

# 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	<b>Preparation and dispatch of samples</b>	<b>Friday 5/03, 11am</b>
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**

# Preparation and dispatch of samples

5 March 2021

**Mathieu Rolland (ANSES)**

**Q&A session with the support of TPS organizers**

JP. Renvoisé (ANSES), C. Harrison (FERA), N. Mehle (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

- Preparation and dispatch of samples
  - Data from TPS organizers
  - Examples: TPS organized on *Bursaphelenchus xylophilus* (AM. Chappé - ANSES) and TPS organized on plum pox virus (on-site testing) (JP. Renvoisé - ANSES)
  - Interviews of TPS organizers
- Q&A session with TPS organizers:  
JP. Renvoisé (ANSES), C. Harrison (FERA), N. Mehle (NIB)

# 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*



*Fusarium circinatum*



citrus tristeza virus



*Bursaphelenchus*  
*xylophilus*



plum pox virus



# 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*



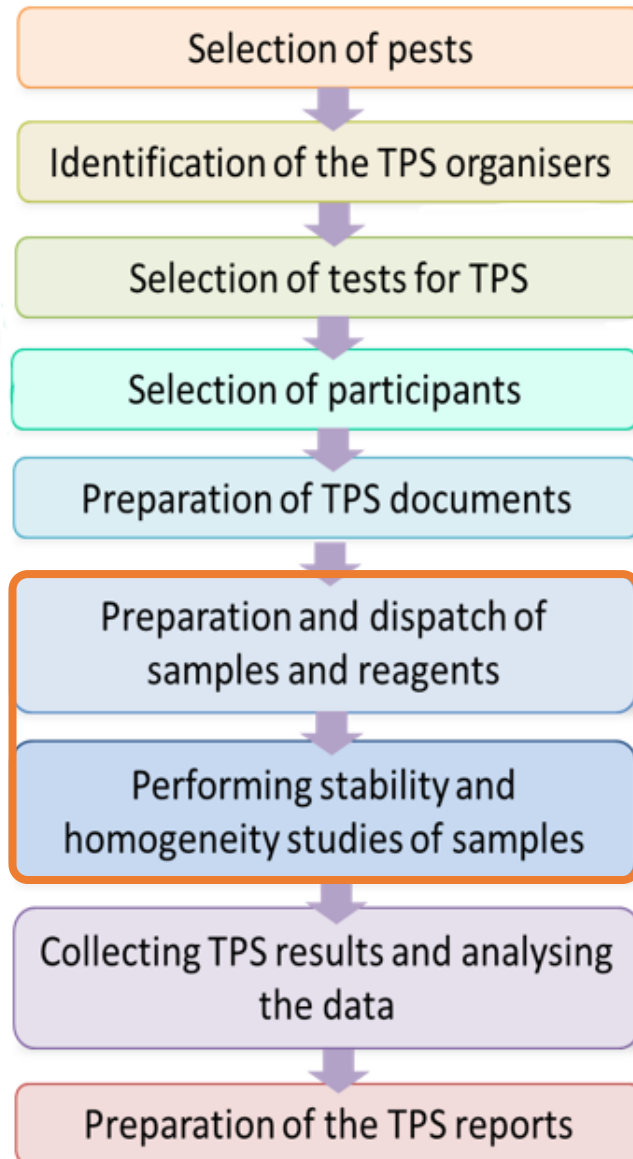
tomato spotted wilt  
tospovirus



*Cryphonectria parasitica*



# TPS organization workflow



**Webinar 4**

Preparation and  
dispatch of samples

Friday 5<sup>th</sup> of  
March, 11am

# Preparation and dispatch of samples

**Scope of the validation (methods, purpose, suitable for, matrix)**

**Design**

**Sample panel composition**

**Description of the different types of samples (positive/negative) and application**

**Production**

**Access to biological material**

**Multiplication of the biological material / spiking / preparation**

**Verification of the status / homogeneity / stability**

**Shipment**

**Coding**

**Regulatory requirements**

**Transport and administrative documents**

# Preparation and dispatch of samples

## Scope of the validation (methods, purpose, suitable for, matrix)

### Design

**Sample panel composition**

**Description of the different types of samples (positive/negative) and application**

### Production

**Access to biological material**

**Multiplication of the biological material / spiking / preparation**

**Verification of the status / homogeneity / stability**

### Shipment

**Coding**

**Regulatory requirements**

**Transport and administrative documents**



# Scope of the validation

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

## SAMPLE TYPE

- Plant extract (wood, leaves, seed, fruit)
- Infected/non-infected plant material
- Plant material with deactivated pathogen
- DNA and RNA extract

