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**Grant agreement N. 773139**

**DELIVERABLE N°6.3**

**Title: Inventory of validation data available**



Validation of diagnostic tests to support plant health



Due date:	Month 4
Actual submission data	<p>Month 6</p> <p>The Description of Work (DOW) stated:  <i>'An inventory of validation data available will be performed and the data will be entered in the EPPO Database on diagnostic expertise (validation section) by EPPO. Additional validation data resulting from the project or available in plant pest diagnostic laboratories will be made publicly and freely available through the EPPO Database on diagnostic expertise (section validation data). All partners concerned will contribute to provide such data. The validation database will be maintained by EPPO after the project.'</i></p> <p>When the project was prepared in 2017 the plans were that the survey would be organised in the spring. The delayed start of the project did not allow this to happen. In addition, to avoid multiple surveys, the Steering Committee agreed that surveys to be organised by the different consortia would be done through a limited number of questionnaires. The present survey was consequently aimed at gathering information on the validation data available for the first test performance study (TPS).</p>
Start date of the project	01-05-2018
Deliverable lead contractor (organization name)	EPPO
Participants (Partners short names)	All partners
Author(s) in alphabetical order	Boutron N, McMullen M, Petter F.
Contact for queries	Madeleine McMullen
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Abstract:

Task 6.3: Improvement of the validation section of the free access EPPO Database on diagnostic expertise.

This report summarises the inventory of validation data performed for the 6 pests included in the first Test performance study (TPS). The validation reports collected will be entered in the EPPO Database on diagnostic expertise (validation section).

Partners involved: all partners

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## **Report - Inventory of validation data available for the 6 pests included in the first TPS.**

When the project was prepared in 2017 the plans were that the survey would be organised in the spring. The delayed start of the project did not allow this to happen. In addition, to avoid multiple surveys, the Steering Committee agreed that surveys to be organised by the different consortia would be done through a limited number of questionnaires. The present survey was consequently aimed at gathering information on the validation data available for the first test performance study (TPS).

The EPPO Secretariat circulated links to the survey on the 2018-07-27 with a deadline of the 2018-09-07 (survey length extended compared to the original plans to allow for the summer vacation).

The survey was sent to 66 laboratories in 31 countries. The laboratories were selected from the EPPO Database on Diagnostic Expertise and these laboratories included experts which had declared expertise on one or more of the 6 pests in the first TPS.

For the pests below Part I of the survey aimed to identify which diagnostic tests are used in the different laboratories (and to collect detailed information on the tests, on the tests themselves and the types of samples and matrices used) and to collect validation data in the form of reports on these tests.

- *Erwinia amylovora*,
- *Pantoea stewartii* subsp. *stewartii*,
- *Citrus tristeza virus*,
- *Plum pox virus*,
- *Fusarium circinatum* and
- *Bursaphelenchus xylophilus*.

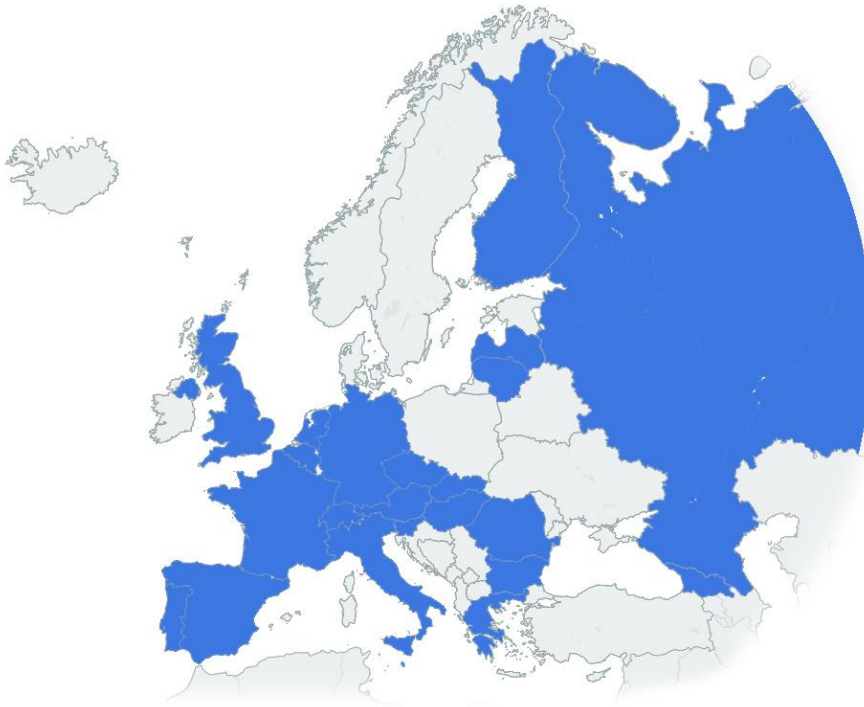
The pdf of the questions in the study are available in Appendix 1.

The full results of the study are available (in an anonymised form) in Appendix 2.

