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Title: Report on improved version of the validation section of the EPPO Database on diagnostic expertise, including new data provided by laboratories



Validation of diagnostic tests to support plant health



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Abstract:

The EPPO Database on Diagnostic expertise (<http://dc.epo.int/index.php>) includes a specific section on validation data for diagnostic tests (<http://dc.epo.int/validationlist.php>). Laboratories can deposit online validation data that they have generated on specific tests and these can be made visible to all users of the database. The validation data can then be retrieved from the database in the form of a PDF file including the description of the test evaluated (pest x matrix x method) and the performance data related to the test (the information is presented in a harmonized format). There is currently no possibility to sort the data except by pest and method.

One of the objectives of VALITEST is to improve the searching capacity of the database to ensure optimal use. This report presents the new features of the validation section that have been identified from the survey and from discussions with users and with VALITEST partners. IT developments are ongoing. The new version of the EPPO Database on diagnostic expertise will be launched in April 2020.

Partners involved: all partners

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TERMS, ABBREVIATIONS AND DEFINITIONS

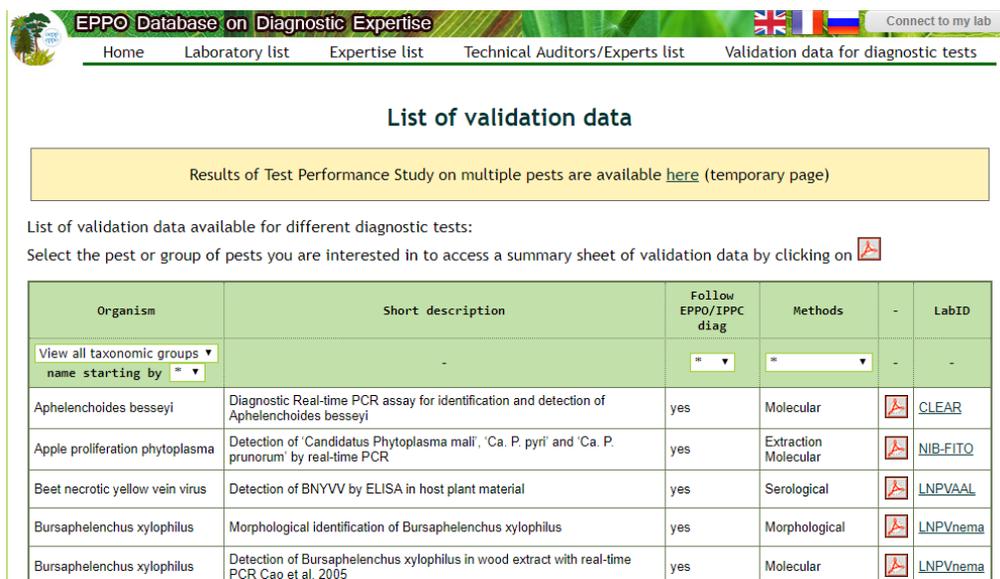
DP: Diagnostic protocol

EPPO: European and Mediterranean Plant Protection Organization

PCR: Polymerase chain reaction

1 Purpose

The EPPO Database on Diagnostic expertise (<http://dc.eppo.int/index.php>) includes a specific section on validation data for diagnostic tests (<http://dc.eppo.int/validationlist.php>). Laboratories can deposit online validation data that they have generated on specific tests and these can be made visible to all users of the database. The validation data can then be retrieved from the database in the form of a PDF file including the description of the test evaluated (pest x matrix x method) and the performance data related to the test (the information is presented in a harmonized format). There is currently no possibility to sort the data except by organism, whether the test follows EPPO/IPPC protocols and method (see screenshot below).