## MATRIX

- Leaves, fruits, wood, seed, shoots, stem material
- Fresh / Freeze-dried
- 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*

## MAX NUMBER OF PARTICIPANTS AND SAMPLES

# Preparation and dispatch of samples

**Scope of the validation (methods, purpose, suitable for, matrix)**

**Design**

**Sample panel composition**

**Description of the different types of samples (positive/negative) and application**

**Production**

**Access to biological material**

**Multiplication of the biological material / spiking / preparation**

**Verification of the status / homogeneity / stability**

**Shipment**

**Coding**

**Regulatory requirements**

**Transport and administrative documents**

# Question to the audience



**How many samples (including controls) would you include in a panel dedicated to a TPS?**

- < 10 samples
- from 10 to 20 samples
- from 20 to 30 samples
- from 30 to 40 samples

# Question to the audience



**According to you, what are the main parameters to define the type of samples to include? (Select maximum 2 answers)**

- **Number of participants**
- **Performance criteria to be assessed**
- **Time and budget constraints**
- **Availability of plant material**

# Sample panel composition

## ➤ Example - TPS Round 1 (VALITEST project)

### Diverse panel compositions

- From 8 to 22 samples
- Different number of dilution points or none
- Different number of replicates depending on the TPS scope

## DIFFICULTIES TO ANALYSE THE RESULTS, TEST PERFORMANCE RESULTS BIAS



### Need of panel composition recommendations for TPS Round 2

- Composition and number of samples
- Number of dilution points
- Number of replicates in a sample panel

# Sample panel composition

## ➤ Sample panel recommendations for TPS Round 2

WP2: Improvement of the validation process. Leader: Dr S. Massart (University of Liège)

Extract from the guidelines developed by WP2 (deliverable 2.1; in development)

Type of samples	Number of biological samples	Number of replicates	Dilution	Note
Negative	2	2	Not applicable	Negative samples should be independent from each other (i.e. not an aliquot of the same sample)
Positive	2	2	One positive sample has a medium concentration and the other one has a low concentration of the target pest (i.e. close to the limit of detection)	Positive samples should be independent from each other (i.e. not an aliquot of the same sample)
Serial dilution	1	the strongest concentration in duplicate and other dilutions in triplicates	Five dilution points with the highest dilution almost not detectable	Positive sample used for the dilution series should be independent from the other positive samples

→ 22 samples in total

# Sample panel composition

## ➤ Sample panel recommendations for TPS

WP2: Improvement of the validation process. Leader: Dr S. Massart (University of Liège)

### Standardized sample panel

→ Harmonization of validation studies

→ Easier and more robust comparison of the data

- Recommendations
- compromise between statistical requirements and practical constraints
  - no obligation, panel composition depends on the performance criteria to be assessed for the test
  - may not be feasible in some cases due to the number of reference material

# Interviews – TPS organisers



**“What was the composition of the panel of samples?”**



# Sample panel composition

- Examples of deviations from WP2 recommendations

WP2 recommendation			22	4 negative samples (2 in duplicate)	4 positive samples (2 in duplicate)	14 (5 dilutions) 1 in duplicate (strongest conc.) and 4 in triplicate
Pest	TPS organiser	Selected methods	Nb of samples	Nb of negative samples	Nb of positive samples (non-diluted)	Nb of positive samples (diluted)
Tomato spotted wilt tospovirus	NIB	conventional and real-time RT-PCR	22	8 (1 in triplicate and 5 independent)	0	14 (3 different isolates: one in 5 dilutions series, one in medium concentration, one in low concentration; all in duplicate)
		DAS-ELISA, on-site tests (LFD)	22	12 (1 in triplicate, 2*2, 6 independent)	0	10 (3 different isolates: one in 3 dilutions series, one in medium concentration, one in low concentration; all in duplicate)
Plum pox virus (on-site testing)	ANSES	LFD RPA, LFD	22	8 (4 in duplicate)	10 (5 in duplicate)	4 (2 dilutions): 2 in duplicate