## Summary of the results of the survey

### Part I

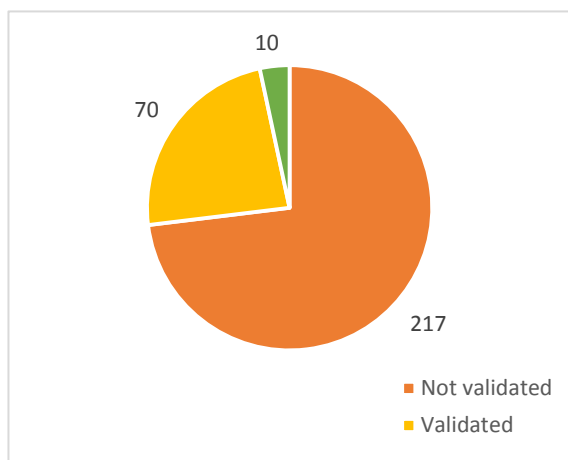
Data on tests were received from 23 countries (AT, BE, BG, CZ, FI, FR, GE, DE, GR, HU, IT, LV, LT, MT, NL, PT, RO, RU, SK, SI, ES, CH, GB) i.e. 74 % of countries contacted.



**Fig. 1.** Map of responding countries.

Forty-three different laboratories provided data, which corresponds to 65% of the laboratories contacted. This was considered a very good level of response.

297 descriptions of tests were provided by laboratories for the 6 pests as follows *Erwinia amylovora* (102), *Pantoea stewartii* subsp. *stewartii* (42), *Citrus tristeza virus* (29), *Plum pox virus*, (47), *Fusarium circinatum* (34) and *Bursaphelenchus xylophilus* (43). For the 297 tests described, 217 tests (73%) were described as not validated, 70 (24%) were described as validated and for 10 (3%) the respondent did not answer this question.



**Fig 2.** Validation status of all the tests described by respondents during Survey 1

Information was received on the following types of tests: plating (58), IF (11), ELISA (40), conventional PCR (60), real-time PCR (45), conventional RT-PCR (17), real-time RT-PCR (17), LAMP (2), other methods applicable for on-site testing (5), other methods applicable for use in the laboratory (42). Details about the tests were also collected.

### Collection of full validation reports

20 tests described were marked as having a validation report already uploaded in the diagnostics database and 17 tests described as having the report ready for upload (to date 10 of these tests had reports marked as ready for upload and were provided to the EPPO Secretariat via the online system, in fact 12 reports were collected as some tests had more than one report provided). Validation data for the reports not yet received will be requested.

The new validation data reports collected during the project are on the following pests/methods/tests:

Pest	Method	Test
<i>Bursaphelenchus xylophilus</i>	Real-time PCR	Real-time PCR SYBR green
<i>Bursaphelenchus xylophilus</i>	Other methods applicable in the laboratory	PCR-sequencing according to PM7/129 - 18S (Holterman <i>et al.</i> 2006 Molecular Biology and Evolution 23, 1792–1800) - 28S (Holterman <i>et al.</i> 2008 Molecular Phylogenetics and Evolution 48, 758–763)
<i>Erwinia amylovora</i>	Plating	NSA, CCT, King B
<i>Erwinia amylovora</i>	Conventional PCR	PCR according to Stoger <i>et al.</i> (2006)
<i>Erwinia amylovora</i>	Real-time PCR	Real-time PCR (Gottsberger, 2010)
<i>Fusarium circinatum</i>	Real-time PCR	<i>Fusarium cirinatum</i> , loos <i>et al</i> , 2009
<i>Pantoea stewartii</i> subsp. <i>stewartii</i>	Other methods applicable in the laboratory	FLASH PCR detection Kit (for fluorescence end-point detection) for detection and identification of <i>P. stewartii</i> subsp. <i>stewartii</i> from samples of plant and seeds
Plum pox virus	ELISA	DAS-ELISA
Plum pox virus	ELISA	DAS-ELISA
Plum pox virus	ELISA	DAS-ELISA

Of the 10 categories of methods described (i.e. plating, IF, ELISA, conventional PCR, real-time PCR, conventional RT-PCR, real-time RT-PCR, LAMP, other methods applicable for on-site testing, other methods applicable for use in the laboratory) new validation reports were collected for 4 different methods: ELISA, conventional PCR, real-time PCR and other methods applicable for use in the laboratory.

Methods for which new validation reports were collected	Number of new validation reports collected.
ELISA	3
Other methods applicable for use in the laboratory	2
Real-time PCR	3
Conventional PCR,	1
Plating	1

New validation reports were collected on tests on 5 of the 6 pests covered in the study. The new validation reports were collected from 6 countries.

Pests for which new validation reports were collected on the tests described	Number of new validation reports collected.
<i>Erwinia amylovora</i> ,	3
<i>Pantoea stewartii</i> subsp. <i>stewartii</i> ,	1
<i>Plum pox virus</i> ,	3
<i>Fusarium circinatum</i>	1
<i>Bursaphelenchus xylophilus</i>	2

Validation data will be collected for tests for other pests (not those included in the first TPS) during the second survey.