EPPO Database on Diagnostic Expertise

Home Laboratory list Expertise list Technical Auditors/Experts list Validation data for diagnostic tests

List of validation data

Results of Test Performance Study on multiple pests are available [here](#) (temporary page)

List of validation data available for different diagnostic tests:
Select the pest or group of pests you are interested in to access a summary sheet of validation data by clicking on 

Organism	Short description	Follow EPPO/IPPC diag	Methods	-	LabID
View all taxonomic groups name starting by <input type="text"/>	-	<input type="text"/>	<input type="text"/>	-	-
Aphelenchoides besseyi	Diagnostic Real-time PCR assay for identification and detection of Aphelenchoides besseyi	yes	Molecular		CLEAR
Apple proliferation phytoplasma	Detection of 'Candidatus Phytoplasma mali', 'Ca. P. pyri' and 'Ca. P. prunorum' by real-time PCR	yes	Extraction Molecular		NIB-FITO
Beet necrotic yellow vein virus	Detection of BNYVV by ELISA in host plant material	yes	Serological		LNPVAAL
Bursaphelenchus xylophilus	Morphological identification of Bursaphelenchus xylophilus	yes	Morphological		LNPVnema
Bursaphelenchus xylophilus	Detection of Bursaphelenchus xylophilus in wood extract with real-time PCR Cao et al. 2005	yes	Molecular		LNPVnema

One of the objectives of VALITEST is to improve the searching capacity of the database to ensure optimal use. In order to evaluate the needs of users a survey was organized. The survey and its results are presented in deliverable 6.2. In this deliverable, we summarize the new features of the validation section that have been identified in this survey and during discussions with the users at EPPO meetings and with the partners (see section 3). These new features are currently used to redesign the new validation section of the EPPO Database on Diagnostic expertise. We also present the strategy that has been decided to transfer the data from the old database to the new redesigned one (see section 4). Finally, the improvement of the validation database is done through the enrichment of the database with new validation data. New data provided by laboratories or obtained within the framework of WP1 have or will be entered in the validation section of the EPPO Database on diagnostic expertise (see section 5).

2 Scope

The new features of the validation section identified in this deliverable are used for IT developments. The new version of the EPPO Database on diagnostic expertise will be available from the 30th of April 2020.

3 New features identified for the improvement of the validation section of the EPPO Database of Diagnostic expertise

3.1 Features identified from the survey

Some improvements of the validation section of the database have been identified from the survey on the needs of users of the EPPO database (**Deliverable 6.2**). In particular, the validation section of the database should:

- Allow searches to be made using key words. The most important identified descriptors that need to be searchable are pest, method, plant species, test, matrix, EPPO-IPPC test.
- Allow combined and flexible queries to be made and, in particular, multiple pest queries
- Allow sorting of information within different methods. Suggestions have been made by users to further divide these molecular and serological methods.
- Allow searches of tests used for detection, identification or both.
- Allow searches to be made on kits (request from companies which are WP7 partners).

All those features will be implemented in the new database.

It is worth noting that performance criteria were also identified as interesting descriptors to search the database. Besides other interesting suggestions were provided by the users such as: detection/identification, name of the test and references.

3.2 Features identified during meetings with users

The need for harmonisation of validation data and the work on the improvement of the validation section of the EPPO database on Diagnostic expertise was discussed in the following meetings:

- EPPO Panel on Diagnostics in Mycology (2018-10)
- EPPO Panel on Diagnostics and Quality Assurance (2019-02)
- EPPO Panel on Diagnostics in Virology and Phytoplasmology (2019-05)
- EPPO Panel on Diagnostics in Nematology (2019-11).

Overall, the experts agreed that more standardization in the way to present validation results would be needed to better compare the validation data provided in the database. For example, it would be helpful if the units used to express performance criteria were harmonized. However, such harmonization seems difficult as the way to express performance criteria may depend on the disciplines as well as on the type of reference material available. Finally, concern was expressed regarding the fact that making the database searchable on multiple performance characteristics would require more fields to be filled when entering data and so more work for the laboratories sharing their data.

3.3 Features identified during meetings with partners

The results of the survey on the needs of users of the EPPO database and the plans for improvements were presented to the partners at the 4th and 5th steering committee and at the mid-term conference.