## TSWV

Cross-reactions of some TSWV specific tests with other similar tospovirus species

**More exclusivity data needed**

→ other tospovirus isolates included in the panel

## PPV-on-site

On-site tests mostly dedicated to symptomatic samples

Diluted samples can not be proved to be similar to symptomatic samples

**Analytical sensitivity not specifically assessed**

**Focus on analytical specificity (inclusivity) and repeatability**

# Preparation and dispatch of samples

**Scope of the validation (methods, purpose, suitable for, matrix)**

**Design**

**Sample panel composition**

**Description of the different types of samples (positive/negative) and application**

**Production**

**Access to biological material**

**Multiplication of the biological material / spiking / preparation**

**Verification of the status / homogeneity / stability**

**Shipment**

**Coding**

**Regulatory requirements**

**Transport and administrative documents**

# Interviews – TPS organisers



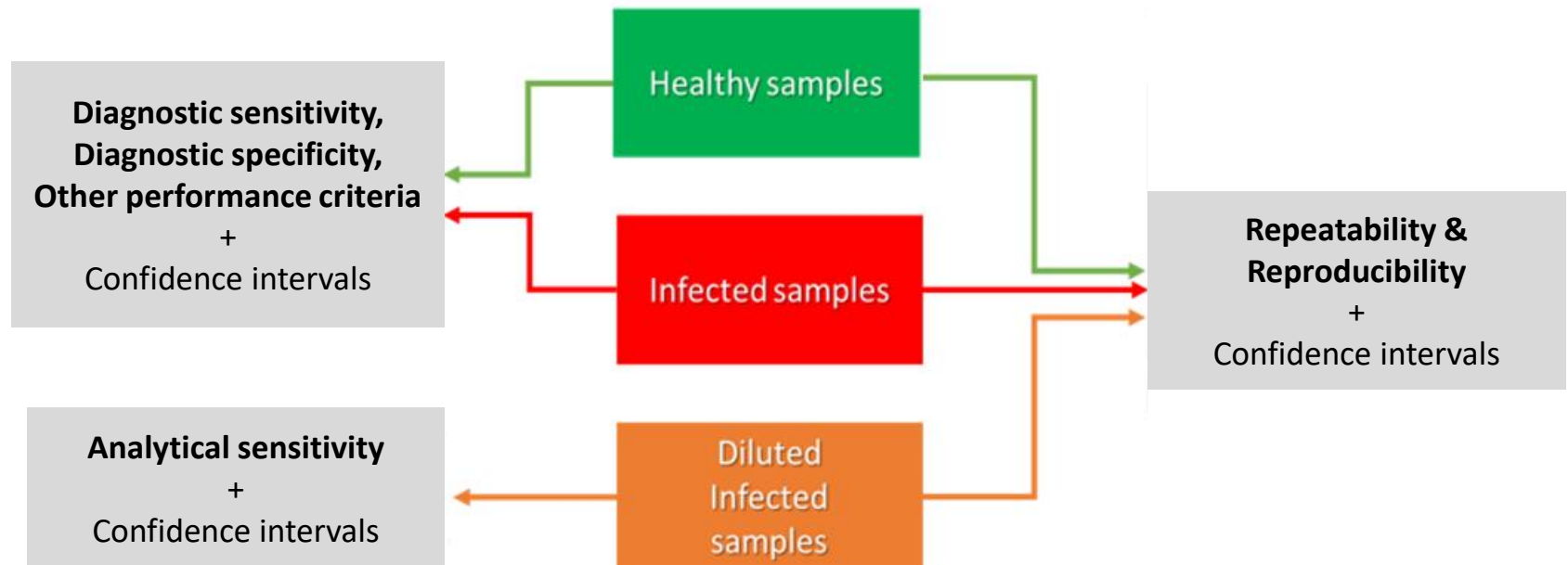
**“Which samples were used to evaluate which criteria?”**

# Description of the different types of samples and application

## ➤ Calculation of the performance criteria of the test:

### Use of the results of each type of sample

WP2: Improvement of the validation process. Leader: Dr S. Massart (University of Liège)



