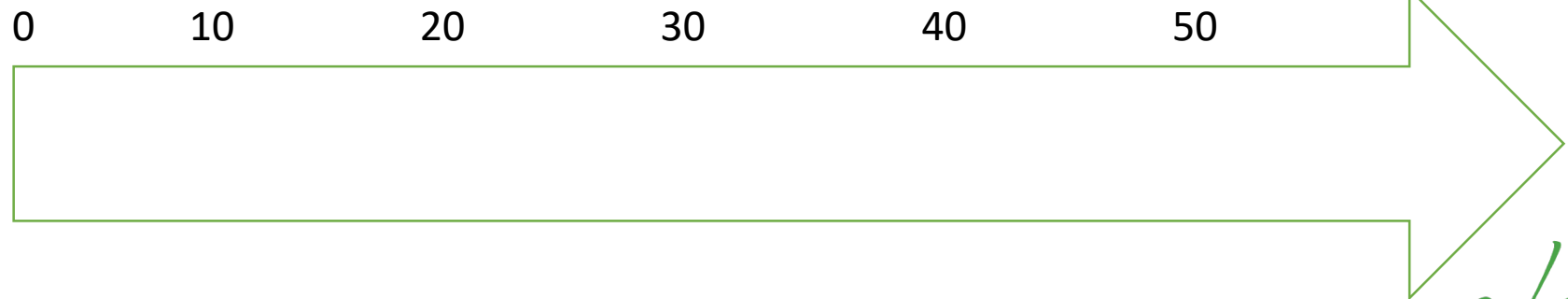
Scope of TPS

Detection and identification of TSWV in symptomatic tomato leaves

No of samples?



No of participants?



Scope of TPS

Detection and identification of TSWV in symptomatic tomato leaves

	Methods							
	ELISA		RT-PCR		real time RT-PCR (RT-qPCR)		methods applicable for on-site	
sample type (DNA, plant material with deactiv. pests, etc.)	Infected/ infected material	non- plant	Infected/ infected material	non- plant	Infected/ infected material	non- plant	Infected/ infected material	non- plant
matrix (type of plant material: seed, leaves, etc.)	Leaves of tomato		Leaves of tomato		Leaves of tomato		Leaves of tomato	
suitable for: symptomatic / asymptomatic sample	symptomatic		symptomatic		symptomatic		symptomatic	
purpose: detection / identification	detection and identification		detection and identification		detection and identification		detection and identification	
type of controls needed (NIC, NAC, PAC, PIC, IC, etc)	PC NC		PAC PIC NAC NIC		PAC PIC NAC NIC IC		PC NC	
no. of samples	22		22		22		22	
max no. of participants	20		20		20		20	

Test selection

Literature search, website of companies:

➤ 76 tests for detection and identification of TSWV:

- 13x ELISA (DAS-, TAS-, B-fast, ELISA with specific single chain antibodies)
- 2x luminex
- 2x tissue-blot immunoassay (TBIA)
- 2x dot-blot immunoassay (DBIA)
- 4x on-site detection (lateral flow devices (LFD), Rapid immune gold)
- 2x dot-blot hybridization
- 36x reverse transcription (RT)-PCR or immunocapture (IC) RT-PCR
- 8x real-time RT-PCR (SYBR green, TaqMan)
- 4x RT- loop-mediated isothermal amplification (LAMP) or IC-RT-LAMP
- 1x RT- thermostable Helicase-Dependent DNA Amplification (RT-HAD)
- 1x hyperspectral Imaging and Outlier Removal Auxiliary Classifier Generative Adversarial Nets (OR-AC-GAN)
- 1x microarray

Test selection

I round

- Literature search, website of companies
- 76 tests (13x ELISA, 4x LFD, 6x dot-blot, 4x RT-LAMP, 36x RT-PCR, 8x RT-qPCR, 1x RT-HAD, 1x OR-AC-GAN, 1x microarray)

II round

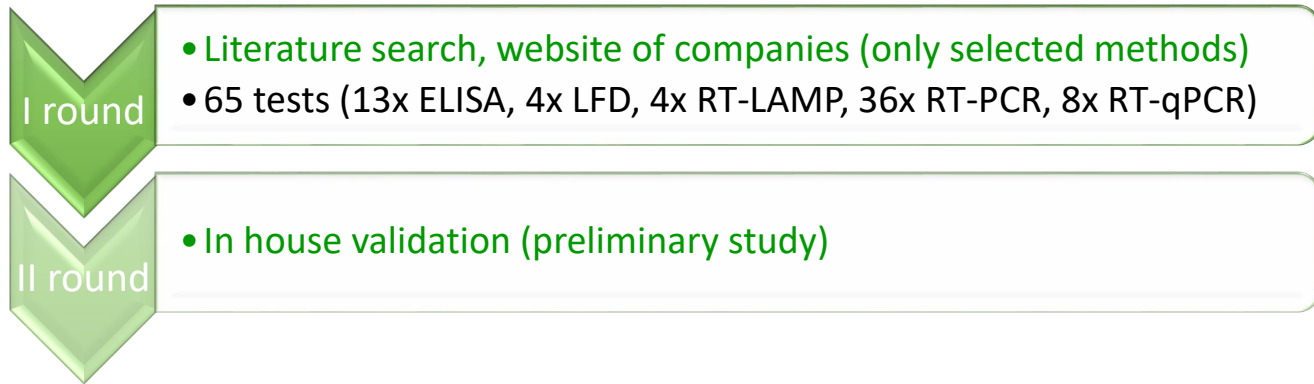
- In house validation (preliminary study)

III round

- TPS

Test selection for preliminary study

????



Which criteria you think are important for selection of tests that will be included in preliminary study (in-house validation)?

Test selection for preliminary study - criteria

Criteria	Lab tests (mark if you consider as important criteria for test selection)	on-site tests (mark if you consider as important criteria for test selection)
Is the target (gene/protein) appropriately selected?		
Available validation data		
Validation data available for selected matrix		
Analytical sensitivity (LOD) (RNA diluted in water)		
Analytical sensitivity in plant material (selected matrix)		
Diagnostic sensitivity (comparison of different tests)		
Analytical specificity: exclusivity + inclusivity		
Selectivity (matrix effect)		
Repeatability (near LOD)		
Reproducibility/ robustness		
Results of interlaboratory comparisons available		

Test selection for preliminary study - criteria

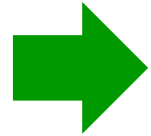
Criteria	Lab tests (mark if you consider as important criteria for test selection)	on-site tests (mark if you consider as important criteria for test selection)
Applicability in different matrixes		
Amount of material which is included in one sample		
Standardized preparation of the reaction (e.g., ready to use reagents)		
Availability and relevance of controls (in the case of kits)		
Available detailed protocols		
Simple test procedure		
Simplicity of data analysis		
User friendly test		
Time needed to complete analysis (quick)		
Easy to multiplex?		
Database/library dependent (for example sequencing,...)		

Test selection for preliminary study - criteria

Criteria	Lab tests (mark if you consider as important criteria for test selection)	on-site tests (mark if you consider as important criteria for test selection)
Stability of chemicals at ambient temperature		
No equipment/ instrument needed		
Test not exclusively developed for a specific instrument		
Cost of obligatory equipment/ instruments (low)		
Any other important criteria for test selection?		

Test selection for preliminary study

Main obstacles:



- **Different approach for validation (-> not possible to compare validation data)**
- **Insufficient validation data**

Things considered (evaluation strategy for selection of tests that have been included for the in house validation)

- *Validation data indicating that a test is not fit for purpose*
- *Tests in legislation, EPPO,..*
- *Widely used tests*
- *Experience of organizers*
- *Specific scope*
- *Availability of test*

Explanation for test selection, case of TSWV

Method	General arguments for selecting the method	Tests selected for preliminary studies	Specific arguments for selecting
ELISA	<p>1. Recommended method listed in EPPO Standards PM7/34(1) and PM7/139(1).</p> <p>2. Method is widely used in diagnostic laboratories</p>	ELISA 1	Test is listed in EPPO Standards PM7/34(1) and PM 7/ 139(1), however only limited validation data are available from the company and in EPPO Standards PM7/34(1) and PM 7/ 139(1).
		ELISA 2	Test is not listed in EPPO Standards PM7/34(1) and PM 7/ 139(1) for TSWV detection and identification and validation data are not available. However, test was included in preliminary study, because company provided kit for preliminary study.
		DAS-ELISA Bioreba	Test is listed in EPPO Standards PM7/34 and PM 7/139(1), however only limited validation data are available from the company and in EPPO Standards PM7/34(1) and PM 7/ 139(1). In addition, company provided kit for preliminary study.
		DAS-ELISA Agdia	Test is listed in EPPO Standards PM7/34 and PM 7/139(1). In addition, company provided kit for preliminary study.
		ELISA 5	Test is listed in EPPO PM 7/ 139(1). Limited validation data are available from the company and in EPPO Standard PM 7/ 139(1). Combines ELISA robustness within the timeframe of PCR.
On-site: LFD	<p>1. Method is mentioned in EPPO Standards PM7/34(1) and PM7/139(1) as a preliminary screening tool.</p> <p>2. Method provides fast on site diagnostic.</p>	ImmunoStrip Agdia	Applicable for fast on site detection.
		AgriStrip Bioreba	Applicable for fast on site detection.

Explanation for test selection, case of TSWV

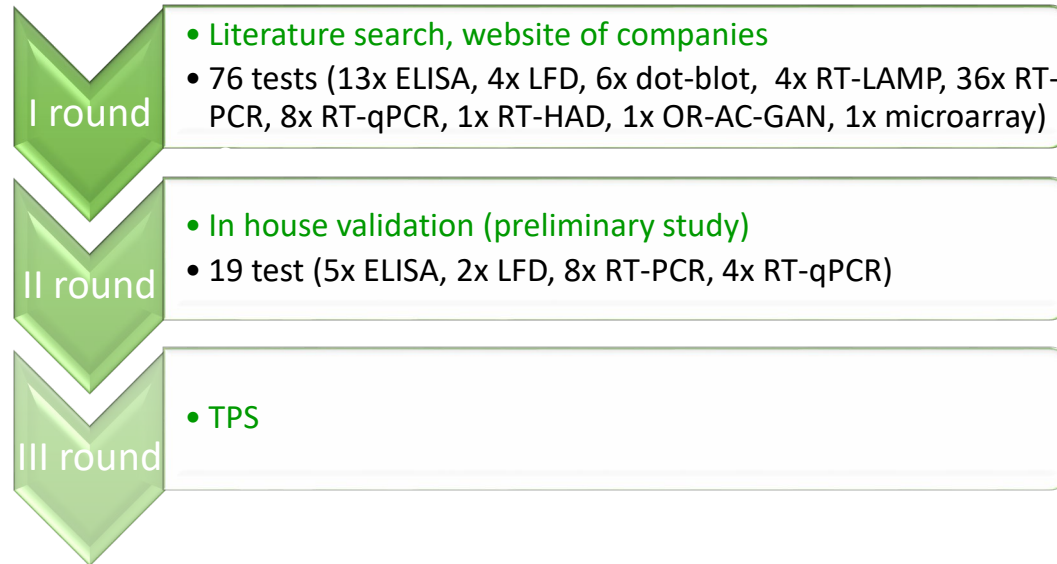
Method	General arguments for selecting the method	Tests selected for preliminary studies	Specific arguments for selecting
Conventional RT-PCR	<p>1. Recommended method listed in EPPO Standards PM7/34(1) and PM7/139(1).</p> <p>2. Method is widely used in diagnostic laboratories.</p>	RT-PCR 1	The only commercially available RT-PCR test for TSWV. Limited validation data available from the company.
		RT-PCR generic for tospoviruses Hassani-Mehraban et al., 2016 (J Virol Methods, 233, 89-96)	<p>Test listed in EPPO PM7/139(1). Limited validation data available from publication.</p> <p>Note: generic primers for viruses of different Tospovirus clades, therefore sequencing is needed for the species identification.</p>
		Hassani-Mehraban et al., 2016 (J Virol Methods, 233, 89-96)	TSWV primers were <i>in silico</i> validated by authors and TPS organizer and have only few mismatches with available TSWV sequences from GenBank. Limited validation data available from publication.
		Mumford et al., 1994 (J Virol Methods, 46(3), 303-311)	Test is widely used (many publications from different labs) and it is listed in EPPO Standard PM7/34 and PM 7/139(1). Limited data available in publication and EPPO Standards PM7/34(1) and PM 7/139(1).
		Zarzyńska-Nowak et al., 2018 (Can J Plant Pathol, 40, 580-586)	TSWV primers were <i>in silico</i> validated by TPS organizer: have only few mismatches with available TSWV sequences from GenBank. Used for detection of TSWV in several different host plants, but limited validation data available in the publication. Test can be used as multiplex, but as singleplex showed higher sensitivity according to publication.
		Fineti-Sialer et al. 2002 (J Plant Pathol, 84(3), 145-152)	Covers NSm gene and originally were used for the detection of TSWV in at least 8 different plant species and didn't cross reacted with other tospoviruses. No other validation data available.
		Vučurović et al. 2012 (Eur J Plant Pathol, 133(4), 935-947)	Primers were designed considering wide range of TSWV isolates and were used in different labs. No validation data available.
		Panno et al. 2012 (J Virol Methods, 186, 152-156)	Covers Gn/Fc gene which is not covered by other primer sets. TSWV primers were <i>in silico</i> validated by TPS organizer: have only few mismatches with available TSWV sequences from GenBank. No other validation data available.

Explanation for test selection, case of TSWV

Method	General arguments for selecting the method	Tests selected for preliminary studies	Specific arguments for selecting
Real-time RT-PCR	1. Recommended method listed in EPPO Standards PM7/34(1) and PM7/139(1). 2. Method is used more and more in diagnostic laboratories.	Boonham et al. 2002 (J Virol Methods, 101(1-2), 37-48)	Test is listed in EPPO Standard PM7/34 and EPPO standard PM 7/139(1). Test is validated, but validation data can not be compared with the validation data of other tests.
		Roberts et al. 2000 (J Virol Methods, 88(1), 1-8)	Test can be used for identification. Limited validation data available.
		Debreczeni et al. 2011 (J Virol Methods, 176(1-2), 32-37)	Test can be used for identification. Limited validation data available.
		Mortimer-Jones et al. 2009 (J Virol Methods, 161, 289-296)	Test can be used for identification. Limited validation data available.

Test selection for TPS

?????



How will you select tests for TPS (plan for preliminary study, criteria for selection of tests for TPS)?