As users, partners mentioned that it is interesting to have a searchable database but time effort to enter the data should stay reasonable.

It was agreed during the mid-term conference to add:

- a general disclaimer that the results that are provided in the database only correspond to a situation at a certain time and under specific conditions
- a specific field so that laboratories entering data can specify whether kits were used according to manufacturer's instruction or not
- examples on the way EPPO would like laboratories to enter their data

- the dates of submission and modification of the reports.

All those features will be implemented in the new database.

4 Improvements of the validation section of the EPPO Database of Diagnostic expertise

The main objective of the improvement of the database is to facilitate retrieval of information. Individual validation data will continue to be retrievable as PDF files but retrieval of sets of data (e.g. multiple tests for one pest or multiple pests) will also be possible via downloads of Excel files.

4.1 Redesign of the EPPO Database on Diagnostic Expertise

As a prerequisite to the improvement of its section on validation, the EPPO Database of Diagnostic expertise is being transferred to a new redesigned and more user-friendly database. The visual layout of this redesigned database is similar to the one used for the VALITEST website in order to ensure the visual continuity with the project (see annex I). This transfer will allow the improvements of all the sections of the website. In particular, the data on laboratories and expertise will be more easily searchable (see annex AI.2 and AI.3).

4.2 IT developments required to include the new features identified in part 3

Table 1 summarise the IT developments that are being made to the validation section of the EPPO Database of Diagnostic expertise to implement the new features mentioned in section 3.

Table 1: Ongoing IT developments

Features	IT developments	Screenshot
<ul style="list-style-type: none"> • Allow searches to be made using key words. The most important identified descriptors that need to be searchable are pest, method, plant species, test, matrix, EPPO-IPPC test. • Allow combined and flexible queries to be made and, in particular, multiple pest queries 	<ul style="list-style-type: none"> • The data will be displayed in the form of a table, one line corresponding to one validation report. Columns will correspond to the most important descriptors identified by the users. Search using key words will be possible for each of these descriptors. It will be possible to search several descriptors at the same time. Individual validation data will continue to be retrievable as PDF files. • Filtered validation data will be downloadable as an excel file to allow further search to be made based on other descriptors (i.e. performance criteria, details regarding the methods ...) 	See annex AI.4
<ul style="list-style-type: none"> • Allow searches of tests used for detection, identification or both. 	<ul style="list-style-type: none"> • A specific field will be added in the form to specify if the aim of the test is detection, identification of both. 	See annex AII.1
<ul style="list-style-type: none"> • Allow sorting of information within different methods. Suggestions have been made by users to further divide these molecular and serological methods. 	<ul style="list-style-type: none"> • Molecular and serological methods will be subdivided further i.e. molecular method will be further divided in: <ul style="list-style-type: none"> - extraction DNA RNA, - molecular Conventional PCR - molecular Conventional RT PCR - molecular real-time PCR - molecular real-time RT PCR - molecular PCR-RFLP - molecular LAMP - molecular other and serological method will be further divided in: <ul style="list-style-type: none"> - serological IF - serological ELISA 	See annex AII.1