Extract from the guidelines developed by WP2 (deliverable 2.1; in development)

# Description of the different types of samples and application

➤ **Example:** TPS plum pox virus (on-site testing) (ANSES)

plum pox virus (on-site testing)	Criteria:
Healthy samples 4 matrices x 2	Diagnostic specificity, Selectivity, Repeatability
Infected samples 5 strains x 2	Diagnostic sensitivity, Inclusivity, Repeatability
Diluted infected samples 2 x 2	Analytical sensitivity, Repeatability
12 Data sets used for statistical analysis	Reproducibility

**Reference:** EPPO guidelines PM7/98 (4), EPPO Bulletin 2018, 48 (3): 387-404

# Preparation and dispatch of samples

**Scope of the validation (methods, purpose, suitable for, matrix)**

**Design**

**Sample panel composition**

**Description of the different types of samples (positive/negative) and application**

**Production**

**Access to biological material**

**Multiplication of the biological material / spiking / preparation**

**Verification of the status / homogeneity / stability**

**Shipment**

**Coding**

**Regulatory requirements**

**Transport and administrative documents**

# Interviews – TPS organisers



**“What was the availability of the biological material?  
What were the difficulties to get/collect the material?”**

# Access to biological material

Example of TPS Round 2 - plum pox virus on-site testing

## FRESH MATERIAL AVAILABILITY



→ Collect and test when the material is available

The best option for on-site tests / difficult in our case

→ Deseasonalize in dedicated facilities

→ Collect and store and check for potential matrix effect

Different storage solutions: freeze, freeze-dry, extract nucleic acids,...

## COLLECT THE RECOMMENDED MATRIX

→ Avoid potential matrix effects

## COLLECT WITH SYMPTOMS

→ For target and non-target pests

→ Collect in the best period

## USE ASYMPTOMATIC PLANT MATERIAL

→ « Healthy » plant material used for selectivity assessment

## USE DILUTIONS

→ Dilutions used for analytical sensitivity assessment



# Preparation and dispatch of samples

**Scope of the validation (methods, purpose, suitable for, matrix)**

**Design**

**Sample panel composition**

**Description of the different types of samples (positive/negative) and application**

**Production**

**Access to biological material**

**Multiplication of the biological material / spiking / preparation**

**Verification of the status / homogeneity / stability**

**Shipment**

**Coding**

**Regulatory requirements**

**Transport and administrative documents**

# Multiplication of the biological material / spiking / preparation

## PLANT MATERIAL

### → COLLECTED

- Seasonality
- Conservation (can be freeze-dried)



LSV La Réunion (ANSES) – citrus tristeza virus

### → PRODUCED

- Depends on the pest
- Some pests can not be cultured or with difficulty



LSV Rennes (ANSES) - Greenhouses for Maintenance of nematodes on plants – RKN nematodes

LSV Nancy (ANSES)  
*Phyllosticta citricarpa*



# Multiplication of the biological material / spiking / preparation

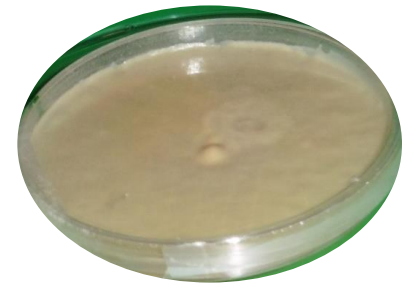
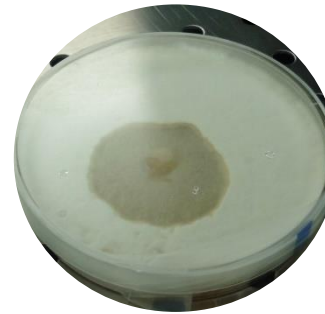
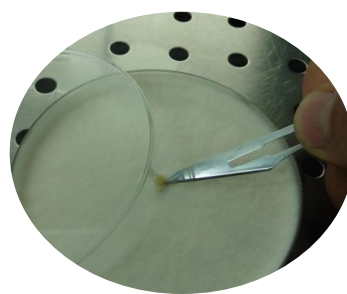
Example in nematology with *Bursaphelenchus xylophilus*