**VALITEST**  
**QUESTIONNAIRE**  
 WP1/WP4/WP6



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

Accurate and reliable detection and identification of plant pests are essential to avoid or reduce economical costs and trade disruptions and to support surveillance activities. Validation is essential to provide information on the performance of the tests. However, most of detection and identification tests are only validated on an intra-laboratory basis or through limited test performance studies (TPS), and there is a need to further harmonize practices. The VALITEST project aims at producing validation data and will include two rounds of TPS. In the first round, TPS will include the following pests: *Erwinia amylovora*, *Pantoea stewartii* subsp. *stewartii*, *Citrus tristeza virus*, *Plum pox virus*, *Fusarium circinatum* and *Bursaphelenchus xylophilus*.

One of the aims of the VALITEST project is to produce validation data by means of test performance studies (TPS) in two different rounds. In the first round, TPS will include the following pests: *Erwinia amylovora*, *Pantoea stewartii*, citrus tristeza virus, plum pox virus, *Fusarium circinatum* and *Bursaphelenchus xylophilus*. The TPS will include combinations of pest/test/matrix, prioritized based on the expertise of the project's consortium. The second round will include other pests and test combinations based on the needs expressed by various stakeholders. We are contacting you as experts in plant pest diagnostics for bacteriology, virology, nematology or mycology. We would like to know which diagnostic tests are used and which validation data already exist in the EPP region for the prioritized pests. We are kindly asking you to provide data on diagnostic tests and validations (Part I). In addition, we are asking you whether you are willing to participate in TPS study (Part II).

### Part I

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

- Erwinia amylovora*
- Pantoea stewartii* subsp. *stewartii*
- Citrus tristeza virus
- Plum pox virus
- Fusarium circinatum*
- Bursaphelenchus xylophilus*
- None of the above (go to part II)

For each of the chosen pest go to the following questions. First Q2 followed by Q3-Q4-Q5-Q6 and repeat this for each chosen pest.

	Next Go to Q2 (except if none of the above then go to II)
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Q2. Which of the following methods do you use for the diagnostic testing of [pest]:

(only the relevant method will appear in the online questionnaire)

- plating
- IF
- ELISA
- conventional PCR
- real-time PCR
- LAMP
- Other methods applicable for on-site testing
- Other method applicable for use in the laboratory

	Next Go to Q3
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XXXXXX is free text

XXXXXX is single choice (radio button)

Q3.

Q3.1 Pest xxxx Plating

Q 3.1a Plating media used (including the reference if available): free text one plating media at a time

Q 3.1b Test used for

Symptomatic samples

Asymptomatic samples

Both symptomatic and asymptomatic samples



Q3.1c Matrix/Matrices used

- Leaves
- Fruit
- Seeds
- Roots
- Herbaceous cuttings
- Woody cuttings
- Wood
- Tubers
- Pure culture
- Soil
- Water
- Other, specify :

Q3.1d Have you validated this [method] test for diagnostic testing of [pest]

Yes (if yes next question will appear)

No (select "add another plating media" or "next")

Q3.1e. Are you willing to share your validation report for the [method] test for diagnostic testing of [pest]. You may already have shared it through the EPPO database on diagnostic expertise. The validation report can be in your own languages.

Yes -> Already uploaded in the 'EPPO Database on diagnostic expertise'

Yes -> Please upload the report(s).

No (select "add another plating media" or "next")

Q3.2 Pest xxxx IF

Q 3.2a Name of kits/antibodies, supplier and catalogue no.: free text

Q 3.2b Test used for

Symptomatic samples

Asymptomatic samples

Both symptomatic and asymptomatic samples

Q3.2c Matrix/Matrices used

- Leaves
- Fruit
- Seeds
- Roots
- Herbaceous cuttings
- Woody cuttings
- Wood
- Tubers
- Pure culture
- Soil
- Water
- Other, specify :

Q3.2d Have you validated this [method] test for diagnostic testing of [pest]

Yes (if yes next question will appear)

No (select "add another kit/antibodies" or "next")

Q3.2e. Are you willing to share your validation report for the [method] test for diagnostic testing of [pest]. You may already have shared it through the EPPO database on diagnostic expertise. The validation report can be in your own languages.

Yes -> Already uploaded in the 'EPPO Database on diagnostic expertise'

Yes -> Please upload the report.

No (select "add another kit/antibodies" or "next")

Q3.3 Pest xxxx ELISA

Q 3.3a description of the test

Indirect ELISA

DAS-ELISA

DASI-ELISA (also called TAS ELISA),

PTA ELISA (direct and indirect)

Tissue print-ELISA or Direct tissue blot immunoassay

Other type of ELISA test: free text

Q 3.3b Name supplier and catalogue no. of kit/antibodies used: free text

Q 3.3c Test used for

Symptomatic samples

Asymptomatic samples

Both symptomatic and asymptomatic samples

Q3.3d Matrix/Matrices used

- Leaves
- Fruit
- Seeds
- Roots
- Herbaceous cuttings
- Woody cuttings
- Wood
- Tubers
- Pure culture
- Soil
- Water
- Other, specify :

Q3.3e Have you validated this [method] test for diagnostic testing of [pest]

Yes (if yes next question should appear)

No (select "add another kit/antibodies" or "next")

Q3.3f. Are you willing to share your validation report for the [method] test for diagnostic testing of [pest]. You may already have shared it through the EPPO database on diagnostic expertise. The validation report can be in your own languages.