Features	IT developments	Screenshot
	<ul style="list-style-type: none"> - serological DAS-ELISA - serological DASI-ELISA - serological PTA ELISA - serological Tissue print-ELISA - serological Lateral Flow Device - serological other. <ul style="list-style-type: none"> • Besides it will be possible to further characterized the type of PCR used using the following characteristics: <ul style="list-style-type: none"> - simplex - duplex - triplex - multiplex (>3) - nested - probe 	See annex All.5
Allow searches to be made on kits.	<ul style="list-style-type: none"> • Several fields will be added in the form regarding the use of kits: <ul style="list-style-type: none"> - yes/no button to indicate whether a kit was used - selection of the kit used. A list of the kits (list maintained by EPPO) will be provided with the possibility to add new kits if the one used is not listed. - yes/no button to indicate whether the kit was used according to manufacturer's instructions with the possibility to specify changes made. 	See annex All.4 and All.5
Harmonization of the way test are described including name of the test and reference	<ul style="list-style-type: none"> • Harmonization of the way tests are described will be ensured by: <ul style="list-style-type: none"> - using standardized fields to describe pests (autocomplete from global database), matrices (list of selection to describe the different type of matrices, autocomplete from global database to select plant species), methods (list of selection, see before) - when adapted from EPPO diagnostic protocols (DP), name of the test used will be selected from a list of all the tests available in DP (list maintained by EPPO). If modifications have been made (yes/no button), they will have to be specified (free text box). - if not adapted from an EPPO DP, reference will have to be provided. • Special field will be created to specify whether the data have been created within the framework of specific research projects. • Harmonization will be also ensured by providing examples and explanation of the required information in the form of pop up box. 	<p>See annex All.1</p> <p>See annex All.3, All.4 and All.5</p> <p>See annex All.1</p> <p>See annex All.1</p>
Harmonization of the way performance criteria are reported	<ul style="list-style-type: none"> • New fields will be created to report performance criteria of each pest. • New fields will be created to better described analytical specificity. 	See annex All.2 an All.6

Features	IT developments	Screenshot
	<ul style="list-style-type: none"> • Harmonization will be also ensured by providing examples and explanation of the required information in the form of pop up box. 	

4.3 Strategy for the transfer of the data from the old database to the new one

The developments described in 4.2 will require:

- to re-enter / re-arrange the data that are already in the database.
- to build two new reference tables: one for kits and one for the name of the tests listed in EPPO DPs. Partners from WP7 will help building the list of kits.

The rearrangement of the data was tested using 7 validation data sheets. Considering the time needed for this transfer, the following strategy was decided:

- All the data from the previous database would be available in an archive section on the new Database website (see annex A1.5).
- The transfer of the data from the old database to the new one will be done step by step going from the most recent validation data to the oldest one.

The reference table for the kits will be built with the help of the EPPO Panel on Diagnostic. Besides, the commercial partners of the project have been asked to provide the list of kits they were selling to EPPO at the mid-term conference to help building this reference table.

5 Enrichment of the validation section of the EPPO Database on Diagnostic expertise

The goal of the validation section of the EPPO Database on Diagnostic expertise is to provide a platform for laboratories to share their validation data. Therefore, the enrichment of this section of the database strongly relies on the regular upload of those data by laboratories. One aim of the VALITEST project is to enrich the database with more validation data. To that end, two strategies have been adopted:

- To make an inventory of the validation data available for the pests included in the TPS organized by WP1 and to collect in this context new validation data to upload in the database.
- To produce new validation data and make them available through the database.

It is worth noting that EPPO publicize the validation section of the EPPO Database on Diagnostic expertise in the different meetings and workshops it organized (Panel on Diagnostics, Workshop for the head of laboratories). In that context, EPPO encouraged laboratories to share their validation data and manage to collect additional reports to upload on the database (see table 2).

5.1 Validation data collected during the project

As described in the **Deliverable 6.3**, 10 validations reports were collected during the survey aiming at making an inventory of validation data available for the 6 pests included in the first TPS. Those reports were uploaded by EPPO in the database by the end of August 2019. Emails were sent to all laboratories that provided reports for their final review and to get their formal approval regarding the publication of the data.

Five out of the ten reports are now available in the database. The publication of one report is pending for final approval by the laboratory. Note that 3 reports were already available in the database. Finally, for one of the reports, the laboratory that provided the data finally decided not to publish them because of traceability issues.

Table 2 summarize the status of the different reports.