(do not forget that our scope is: detection and identification of TSWV in symptomatic tomato leaves)

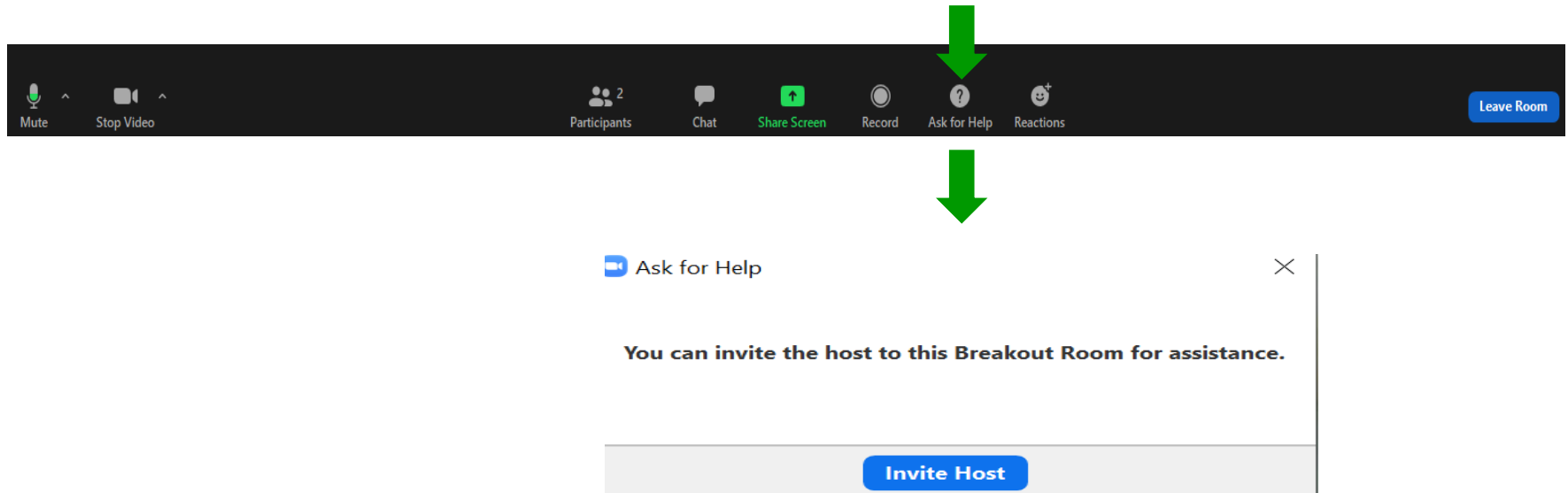
Group 1:
Serological tests (lab)
Rene
Eva
Maria

Group 2:
Molecular tests (lab)
Karin
Maria Leonor
Valeria
Vilma

Group 3:
On-site tests
Martijn
Despoina
Blanca

If you will need our help?

-> Ask for help



One from each group should make short notes in a word document -> when we will be back: copy notes in ppt!

Decide who will explain us your thoughts!

Test selection for TPS

Group 1:
Serological
tests (lab)

Test selection for TPS

Group 2:
Molecular
tests (lab)

Test selection for TPS

Group 3:
On-site tests

Evaluation of the possibility to use the same buffers for all ELISA tests

Why?: to be able to prepare enough homogeneous material for all tests (that the same extract could be used for serological and for molecular tests), to lower the costs

Suggested modification: extraction buffer PBST-Tween + 2% PVP + 0.2% BSA, and coating buffer, wash buffer, substrate buffer and alkaline phosphatase as recommended in Appendix 3 of EPPO PM 7/125

->dilution series of 1 TSWV isolate, two other tospovirus isolates (INSV, CSNV)

Evaluation of the possibility to use the same buffers for all ELISA tests

Analytical sensitivity and specificity of ELISA Agdia

Sample		Agdia buffers	buffers suggested by TPS organizer
Virus-isolate	dilution		
CSNV-PV-0529	undiluted	neg	neg
INSV-PV-0281	undiluted	neg	neg
TSWV-PV-0182	undiluted	pos	pos
	10x	pos	pos
	100x	pos	pos
	1000x	pos	pos
	10000x	pos	pos*
healthy tomato		neg	neg
buffer control		neg	neg
PC-kit		pos	pos

*Sample was positive only after 2h of incubation

Analytical sensitivity and specificity of ELISA Bioreba

Sample		Bioreba buffers	buffers suggested by TPS organizer
Virus-isolate	dilution		
CSNV-PV-0529	undiluted	pos	pos
INSV-PV-0281	undiluted	neg	neg
TSWV-PV-0182	undiluted	pos	pos
	10x	pos	pos
	100x	pos	pos
	1000x	pos	pos
	10000x	pos	pos
healthy tomato		neg	neg
buffer control		neg	neg
PC-kit		pos	pos
NC-kit		neg	neg

-> decision: using buffers which are recommended by the producers!

Communicate with the producers of kits!!!

Preliminary study (19 tests)

- ✓ ***analytical sensitivity:*** series of 10-fold dilutions of two different TSWV isolates
- ✓ ***analytical specificity:*** 2 TSWV isolates, 6 other tospoviruses



- ✓ ***8 tests selected***

Preliminary study: results of ELISA

Sample		ELISA	ELISA	ELISA 2	ELISA 5	ELISA 1
Virus-isolate	dilution	Agdia	Bioreba			
ANSV- PV-1027	undiluted	pos	pos	pos	pos	pos
CSNV-PV-0529	undiluted	neg	pos	pos	neg	pos
GRSV- PV-0205	undiluted	pos	pos	pos	pos	pos
INSV-PV-0281	undiluted	neg	neg	neg	neg	neg
MSMV VE440	undiluted	neg	neg	neg	neg	neg
TCSV-PV-0390	undiluted	pos	pos	pos	pos	pos
TSWV-PV-0182	undiluted	pos	pos	pos	pos	pos
	10x	pos	pos	pos	pos	pos
	100x	pos	pos	pos	pos	pos*
	1000x	pos	pos	pos	neg	neg
	10000x	pos	pos	neg	neg	neg
	100000x	neg	neg	neg	neg	neg
	1000000x	neg	neg	neg	neg	neg
TSWV-PV-0389	undiluted	pos	pos	pos	pos	nt
	10x	pos	pos	pos	pos	nt
	100x	pos	pos	pos	pos	nt
	1000x	pos	pos	pos	pos	nt
	10000x	pos	pos	pos*	neg	nt
	100000x	neg	neg	neg	neg	nt
	1000000x	neg	neg	neg	neg	nt
	10000000x	neg	neg	neg	neg	nt
	100000000x	neg	neg	neg	neg	nt
1000000000x	neg	neg	neg	neg	nt	
healthy tomato		neg	neg	neg	neg	neg

*weak signal



Preliminary study: results of LFD

Sample		Immunostrip Agdia	AgriStrip Bioreba
Virus-isolate	dilution		
ANSV- PV-1027	undiluted	pos	pos*
CSNV-PV-0529	undiluted	neg	pos*
GRSV- PV-0205	undiluted	pos	pos*
INSV-PV-0281	undiluted	neg	neg
MSMV VE440	undiluted	neg	neg
TCSV-PV-0390	undiluted	pos	pos
TSWV-PV-0182	undiluted	pos	pos
	10x	pos	pos
	100x	pos	pos*
	1000x	neg	neg
	10000x	neg	neg
TSWV-PV-0389	undiluted	pos	pos
	10x	pos	pos
	100x	pos	pos
	1000x	pos	pos*
	10000x	neg	neg
	100000x	neg	neg
healthy tomato		neg	neg



*weak signal

Preliminary study: results of RT-PCR

Sample		RT-PCR 1	RT-PCR generic for tospoviruses Hassani- Mehraban et al., 2016**	Hassani- Mehraba n et al., 2016**	Mumford et al., 1994**	Zarzyńska- Nowak et al., 2018**	Fineti- Sialer et al. 2002**	Vučurović et al. 2012**	Panno et al. 2012**
Virus-isolate	dilution								
ANSV-PV-1027	undiluted	neg	pos	neg	neg	pos*	neg	pos	pos*
CSNV-PV-0529	undiluted	neg	pos*/neg	neg	pos*	neg	pos*	pos	neg
GRSV-PV-0205	undiluted	neg	pos	neg	pos	neg	neg	neg/pos	neg
INSV-PV-0281	undiluted	neg	pos*	neg	neg	neg	neg	neg	neg
MSMV VE440	undiluted	neg	pos*	neg	pos	neg	neg	neg	neg
TCSV-PV-0390	undiluted	pos*	pos	neg	pos*	pos	neg	pos	neg
TSWV-PV-0182	undiluted	pos	pos	pos	pos	pos	pos	pos	pos
	10x	pos	pos	pos	pos	pos	pos	pos	pos
	100x	pos	pos	pos	pos	pos/pos*	pos	pos	pos
	1000x	neg	pos*	pos	pos	pos	pos	pos	pos
	10000x	neg	pos*	pos*	pos	pos	pos	pos	pos
	100000x	neg	neg	pos*	pos*	pos*	pos	pos	pos*
	1000000x	neg	neg	neg	neg	neg	neg	neg/pos*	neg/pos
TSWV-PV-0389	undiluted	nt	nt	nt	nt	nt	nt	nt	nt
	10x	nt	nt	pos	nt	pos	pos	nt	pos
	100x	nt	nt	pos	nt	pos	pos	nt	pos
	1000x	nt	nt	pos	nt	pos	pos	nt	pos
	10000x	nt	nt	pos*	nt	pos*	pos*	nt	pos
	100000x	nt	nt	pos*	nt	neg	neg	nt	pos*
	1000000x	nt	nt	neg	nt	neg	neg	nt	neg
	10000000x	nt	nt	neg	nt	neg	neg	nt	neg
	100000000x	nt	nt	neg	nt	neg	neg	nt	neg
	1000000000x	nt	nt	neg	nt	neg	neg	nt	neg
healthy tomato		neg	neg	neg	neg	neg	neg	neg	neg



*weak signal

**one-step RT-PCR kit, Qiagen



Preliminary study: results of RT-qPCR

Sample		Boonham et al. 2002**	Roberts et al. 2000**	Mortimer-Jones et al. 2009**	Debreczeni et al. 2011**
Virus-isolate	dilution				
ANSV- PV-1027	undiluted	neg	neg	neg	neg*
CSNV-PV-0529	undiluted	neg	neg	neg	neg
GRSV- PV-0205	undiluted	neg	neg	neg	pos
INSV-PV-0281	undiluted	neg	neg	neg	neg
MSMV VE440	undiluted	neg*	neg	neg	neg
TCSV-PV-0390	undiluted	neg*	neg	neg	pos
TSWV-PV-0182	undiluted	pos	pos	pos	pos
	10x	pos	pos	pos	pos
	100x	pos	pos	pos	pos
	1000x	pos	pos	pos	pos
	10000x	pos	pos	pos	pos
	100000x	pos	pos	pos	pos
	1000000x	pos	pos	pos/neg*	pos
TSWV-PV-0389	undiluted	nt	nt	nt	nt
	10x	pos	pos	pos	pos
	100x	pos	pos	pos	pos
	1000x	pos	pos	pos	pos
	10000x	pos	pos	pos	pos
	100000x	pos	pos	pos	pos
	1000000x	pos	pos	pos	neg*
	10000000x	neg*	neg*/neg	neg*	neg*
	100000000x	neg	neg	neg*	neg*/neg
	1000000000x	neg	neg	neg	neg
healthy tomato		neg	neg	neg	neg



* Cq>35

**AgPath-IDTM One-step RT-PCR kit, Ambion (Thermo Scientific)



RNA extraction

Questionnaire sent to potential TPS participants

-> the most often used RNA extraction procedure by potential TPS participants:

Qiagen RNeasy Plant Mini Kit

In-house validation for tests selected for TPS

Which performance characteristics you think can be evaluated in-house (in one lab)?

PERFORMANCE CHARACTERISTICS	Mark if you think can be evaluated in-house
ANALYTICAL SENSITIVITY	
ANALYTICAL SPECIFICITY (inclusivity + exclusivity)	
REPRODUCIBILITY	
REPETABILITY	
SELECTIVITY	

In-house validation for tests selected for TPS (TSWV example)

Virus-isolate	ELISA Agdia	ELISA Bireba	LFD Agdia	LFD Bioreba	RT-PCR Hassani-Merhaban	RT-qPCR Boomham	RT-qPCR Roberts et al.	RT-qPCR Mortimer-Jones
TSWV-PV-0204	pos	pos	pos	pos	pos	pos	pos	pos
TSWV-USA, tomato	pos	pos	pos	pos	pos	pos	pos	pos
TSWV-108-19, Serbia, tomato***	nt	nt	nt	nt	pos	pos	pos	pos
TSWV-109-19, Serbia, tomato***	nt	nt	nt	nt	pos	pos	pos	pos
TSWV-France 77, chilli pepper***	pos	pos	pos	pos	pos	pos	pos	pos
TSWV-Italy, pepper, 2011	pos	pos	pos	pos	pos	pos	pos	pos
TSWV-Italy, tomato	pos	pos	pos	pos	pos	pos	pos	pos
TSWV-Italy, hot pepper	pos	pos	pos	pos	pos	pos	pos	pos
TSWV-Italy, pepper, 2015	pos	pos	pos	pos	pos	pos	pos	pos
TSWV-Italy, lisianthus	pos	pos	pos	pos	pos	pos	pos	pos
TSWV-NPPO-NL: 21007721, The Netherlands, ligularia	pos	pos	pos	pos	pos	pos	pos	pos
TSWV-PV-0182	pos	pos	pos	pos	pos	pos	pos	pos
TSWV-PV-0182, 10x diluted	pos	pos	pos	pos	pos	pos	pos	pos
TSWV-PV-0182, 100x diluted	pos	pos	pos	pos*	pos	pos	pos	pos
TSWV-PV-0182, 1000x diluted	pos	pos	neg	neg	pos	pos	pos	pos
TSWV-PV-0182, 10000x diluted	pos	pos	neg	neg	pos*	pos	pos	pos
TSWV-PV-0182, 100000x diluted	neg	neg	nt	nt	pos*	pos	pos	pos
TSWV-PV-0182, 1000000x diluted	neg	neg	nt	nt	neg	pos	pos	sus
TSWV-PV-0389	pos	pos	pos	pos	nt	nt	nt	nt
TSWV-PV-0389, 10x diluted	pos	pos	pos	pos	pos	pos	pos	pos
TSWV-PV-0389, 100x diluted	pos	pos	pos	pos	pos	pos	pos	pos
TSWV-PV-0389, 1000x diluted	pos	pos	pos	pos*	pos	pos	pos	pos
TSWV-PV-0389, 10000x diluted	pos	pos	neg	neg	pos*	pos	pos	pos
TSWV-PV-0389, 100000x diluted	neg	neg	neg	neg	pos*	pos	pos	pos
TSWV-PV-0389, 1000000x diluted	neg	neg	nt	nt	neg	pos	pos	pos
TSWV-PV-0389, 10000000x diluted	neg	neg	nt	nt	neg	neg**	neg**	neg**
TSWV-PV-0389, 100000000x diluted	neg	neg	nt	nt	neg	neg	neg	neg**
TSWV-PV-0389, 1000000000x diluted	neg	neg	nt	nt	neg	neg	neg	neg
TSWV-PV-1175, 100x diluted	pos	pos	pos	pos	pos	pos	pos	pos
TSWV-PV-1175, 1000x diluted	pos	pos	pos	pos	pos	pos	pos	pos
TSWV-PV-1175, 10000x diluted	pos	pos	pos	neg	pos	pos	pos	pos
TSWV-PV-1175, 100000x diluted	neg	neg	neg	neg	pos	pos	pos	pos
TSWV-PV-1175, 1000000x diluted	neg	neg	neg	neg	pos	pos	pos	pos
TSWV-PV-0393, 10x diluted	pos	pos	pos	pos	pos	pos	pos	pos

In-house validation for tests selected for TPS (TSWV example)

Virus-isolate	ELISA Agdia	ELISA Bireba	LFD Agdia	LFD Bioreba	RT-PCR Hassani-Merhaban	RT-qPCR Boomham	RT-qPCR Roberts et al.	RT-qPCR Mortimer-Jones
ANSV-PV-1027	pos	pos	pos	pos*	neg	neg	neg	neg
CaCV-PV-0864	neg	neg	neg	neg	neg	neg**	neg	neg
CSNV-PV-0529	neg	pos	neg	pos*	neg	neg	neg	neg
CSNV-PV-1219	neg	pos	neg	pos*	neg	neg	neg	neg
GRSV-PV-0205	pos	pos	pos	pos*	neg	neg	neg	neg
INSV-PV-0280	neg	neg	neg	neg	neg	neg	neg	neg
INSV-PV-0281	neg	neg	neg	neg	neg	neg	neg	neg
INSV-PV-0485	neg	neg	neg	neg	neg	neg	neg	neg
INSV-PV-1123	neg	pos*	neg	neg	neg	neg	neg	neg
INSV-PV-1189	neg	pos	neg	neg	neg	neg**	neg	neg
IYSV-PV-0528	neg	neg	neg	neg	neg	neg	neg	neg**
MSMV-VE440	neg	neg	neg	neg	neg	neg**	neg	neg
TCSV-PV-0390	pos	pos	pos	pos	neg	neg**	neg	neg
TCSV-PV-0391	pos	pos	pos	pos*	neg	neg	neg	neg
TYRV-PV-0526	neg	pos	neg	neg	neg	neg	neg	neg
TYRV-PV-0532	neg	pos	neg	pos*	neg	neg	neg	neg
WSMoV-PV-0283	neg	neg	neg	neg	neg	neg	neg	neg

*-week reaction, close to the limit of detection

** Cq above cut-off value. For all RT-qPCRs Cq cut-off value of 35 was used.

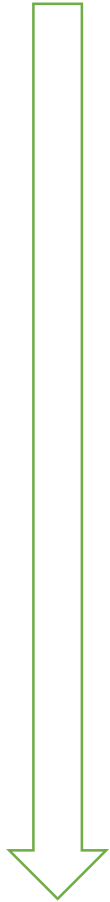
***resistance breaking strain

sus-one replicate positive, one negative (close to the limit of detection)

Selection of TPS participants

Invitation letter to potential participants: 92

Content of the invitation letter?