Yes -> Already uploaded in the 'EPPO Database on diagnostic expertise'

Yes -> Please upload the report.

No (select "add another kit/antibodies" or "next")

Q3.4 Pest xxxx Conventional PCR

Q 3.4a Name of Conventional PCR test used (including the reference if available): free text one test at a time

Q 3.4b Name of Primer and Probes: free text

Q 3.4c Name of polymerase/kit, supplier and catalogue no.: free text

Q 3.4d Test used for

Symptomatic samples

Asymptomatic samples

Both symptomatic and asymptomatic samples

Q3.4e Matrix/Matrices used

- Leaves
- Fruit
- Seeds
- Roots
- Herbaceous cuttings
- Woody cuttings
- Wood
- Tubers
- Pure culture
- Soil
- Water
- Other, specify :

Q3.4f Have you validated this [method] test for diagnostic testing of [pest]

Yes (if yes next question will appear)

No (select "add another Conventional PCR" or "next")

Q3.4g. Are you willing to share your validation report for the [method] test for diagnostic testing of [pest]. You may already have shared it through the EPPO database on diagnostic expertise. The validation report can be in your own languages.

Yes -> Already uploaded in the 'EPPO Database on diagnostic expertise'

Yes -> Please upload the report.

No (select "add another Conventional PCR" or "next")

Q3.5 Pest xxxx Conventional RT PCR

Q 3.5a Name of Conventional RT PCR test used (including the reference if available): free text one test at a time

Q 3.5b Name of Primer and Probes: free text

Q 3.5c Name of polymerase/kit, supplier and catalogue no.: free text

Q 3.5d Test used for

Symptomatic samples

Asymptomatic samples

Both symptomatic and asymptomatic samples

Q3.1e Matrix/Matrices used

- Leaves
- Fruit
- Seeds
- Roots
- Herbaceous cuttings
- Woody cuttings
- Wood
- Tubers
- Pure culture
- Soil
- Water
- Other, specify :

Q3.5f Have you validated this [method] test for diagnostic testing of [pest]

Yes (if yes next question should appear)

No (select "add another Conventional RT PCR" or "next")

Q3.5g. Are you willing to share your validation report for the [method] test for diagnostic testing of [pest]. You may already have shared it through the EPPO database on diagnostic expertise. The validation report can be in your own languages.

Yes -> Already uploaded in the 'EPPO Database on diagnostic expertise'

Yes -> Please upload the report.

No (select "add another Conventional RT PCR" or "next")

Q3.6 Pest xxxx real-time PCR

Q 3.6a Name of real-time PCR test used (including the reference if available): free text one test at a time

Q 3.6b Name of Primer and Probes: free text

Q 3.6c Name of polymerase/kit, supplier and catalogue no.: free text

Q 3.6d Test used for

Symptomatic samples

Asymptomatic samples

Both symptomatic and asymptomatic samples

Q3.6e Matrix/Matrices used

- Leaves
- Fruit
- Seeds
- Roots
- Herbaceous cuttings
- Woody cuttings
- Wood
- Tubers
- Pure culture
- Soil
- Water
- Other, specify :

Q3.6f Have you validated this [method] test for diagnostic testing of [pest]

Yes (if yes next question should appear)

No (select "add another real-time PCR" or "next")

Q3.6g. Are you willing to share your validation report for the [method] test for diagnostic testing of [pest]. You may already have shared it through the EPPO database on diagnostic expertise. The validation report can be in your own languages.

Yes -> Already uploaded in the 'EPPO Database on diagnostic expertise'

Yes -> Please upload the report.

No (select "add another real-time PCR" or "next")

Q3.7 Pest xxxx real-time RT PCR

Q 3.7a Name of real-time RT PCR test used (including the reference if available): free text one test at a time

Q 3.7b Name of Primer and Probes: free text

Q 3.7c Name of polymerase/kit, supplier and catalogue no.: free text

Q 3.7d Test used for

Symptomatic samples

Asymptomatic samples

Both symptomatic and asymptomatic samples

Q3.7e Matrix/Matrices used

- Leaves

- Fruit
- Seeds
- Roots
- Herbaceous cuttings
- Woody cuttings
- Wood
- Tubers
- Pure culture
- Soil
- Water
- Other, specify :

Q3.7f Have you validated this [method] test for diagnostic testing of [pest]

Yes (if yes next question should appear)

No (select "add another real-time RT PCR" or "next")

Q3.7g. Are you willing to share your validation report for the [method] test for diagnostic testing of [pest]. You may already have shared it through the EPPO database on diagnostic expertise. The validation report can be in your own languages.

Yes -> Already uploaded in the 'EPPO Database on diagnostic expertise'

Yes -> Please upload the report.