Table 2: Status of the validation data reports collected during the project:

Pest	Method	Status of the report
Reports collected during surveys		
<i>Bursaphelenchus xylophilus</i>	Real-time PCR	Available since the 20/11/2019
<i>Bursaphelenchus xylophilus</i>	Other methods applicable in the laboratory	Already available in the EPPO database
<i>Erwinia amylovora</i>	Plating	Available since the 17/09/2019
<i>Erwinia amylovora</i>	Conventional PCR	Already available in the EPPO database
<i>Erwinia amylovora</i>	Real-time PCR	Already available in the EPPO database
<i>Fusarium circinatum</i>	Real-time PCR	Entered in the EPPO database – waiting for final approval by the laboratory
<i>Pantoea stewartii</i> subsp. <i>stewartii</i>	Other methods applicable in the laboratory	Available since the 20/11/2019
Plum pox virus	ELISA	Available since the 13/09/2019
Plum pox virus	ELISA	Available since the 24/09/2019
Plum pox virus	ELISA	The laboratory decided that the validation data should not be made public because of traceability issues
Other reports collected		
<i>Potato spindle tuber viroid</i>	Real-time PCR	Available since the 13/09/2019

5.2 Validation data obtained from the TPS organised by WP1

Validation data obtained in the framework of WP1 will be made available in the validation section of the EPPO Database on Diagnostic expertise once the new database has been created. This will avoid the transfer of those data from the old database to the new one. Besides, it will allow TPS organizers to test the new database and EPPO to make adjustments based on their experience.

6 Further steps

Before the publication of the database in April 2020, the following activities will be organized:

- A webinar will be planned in early 2020 to present the new Validation data section and gather feedback. This webinar will focus on VALITEST partners first. A second Webinar may be organized with non partners (depending on the outcome of the first Webinar).
- VALITEST partners will be asked to enter validation data in the new designed section of validation data, as well as non-partners volunteers from EPPO Panels. This life size test of the redesigned database will allow to gather feedback and improve the database before its publication.

7 Conclusion

The improvement of the database on diagnostic expertise including the section on validation data is well on track and the publication of the new database is envisaged as scheduled at the end of April 2020.

ANNEX I: Screenshots of the new EPPO Database on Diagnostic Expertise

Screenshots are as retrieved on the 21/11/2019 and are provided to reflect the advancement of the work performed. They do not reflect the finalized version of the database. Further IT development are still in progress.

AI.1 Home page



EPPO database on Diagnostic Expertise

- HOME
- Laboratory List
- Expertise List
- Technical Auditors/Experts list
- Validation data for diagnostic tests
- Connect to my Lab



This database provides an inventory of the diagnostic expertise available in the EPPO region. Its aim is to cover the expertise on regulated pests (i.e. pests of EPPO A1 and A2 Lists, pests mentioned in EPPO Standards PM4: Production of Healthy Plants for Planting), pests possibly presenting a risk to EPPO member countries (EPPO Alert List) and plants of the EPPO List of invasive alien plants. This database does not include common pests which are widely distributed in the EPPO region. The EPPO Secretariat is maintaining the database but please note that all information included in the database is based on individual expert's own declarations of their expertise. This database had been established as a follow-up action of the EPPO Council Colloquium in Madeira in 2004-09 where the declaration "Plant Health Endangered - State of Emergency" was adopted.

How to search the database

Data can be searched by laboratory (Laboratory List), by expertise on specific pests (Expertise List) or by technical auditors or technical experts (Technical auditors/experts List).

AI.2 Laboratory list



EPPO database on Diagnostic Expertise

- HOME
- Laboratory List
- Expertise List
- Technical Auditors/Experts list
- Validation data for diagnostic tests
- Connect to my Lab



LABORATORY LIST

Country	Name	Quality Prog	ISO	TPS	PT	Training
Austria	AGES Institute of Sustainable Plant Production Vienna	YES	YES	YES	YES	YES
Austria	Department of Forest Protection Wien	YES	NO	YES	YES	NO
Azerbaijan	Republic Plant Quarantine Expertise Laboratory Baku	YES	NO	NO	NO	YES
Belarus	department of quarantine expertise Minsk	YES	NO	NO	YES	YES
Belgium	Federal Agency for the Safety of the Food Chain - Labo Gembloux (LFSAGx) Gembloux	YES	YES	YES	YES	NO
Belgium	Federal Agency for the Safety of the Food Chain - labo Melle Melle	YES	YES	YES	YES	NO