Max number of participants: 20

Selection of TPS participants

Invitation letter to potential participants: 92

Content of the invitation letter:

- Aim
- Expected timeline
- Scope of TPS
- methods, max number of tests within each method
(not necessary to perform all methods, whereas it was necessary to perform all tests for the selected method)
- sample type, number of samples
- Estimation of costs for participants
- Critical selection criteria, which need to be fulfilled for participation in TPS ???

Max number of participants: 20

Selection of TPS participants

Critical selection criteria, which need to be fulfilled for participation in TPS :

Criteria	Importance:	High	Medium	Low
TPS time schedule compatible participant's availability				
Ability/willing to perform all methods				
Technical expertise for the pest group (e.g. virology)				
Expertise in the use of the method (e.g. ELISA, qPCR, etc.)				
Authorized by the national competent authority to work with the TSWV and with other tospoviruses (including CSNV)				
Possibility to obtain an import document or Letter of Authority				
Possibility to obtain an import document or Letter of Authority within 4 weeks				
Previous participation in TPS or PT				
Available equipment that are need to perform methods				
No constraints for delivery				
No problems or limitations with delivery on dry ice				
Has committed to perform the test and deliver results in the time frame defined				
Traceability in place / QA in place				



Contract for TPS participants



Contract for TPS participants

- Identification of the TPS (TPS code, organizer – name, contact)
- Participant's name, contact info, address
- Which method/s (all the tests related to the methods it is willing to perform!)
- Details about **sample panel**: type of material, quantity, scientific name of material including the concerned pests, expeditor of the panel
- **Regulatory requirements** (e.g., LoA) needed (if yes, when it should be provided to TPS organizer)
- **When samples will be dispatch** (will be participant available to receive the samples?)
- Agreement to perform analyses **according the instructions, within the deadline** (any modification, difficulties should be reported)
- Info about transmission of the results (**anonymously**)
- **Confidentiality and collusion** (coding of the samples kept confidential till the end of TPS; to not communicate with other participants; results used anonymously for scientific purpose)
- What to do with **samples after TPS** (can be used for other purposes?)
- That participants must have adopted measures to **prevent the risk of unintentional release of pests**
- **GDPR disclaimer** (categories of personal data, safety of important personal data, retention of the personal data, rights of participants, applicable law)

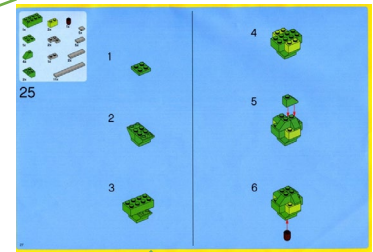
Technical instructions



Build a house!

Build a house with red roof!

Build a house with red roof, garage and with garden!



X

X

X



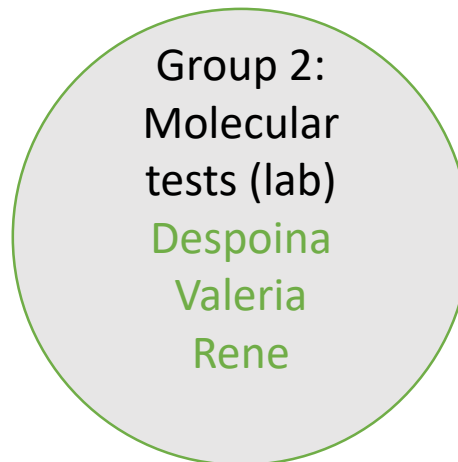
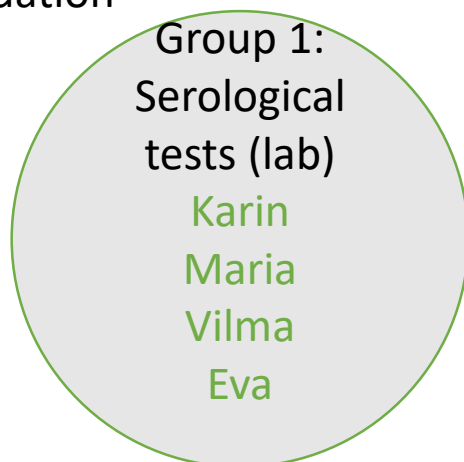
Test panel composition

Propose test panel composition!

- How many different TSWV isolates? How many replicates?
- Will you include healthy samples? If yes, which? How many replicates?
- Will you include non target viruses? If yes, which? How many replicates/isolates?
- Will you include serial dilution of a positive sample? If yes, how many dilution points and how many replicates?

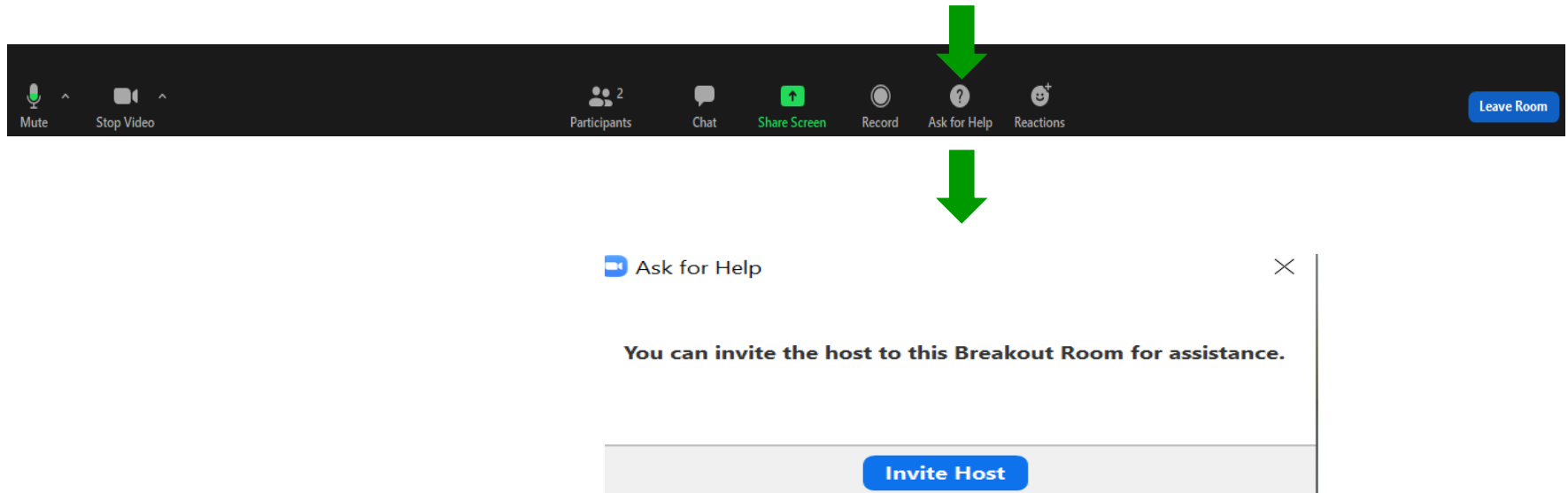
Have in mind:

- Our scope is detection and identification of TSWV in symptomatic tomato leaves
- Methods that should be evaluated: ELISA, RT-PCR, RT-qPCR, on-site tests
- Max number of samples 22
- Performance characteristics of tests you would like to evaluate during TPS
- We already got some data about performance characteristics of tests during in-house validation



If you will need our help?

-> Ask for help



One from each group should make short notes in a word document -> when we will be back: copy notes in ppt!

Decide who will explain us your thoughts!

Test panel composition

Group 1:
Serological
tests (lab)

Test panel composition

Group 2:
Molecular
tests (lab)

Test panel composition

Group 3:
On-site tests

Test panel composition (TPS TSWV example)

	type of sample (isolate designation in DSMZ collection)	Sample designation					Health status of TSWV
		Molecular	LFD Agdia	LFD Bioreba	ELISA Agdia	ELISA Bioreba	
healthy tomato	healthy tomato 1	S-7	S-15	S-4	S-4	S-8	neg
	healthy tomato 1	S-17	S-20	S-10	S-15	S-10	neg
	healthy tomato 2	S-21	S-10	S-3	S-6	S-9	neg
other tospoviruses	ANSV (PV-1027)	S-12	S-11	S-7	S-19	S-14	neg
	CSNV2 (PV-0529/G 25.37)	S-15	S-3	S-21	S-7	S-19	neg
	GRSV (PV-0205/SA05)	S-2	S-13	S-14	S-16	S-11	neg
	INSV2 (PV-0281)	S-13	S-7	S-2	S-9	S-3	neg
	TCSV (PV-0390/VE 225)	S-10	S-21	S-15	S-12	S-7	neg
TSWV dilution series	TSWV (PV1175) 1000000x	S-18	S-2	S-5	S-14	S-4	pos
	TSWV (PV1175) 1000000x	S-20	S-18	S-22	S-20	S-12	pos
	TSWV (PV1175) 100000x	S-1	S-17	S-6	S-10	S-6	pos
	TSWV (PV1175) 100000x	S-19	S-22	S-9	S-11	S-21	pos
	TSWV (PV1175) 10000x	S-11	S-8	S-13	S-13	S-2	pos
	TSWV (PV1175) 10000x	S-14	S-9	S-19	S-21	S-22	pos
	TSWV (PV1175) 1000x	S-3	S-1	S-1	S-18	S-13	pos
	TSWV (PV1175) 1000x	S-9	S-16	S-16	S-22	S-15	pos
	TSWV (PV1175) 100x	S-6	S-4	S-12	S-5	S-1	pos
TSWV (PV1175) 100x	S-22	S-6	S-20	S-8	S-18	pos	
TSWV medium concentration	TSWV (PV-0182) 1000x diluted	S-8	nt	nt	nt	nt	pos
	TSWV (PV-0182) 1000x diluted	S-4	nt	nt	nt	nt	pos
	TSWV (PV-0182) 100x diluted	nt	nt	nt	S-1	S-5	pos
	TSWV (PV-0182) 100x diluted	nt	nt	nt	S-2	S-17	pos
	TSWV (PV-0182) 10x diluted	nt	S-14	S-8	nt	nt	pos
	TSWV (PV-0182) 10x diluted	nt	S-19	S-17	nt	nt	pos
TSWV at concentration close to limit of detection	TSWV (PV-0389) 100000x diluted	S-5	nt	nt	nt	nt	pos
	TSWV (PV-0389) 100000x diluted	S-16	nt	nt	nt	nt	pos
	TSWV (PV-0389) 10000x diluted	nt	nt	nt	S-3	S-16	pos
	TSWV (PV-0389) 10000x diluted	nt	nt	nt	S-17	S-20	pos
	TSWV (PV-0389) 1000x diluted	nt	S-5	S-11	nt	nt	pos
	TSWV (PV-0389) 1000x diluted	nt	S-12	S-18	nt	nt	pos
controls	healthy tomato 2	NC/NIC	NC	NC	NC	NIC	neg
	TSWV (PV0393) 10x diluted	PC/PIC	PC	PC	PC	PIC	pos
	no template control*	NAC	nt	nt	nt	nt	neg
	total RNA (PV-0182 and PV-0389)	PAC	nt	nt	nt	nt	pos
	* not provided for participants						

Reference material/ samples included in TPS

What are important characteristics/ descriptors/ criteria of reference material/ samples included in TPS?

Reference material/ samples included in TPS

Descriptor	Value	Minimum criterion	Description
Intended use	should be defined (in this case it equals preparation of RM for the scope of the individual test or TPS)	yes	RM for the TPS on detection and identification of tomato spotted wilt tospovirus in symptomatic tomato leaf material
Identity	identified to the level of internationally recognized diagnostic protocols (mention tests and outcome)	yes	All isolates used for TPS were obtained from German Collection of Microorganisms and Cell Cultures GmbH (DSMZ). DSMZ made isolate characterisation. However, the identity of the virus isolates used in TPS has been confirmed also in the TPS organizer laboratory by Sanger sequencing of PCR products and by all tests included in TPS.

Reference material/ samples included in TPS

Descriptor	Value	Minimum criterion	Description
Traceability	traceability to a specimen from a reference culture collection	no	Yes. DSMZ collection : TSWV isolates: PV-0182, PV-0389, PV1175, PV0393 ; Other tospovirus isolates: PV-0390, PV-0529, PV-1027, PV-0281, PV-0205
	traceability to a specimen from a working culture collection	no	/
	traceability provided for the target pest and matrix used (the latter if relevant)	yes	Samples were prepared by mixing virus isolates (see above) with healthy tomato cv. Money Maker grown under green-house conditions . The status of these tomato plants were confirmed by all tests included in TPS.

Reference material/ samples included in TPS

Descriptor	Value	Minimum criterion	Description
Commutability level	<input type="checkbox"/> naturally infested plant material	no	/
	<input type="checkbox"/> artificially infested plant material	no	✓
	<input type="checkbox"/> spiked plant material	no	✓
	<input type="checkbox"/> purified organisms	no	/
	<input type="checkbox"/> total nucleic acids from a sample (target organism in background)	no	/
	<input type="checkbox"/> purified nucleic acids	no	/
	<input type="checkbox"/> synthetic nucleic acids	yes	/

Reference material/ samples included in TPS

Descriptor	Value	Minimum criterion	Description
Homogeneity	homogenous Provide test & test results	yes	Plant material were homogenized and several aliquots were prepared . The homogeneity of each batch was confirmed by testing some randomly chosen aliquots with all tests included in TPS (before sending to the participants) .

Reference material/ samples included in TPS

Descriptor	Value	Minimum criterion	Description
Stability	stable		Stability testing was conducted at several time points in conditions that mimic transport and storage conditions. This has been done on randomly chosen aliquots of each batch of samples (before sending to the participants).
	stability - short term	yes	
	stability - long term	no	Done at the time when first participant did analysis
	stability - long term	no	Tested on the deadline to perform analysis. Due “Covid-19” situation, the stability testing was done at several time points also after deadline to perform analysis (till July 1 st , 2020).