## MULTIPLICATION OF BIOLOGICAL MATERIAL

*Bursaphelenchus xylophilus*: a fungivorous species



LSV Rennes (ANSES)

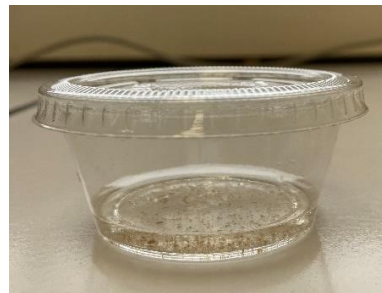


Mycelium of non sporulent  
*Botryotinia fuckeliana*  
Previous *Botrytis cinerea*

Incubation : 20° C

## SPIKING

wood extracts spiked with  
nematodes



LSV Rennes (ANSES)

# Preparation and dispatch of samples

**Scope of the validation (methods, purpose, suitable for, matrix)**

**Design**

**Sample panel composition**

**Description of the different types of samples (positive/negative) and application**

**Production**

**Access to biological material**

**Multiplication of the biological material / spiking / preparation**

**Verification of the status / homogeneity / stability**

**Shipment**

**Coding**

**Regulatory requirements**

**Transport and administrative documents**

# Check of the status / homogeneity / stability

## HOMOGENEITY / ASSIGNED VALUE

- Results should not be influenced by the preparation of samples
- Avoid an uneven distribution of the pest in the samples

→ Homogeneity is a crucial point to be met

### → Test samples for homogeneity

- Decisions:
- test used for homogeneity evaluation
  - nb of units tested per production lot
  - homogeneity decision rules

### → Determine the assigned value of samples

### → Choose homogeneous sample lots for panels constitution

## STABILITY

### → Store dedicated samples in the same conditions as for participants (TPS)

### → Set a short term deadline for analyzing samples

### → Test samples using the same test as for homogeneity

### → Choose stable lots for statistical analysis

# Preparation and dispatch of samples

**Scope of the validation (methods, purpose, suitable for, matrix)**

**Design**

**Sample panel composition**

**Description of the different types of samples (positive/negative) and application**

**Production**

**Access to biological material**

**Multiplication of the biological material / spiking / preparation**

**Verification of the status / homogeneity / stability**



**Webinar 5**

Production of reference material for TPS

Wednesday 10<sup>th</sup> of March, 2pm

# Preparation and dispatch of samples

**Scope of the validation (methods, purpose, suitable for, matrix)**

**Design**

**Sample panel composition**

**Description of the different types of samples (positive/negative) and application**

**Production**

**Access to biological material**

**Multiplication of the biological material / spiking / preparation**

**Verification of the status / homogeneity / stability**

**Shipment**

**Coding**

**Regulatory requirements**

**Transport and administrative documents**

# Coding

## ➤ Randomization of samples (per panel)

- To ensure a fully blind testing of samples
- Individual code of samples randomly chosen
- Different for each panel of samples and for each participant

## ➤ Example of TPS Round 2: plum pox virus on-site testing

**PPV2-P04-B**  
**18**

Sample N° 18 of panel N° P04 to be analysed with AgriStrip® test from Bioreba

**-TPS code:** PPV2

**-Panel code:** in the form “PXX” where XX is a two-digit number

**-Tests to be performed:**

“A” for the ImmunoStrip® and/or Amplify RP® Acceler8 test from Agdia

“B” for the AgriStrip® test from Bioreba

**-Sample code:** from 01 to 22



LSV Clermont-Ferrand (ANSES)



# Preparation and dispatch of samples

**Scope of the validation (methods, purpose, suitable for, matrix)**

**Design**

**Sample panel composition**

**Description of the different types of samples (positive/negative) and application**

**Preparation**

**Access to biological material**

**Production / multiplication of the biological material / spiking**

**Verification of the status / homogeneity / stability**

**Shipment**

**Coding**

**Regulatory requirements**

**Transport and administrative documents**

# Regulatory requirements

➤ Example of TPS – *Bursaphelenchus xylophilus*

## METHODS

## TYPE OF SAMPLES

Real-time PCR  
LAMP



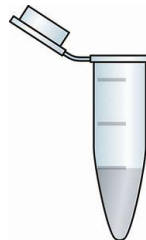
DNA extracts

Wood extracts spiked  
with nematodes  
→ Living material



**Required LoA**  
(Letter of Authority)  
**or import**  
**permit**

Conventional PCR



DNA extracts



**No requirement**

# Regulatory requirements

## Extract of Participant's contract (VALITEST project)

5) **Regulatory requirements** concerning the plant health regulatory of your country (territory) to allow the sending of the samples:

No requirements

Requirements<sup>(3)</sup> :  the parcel must be accompanied by a LOA<sup>(4)</sup>

other : precise : .....