AI.3 Expertise list



EPPO database on Diagnostic Expertise

HOME Laboratory List Expertise List Technical Auditors/Experts list Validation data for diagnostic tests Connect to my Lab



EXPERTISE LIST

You can select one or several organisms :

Agrilus anxius, Agrilus planipennis

Display results

23 results for this selection

Expert	Level	Organism	Morphological	Serological	Molecular	Bioassay	Biochemical	Fatty acid profiling	Microbiology	Years of experience
ALLEN Duncan (United Kingdom) as order: Coleoptera	order	Agrilus anxius	YES	NO	NO	NO	NO	NO	NO	10
ALLEN Duncan (United Kingdom) as order: Coleoptera	order	Agrilus planipennis	YES	NO	NO	NO	NO	NO	NO	10
BUBNOVA ANNA (Belarus)	species	Agrilus planipennis	YES	NO	NO	NO	NO	NO	NO	4
DE GROOT Maarten (Slovenia)	species	Agrilus anxius	YES	NO	NO	NO	NO	NO	NO	5
DE GROOT Maarten (Slovenia)	species	Agrilus planipennis	YES	NO	NO	NO	NO	NO	NO	5

AI.4 Validation data for diagnostic tests (new data)



EPPO database on Diagnostic Expertise

HOME Laboratory List Expertise List Technical Auditors/Experts list Validation data for diagnostic tests Connect to my Lab



LIST OF VALIDATION DATA

Report reference	Report date	Organism(s)	Methods	Matrix(ces)	plant species	Follow EPPO/IPPC diag	PDF	LabID
(10 - id-lab: 82) Not specified	2012-03-01	Erwinia amylovora	Extraction, Isolation	Leaves, Shoots	Rosaceae	yes		DAMIEN
(71 - id-lab: 42) Potato Council Project Report 2009/15: Validation of quantitative DNA detection systems for PCN. Ref: R287	2013-09-01	Globodera pallida, Globodera rostochiensis	Extraction, Extraction DNA, RNA, Molecular real time PCR	Specimen	Archidendron jiringa, Frankia	yes		DAMIEN
(82 - id-lab: 78) Validation report on the testing of phytoplasma of the apple proliferation group using real time PCR	2014-08-28	Phytoplasma mali, Phytoplasma prunorum, Phytoplasma pyri	Extraction DNA, RNA, Molecular real time PCR	Leaves, Roots, Woody cuttings	Malus domestica, Prunus sp., Pyrus communis	yes		DAMIEN
(154 - id-lab: 205) Not specified	2011-05-31	Pantoea stewartii	Serological IF	Other	Zea mays	yes		DAMIEN
(188 - id-lab: 59) F18_V08; F18_V12	2018-02-12	Tomato spotted wilt tospovirus	Extraction DNA, RNA, Molecular real time RT-PCR, Serological DASH-ELISA	Leaves	Solanum lycopersicum	yes		DAMIEN
(186 - id-lab: 59) F18_N01	2008-12-31	Meloidogyne	Extraction, Morphological	Soil, Tubers		yes		DAMIEN

Note that all columns are searchable.