Reference material/ samples included in TPS

Descriptor	Value	Minimum criterion	Description
Purity	absence of non-targets	no	/
	absence of interfering non-targets	no	/
	known ratio of target vs. non-target interfering with the test - high	no	/
	known ratio of target vs. non-target interfering with the test - medium	no	/
	known ratio of target vs. non-target interfering with the test - low	yes	Purity of isolates have been tested by DSMZ. No additional tests regarding purity of isolates have been carried out by TPS organizer (only Sanger sequencing of tospovirus specific PCR product have been done).

Reference material/ samples included in TPS

Descriptor	Value	Minimum criterion	Description
Assigned value	absolute concentration known	no	no
	level of concentration known (high/medium/low)	no	Estimated by real-time PCR analyses
	qualitative status known (above LOD level)	no	The minimum level of detection which guarantee all repetitions are positive.
	originating from plants with known health statues with a recent test result (a given period of time depends on the plant-pest combination and previous experience)	yes	All assigned values have been determined by DSMZ and confirmed by TPS organizer

TPS TSWV example: health status vs. expected results

	type of sample (isolate designation in DSMZ collection)	Sample designation					Health status of TSWV	Expected result for TSWV		
		Molecular	LFD Agdia	LFD Bioreba	ELISA Agdia	ELISA Bioreba		Molecular	LFD	ELISA
healthy tomato	healthy tomato 1	S-7	S-15	S-4	S-4	S-8	neg	neg	neg	neg
	healthy tomato 1	S-17	S-20	S-10	S-15	S-10	neg	neg	neg	neg
	healthy tomato 2	S-21	S-10	S-3	S-6	S-9	neg	neg	neg	neg
other tospoviruses	ANSV (PV-1027)	S-12	S-11	S-7	S-19	S-14	neg	neg	neg	neg
	CSNV2 (PV-0529/G 25.37)	S-15	S-3	S-21	S-7	S-19	neg	neg	neg	neg
	GRSV (PV-0205/SA05)	S-2	S-13	S-14	S-16	S-11	neg	neg	neg	neg
	INSV2 (PV-0281)	S-13	S-7	S-2	S-9	S-3	neg	neg	neg	neg
	TCSV (PV-0390/VE 225)	S-10	S-21	S-15	S-12	S-7	neg	neg	neg	neg
TSWV dilution series	TSWV (PV1175) 1000000x	S-18	S-2	S-5	S-14	S-4	pos	pos	neg	neg
	TSWV (PV1175) 1000000x	S-20	S-18	S-22	S-20	S-12	pos	pos	neg	neg
	TSWV (PV1175) 100000x	S-1	S-17	S-6	S-10	S-6	pos	pos	neg	neg
	TSWV (PV1175) 100000x	S-19	S-22	S-9	S-11	S-21	pos	pos	neg	neg
	TSWV (PV1175) 10000x	S-11	S-8	S-13	S-13	S-2	pos	pos	pos	pos
	TSWV (PV1175) 10000x	S-14	S-9	S-19	S-21	S-22	pos	pos	pos	pos
	TSWV (PV1175) 1000x	S-3	S-1	S-1	S-18	S-13	pos	pos	pos	pos
	TSWV (PV1175) 1000x	S-9	S-16	S-16	S-22	S-15	pos	pos	pos	pos
	TSWV (PV1175) 100x	S-6	S-4	S-12	S-5	S-1	pos	pos	pos	pos
TSWV medium concentration	TSWV (PV-0182) 1000x diluted	S-22	S-6	S-20	S-8	S-18	pos	pos	pos	pos
	TSWV (PV-0182) 1000x diluted	S-8	nt	nt	nt	nt	pos	pos	nt	nt
	TSWV (PV-0182) 100x diluted	S-4	nt	nt	nt	nt	pos	pos	nt	nt
	TSWV (PV-0182) 100x diluted	nt	nt	nt	S-1	S-5	pos	nt	nt	pos
	TSWV (PV-0182) 10x diluted	nt	nt	nt	S-2	S-17	pos	nt	nt	pos
	TSWV (PV-0182) 10x diluted	nt	S-14	S-8	nt	nt	pos	nt	pos	nt
TSWV at concentration close to limit of detection	TSWV (PV-0182) 10x diluted	nt	S-19	S-17	nt	nt	pos	nt	pos	nt
	TSWV (PV-0389) 100000x diluted	S-5	nt	nt	nt	nt	pos	pos	nt	nt
	TSWV (PV-0389) 100000x diluted	S-16	nt	nt	nt	nt	pos	pos	nt	nt
	TSWV (PV-0389) 10000x diluted	nt	nt	nt	S-3	S-16	pos	nt	nt	pos
	TSWV (PV-0389) 10000x diluted	nt	nt	nt	S-17	S-20	pos	nt	nt	pos
	TSWV (PV-0389) 1000x diluted	nt	S-5	S-11	nt	nt	pos	nt	pos	nt
controls	TSWV (PV-0389) 1000x diluted	nt	S-12	S-18	nt	nt	pos	nt	pos	nt
	healthy tomato 2	NC/NIC	NC	NC	NC	NIC	neg	neg	neg	neg
	TSWV (PV0393) 10x diluted	PC/PIC	PC	PC	PC	PIC	pos	pos	pos	pos
	no template control*	NAC	nt	nt	nt	nt	neg	neg	nt	nt
	total RNA (PV-0182 and PV-0389)	PAC	nt	nt	nt	nt	pos	pos	nt	nt
	* not provided for participants									

Randomization, distribution of the samples, consumables

For each participants (21):

- 1-5 batches (2xELISA, 2xLFD, 1x molecular tests) with 22 tests items
- Controls (NC, PC, PIC, NIC, PAC)
- Primers mix
- Primers and probes mix

Stability and homogeneity confirmed as for samples!





Collection of participants results

- Uniform TPS result form for all participants and for all tests
- Collect as much as possible data you might need
- Clearly specify how data should be entered (e.g., only „pos/ neg/ inconclusive“ can be entered by participants)
- Form should be compatible with file where you will collect data of all participants (e.g., copy->paste)

Collection of participants results

example:

							
This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N° 773139							
TPS result form							
TPS code	TSWV			Target organism	Tomato spotted wilt tospovirus		
Method	Real-time RT-PCR			Test	Test adapted from Mortimer-Jones et al. , 2009		
Identification of the participating laboratory							
Name of the laboratory							
Panel code							
Implementation of the test							
Starting date for performing the analyses							
Ending date for performing the analyses							
Details about test running/ analysis							
		Supplier	Name and reference				
One step amplification kit							
OR							
Reverse-transcription kit							
Amplification kit							
dNTP							
Real-time thermalcycler		Brand	Model	Wavelength of filters			
Specify any deviation from the real-time RT-PCR described in the TPS technical sheet (e.g. amplification conditions)							
Remarks, comments, difficulties encountered when carrying out the real-time RT-PCR protocol							

Results obtained from the controls

Controls	Repetition (well)	Cq value per well	Average Cq value per control	Qualitative result	Comments (if necessary)
Negative isolation control (NIC)	1		#DIV/0!		
	2				
Positive isolation control (PIC)	1		#DIV/0!		
	2				
Negative amplification control (NAC)	1		#DIV/0!		
	2				
Positive amplification control (PAC)	1		#DIV/0!		
	2				
Other (precise):	1		#DIV/0!		
	2				
Other (precise):	1		#DIV/0!		
	2				

Determination of the threshold

Determination of the threshold		<i>if manual, precise the decision-making rules:</i>	
Value of the threshold			
If necessary : Value of the cut-off			

Results obtained from the samples

Sample reference: Molecular tests	Repetition (well)	Cq value per well	Average Cq value per sample	Qualitative result	Comments (if necessary)
S-1	1 2		#DIV/0!		
S-2	1 2		#DIV/0!		
S-3	1 2		#DIV/0!		
S-4	1 2		#DIV/0!		
S-5	1 2		#DIV/0!		
S-6	1 2		#DIV/0!		
S-7	1 2		#DIV/0!		
S-8	1 2		#DIV/0!		
S-9	1 2		#DIV/0!		
S-10	1 2		#DIV/0!		
S-11	1 2		#DIV/0!		
S-12	1 2		#DIV/0!		
S-13	1 2		#DIV/0!		
S-14	1 2		#DIV/0!		
S-15	1 2		#DIV/0!		
S-16	1 2		#DIV/0!		
S-17	1 2		#DIV/0!		
S-18	1 2		#DIV/0!		
S-19	1 2		#DIV/0!		
S-20	1 2		#DIV/0!		
S-21	1 2		#DIV/0!		
S-22	1 2		#DIV/0!		

Join a copy of the real-time RT-PCR report with amplification curves!

Validation by the TPS participant

Date		Name	
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TPS results analysis

Should we take into account all results we got from TPS participants?

yes

no

TPS results analysis

Criteria to define outliers (which results should be excluded)?



Assessment of validity of results (controls)

Control	Total (N°)	Concordant		Non-concordant (%)			
		(N°)	%	FN	FP	INC	not done
ELISA	60	60	100	0	0	0	0
Agdia-PC	15	15	100	0	0	0	0
Bioreba-PC	15	15	100	0	0	0	0
Agdia-NC	15	15	100	0	0	0	0
Bioreba NC	15	15	100	0	0	0	0
LFD	50	50	100	0	0	0	0
Agdia-PC	13	13	100	0	0	0	0
Bioreba-PC	12	12	100	0	0	0	0
Agdia-NC	13	13	100	0	0	0	0
Bioreba NC	12	12	100	0	0	0	0
Molecular	240	236	98.3	1.25	0	0	0.4
RT-PCR Hassani Merhaban-PIC	15	14	93.3	6.7	0	0	0
RT-PCR Hassani Merhaban-NIC	15	14	93.3	0	0	0	6.7
RT-PCR Hassani Merhaban-PAC	15	14	93.3	6.7	0	0	0
RT-PCR Hassani Merhaban-NAC	15	15	100	0	0	0	0
RT-qPCR Boonham-PIC	15	15	100	0	0	0	0
RT-qPCR Boonham-NIC	15	15	100	0	0	0	0
RT-qPCR Boonham-PAC	15	15	100	0	0	0	0
RT-qPCR Boonham-NAC	15	15	100	0	0	0	0
RT-qPCR Roberts-PIC	15	15	100	0	0	0	0
RT-qPCR Roberts-NIC	15	15	100	0	0	0	0
RT-qPCR Roberts-PAC	15	15	100	0	0	0	0
RT-qPCR Roberts-NAC	15	15	100	0	0	0	0
RT-qPCR Mortimere Jones-PIC	15	15	100	0	0	0	0
RT-qPCR Mortimere Jones-NIC	15	15	100	0	0	0	0
RT-qPCR Mortimere Jones-PAC	15	14	93.3	6.7	0	0	0
RT-qPCR Mortimere Jones-NAC	15	15	100	0	0	0	0

outlier identification (wrong controls)

-> excluded: RT-PCR 2 labs; RT-qPCR Mortimer Jones 1 lab

Assessment of validity of results (healthy tomato samples)

ELISA Agdia	Sample type	L01	L02	L03	L04	L05	L06	L07	L08	L09	L10	L11	L12	L13	L14	L15	L16	L17	L18	L19	L20	L21
S-4	healthy tomato 1			0	0		0	0		2	0		0	0	0	0		0	0	0	0	0
S-15	healthy tomato 1			0	0		0	0		0	0		0	0	0	0		0	0	0	0	0
S-6	healthy tomato 2			0	0		0	0		0	0		0	0	0	0		0	0	0	0	0

ELISA Bioreba	Sample type	L01	L02	L03	L04	L05	L06	L07	L08	L09	L10	L11	L12	L13	L14	L15	L16	L17	L18	L19	L20	L21
S-8	healthy tomato 1			0	0		0	0		0	0		0	0		0		0	0	0	0	0
S-10	healthy tomato 1			0	0		0	0		2	0		0	0		0		0	0	0	0	0
S-9	healthy tomato 2			0	0		0	0		0	0		0	0		0		0	0	0	0	0

LFD Agdia	Sample type	L01	L02	L03	L04	L05	L06	L07	L08	L09	L10	L11	L12	L13	L14	L15	L16	L17	L18	L19	L20	L21
S-15	healthy tomato 1			0	0	0		0		0	0		0		0			0	0	0	0	0
S-20	healthy tomato 1			0	0	0		0		0	0		0		0			0	0	0	0	0
S-10	healthy tomato 2			0	0	0		0		0	0		0		0			0	0	0	0	0

LFD Bioreba	Sample type	L01	L02	L03	L04	L05	L06	L07	L08	L09	L10	L11	L12	L13	L14	L15	L16	L17	L18	L19	L20	L21
S-4	healthy tomato 1			0	0	0		0		0	0		0					0	0	0	0	0
S-10	healthy tomato 1			1	0	0		0		0	0		0					0	0	0	0	0
S-3	healthy tomato 2			0	0	0		0		0	0		0					0	0	0	0	0

Assessment of validity of results (healthy tomato samples)

RT-PCR	Sample type	L01	L02	L03	L04	L05	L06	L07	L08	L09	L10	L11	L12	L13	L14	L15	L16	L17	L18	L19	L20	L21
S-7	healthy tomato 1	0	0	0	0	0		0		0	0		0	0	0		0		0	0	2	0
S-17	healthy tomato 1	0	0	0	0	0		0		0	0		0	0	0		0		0	0	0	0
S-21	healthy tomato 2	0	0	0	0	0		1		0	0		0	0	0		0		0	0	0	0

RT-qPCR Boonham	Sample type	L01	L02	L03	L04	L05	L06	L07	L08	L09	L10	L11	L12	L13	L14	L15	L16	L17	L18	L19	L20	L21
S-7	healthy tomato 1	0		1	1	0		0		2	0	0	0	0	0	0	0	0	0	0	0	0
S-17	healthy tomato 1	0		1	0	0		0		1	0	0	0	0	0	0	0	0	0	0	0	0
S-21	healthy tomato 2	1		1	0	0		1		1	0	0	0	0	0	0	0	0	0	0	0	0

RT-qPCR Roberts	Sample type	L01	L02	L03	L04	L05	L06	L07	L08	L09	L10	L11	L12	L13	L14	L15	L16	L17	L18	L19	L20	L21
S-7	healthy tomato 1	0		1	1	0		0		0	0		0	0	0	0	0	0	0	0	0	0
S-17	healthy tomato 1	0		1	0	0		0		0	0		0	0	0	0	0	0	0	0	0	0
S-21	healthy tomato 2	1		1	0	0		1		1	0		0	0	0	0	0	0	0	0	2	0

RT-qPCR Mortimer Jones	Sample type	L01	L02	L03	L04	L05	L06	L07	L08	L09	L10	L11	L12	L13	L14	L15	L16	L17	L18	L19	L20	L21
S-7	healthy tomato 1	0		1	1	0		0		2	0		0	0	0	0	0	0	0	0	0	0
S-17	healthy tomato 1	0		1	0	0		0		2	0		0	0	0	0	0	0	0	0	0	0
S-21	healthy tomato 2	1		1	0	0		1		1	0		0	0	0	0	0	0	0	0	0	0

Additional outlier: ≥ 2 FP results for healthy tomato samples

Assessment of validity of datasets

Method and test	Number of submitted data sets	Number of valid data sets	
		(N°)	Percentage
ELISA			
Agdia	16	16	100
Bioreba	14	14	100
LFD			
Agdia	13	13	100
Bioreba	12	12	100
Molecular RT-PCR			
Hassani-Mehraban et al., 2016	15	13	86.7
Molecular real time RT-PCR			
Boonham et al. 2002	15	13	86.7
Roberts et al. 2000	15	14	93.3
Mortimer-Jones et al. 2009	15	13	86.7
Total	115	108	93.9