No (select "add another real-time RT PCR" or "next")

Q3.8 Pest xxxx LAMP

Q 3.8a Name of LAMP test used (including the reference if available): free text one test at a time

Q 3.8b Name supplier and catalogue no. of polymerase/kit used: free text

Q 3.8c Test used for

Symptomatic samples

Asymptomatic samples

Both symptomatic and asymptomatic samples

Q3.8d Matrix/Matrices used

- Leaves
- Fruit
- Seeds
- Roots
- Herbaceous cuttings
- Woody cuttings
- Wood
- Tubers
- Pure culture
- Soil
- Water
- Other, specify :

Q3.8e Have you validated this [method] test for diagnostic testing of [pest]

Yes (if yes next question will appear)

No (select "add another LAMP test" or "next")

Q3.8f. Are you willing to share your validation report for the [method] test for diagnostic testing of [pest]. You may already have shared it through the EPPO database on diagnostic expertise. The validation report can be in your own languages.

Yes -> Already uploaded in the 'EPPO Database on diagnostic expertise'

Yes -> Please upload the report.

No (select "add another LAMP test" or "next")

Q3.9 Pest xxxx Other methods that can be used on-site

Q 3.9a Name of the test used (including the reference if available): free text one test at a time

Q 3.9b Name supplier and catalogue no. (put not applicable, if not relevant): free text

Q 3.9c Test used for

Symptomatic samples

Asymptomatic samples

Both symptomatic and asymptomatic samples

Q3.9d Matrix/Matrices used

- Leaves
- Fruit
- Seeds
- Roots
- Herbaceous cuttings
- Woody cuttings
- Wood

- Tubers
- Pure culture
- Soil
- Water
- Other, specify :

Q3.9e Have you validated this [method] test for diagnostic testing of [pest]

Yes (if yes next question should appear)

No (select "add an additional Other method that can be used on-site" or "next")

Q3.9f. Are you willing to share your validation report for the [method] test for diagnostic testing of [pest]. You may already have shared it through the EPPO database on diagnostic expertise. The validation report can be in your own languages.

Yes -> Already uploaded in the 'EPPO Database on diagnostic expertise'

Yes -> Please upload the report.

No (select "add an additional Other method that can be used on-site" or "next")

Q3.10 Pest xxxx Other methods that can be used in the laboratory

Q 3.10a Name of the test used (including the reference if available): free text one test at a time

Q 3.10b Name supplier and catalogue no. (put not applicable, if not relevant): free text

Q 3.10c Test used for

Symptomatic samples

Asymptomatic samples

Both symptomatic and asymptomatic samples

Q3.10d Matrix/Matrices used

- Leaves
- Fruit
- Seeds
- Roots
- Herbaceous cuttings
- Woody cuttings
- Wood
- Tubers
- Pure culture
- Soil
- Water
- Other, specify :

Q3.10e Have you validated this [method] test for diagnostic testing of [pest]

Yes (if yes next question should appear)

No (select "add an additional Other method that can be used in the laboratory" or "next")

Q3.10f. Are you willing to share your validation report for the [method] test for diagnostic testing of [pest]. You may already have shared it through the EPPO database on diagnostic expertise. The validation report can be in your own languages.

Yes -> Already uploaded in the 'EPPO Database on diagnostic expertise'

Yes -> Please upload the report.

No (select "add an additional Other method that can be used in the

## Part II

The following questions are included to make an inventory of possible participants in the first round of test performance studies which will be launched in the end of 2018. Samples are planned to be sent in March 2019 and data will be collected in June 2019. Studies will be performed on six pests using different diagnostic tests, which still need to be selected. As participating laboratory, you will receive a set of samples, protocols and the selected chemicals/reagents. However, all other costs, which occur in the course of the study, should be covered by your laboratory. The results will be reported in a confidential manner.

Q4. For which of the the following pest-method combinations are you or your institute potentially interested in participating in a test performance study?

Please fill in your contact information in case we need some extra information about the diagnostic tests and for the possible participation in any test performance study. Your contact details will only be used in the frame of the VALITEST project.

Name:

e-mail:





74	209	France	Bursaphelenchus	real-time PCR	François (Francois et al. 2008)	BSatF, BsatR et BsatS	LightCycler® 480 Probes Master kit	Both symptomatic and asymptomatic samples	Otherwood or wood products	Yes	Yes - repo
131	211	France	Plum pox virus	ELISA	DAS-ELISA		SRA - 31505, AGDIA	Both symptomatic and asymptomatic samples	Leaves, Herbaceous cuttings	Yes	Yes - repo
133	211	France	Plum pox virus	real-time RT-PCR	MA 043 - Olmos et al. 2007	P241 - P316D - P316 M - PPV-1	Multiscribe Reverse Transcriptase	Both symptomatic and asymptomatic samples	Leaves, Herbaceous cuttings	Yes	
129	211	France	Citrus tristeza virus	ELISA	DAS-ELISA		CT-SRA, SEDIAG	Both symptomatic and asymptomatic samples	Leaves	No	
58	212	France	Fusarium circinatum	plating	DCPA medium (according to EPPO PM 7/91(1): Gibberella circinata)			Both symptomatic and asymptomatic samples	Leaves, Roots, Herbaceous cuttings	Yes	Yes - repo
56	212	France	Fusarium circinatum	plating	DCPA medium (according to EPPO PM 7/91(1): Gibberella circinata)			Both symptomatic and asymptomatic samples	Roots, Herbaceous cuttings	Yes	Yes - repo
57	212	France	Fusarium circinatum	real-time PCR	real-time PCR (loos et al. 2007)	FCIR-F, FCIR-R, FCIR-P	qPCR Core kit No Rox (Eurogentec)	Asymptomatic samples	Seeds	Yes	Yes - repo
59	212	France	Fusarium circinatum	Other methods	Sequence analysis of several phylogenetic markers (EF1alpha, RPB2)			Both symptomatic and asymptomatic samples	Pure culture	No	
99	214	Malta	Fusarium circinatum	plating	Potato Dextrose agar & Water agar			Both symptomatic and asymptomatic samples	Woody cuttings, Wood	No	
97	214	Malta	Citrus tristeza virus	ELISA	Tissue print-ELISA or Direct tissue blot immunoassay		Plantprint N/A	Both symptomatic and asymptomatic samples	Herbaceous cuttings, Wood	No	
98	214	Malta	Plum pox virus	ELISA	DAS-ELISA		LOEWE 07186C/480	Asymptomatic samples	Leaves	No	
295	216	Slovenia	Pantoea stevensii	IF			Linaris	Symptomatic samples	Pure culture	No	
291	216	Slovenia	Erwinia amylovora	plating	King's B, CCT			Both symptomatic and asymptomatic samples	Leaves, Fruit, Woody cuttings	Yes	Yes - repo
292	216	Slovenia	Erwinia amylovora	real-time PCR	Real-time PCR described by (Pirc, 2009)	ITS, AmsC (Pirc, 2009)		Both symptomatic and asymptomatic samples	Leaves, Fruit, Woody cuttings	Yes	Yes - repo
293	216	Slovenia	Erwinia amylovora	Other methods	Agglutination, pathogenicity test, enrichment		Applied Biosystems mastermix not relevant	Both symptomatic and asymptomatic samples	Leaves, Fruit, Woody cuttings	Yes	No
294	216	Slovenia	Pantoea stevensii	plating	General media			Symptomatic samples	Leaves	No	
296	216	Slovenia	Pantoea stevensii	conventional PCR	AGES - 16S rRNA (EPP0)	AGES - 16S rRNA	/	Symptomatic samples	Leaves, Seeds, Pure culture	Yes	No
297	216	Slovenia	Pantoea stevensii	real-time PCR	Tambong, 2008 - cpsD	Tambong, 2008 - cpsD	Applied Biosystems mastermix	Both symptomatic and asymptomatic samples	Leaves, Seeds, Pure culture	Yes	Yes - repo
298	216	Slovenia	Pantoea stevensii	Other methods	MALDI-TOF			Symptomatic samples	Pure culture	No	No
128	218	Netherlands	Bursaphelenchus	Other methods	PCR-sequencing according to PM7/129 - 18S (Holterman et al. 2008)		Phusion® High-Fidelity DNA Polymerase	Both symptomatic and asymptomatic samples	Othersingle fished nematode	Yes	Yes - repo
263	218	Netherlands	Erwinia amylovora	IF			Serum IPO 7043-7072 (IPO is now no application)	Both symptomatic and asymptomatic samples	Pure culture	No	
189	218	Netherlands	Erwinia amylovora	Other methods	Pathogenicity test on young fruits of Pyrus			Both symptomatic and asymptomatic samples	Pure culture	No	
262	218	Netherlands	Erwinia amylovora	real-time PCR	Real-time PCR described by (Ams116F, Ams189R, Ams141T)		TaqMan Universal PCR Master Mix II	Both symptomatic and asymptomatic samples	Pure culture	No	
127	218	Netherlands	Bursaphelenchus	real-time PCR	Real-time PCR SYBR green		Bursaphelenchus xylophilus specific	Both symptomatic and asymptomatic samples	Other1-10 fished nematode	Yes	Yes - repo