AI.5 Validation data for diagnostic tests (archive)



EPPPO database
on Diagnostic Expertise

HOME Laboratory List Expertise List Technical Auditors/Experts list Validation data for diagnostic tests Connect to my Lab



LIST OF VALIDATION DATA

Organism	Short description	Follow EPPPO/PPC diag	Methods	PDF	LabID
Acidovorax citrulli	Detection of Acidovorax citrulli by PCR in seeds	yes	molecular		NAKTUINBOUW
Aphelenchoides besseyi	Diagnostic Real-time PCR assay for identification and detection of Aphelenchoides besseyi	yes	molecular		CLEAR
Apple proliferation phytoplasma	Detection of 'Candidatus Phytoplasma mali', 'Ca. P. pyri' and 'Ca. P. prunorum' by real-time PCR	yes	extraction molecular		NIB-FITO
Beet necrotic yellow vein virus	Detection of BNYYV by ELISA in host plant material	yes	serological		LNPVAAL
Bursaphelenchus xylophilus	Detection of Bursaphelenchus xylophilus in wood extract with PCR RFLP Burgersmeister et al. 2005	yes	molecular		LNPVnema
Bursaphelenchus xylophilus	Morphological identification of Bursaphelenchus xylophilus	yes	morphological		LNPVnema
Bursaphelenchus xylophilus	Specific PCR Leal et al. 2005	yes	molecular		LNPVnema
Bursaphelenchus xylophilus	Identification of Bursaphelenchus xylophilus by nested PCR (Takeuchi et al. 2005)	yes	molecular		LNPVnema
Bursaphelenchus xylophilus	Duplex PCR based identification of Bursaphelenchus xylophilus Jiang et al. 2005	yes	molecular		LNPVnema
Bursaphelenchus xylophilus	Identification of Bursaphelenchus xylophilus by specific PCR Castagnone et al. 2005	yes	molecular		LNPVnema
Bursaphelenchus xylophilus	Identification of Bursaphelenchus xylophilus by species specific PCR	yes	molecular		LNPVnema
Bursaphelenchus xylophilus	Detection of Bursaphelenchus xylophilus in wood extract with real-time PCR Cao et al. 2005	yes	molecular		LNPVnema

Note that all columns are searchable.

ANNEX II: Screenshots of new forms to enter validation data in the database

All.1 Page 1 - Basic information and description of the test

LabID
DAMIEN

DAMIEN

Enter **validation data** ★ Required

Basic information regarding the validation report:

Date of the validation report ★

Name/code of the validation report: ★

Validation process according to EPPO Standard PM 7/98? Yes No

Is the lab accredited for this test? Yes No

Was the validated data generated in the framework of a project? No
 Yes, Euphresco project
 Yes, other project

Description of the test:

Pest(s)

Organism(s) ★

detection/identification detection
 identification
 detection and identification

Matrix(ces)

Plant species ★

Matrix(ces) tested - ★

Specify the matrix(ces):

Method(s)

Method(s) ▲ ★

[Save and continue](#)

All.1 Page 2: Description of the test and performance criteria

LabID
DAMIEN

DAMIEN

Validation data

Description of the test:

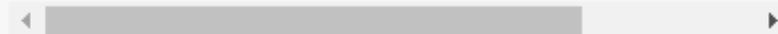
actions	Method(s)	Kit(s)	molecular amplification type	Reference of the test description
Add information	Extraction			Antioxidant maceration buffer (Gorris et al., 1996)
Add information	Extraction DNA RNA	yes QIAmp DNA mini kit		
Add information	Molecular real time PCR	no	Probe	Taqman realtime PCR developed by FERA

Add another method

Are the validation data referred to in EPPD diagnostic protocol? Yes No

Performance criteria & test performance study:

Actions	Organism(s)	Analytical sensitivity	Diagnostic sensitivity	Analytical specificity	Diagnostic specificity	Reproducibility
Add information	Globodera pallida	DNA from single cyst detectable at 1000 fold dilut ...	100% ... The TaqMan assay was compared to the standard conv	Inclusive: 20+ strains of G. pallida (see validation report) ... exclusive: Strains of G. tabacum (see validation report) Str ...	G. pallida 87.1% The testing gave no false negat ... The TaqMan assay was compared to the standard conv ...	The testing has been successful carried out by multiple users on all e ...
Add information	Globodera rostochiensis	DNA from single cyst detectable at 1000 fold dilut ...	100% ... The TaqMan assay was compared to the standard conv	Inclusive: 20+ strains of G. rostochiensis (see validation re ... exclusive: Strains of G. tabacum (see validation report) Str ...	G. rostochiensis 93.75% The testing gave no false ... The TaqMan assay was compared to the standard conv ...	The testing has been successful carried out by multiple users on all e ...