Analysis for each of the tests

Test: ELISA Agdia

No. of datasets: 16

No. of data points: 352

Test items	Sample type	INC	TN	FP	FN	TP	INC %	TN %	FP %	FN %	TP %	Concordant	Non-concordant	Concordant %	Non-concordant %
S-4	healthy tomato 1	1	15	0			6,3	93,8	0,0	0,0	0,0	15,0	1,0	93,8	6,3
S-15	healthy tomato 1	0	16	0			0,0	100,0	0,0	0,0	0,0	16,0	0,0	100,0	0,0
S-6	healthy tomato 2	0	16	0			0,0	100,0	0,0	0,0	0,0	16,0	0,0	100,0	0,0
S-19	ANSV (PV-1027)	0	10	6			0,0	62,5	37,5	0,0	0,0	10,0	6,0	62,5	37,5
S-7	CSNV2 (PV-0529/G 25.37)	0	16	0			0,0	100,0	0,0	0,0	0,0	16,0	0,0	100,0	0,0
S-16	GRSV (PV-0205/SA05)	2	8	6			12,5	50,0	37,5	0,0	0,0	8,0	8,0	50,0	50,0
S-9	INSV2 (PV-0281)	1	15	0			6,3	93,8	0,0	0,0	0,0	15,0	1,0	93,8	6,3
S-12	TCSV (PV-0390/VE 225)	0	16	0			0,0	100,0	0,0	0,0	0,0	16,0	0,0	100,0	0,0
S-14	TSWV (PV1175) 1000000x	0	15	1			0,0	93,8	6,3	0,0	0,0	15,0	1,0	93,8	6,3
S-20	TSWV (PV1175) 1000000x	0	16	0			0,0	100,0	0,0	0,0	0,0	16,0	0,0	100,0	0,0
S-10	TSWV (PV1175) 100000x	1	15	0			6,3	93,8	0,0	0,0	0,0	15,0	1,0	93,8	6,3
S-11	TSWV (PV1175) 100000x	1	14	1			6,3	87,5	6,3	0,0	0,0	14,0	2,0	87,5	12,5
S-13	TSWV (PV1175) 10000x	0			2	14	0,0	0,0	0,0	12,5	87,5	14,0	2,0	87,5	12,5
S-21	TSWV (PV1175) 10000x	1			1	14	6,3	0,0	0,0	6,3	87,5	14,0	2,0	87,5	12,5
S-18	TSWV (PV1175) 1000x	0		0		16	0,0	0,0	0,0	0,0	100,0	16,0	0,0	100,0	0,0
S-22	TSWV (PV1175) 1000x	0		0		16	0,0	0,0	0,0	0,0	100,0	16,0	0,0	100,0	0,0
S-5	TSWV (PV1175) 100x	0		0		16	0,0	0,0	0,0	0,0	100,0	16,0	0,0	100,0	0,0
S-8	TSWV (PV1175) 100x	0			1	15	0,0	0,0	0,0	6,3	93,8	15,0	1,0	93,8	6,3
S-1	TSWV (PV-0182) 100x diluted	0		0		16	0,0	0,0	0,0	0,0	100,0	16,0	0,0	100,0	0,0
S-2	TSWV (PV-0182) 100x diluted	0		0		16	0,0	0,0	0,0	0,0	100,0	16,0	0,0	100,0	0,0
S-3	TSWV (PV-0389) 10000x diluted	0		0		16	0,0	0,0	0,0	0,0	100,0	16,0	0,0	100,0	0,0
S-17	TSWV (PV-0389) 10000x diluted	0		0		16	0,0	0,0	0,0	0,0	100,0	16,0	0,0	100,0	0,0
Total		7	172	14	4	155	2,0	48,9	4,0	1,1	44,0	327,0	25,0	92,9	7,1

Be aware expected results ≠ health status!

TP = true positive, TN = true negative, FN = false negative, FP = false positive, INC = inconclusive.



Test: ELISA Bioreba

No. of datasets: 14

No. of data points: 308

Test items	Sample type	INC	TN	FP	FN	TP	INC %	TN %	FP %	FN %	TP %	Concordant	Non-concordant	Concordant %	Non-concordant %
S-4	healthy tomato 1	0	14	0			0,0	100,0	0,0	0,0	0,0	14,0	0,0	100,0	0,0
S-15	healthy tomato 1	1	13	0			7,1	92,9	0,0	0,0	0,0	13,0	1,0	92,9	7,1
S-6	healthy tomato 2	0	14	0			0,0	100,0	0,0	0,0	0,0	14,0	0,0	100,0	0,0
S-19	ANSV (PV-1027)	0	2	12			0,0	14,3	85,7	0,0	0,0	2,0	12,0	14,3	85,7
S-7	CSNV2 (PV-0529/G 25.37)	1	2	11			7,1	14,3	78,6	0,0	0,0	2,0	12,0	14,3	85,7
S-16	GRSV (PV-0205/SA05)	0	1	13			0,0	7,1	92,9	0,0	0,0	1,0	13,0	7,1	92,9
S-9	INSV2 (PV-0281)	0	14	0			0,0	100,0	0,0	0,0	0,0	14,0	0,0	100,0	0,0
S-12	TCSV (PV-0390/VE 225)	2	2	10			14,3	14,3	71,4	0,0	0,0	2,0	12,0	14,3	85,7
S-14	TSWV (PV1175) 1000000x	0	14	0			0,0	100,0	0,0	0,0	0,0	14,0	0,0	100,0	0,0
S-20	TSWV (PV1175) 1000000x	0	13	1			0,0	92,9	7,1	0,0	0,0	13,0	1,0	92,9	7,1
S-10	TSWV (PV1175) 100000x	0	14	0			0,0	100,0	0,0	0,0	0,0	14,0	0,0	100,0	0,0
S-11	TSWV (PV1175) 100000x	0	14	0			0,0	100,0	0,0	0,0	0,0	14,0	0,0	100,0	0,0
S-13	TSWV (PV1175) 10000x	0			2	12	0,0	0,0	0,0	14,3	85,7	12,0	2,0	85,7	14,3
S-21	TSWV (PV1175) 10000x	0			3	11	0,0	0,0	0,0	21,4	78,6	11,0	3,0	78,6	21,4
S-18	TSWV (PV1175) 1000x	0			0	14	0,0	0,0	0,0	0,0	100,0	14,0	0,0	100,0	0,0
S-22	TSWV (PV1175) 1000x	0			0	14	0,0	0,0	0,0	0,0	100,0	14,0	0,0	100,0	0,0
S-5	TSWV (PV1175) 100x	0			0	14	0,0	0,0	0,0	0,0	100,0	14,0	0,0	100,0	0,0
S-8	TSWV (PV1175) 100x	0			0	14	0,0	0,0	0,0	0,0	100,0	14,0	0,0	100,0	0,0
S-1	TSWV (PV-0182) 100x diluted	0			0	14	0,0	0,0	0,0	0,0	100,0	14,0	0,0	100,0	0,0
S-2	TSWV (PV-0182) 100x diluted	0			0	14	0,0	0,0	0,0	0,0	100,0	14,0	0,0	100,0	0,0
S-3	TSWV (PV-0389) 10000x diluted	0			0	14	0,0	0,0	0,0	0,0	100,0	14,0	0,0	100,0	0,0
S-17	TSWV (PV-0389) 10000x diluted	0			0	14	0,0	0,0	0,0	0,0	100,0	14,0	0,0	100,0	0,0
Total		4	117	47	5	135	1,3	38,0	15,3	1,6	43,8	252,0	56,0	81,8	18,2

Be aware expected results \neq health status!



TP = true positive, TN = true negative, FN = false negative, FP = false positive, INC = inconclusive.

Test: LFD Agdia

No. of datasets: 13

No. of data points: 286

Test items	Sample type	INC	TN	FP	FN	TP	INC %	TN %	FP %	FN %	TP %	Concordant	Non-concordant	Concordant %	Non-concordant %
S-15	healthy tomato 1	0	13	0			0,0	100,0	0,0	0,0	0,0	13,0	0,0	100,0	0,0
S-20	healthy tomato 1	0	13	0			0,0	100,0	0,0	0,0	0,0	13,0	0,0	100,0	0,0
S-10	healthy tomato 2	0	13	0			0,0	100,0	0,0	0,0	0,0	13,0	0,0	100,0	0,0
S-11	ANSV (PV-1027)	0	1	12			0,0	7,7	92,3	0,0	0,0	1,0	12,0	7,7	92,3
S-3	CSNV2 (PV-0529/G 25.37)	0	13	0			0,0	100,0	0,0	0,0	0,0	13,0	0,0	100,0	0,0
S-13	GRSV (PV-0205/SA05)	0	0	13			0,0	0,0	100,0	0,0	0,0	0,0	13,0	0,0	100,0
S-7	INSV2 (PV-0281)	0	13	0			0,0	100,0	0,0	0,0	0,0	13,0	0,0	100,0	0,0
S-21	TCSV (PV-0390/VE 225)	0	0	13			0,0	0,0	100,0	0,0	0,0	0,0	13,0	0,0	100,0
S-2	TSWV (PV1175) 1000000x	0	13	0			0,0	100,0	0,0	0,0	0,0	13,0	0,0	100,0	0,0
S-18	TSWV (PV1175) 1000000x	0	13	0			0,0	100,0	0,0	0,0	0,0	13,0	0,0	100,0	0,0
S-17	TSWV (PV1175) 100000x	0	13	0			0,0	100,0	0,0	0,0	0,0	13,0	0,0	100,0	0,0
S-22	TSWV (PV1175) 100000x	0	12	1			0,0	92,3	7,7	0,0	0,0	12,0	1,0	92,3	7,7
S-8	TSWV (PV1175) 10000x	1			2	10	7,7	0,0	0,0	15,4	76,9	10,0	3,0	76,9	23,1
S-9	TSWV (PV1175) 10000x	1			2	10	7,7	0,0	0,0	15,4	76,9	10,0	3,0	76,9	23,1
S-1	TSWV (PV1175) 1000x	0			0	13	0,0	0,0	0,0	0,0	100,0	13,0	0,0	100,0	0,0
S-16	TSWV (PV1175) 1000x	1			0	12	7,7	0,0	0,0	0,0	92,3	12,0	1,0	92,3	7,7
S-4	TSWV (PV1175) 100x	0			1	12	0,0	0,0	0,0	7,7	92,3	12,0	1,0	92,3	7,7
S-6	TSWV (PV1175) 100x	0			0	13	0,0	0,0	0,0	0,0	100,0	13,0	0,0	100,0	0,0
S-14	TSWV (PV-0182) 10x diluted	0			0	13	0,0	0,0	0,0	0,0	100,0	13,0	0,0	100,0	0,0
S-19	TSWV (PV-0182) 10x diluted	0			0	13	0,0	0,0	0,0	0,0	100,0	13,0	0,0	100,0	0,0
S-5	TSWV (PV-0389) 1000x diluted	0			0	13	0,0	0,0	0,0	0,0	100,0	13,0	0,0	100,0	0,0
S-12	TSWV (PV-0389) 1000x diluted	0			0	13	0,0	0,0	0,0	0,0	100,0	13,0	0,0	100,0	0,0
Total		3	117	39	5	122	1,0	40,9	13,6	1,7	42,7	239,0	47,0	83,6	16,4

Be aware expected results ≠ health status!

TP = true positive, TN = true negative, FN = false negative, FP = false positive, INC = inconclusive.



Test: LFD Bioreba
 No. of datasets: 12
 No. of data points: 264

Test items	Sample type	INC	TN	FP	FN	TP	INC %	TN %	FP %	FN %	TP %	Concordant	Non-concordant	Concordant %	Non-concordant %
S-15	healthy tomato 1	0	12	0			0,0	100,0	0,0	0,0	0,0	12,0	0,0	100,0	0,0
S-20	healthy tomato 1	0	11	1			0,0	91,7	8,3	0,0	0,0	11,0	1,0	91,7	8,3
S-10	healthy tomato 2	0	12	0			0,0	100,0	0,0	0,0	0,0	12,0	0,0	100,0	0,0
S-11	ANSV (PV-1027)	0	11	1			0,0	91,7	8,3	0,0	0,0	11,0	1,0	91,7	8,3
S-3	CNSV2 (PV-0529/G 25.37)	0	12	0			0,0	100,0	0,0	0,0	0,0	12,0	0,0	100,0	0,0
S-13	GRSV (PV-0205/SA05)	0	10	2			0,0	83,3	16,7	0,0	0,0	10,0	2,0	83,3	16,7
S-7	INSV2 (PV-0281)	0	12	0			0,0	100,0	0,0	0,0	0,0	12,0	0,0	100,0	0,0
S-21	TCSV (PV-0390/VE 225)	0	12	0			0,0	100,0	0,0	0,0	0,0	12,0	0,0	100,0	0,0
S-2	TSWV (PV1175) 1000000x	0	12	0			0,0	100,0	0,0	0,0	0,0	12,0	0,0	100,0	0,0
S-18	TSWV (PV1175) 1000000x	0	12	0			0,0	100,0	0,0	0,0	0,0	12,0	0,0	100,0	0,0
S-17	TSWV (PV1175) 100000x	0	12	0			0,0	100,0	0,0	0,0	0,0	12,0	0,0	100,0	0,0
S-22	TSWV (PV1175) 100000x	0	12	0			0,0	100,0	0,0	0,0	0,0	12,0	0,0	100,0	0,0
S-8	TSWV (PV1175) 10000x	0			12	0	0,0	0,0	0,0	100,0	0,0	0,0	12,0	0,0	100,0
S-9	TSWV (PV1175) 10000x	0			12	0	0,0	0,0	0,0	100,0	0,0	0,0	12,0	0,0	100,0
S-1	TSWV (PV1175) 1000x	0			0	12	0,0	0,0	0,0	0,0	100,0	12,0	0,0	100,0	0,0
S-16	TSWV (PV1175) 1000x	0			0	12	0,0	0,0	0,0	0,0	100,0	12,0	0,0	100,0	0,0
S-4	TSWV (PV1175) 100x	0			0	12	0,0	0,0	0,0	0,0	100,0	12,0	0,0	100,0	0,0
S-6	TSWV (PV1175) 100x	0			0	12	0,0	0,0	0,0	0,0	100,0	12,0	0,0	100,0	0,0
S-14	TSWV (PV-0182) 10x diluted	0			0	12	0,0	0,0	0,0	0,0	100,0	12,0	0,0	100,0	0,0
S-19	TSWV (PV-0182) 10x diluted	0			0	12	0,0	0,0	0,0	0,0	100,0	12,0	0,0	100,0	0,0
S-5	TSWV (PV-0389) 1000x diluted	0			0	12	0,0	0,0	0,0	0,0	100,0	12,0	0,0	100,0	0,0
S-12	TSWV (PV-0389) 1000x diluted	0			0	12	0,0	0,0	0,0	0,0	100,0	12,0	0,0	100,0	0,0
Total		0	140	4	24	96	0,0	53,0	1,5	9,1	36,4	236,0	28,0	89,4	10,6

Be aware expected results ≠ health status!