(3) **IMPORTANT**, if requirements are specified and if the TPS organizer doesn't have the necessary documents before the date set for sending the samples, the legislation will not allow to send the parcel of samples and the participation of the laboratory will be compromised.

(4) Letter of Authority authorising the circulation of a regulated pest in the European Union.

*Note: for some countries, an import permit might be required*

**If required, regulatory documents should be received before sending the samples**

→ Legislation will not allow to send the parcel of samples

→ Participation in the TPS will be compromised

# Preparation and dispatch of samples

**Scope of the validation (methods, purpose, suitable for, matrix)**

**Design**

**Sample panel composition**

**Description of the different types of samples (positive/negative) and application**

**Production**

**Access to biological material**

**Multiplication of the biological material / spiking / preparation**

**Verification of the status / homogeneity / stability**

**Shipment**

**Coding**

**Regulatory requirements**

**Transport and administrative documents**

# Transport and administrative documents

## Example: TPS – *Bursaphelenchus xylophilus*

### Per panel for conventional PCR

- 10 DNA extracts (~100 µL of DNA in 0.5mL microtubes)

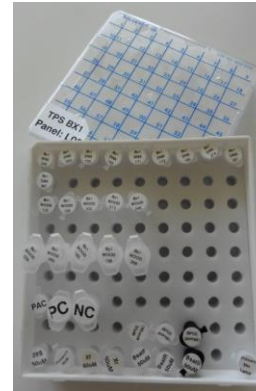
### Per panel for real-time PCR or LAMP

- 10 DNA extracts (~100 µL of DNA in 0.5mL microtubes)

- 5 DNA extracts (~50 µL of DNA in 0.5mL microtubes)

5 spiked wood extracts (1.4mL in 1.5mL microtubes)

+ primers, probes and controls



## Example: TPS – PPV on-site testing

### Per panel:

10 ground freeze-dried leaves (2mL tubes)

12 ground freeze-dried leaves (crushing bags)

+ control


### Specific consumables:

2 specific panels for tests requiring specific bags and buffers



# Transport and administrative documents

- TPS technical sheet describing the experimental protocols to be performed
- TPS instruction sheet describing the instructions to be followed at each step in the implementation of the TPS
- Acknowledgement of receipt of the samples
- TPS result form to record the TPS results


 **Test Performance Study (TPS)**  
Technical sheet

---

**Identification of the TPS**  
TPS code: Bx1

	Methods for <i>Bursaphelenchus xylophilus</i>		
	conventional PCR	real-time PCR	LAMP
Sample type	DNA	Wood extract, DNA	Wood extract, DNA
No. of samples			
Number of tests to be evaluated			

1) real-time PCR and 1

 **TPS Instruction sheet**

---


**Identification of the TPS**  
TPS code: Bx1

**1) Receipt of the parcel**

Check the content and conformity of the package and complete the acknowledgement of receipt.

The package will contain 1 panel consisting of 10 or 20 samples according to the evaluated methods, primers, probes and controls.



NA extracts (~100 µL of  
 consisting of 20 samples:  
 ctly for further analysis,  
 ctly for further analysis  
 traction before further

 **Acknowledgement of receipt of the panel of samples**

---

To be returned upon receiving the samples by e-mail to:  
 hanna.mouaziz@anses.fr  
 anne-marie.chappe@anses.fr