Any other information considered useful:

The *Globodera pallida* probe is known to cross-react slightly with *Globodera rostochiensis* DNA. The cross reaction will show as a slight increase in delta Rn in the FAM channel (*G. pallida*) as the delta Rn increases exponentially in the TET channel (*G. rostochiensis*). This cross reaction is only observed when a sample is positive for *G. rostochiensis*.

Save and continue

All.3 Further description of the tests: extraction methods

LabID
DAMIEN

DAMIEN

Validation data

Description of the test:

Method : Method for extraction/isolation/baiting of target organism from matrix (except nucleic acid extraction)

Precision regarding the test: Conventional flotation method to isolate cysts (Wye washer, following EPPO diagnostic protocol PM 7/40 (3))

Reference of the test description

As or adapted from in the diagnostic protocol Yes No

Diagnostic Protocol reference PM 7/040 (Version 4) EPPO Diagnostic Protocol for Glo

Choose from DP

Is the test modified compared to PM7 Yes No

All.4 Further description of the tests: extraction DNA/RNA

LabID
DAMIEN

DAMIEN

Validation data

Description of the test:
Method : Nucleic acid extraction

Precision regarding the test:

Specify whether a kit is used Yes No

If yes, specify the kit used:

Did you use the kit following the manufacturer's instructions? Yes No

Please specify:

Reference of the test description

As or adapted from in the diagnostic protocol Yes No

Diagnostic Protocol reference ★

Choose from DP ★

Is the test modified compared to PM7 Yes No

Save and continue

All.5 Further description of the tests: PCR methods

LabID
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DAMIEN

Validation data

Description of the test:

Method : Molecular method : real-time PCR (qPCR)

Precision regarding the test:

Specify whether a kit is used Yes No

Molecular amplification type:

Reference of the test description

As or adapted from in the diagnostic protocol Yes No

Diagnostic Protocol reference ★

Choose from DP ★

Is the test modified compared to PM7 Yes No

Save and continue

All.6 Performance criteria

Organism: *Globodera pallida* (HETDPA)

Performance Criteria

Analytical sensitivity

What is the smallest amount of target that can be detected reliably?:

DNA from single cyst detectable at 1000 fold dilution

Diagnostic sensitivity

Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98:

100%

Specify the standard test(s):

The TaqMan assay was compared to the standard conventional PCR assay of Bulman & Marshall, 1997.

Analytical specificity - inclusivity

Number of strains/populations of target organisms tested (please provide a list):

20+ strains of *G. pallida* (see validation report)

Specificity value:

Analytical specificity - exclusivity

Number of non-target organisms tested (please provide a list):

Strains of *G. tabacum* (see validation report)
Strains of *G. achillae/millefolii* (see validation report)

Specificity value:

Cross reacts with (specify the species)📌

× *Globodera tabacum tabacum* (GLOBTT) × ★

Diagnostic specificity

Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test:

G. pallida 87.1%
The testing gave no false negatives for either species.

Specify the test(s):

The TaqMan assay was compared to the standard conventional PCR assay of Bulman & Marshall, 1997 and morphological identification.

Reproducibility

Provide the calculated % of agreement for a given level of the pest (see PM 7/98):

The testing has been successful carried out by multiple users on all equipment over several days

Repeatability

Provide the calculated % of agreement for a given level of the pest (see PM 7/98):

Positive controls used on every run produce repeatable results.

Test performance study

Test performance study Yes No

Include brief details of the test performance study and its output. If available, provide a link to a published article/report:

100% correct identification results over recent proficiency tests.

Save and continue