Test: RT-PCR

No. of datasets: 13

No. of data points: 286

Test items	Sample type	INC	TN	FP	FN	TP	INC %	TN %	FP %	FN %	TP %	Concordant	Non-concordant	Concordant %	Non-concordant %
S-7	healthy tomato 1	1	12	0			7,7	92,3	0,0	0,0	0,0	12,0	1,0	92,3	7,7
S-17	healthy tomato 1	0	13	0			0,0	100,0	0,0	0,0	0,0	13,0	0,0	100,0	0,0
S-21	healthy tomato 2	0	12	1			0,0	92,3	7,7	0,0	0,0	12,0	1,0	92,3	7,7
S-12	ANSV (PV-1027)	0	13	0			0,0	100,0	0,0	0,0	0,0	13,0	0,0	100,0	0,0
S-15	CSNV2 (PV-0529/G 25.37)	0	13	0			0,0	100,0	0,0	0,0	0,0	13,0	0,0	100,0	0,0
S-2	GRSV (PV-0205/SA05)	0	13	0			0,0	100,0	0,0	0,0	0,0	13,0	0,0	100,0	0,0
S-13	INSV2 (PV-0281)	0	13	0			0,0	100,0	0,0	0,0	0,0	13,0	0,0	100,0	0,0
S-10	TCSV (PV-0390/VE 225)	0	12	1			0,0	92,3	7,7	0,0	0,0	12,0	1,0	92,3	7,7
S-18	TSWV (PV1175) 1000000x	1			1	11	7,7	0,0	0,0	7,7	84,6	11,0	2,0	84,6	15,4
S-20	TSWV (PV1175) 1000000x	1			3	9	7,7	0,0	0,0	23,1	69,2	9,0	4,0	69,2	30,8
S-1	TSWV (PV1175) 100000x	0			1	12	0,0	0,0	0,0	7,7	92,3	12,0	1,0	92,3	7,7
S-19	TSWV (PV1175) 100000x	0			1	12	0,0	0,0	0,0	7,7	92,3	12,0	1,0	92,3	7,7
S-11	TSWV (PV1175) 10000x	0			0	13	0,0	0,0	0,0	0,0	100,0	13,0	0,0	100,0	0,0
S-14	TSWV (PV1175) 10000x	0			0	13	0,0	0,0	0,0	0,0	100,0	13,0	0,0	100,0	0,0
S-3	TSWV (PV1175) 1000x	0			0	13	0,0	0,0	0,0	0,0	100,0	13,0	0,0	100,0	0,0
S-9	TSWV (PV1175) 1000x	0			0	13	0,0	0,0	0,0	0,0	100,0	13,0	0,0	100,0	0,0
S-6	TSWV (PV1175) 100x	0			0	13	0,0	0,0	0,0	0,0	100,0	13,0	0,0	100,0	0,0
S-22	TSWV (PV1175) 100x	0			2	11	0,0	0,0	0,0	15,4	84,6	11,0	2,0	84,6	15,4
S-8	TSWV (PV-0182) 1000x diluted	0			0	13	0,0	0,0	0,0	0,0	100,0	13,0	0,0	100,0	0,0
S-4	TSWV (PV-0182) 1000x diluted	0			0	13	0,0	0,0	0,0	0,0	100,0	13,0	0,0	100,0	0,0
S-5	TSWV (PV-0389) 100000x diluted	1			3	9	7,7	0,0	0,0	23,1	69,2	9,0	4,0	69,2	30,8
S-16	TSWV (PV-0389) 100000x diluted	0			3	10	0,0	0,0	0,0	23,1	76,9	10,0	3,0	76,9	23,1
Total		4	101	2	14	165	1,4	35,3	0,7	4,9	57,7	266,0	20,0	93,0	7,0

Note: expected results = health status

TP = true positive, TN = true negative, FN = false negative, FP = false positive, INC = inconclusive.



	Sample	rep	L01	L03	L04	L05	L07	L09	L10	L11	L12	L13	L15	L17	L19	L20	L21
healthy tomato 1	S-7	1	undet	33	undet/31	undet	undet	24 ^f	39/38	undet	undet	39	undet	undet	36	undet	37
healthy tomato 1		2	undet	33	38/31	undet	undet	29 ^f	undet/undet	undet	undet	38	undet	undet	36	undet	37
healthy tomato 1	S-17	1	37	33	38/undet	undet	36.5	29	39	undet	undet	36	undet	undet	undet	undet	36
healthy tomato 1		2	43	33	undet/undet	undet	36	28	39	undet	undet	36	undet	undet	36	undet	undet
healthy tomato 2	S-21	1	32	33	undet/undet	42	17	29	undet/undet	undet	undet	40	undet	undet	35	37	undet
healthy tomato 2		2	32	33	39/38	40	17	29	39/38	undet	undet	37	undet	undet	35	36	42
ANSV (PV-1027)	S-12	1	undet	undet	undet	undet	undet	undet	undet	undet	undet	undet	undet	undet	undet	undet	undet
ANSV (PV-1027)		2	undet	undet	undet	undet	undet	undet	undet	undet	undet	undet	undet	undet	undet	undet	undet
CSNV2 (PV-0529/G 25.37)	S-15	1	undet	undet	undet	undet	undet	undet	undet	undet	undet	undet	undet	undet	undet ^e	undet	undet
CSNV2 (PV-0529/G 25.37)		2	undet	undet	undet	undet	undet	undet	undet	undet	undet	undet	undet	undet	undet	undet	undet
GRSV (PV-0205/SA05)	S-2	1	32	undet	38/undet	undet	undet	29 ^e	44/undet	undet	undet	undet	undet	undet	undet	undet	undet
GRSV (PV-0205/SA05)		2	33	undet	38/undet	undet	undet	29 ^e	42/undet	undet	undet	undet	undet	undet	undet	undet	undet
INSV2 (PV-0281)	S-13	1	undet	30	undet/undet	40	38	35 ^e	undet	undet	undet	38	undet	36	35	undet	undet
INSV2 (PV-0281)		2	36	30	37/39	40	39	35 ^e	undet	undet	undet	38	undet	undet	36	undet	undet
TCSV (PV-0390/VE 225)	S-10	1	37	undet	undet	undet	undet	22 ^e	undet	undet	undet	undet	undet	undet	undet	undet	undet
TCSV (PV-0390/VE 225)		2	38	undet	undet	undet	undet	23 ^e	undet	undet	undet	undet	undet	undet	undet	undet	undet
TSWV (PV1175) 1000000x	S-18	1	28	28	34	28	31	26	32	29	30	30	29	28	27	30	27
TSWV (PV1175) 1000000x		2	28	28	34	28	31	26	31	29	30	30	29	28	27	30	27
TSWV (PV1175) 1000000x	S-20	1	29	28	36 ^h	29	30	27	30	29	30	31	29	29	28	30	28
TSWV (PV1175) 1000000x		2	29	28	35 ^h	29	30	27	31	29	30	31	29	29	28	30	28
TSWV (PV1175) 100000x	S-1	1	24	24	29	25	29	22	28	b	26	26	25	25	25	26	24
TSWV (PV1175) 100000x		2	24	24	29	25	26	23	28	25	26	26	25	24	24	26	24
TSWV (PV1175) 100000x	S-19	1	25	24	30	25	27	23	29	25	26	27	26	25	24	27	24
TSWV (PV1175) 100000x		2	25	25	31	25	27	24	27	25	26	26	26	25	24	27	24
TSWV (PV1175) 10000x	S-11	1	22	21	27	22	24	19	26	22	23	24	23	21	21	23	21
TSWV (PV1175) 10000x		2	22	21	27	22	24	19	26	22	23	24	22	21	21	23	21
TSWV (PV1175) 10000x	S-14	1	22	21	27	22	24	20	25	22	23	24	23	22	21	24	21
TSWV (PV1175) 10000x		2	22	21	27	22	24	20	24	22	22	24	23	22	21	24	21
TSWV (PV1175) 1000x	S-3	1	18	18	22	18	23	16	23	19	19	20	19	18	18	19	17
TSWV (PV1175) 1000x		2	18	18	23	18	20	16	22	19	20	20	19	18	18	20	17
TSWV (PV1175) 1000x	S-9	1	18	18	24	19	20	21	21	19	19	20	19	18	18	20	17
TSWV (PV1175) 1000x		2	18	18	25	20	20	21	21	19	19	20	19	18	17	20	17
TSWV (PV1175) 100x	S-6	1	15	15	21	15	17	13	19	15	16	17	16	15	14	16	14
TSWV (PV1175) 100x		2	15	15	21	16	17	13	18	16	16	17	16	15	15	17	14
TSWV (PV1175) 100x	S-22	1	15	14	22	16	36	17	17	15	16	17	16	15	17	17	15
TSWV (PV1175) 100x		2	15	14	22	16	35.3	17	17	16	16	17	16	15	17	17	15
TSWV (PV-0182) 1000x diluted	S-8	1	18	18	23	19	20	15	21	18	19	20	18	17	17	19	16
TSWV (PV-0182) 1000x diluted		2	18	18	23	19	20	15	21	18	19	20	18	17	17	19	16
TSWV (PV-0182) 1000x diluted	S-4	1	18	18	22	18	20	16	22	18	18	19	18	17	17	19	16
TSWV (PV-0182) 1000x diluted		2	18	18	22	18	20	16	21	18	19	20	18	17	17	19	16
TSWV (PV-0389) 100000x diluted	S-5	1	29	28	33	28	30	24	31	28	29	30	30	27	27	30	26
TSWV (PV-0389) 100000x diluted		2	28	28	33	28	30	24	31	29	29	30	29	27	27	29	26
TSWV (PV-0389) 100000x diluted	S-16	1	29	27	35 ^h	30	29	24	32	29	29	30	29	28	28	30	28
TSWV (PV-0389) 100000x diluted		2	29	27	34	30	29	24	31	29	29	30	29	28	28	30	28
Negative isolation control (NIC)		1	38	undet	undet	undet	38	undet	undet	undet	undet	36	undet	undet	undet	42 ^e	undet
Negative isolation control (NIC)		2	undet	undet	undet	undet	38	undet	undet	undet	38	undet	undet	undet	38	undet	undet
Positive isolation control (PIC)		1	11	28	16	12	13	23	14	11	12	13	11	10	12	13	11
Positive isolation control (PIC)		2	11	28	16	12	13	24	13	11	12	12	12	10	12	13	11
Negative amplification control (NAC)		1	undet	39	undet	undet	undet	undet	undet	undet	undet	undet	undet	undet	undet	38	undet
Negative amplification control (NAC)		2	undet	37	undet	undet	undet	undet	undet	undet	undet	undet	undet	undet	undet	41	undet
Positive amplification control (PAC)		1	25	25	30	11	27	8	26	26	26	26	25	26	23	26	24
Positive amplification control (PAC)		2	25	25	30	11	27	10	26	25	26	26	25	26	23	26	24
participant's way of the evaluation of the results	cut-off value (yes/no)		yes	no	yes ^d	yes ^d	yes ^d	no	no	no	no	no	no	no	yes	no	no
	Cq cut-off value		35	/	38.22	40	>37	/	/	/	/	/	/	/	32	/	/
	Cq values for inconclusive results		/	/	undet/ ~38 ^a	/	35-37	high dif. between rep. ^f	undet/ >38 ^a	/	undet/ >38 ^a	/	/	undet/ >36 ^a	/	/	undet/ >36 ^a

Test: RT-qPCR Boonham et al.

No. of datasets: 13

No. of data points: 286

Test items	Sample type	INC	TN	FP	FN	TP	INC %	TN %	FP %	FN %	TP %	Concordant	Non-concordant	Concordant %	Non-concordant %
S-7	healthy tomato 1	0	12	1			0,0	92,3	7,7	0,0	0,0	12,0	1,0	92,3	7,7
S-17	healthy tomato 1	0	13	0			0,0	100,0	0,0	0,0	0,0	13,0	0,0	100,0	0,0
S-21	healthy tomato 2	0	11	2			0,0	84,6	15,4	0,0	0,0	11,0	2,0	84,6	15,4
S-12	ANSV (PV-1027)	0	13	0			0,0	100,0	0,0	0,0	0,0	13,0	0,0	100,0	0,0
S-15	CSNV2 (PV-0529/G 25.37)	0	13	0			0,0	100,0	0,0	0,0	0,0	13,0	0,0	100,0	0,0
S-2	GRSV (PV-0205/SA05)	0	12	1			0,0	92,3	7,7	0,0	0,0	12,0	1,0	92,3	7,7
S-13	INSV2 (PV-0281)	0	13	0			0,0	100,0	0,0	0,0	0,0	13,0	0,0	100,0	0,0
S-10	TCSV (PV-0390/VE 225)	0	13	0			0,0	100,0	0,0	0,0	0,0	13,0	0,0	100,0	0,0
S-18	TSWV (PV1175) 1000000x	0			0	13	0,0	0,0	0,0	0,0	100,0	13,0	0,0	100,0	0,0
S-20	TSWV (PV1175) 1000000x	0			0	13	0,0	0,0	0,0	0,0	100,0	13,0	0,0	100,0	0,0
S-1	TSWV (PV1175) 100000x	0			0	13	0,0	0,0	0,0	0,0	100,0	13,0	0,0	100,0	0,0
S-19	TSWV (PV1175) 100000x	0			0	13	0,0	0,0	0,0	0,0	100,0	13,0	0,0	100,0	0,0
S-11	TSWV (PV1175) 10000x	0			0	13	0,0	0,0	0,0	0,0	100,0	13,0	0,0	100,0	0,0
S-14	TSWV (PV1175) 10000x	0			0	13	0,0	0,0	0,0	0,0	100,0	13,0	0,0	100,0	0,0
S-3	TSWV (PV1175) 1000x	0			0	13	0,0	0,0	0,0	0,0	100,0	13,0	0,0	100,0	0,0
S-9	TSWV (PV1175) 1000x	0			0	13	0,0	0,0	0,0	0,0	100,0	13,0	0,0	100,0	0,0
S-6	TSWV (PV1175) 100x	0			0	13	0,0	0,0	0,0	0,0	100,0	13,0	0,0	100,0	0,0
S-22	TSWV (PV1175) 100x	0			1	12	0,0	0,0	0,0	7,7	92,3	12,0	1,0	92,3	7,7
S-8	TSWV (PV-0182) 1000x diluted	0			0	13	0,0	0,0	0,0	0,0	100,0	13,0	0,0	100,0	0,0
S-4	TSWV (PV-0182) 1000x diluted	0			0	13	0,0	0,0	0,0	0,0	100,0	13,0	0,0	100,0	0,0
S-5	TSWV (PV-0389) 1000000x diluted	0			0	13	0,0	0,0	0,0	0,0	100,0	13,0	0,0	100,0	0,0
S-16	TSWV (PV-0389) 1000000x diluted	0			0	13	0,0	0,0	0,0	0,0	100,0	13,0	0,0	100,0	0,0
Total		0	100	4	1	181	0,0	35,0	1,4	0,3	63,3	281,0	5,0	98,3	1,7

Note: expected results = health status

TP = true positive, TN = true negative, FN = false negative, FP = false positive, INC = inconclusive.



Test: RT-qPCR Roberts et al.