**Identification of the TPS**  
 Pest name: *Bursaphelenchus xylophilus*  
 TPS code: Bx1

  **TPS result form**

TPS code  Target organism

**Identification of the participating laboratory**

Name of the laboratory	
Panel code	

# Interviews – TPS organisers



**“Did you encounter any logistic or regulatory problem?”**

# LESSONS LEARNT

## MAIN DIFFICULTIES

- **Issues with seasonality of plant material**
  - collection of plant material in advance
  - storage in order to ensure stability
- **Difficulties to have enough available biological material**
  - host infected artificially
  - pure culture and extracted DNA
- **Difficulties to find effective homogenisation method**
- **Difficulties to get the LoA on time**

## IMPROVEMENT

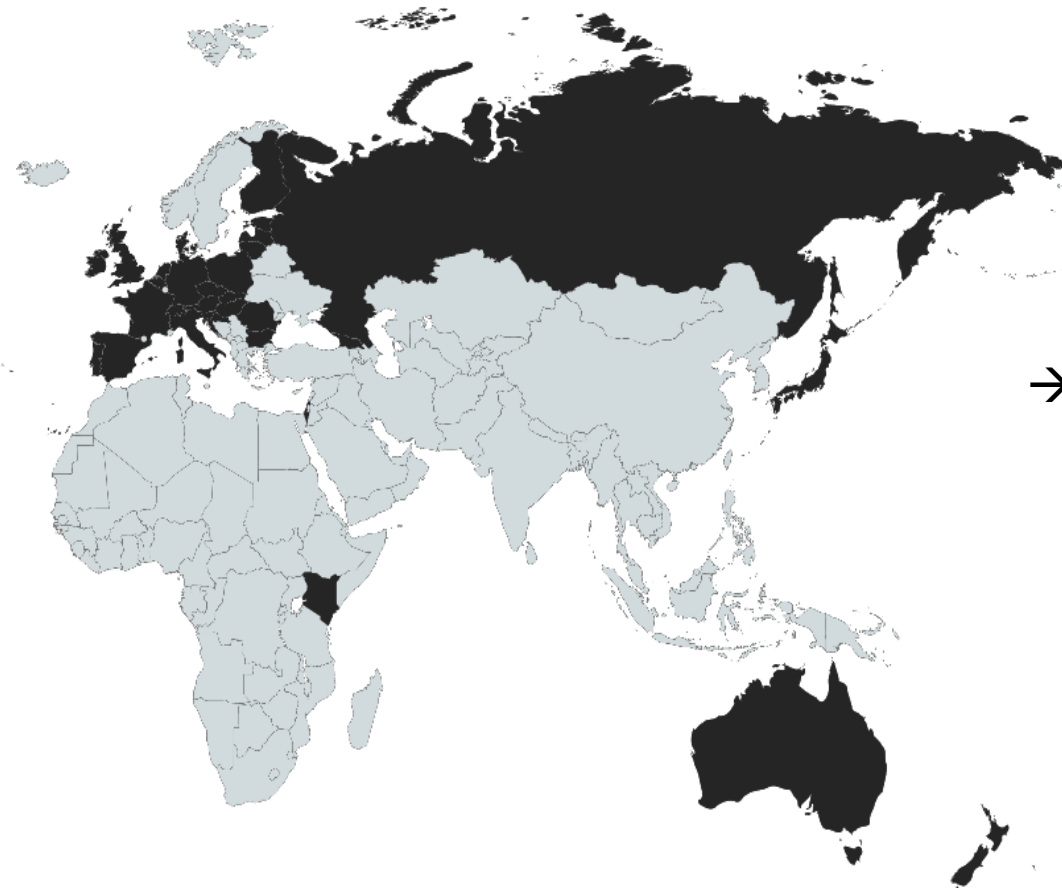
- **More time between sending the contract and sending the samples**
  - to order reagents
  - to get the LoA

TPS Round 1 (1 month) → TPS Round 2 (2 months)



# Conclusion – Dispatch of samples

## ➤ Representation of Dispatch of samples



→ Around 9700 samples prepared and dispatched worldwide

# Thank you for your attention!

## Any question?



The content of this presentation represents the views of the author only and is his/her sole responsibility; it cannot be considered to reflect the views of the European Commission and/or the Research Executive Agency or any other body of the European Union. The European Commission and the Agency do not accept any responsibility for use that may be made of the information it contains.



# 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	<b>Production of reference material for TPS</b>	<b>Wednesday 10/03, 2pm</b>
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