264	218	Netherlands	Fusarium circinatum	plating	DCPA (Seeds, asymptomatic); KA, SNA, WA, PDA, DCPA (Wood, symptomatic); SNA, PDA (Purification)		V-PPV-CO and V-PPV-AP, Prime d	Symptomatic samples	Leaves	No	No
260	218	Netherlands	Plum pox virus	ELISA	DAS-ELISA			Symptomatic samples	Leaves	No	
187	218	Netherlands	Erwinia amylovora	plating	King's B, Levan, CCT (only for asymptomatic samples, which are very rare)			Symptomatic samples	Fruit, Herbaceous cuttings	No	
261	218	Netherlands	Citrus tristeza virus	ELISA	DAS-ELISA		Citrus tristeza virus IgG/Citrus tristeza virus	Symptomatic samples	Leaves, Fruit	No	
322	221	Lithuania	Plum pox virus	ELISA	DAS-ELISA		Plum pox virus Reagent set, BIORAD	Both symptomatic and asymptomatic samples	Leaves, Fruit, Woody cuttings	No	
321	221	Lithuania	Erwinia amylovora	Other methods	pathogenicity, hypersensitivity, EPPO PM 7/20 (2)			Both symptomatic and asymptomatic samples	Leaves, Fruit, Woody cuttings	No	
325	221	Lithuania	Fusarium circinatum	real-time PCR	Real-time PCR (loos et al., 2007)	FCIR-F, FCIR-R, FCIR-P	TaqMan Universal Master Mix II	Both symptomatic and asymptomatic samples	Pure culture	No	
324	221	Lithuania	Fusarium circinatum	plating	DCPA, PDA, SNA, EPPO PM7/91(1)			Both symptomatic and asymptomatic samples	Seeds, Roots, Woody cuttings	Yes	No
326	221	Lithuania	Bursaphelenchus	Other methods	Baermann, morphological-morphometric tests, EPPO PM 7/20 (2)			Symptomatic samples	Wood, WPM	No	
318	221	Lithuania	Erwinia amylovora	plating	CCT, Levan, King's B			Both symptomatic and asymptomatic samples	Leaves, Fruit, Woody cuttings	No	
319	221	Lithuania	Erwinia amylovora	IF			IVIA (Spain) monoclonal	Symptomatic samples	Leaves, Fruit, Woody cuttings	No	
348	221	Lithuania	Erwinia amylovora	real-time PCR	Real-time PCR (Pirc M, Ravitsky A, Ams116F, Ams189R, Ams141T)		TaqMan Universal Master Mix II	Both symptomatic and asymptomatic samples	Leaves, Fruit, Woody cuttings	No	
320	221	Lithuania	Erwinia amylovora	real-time PCR	Real-time PCR (Gottsberger et al. 2007)	hpEaF, hpEaR, hpEaP	TaqMan Universal Master Mix II	Both symptomatic and asymptomatic samples	Leaves, Fruit, Woody cuttings	No	
327	221	Lithuania	Bursaphelenchus	Other methods	PCR-RFLP, Burgermeister W, Braasch H, Metge K, Gu J, Seifried M		Primer set: forward (Ferris et al., 2006)	Symptomatic samples	Other nematodes	No	
323	221	Lithuania	Plum pox virus	conventional RT-PCR	Conventional RT PCR (Levy, 2004)	3'NCR sense, 3'NCR antisense	Taq DNA polymerase (recombinant)	Both symptomatic and asymptomatic samples	Leaves, Fruit, Woody cuttings	No	
376	222	Germany	Fusarium circinatum	real-time PCR	Schweigkofler et al., 2004	Circ1A, Circ4A	qPCR Mastermix SybrGreen (Applied Biosystems)	Both symptomatic and asymptomatic samples	Leaves, Seeds	No	
373	222	Germany	Erwinia amylovora	conventional PCR	Beresswill et al. (1992)	A: 5'-CGG TTT TTA ACG CTC GTG G	Promega GoTaq G2	Both symptomatic and asymptomatic samples	Leaves, Woody cuttings, Pure culture	No	
375	222	Germany	Pantoea stevensii	real-time PCR	Tambong et al., 2007	cpRT74F, cps177R, cps133 (p)	InnuMix qPCR Mastermix (Analytik Jena)	Asymptomatic samples	Seeds	Yes	
374	222	Germany	Pantoea stevensii	IF			Erwinia stewartii monoclonal (Linaris)	Asymptomatic samples	Seeds	Yes	
134	222	Germany	Erwinia amylovora	plating	NSA			Symptomatic samples	Leaves, Fruit, Pure culture	No	
177	224	Germany	Plum pox virus	ELISA	DAS-ELISA		IgG 150512, conj 150522, BIOREBE	Both symptomatic and asymptomatic samples	Leaves, Fruit	Yes	Yes - repo
135	224	Germany	Erwinia amylovora	conventional PCR	Stoger et al., 2006	PEANT1 / PEANT 2		Asymptomatic samples	Leaves, Fruit, Woody cuttings	No	
179	224	Germany	Plum pox virus	conventional RT-PCR	Wetzal et al, 1991	P1 / P2	One Step Kit, Qiagen 210212	Both symptomatic and asymptomatic samples	Leaves, Fruit	No	
176	224	Germany	Erwinia amylovora	conventional PCR	Stoger et al., 2006	PEANT 1 / PEANT 2	Extract-N-Amp Plant PCR kit, Sigma	Both symptomatic and asymptomatic samples	Leaves, Fruit, Woody cuttings	No	
125	225	United Kingdom	Erwinia amylovora	real-time PCR	Real-time PCR (Gottsberger et al. 2007)	hpEaF, hpEaR, hpEaP	JUMPSTART TAQ READYMIX FOR PCR	Both symptomatic and asymptomatic samples	Herbaceous cuttings, Wood	No	