No. of datasets: 14

No. of data points: 308

Test items	Sample type	INC	TN	FP	FN	TP	INC %	TN %	FP %	FN %	TP %	Concordant	Non-concordant	Concordant %	Non-concordant %
S-7	healthy tomato 1	0	13	1			0,0	92,9	7,1	0,0	0,0	13,0	1,0	92,9	7,1
S-17	healthy tomato 1	0	14	0			0,0	100,0	0,0	0,0	0,0	14,0	0,0	100,0	0,0
S-21	healthy tomato 2	1	10	3			7,1	71,4	21,4	0,0	0,0	10,0	4,0	71,4	28,6
S-12	ANSV (PV-1027)	1	13	0			7,1	92,9	0,0	0,0	0,0	13,0	1,0	92,9	7,1
S-15	CSNV2 (PV-0529/G 25.37)	1	13	0			7,1	92,9	0,0	0,0	0,0	13,0	1,0	92,9	7,1
S-2	GRSV (PV-0205/SA05)	0	13	1			0,0	92,9	7,1	0,0	0,0	13,0	1,0	92,9	7,1
S-13	INSV2 (PV-0281)	0	14	0			0,0	100,0	0,0	0,0	0,0	14,0	0,0	100,0	0,0
S-10	TCSV (PV-0390/VE 225)	0	12	2			0,0	85,7	14,3	0,0	0,0	12,0	2,0	85,7	14,3
S-18	TSWV (PV1175) 1000000x	0			0	14	0,0	0,0	0,0	0,0	100,0	14,0	0,0	100,0	0,0
S-20	TSWV (PV1175) 1000000x	0			0	14	0,0	0,0	0,0	0,0	100,0	14,0	0,0	100,0	0,0
S-1	TSWV (PV1175) 100000x	0			0	14	0,0	0,0	0,0	0,0	100,0	14,0	0,0	100,0	0,0
S-19	TSWV (PV1175) 100000x	0			0	14	0,0	0,0	0,0	0,0	100,0	14,0	0,0	100,0	0,0
S-11	TSWV (PV1175) 10000x	0			0	14	0,0	0,0	0,0	0,0	100,0	14,0	0,0	100,0	0,0
S-14	TSWV (PV1175) 10000x	0			0	14	0,0	0,0	0,0	0,0	100,0	14,0	0,0	100,0	0,0
S-3	TSWV (PV1175) 1000x	0			0	14	0,0	0,0	0,0	0,0	100,0	14,0	0,0	100,0	0,0
S-9	TSWV (PV1175) 1000x	0			0	14	0,0	0,0	0,0	0,0	100,0	14,0	0,0	100,0	0,0
S-6	TSWV (PV1175) 100x	0			0	14	0,0	0,0	0,0	0,0	100,0	14,0	0,0	100,0	0,0
S-22	TSWV (PV1175) 100x	0			1	13	0,0	0,0	0,0	7,1	92,9	13,0	1,0	92,9	7,1
S-8	TSWV (PV-0182) 1000x diluted	0			0	14	0,0	0,0	0,0	0,0	100,0	14,0	0,0	100,0	0,0
S-4	TSWV (PV-0182) 1000x diluted	0			0	14	0,0	0,0	0,0	0,0	100,0	14,0	0,0	100,0	0,0
S-5	TSWV (PV-0389) 100000x diluted	0			0	14	0,0	0,0	0,0	0,0	100,0	14,0	0,0	100,0	0,0
S-16	TSWV (PV-0389) 100000x diluted	0			0	14	0,0	0,0	0,0	0,0	100,0	14,0	0,0	100,0	0,0
Total		3	102	7	1	195	1,0	33,1	2,3	0,3	63,3	297,0	11,0	96,4	3,6

Note: expected results = health status

TP = true positive, TN = true negative, FN = false negative, FP = false positive.



Test: RT-qPCR Mortimer Jones et al.

No. of datasets: 13

No. of data points: 286

Test items	Sample type	INC	TN	FP	FN	TP	INC %	TN %	FP %	FN %	TP %	Concordant	Non-concordant	Concordant %	Non-concordant %
S-7	healthy tomato 1	0	12	1			0,0	92,3	7,7	0,0	0,0	12,0	1,0	92,3	7,7
S-17	healthy tomato 1	0	13	0			0,0	100,0	0,0	0,0	0,0	13,0	0,0	100,0	0,0
S-21	healthy tomato 2	0	11	2			0,0	84,6	15,4	0,0	0,0	11,0	2,0	84,6	15,4
S-12	ANSV (PV-1027)	0	13	0			0,0	100,0	0,0	0,0	0,0	13,0	0,0	100,0	0,0
S-15	CSNV2 (PV-0529/G 25.37)	0	12	1			0,0	92,3	7,7	0,0	0,0	12,0	1,0	92,3	7,7
S-2	GRSV (PV-0205/SA05)	0	12	1			0,0	92,3	7,7	0,0	0,0	12,0	1,0	92,3	7,7
S-13	INSV2 (PV-0281)	0	13	0			0,0	100,0	0,0	0,0	0,0	13,0	0,0	100,0	0,0
S-10	TCSV (PV-0390/VE 225)	0	13	0			0,0	100,0	0,0	0,0	0,0	13,0	0,0	100,0	0,0
S-18	TSWV (PV1175) 1000000x	0			0	13	0,0	0,0	0,0	0,0	100,0	13,0	0,0	100,0	0,0
S-20	TSWV (PV1175) 1000000x	0			0	13	0,0	0,0	0,0	0,0	100,0	13,0	0,0	100,0	0,0
S-1	TSWV (PV1175) 1000000x	0			0	13	0,0	0,0	0,0	0,0	100,0	13,0	0,0	100,0	0,0
S-19	TSWV (PV1175) 1000000x	0			0	13	0,0	0,0	0,0	0,0	100,0	13,0	0,0	100,0	0,0
S-11	TSWV (PV1175) 100000x	0			0	13	0,0	0,0	0,0	0,0	100,0	13,0	0,0	100,0	0,0
S-14	TSWV (PV1175) 10000x	0			0	13	0,0	0,0	0,0	0,0	100,0	13,0	0,0	100,0	0,0
S-3	TSWV (PV1175) 1000x	0			0	13	0,0	0,0	0,0	0,0	100,0	13,0	0,0	100,0	0,0
S-9	TSWV (PV1175) 1000x	0			0	13	0,0	0,0	0,0	0,0	100,0	13,0	0,0	100,0	0,0
S-6	TSWV (PV1175) 100x	0			0	13	0,0	0,0	0,0	0,0	100,0	13,0	0,0	100,0	0,0
S-22	TSWV (PV1175) 100x	0			1	12	0,0	0,0	0,0	7,7	92,3	12,0	1,0	92,3	7,7
S-8	TSWV (PV-0182) 1000x diluted	0			0	13	0,0	0,0	0,0	0,0	100,0	13,0	0,0	100,0	0,0
S-4	TSWV (PV-0182) 1000x diluted	0			0	13	0,0	0,0	0,0	0,0	100,0	13,0	0,0	100,0	0,0
S-5	TSWV (PV-0389) 1000000x diluted	0			0	13	0,0	0,0	0,0	0,0	100,0	13,0	0,0	100,0	0,0
S-16	TSWV (PV-0389) 1000000x diluted	0			0	13	0,0	0,0	0,0	0,0	100,0	13,0	0,0	100,0	0,0
Total		0	99	5	1	181	0,0	34,6	1,7	0,3	63,3	280,0	6,0	97,9	2,1

Note: expected results = health status

TP = true positive, TN = true negative, FN = false negative, FP = false positive, INC = inconclusive.



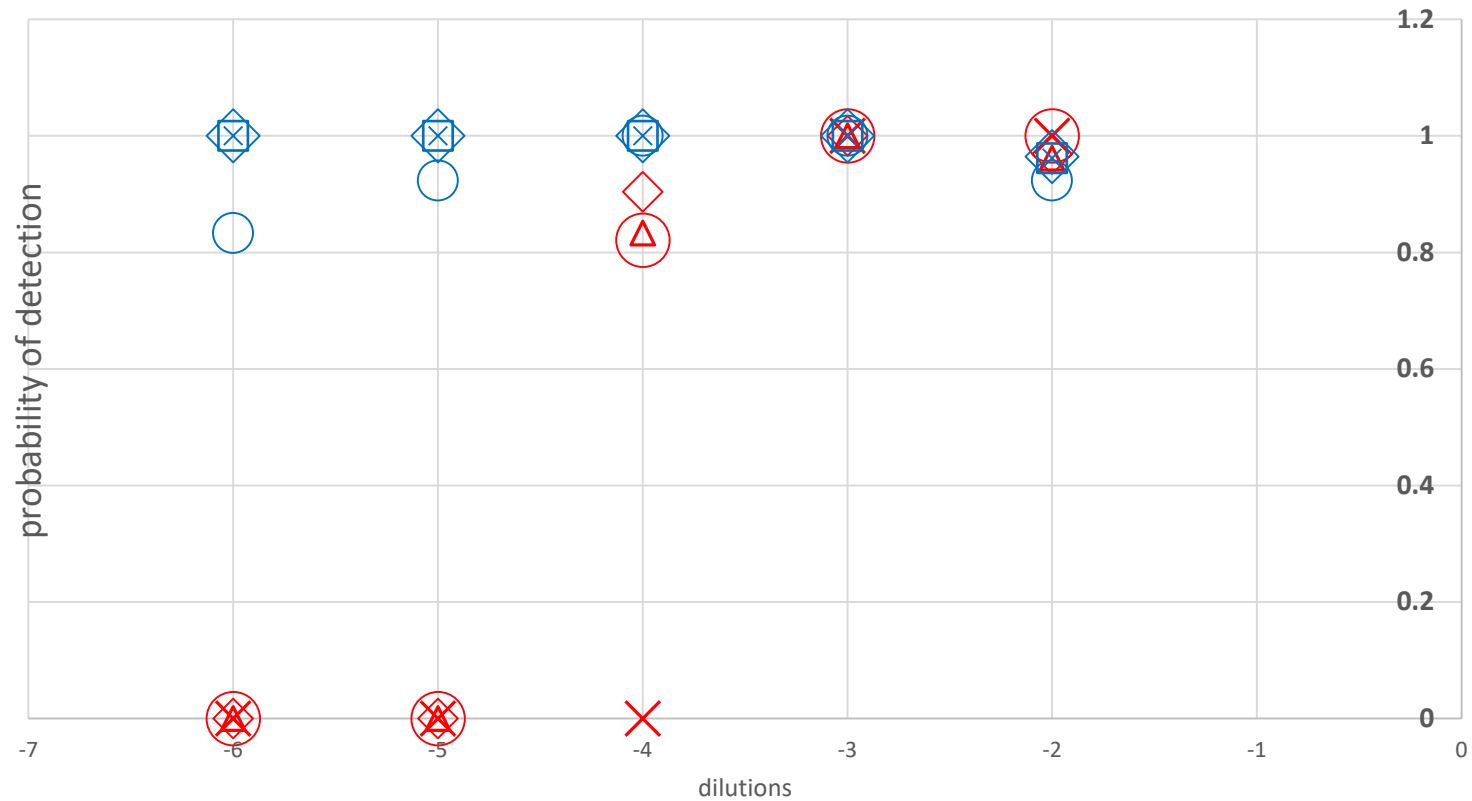
TPS results analysis

Which validation data can be obtained from our TPS (TSWV example)?

	type of sample (isolate designation in DSMZ collection)
healty tomato	healthy tomato 1
	healthy tomato 1
	healthy tomato 2
other tospoviruses	ANSV (PV-1027)
	CSNV2 (PV-0529)
	GRSV (PV-0205)
	INSV2 (PV-0281)
	TCSV (PV-0390)
TSWV dilution series	TSWV (PV-1175) 1000000x diluted
	TSWV (PV-1175) 1000000x diluted
	TSWV (PV-1175) 100000x diluted
	TSWV (PV-1175) 100000x diluted
	TSWV (PV-1175) 10000x diluted
	TSWV (PV-1175) 10000x diluted
	TSWV (PV-1175) 1000x diluted
	TSWV (PV-1175) 1000x diluted
	TSWV (PV-1175) 100x diluted
	TSWV (PV-1175) 100x diluted
TSWV medium concentration	TSWV (PV-0182) 1000x diluted
	TSWV (PV-0182) 1000x diluted
	TSWV (PV-0182) 100x diluted
	TSWV (PV-0182) 100x diluted
	TSWV (PV-0182) 10x diluted
	TSWV (PV-0182) 10x diluted
TSWV at concentration close to limit of detection	TSWV (PV-0389) 100000x diluted
	TSWV (PV-0389) 100000x diluted
	TSWV (PV-0389) 10000x diluted
	TSWV (PV-0389) 10000x diluted
	TSWV (PV-0389) 1000x diluted

PERFORMANCE CHARACTERISTICS	Mark if you think can be obtained from our TPS data
ANALYTICAL SENSITIVITY	
INCLUSIVITY	
EXCLUSIVITY	
REPRODUCIBILITY	
REPETABILITY	
SELECTIVITY	

Analytical sensitivity



◇ ELISA_Agdia

○ ELISA_Bioreba

△ LFD_Agdia

× LFD_Bioreba

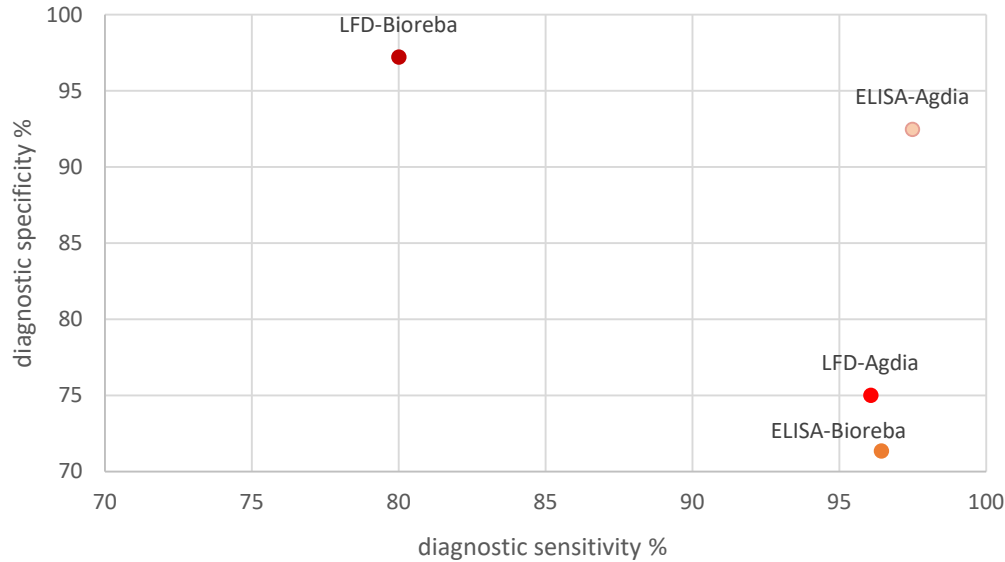
○ RT-PCR_Hassani-Merhaban

□ RT-qPCR_Boonham

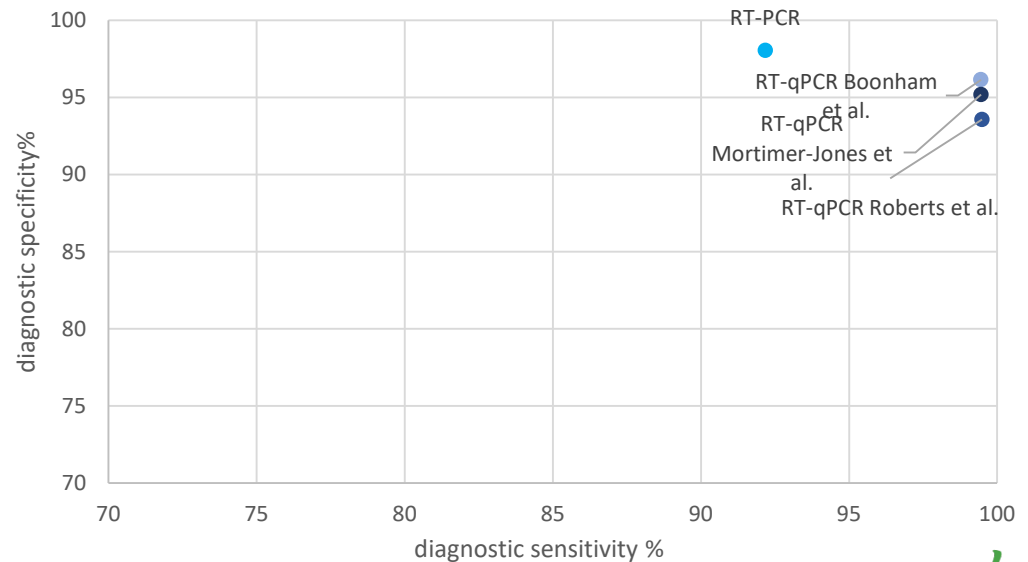
◇ RT-qPCR_Roberts

× RT-qPCR_Mortimer-Jones

Diagnostic specificity vs. diagnostic sensitivity



Be aware expected results \neq health status!

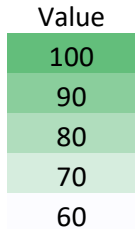


TP = true positive, TN = true negative, FN = false negative, FP = false positive. Diagnostic sensitivity (SE) - an estimation of the ability of the method to detect the target, $SE=100 \times TP / (TP+FN)$; Diagnostic specificity (SP) - an estimation of the ability of the method not to detect the non-target, $SP= 100 \times TN / (FP+TN)$.

Repeatability (%)

Test	L01	L02	L03	L04	L05	L06	L07	L08	L09	L10	L11	L12	L13	L14	L15	L16	L17	L18	L19	L20	L21	Average
ELISA_Agdia			100	100		100	100		77.8	100	100	100	100	100	100		88.9	100	100	66.7	100	95.8
ELISA_Bioreba			100	100		100	100		88.9	100	100	100			100		88.9	100	100	88.9	100	97.6
LFD_Agdia			100	100	100		100		100	100		100		77.8			100	100	100	100	100	98.3
LFD_Bioreba			88.9	100	100		100		100	100		100					100	100	100	100	100	99.1
RT-PCR_Hassani-Merhaban	100		100	77.8	100		77.8		88.9	100	100	100					77.8		100	77.8	100	92.3
RT-qPCR_Boonham	88.9			88.9	100		77.8			100	100	100	100		100		100		100	100	100	96.6
RT-qPCR_Roberts	88.9			88.9	100		77.8		88.9	100	100	100	100		100		100		100	88.9	100	94.8
RT-qPCR_Mortimer-Jones	88.9			88.9	100		77.8			100	100	100	100		100		100		100	100	100	96.0
Average	91.67		97.78	93.06	100	100	88.89		90.74	100	100	100	100	88.89	100		94.44	100	100	90.28	100	

 Outlier
  not participated

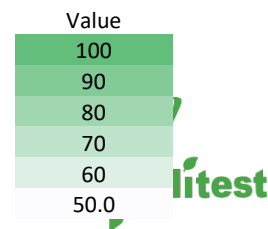


Repeatability=number of samples where both repetitions had same result/total number of samples
 (For calculation of the repeatability, samples with other tospoviruses has been excluded since tested only in one replicate)

Reproducibility (%)

	type of sample (isolate designation in DSMZ collection)	ELISA_Agdia	ELISA_Bioreba	LFD_Agdia	LFD_Bioreba	RT-PCR_Hassani-Merhaban	RT-qPCR_Boonham	RT-qPCR_Roberts	RT-qPCR_Mortimer-Jones
healthy tomato	healthy tomato 1	93.8	100.0	100.0	100.0	92.3	92.3	92.9	92.3
	healthy tomato 1	100.0	92.9	100.0	91.7	100.0	100.0	100.0	100.0
	healthy tomato 2	100.0	100.0	100.0	100.0	92.3	84.6	71.4	84.6
other tospoviruses	ANSV (PV-1027)	62.5	85.7	92.3	91.7	100.0	100.0	92.9	100.0
	CSNV2 (PV-0529)	100.0	85.7	100.0	100.0	100.0	100.0	92.9	92.3
	GRSV (PV-0205)	50.0	92.9	100.0	83.3	100.0	92.3	92.9	92.3
	INSV2 (PV-0281)	93.8	100.0	100.0	100.0	100.0	100.0	100.0	100.0
	TCSV (PV-0390)	100.0	85.7	100.0	100.0	92.3	100.0	85.7	100.0
TSWV dilution series	TSWV (PV-1175) 1000000x diluted	93.8	100.0	100.0	100.0	84.6	100.0	100.0	100.0
	TSWV (PV-1175) 1000000x diluted	100.0	92.9	100.0	100.0	69.2	100.0	100.0	100.0
	TSWV (PV-1175) 100000x diluted	93.8	100.0	100.0	100.0	92.3	100.0	100.0	100.0
	TSWV (PV-1175) 100000x diluted	87.5	100.0	92.3	100.0	92.3	100.0	100.0	100.0
	TSWV (PV-1175) 10000x diluted	87.5	85.7	76.9	100.0	100.0	100.0	100.0	100.0
	TSWV (PV-1175) 10000x diluted	87.5	78.6	76.9	100.0	100.0	100.0	100.0	100.0
	TSWV (PV-1175) 1000x diluted	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
	TSWV (PV-1175) 1000x diluted	100.0	100.0	92.3	100.0	100.0	100.0	100.0	100.0
	TSWV (PV-1175) 100x diluted	100.0	100.0	92.3	100.0	100.0	100.0	100.0	100.0
TSWV medium concentration	TSWV (PV-0182) 1000x diluted	93.8	100.0	100.0	100.0	84.6	92.3	92.9	92.3
	TSWV (PV-0182) 1000x diluted	nt	nt	nt	nt	100.0	100.0	100.0	100.0
	TSWV (PV-0182) 100x diluted	100.0	100.0	nt	nt	nt	nt	nt	nt
	TSWV (PV-0182) 100x diluted	100.0	100.0	nt	nt	nt	nt	nt	nt
	TSWV (PV-0182) 10x diluted	nt	nt	100.0	100.0	nt	nt	nt	nt
	TSWV (PV-0182) 10x diluted	nt	nt	100.0	100.0	nt	nt	nt	nt
TSWV at concentration close to limit of detection	TSWV (PV-0389) 100000x diluted	nt	nt	nt	nt	69.2	100.0	100.0	100.0
	TSWV (PV-0389) 100000x diluted	nt	nt	nt	nt	76.9	100.0	100.0	100.0
	TSWV (PV-0389) 10000x diluted	100.0	100.0	nt	nt	nt	nt	nt	nt
	TSWV (PV-0389) 10000x diluted	100.0	100.0	nt	nt	nt	nt	nt	nt
	TSWV (PV-0389) 1000x diluted	nt	nt	100.0	100.0	nt	nt	nt	nt
	TSWV (PV-0389) 1000x diluted	nt	nt	100.0	100.0	nt	nt	nt	nt
Average		92.9	95.5	96.5	98.5	93.0	98.3	96.4	97.9

Reproducibility=number recurring result per sample/total number of results per sample



Conclusions from TPS (TSWV example)

- All tests are fit for purpose
- Real time RT-PCRs had the highest diagnostic sensitivity
- RT-PCR had the highest diagnostic specificity
- ELISA – Agdia vs Bioreba: similar sensitivity, Agdia higher specificity
- On-site tests - Agdia vs Bioreba: Agdia more sensitive, but less specific; Bioreba more specific, but less sensitive.
- Repeatability for all tests: > 92%
- Reproducibility for all tests: > 92%

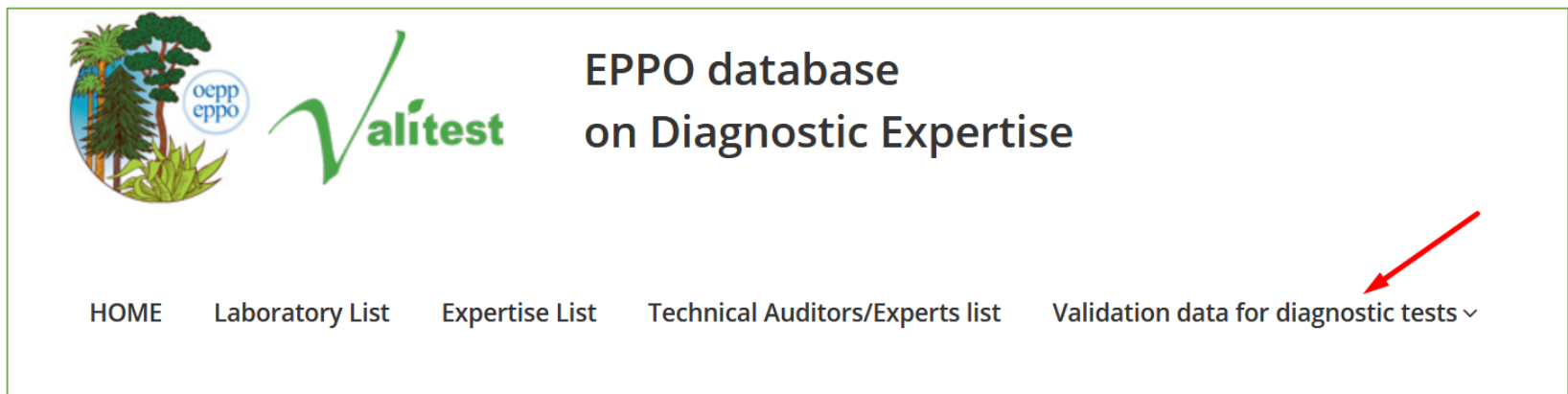
Disclaimer: the results only reflect the specific study case and reflects only the results on reagents at the time when they were included in the study!



Share validation data!

- Detail report to all participants!!!
- Article (optional)
- Request:

https://dc.eppo.int/validation_data/validationlist

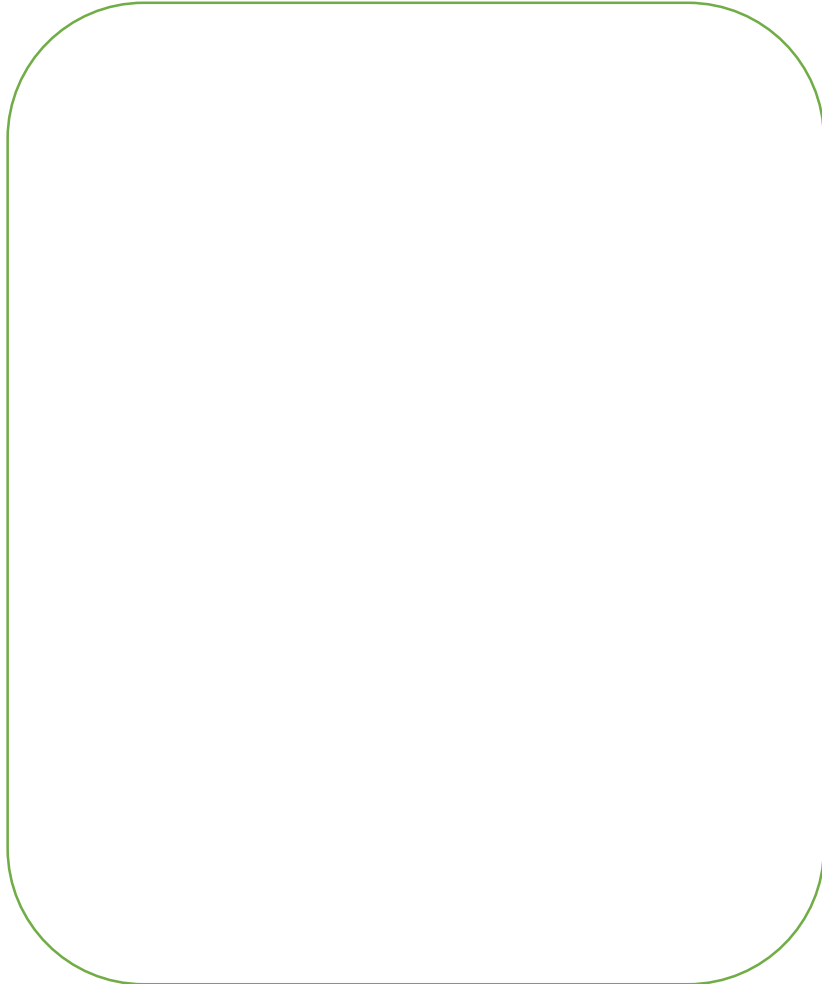


The screenshot shows the top navigation bar of the EPPO database website. On the left, there is a circular logo with a globe and trees, and the text 'oepp eppo'. Next to it is the 'valitest' logo, which features a green checkmark and the word 'valitest'. To the right of the logos, the text reads 'EPPO database on Diagnostic Expertise'. Below this, there is a horizontal menu with five items: 'HOME', 'Laboratory List', 'Expertise List', 'Technical Auditors/Experts list', and 'Validation data for diagnostic tests'. A red arrow points to the 'Validation data for diagnostic tests' item, which has a small downward-pointing chevron next to it.

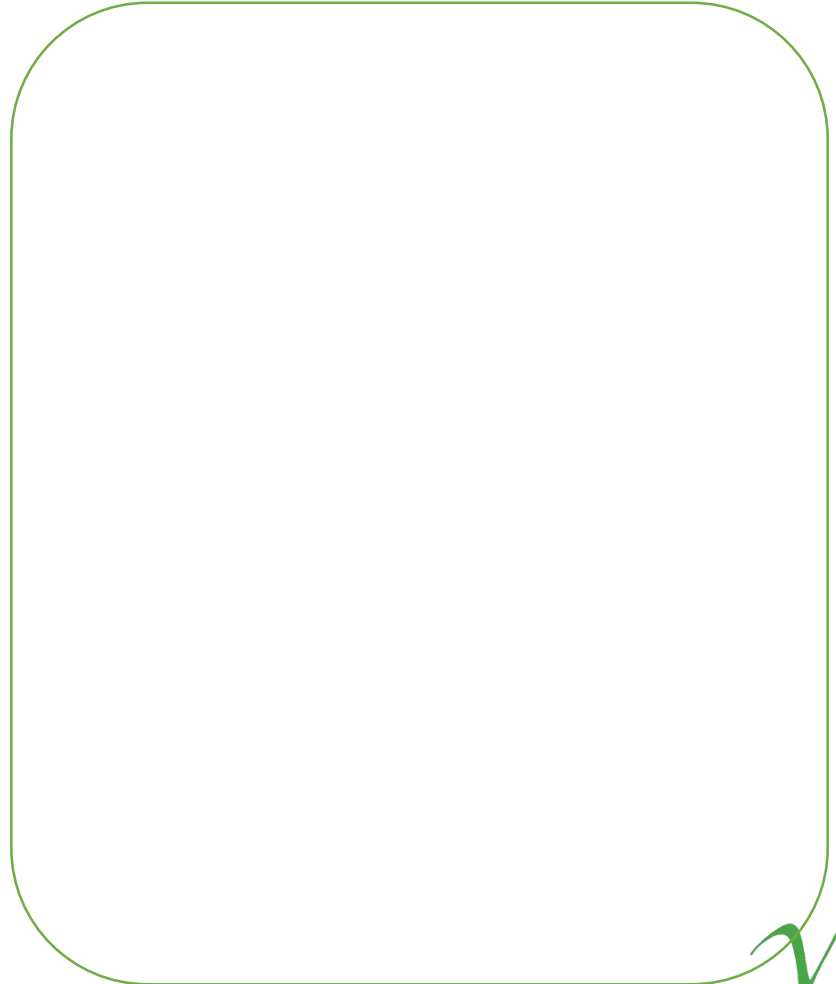
Wrap up

Questions?

Your conclusions about TPS



Your thoughts about that training session



Thank you!



This project (Validation of diagnostic tests to support plant health) has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N° 773139