123	225	United Kingdom	Erwinia amylovora	plating	Levan Medium, King's B medium (King et al., 1954).		PM 7/20 (2)* Erwinia amylovora	Both symptomatic and asymptomatic samples	Herbaceous cuttings, Wood	No	
124	225	United Kingdom	Erwinia amylovora	conventional PCR	Nested PCR (Llop et al., 2007)	Llop - PEANT1, PEANT2, AJ75	JUMPSTART(TM) REDTAQ(R) REAL TIME PCR	Both symptomatic and asymptomatic samples	Herbaceous cuttings, Wood	No	
242	226	Germany	Bursaphelenchus	Other methods	microscopy			Symptomatic samples	Wood	No	
48	228	Czechia	Bursaphelenchus	Other methods	Baermann funnel			Both symptomatic and asymptomatic samples	Wood	No	
204	228	Czechia	Pantoea stevensii	conventional PCR	Coplin and Majerczak 2002	ES16, ESIG2c	Biotools DNA polymerase	Both symptomatic and asymptomatic samples	Pure culture	Yes	Yes - repo
199	228	Czechia	Erwinia amylovora	conventional PCR	Beresswill et al 1992	A, B	DNA polymerase, Biotools	Both symptomatic and asymptomatic samples	Leaves, Fruit, Herbaceous cuttings	Yes	Yes - repo
347	228	Czechia	Plum pox virus	Other methods	Immunocapture PCR method (Wetzal et al. 1992)		Promega reverse transcriptase, Biorad	Both symptomatic and asymptomatic samples	Leaves, Fruit	No	
354	228	Czechia	Fusarium circinatum	plating	PDA			Symptomatic samples	Seeds, Roots, Woody cuttings	No	
355	228	Czechia	Fusarium circinatum	conventional PCR	Conventional PCR (W. Schwabe et al. 2007)	CIRC1A, CIRC4A	Biotools, TH polymerase	Symptomatic samples	Pure culture	No	
198	228	Czechia	Erwinia amylovora	plating	NA, KING, CCT			Both symptomatic and asymptomatic samples	Leaves, Herbaceous cuttings	No	
346	228	Czechia	Plum pox virus	ELISA	DAS-ELISA		Bioreba	Both symptomatic and asymptomatic samples	Leaves, Fruit	Yes	Yes - repo
202	228	Czechia	Pantoea stevensii	plating	NA, KING			Both symptomatic and asymptomatic samples	Leaves, Seeds, Herbaceous cuttings	No	
200	228	Czechia	Erwinia amylovora	Other methods	Pathogenicity test			Both symptomatic and asymptomatic samples	Pure culture	No	
219	231	Switzerland	Bursaphelenchus	real-time PCR	Real-time PCR for direct detection of BsatF, BsatRV, BsatS, 18S units		qPCR core kit NO ROX Hotgoldstar	Both symptomatic and asymptomatic samples	Woody cuttings, Wood	No	
367	232	Italy	Plum pox virus	ELISA	DASI-ELISA (also called TAS ELISA)		PPV Universal ELISA; capture poly	Both symptomatic and asymptomatic samples	Leaves, Fruit, Herbaceous cuttings	Yes	No
372	232	Italy	Erwinia amylovora	real-time PCR	Real-time PCR Taqman (Salvi et al. 2007)	P291F, P291R, P291M	SsoAdvanced™ Universal Probes	Both symptomatic and asymptomatic samples	Leaves, Fruit, Herbaceous cuttings	No	
371	232	Italy	Erwinia amylovora	ELISA	DAS-ELISA		Kit Erwinia amylovora; LOEWE®;	Both symptomatic and asymptomatic samples	Leaves, Fruit, Herbaceous cuttings	No	
369	232	Italy	Plum pox virus	real-time RT-PCR	Real-time RT-PCR Taqman	P241, P316M, P316D, TaqMan	SsoAdvanced™ Universal Probes	Both symptomatic and asymptomatic samples	Leaves, Fruit, Herbaceous cuttings	No	
368	232	Italy	Plum pox virus	conventional RT-PCR	Saggio RT-PCR (Wetzal et al. 1991 and 2007)	P1 and P2	GoTaq® DNA Polymerase; PROMEGA	Both symptomatic and asymptomatic samples	Leaves, Fruit, Herbaceous cuttings	No	
359	232	Italy	Citrus tristeza virus	ELISA	DAS-ELISA		CTV Tristeza ELISA, capture poly	Both symptomatic and asymptomatic samples	Leaves, Herbaceous cuttings	Yes	No
363	232	Italy	Citrus tristeza virus	conventional RT-PCR	Amplification by RT-PCR (OPIN1-PIN2 primers)		GoTaq® DNA Polymerase; PROMEGA	Both symptomatic and asymptomatic samples	Leaves, Herbaceous cuttings	Yes	
370	232	Italy	Erwinia amylovora	plating	NSA and CCT medium (Bulletin OEPP/EPPO Bulletin (2013) 43 (1), 21-45)			Both symptomatic and asymptomatic samples	Leaves, Fruit, Herbaceous cuttings	No	
366	232	Italy	Citrus tristeza virus	real-time RT-PCR	Real-time reverse transcription PCR	P25F (forward); P25R (reverse)	SsoAdvanced™ Universal Probes	Asymptomatic samples	Leaves, Herbaceous cuttings	No	