EUROPEAN AND MEDITERRANEAN PLANT PROTECTION ORGANIZATION
ORGANISATION EUROPEENNE ET MEDITERRANEENNE POUR LA PROTECTION DES PLANTES
Summary sheet of validation data for a diagnostic test

The EPPO Standard PM 7/98 *Specific requirements for laboratories preparing accreditation for a plant pest diagnostic activity* describes how validation should be conducted. It also includes definitions of performance criteria.

Laboratory contact details	Naktuinbouw Sotaweg 22, 2371 GD Roelofarendsveen, Netherlands
Short description of the test	Detection of Phytophthora ramorum by Molecular real time PCR in leaf material.
Date, reference of the validation report	2023-02-27 - Validation report Phytophthora ramorum v3.1
Validation process according to EPPO Standard PM7/98?	yes
Is the lab accredited for this test?	yes
Was the validated data generated in the framework of a project?	no
Description of the test	
Organism(s)	Phytophthora ramorum (PHYTRA)
Detection / identification	detection
Method(s)	Molecular real time PCR
Method: Molecular real time PCR	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	no
New test being considered for inclusion in the next version of the EPPO diagnostic protocol?	yes
As or adapted from an IPPC diagnostic protocol	no
Reference of the test	Hayden et al., 2006
Is the test modified compared to the reference test	yes Only primers pram5, pram6 and probe pram7 are used in the PCR assay. Primer/Probe concentration (0.3 µM F/R primers; 0.1 µM probe); PerfeCTa MultiPlex qPCR ToughMix (Quantabio). PCR conditions: Initial denaturation 95 °C for 10 min; then 40 cycles of denaturation 95 °C for 15 s; annealing/extension 63 °C for 1 min. The fluorescence of the reporter dye is monitored at the end of each annealing/extension step. Final volume of the reaction 25 µL; 5 µL DNA template.

Kit	
Is a kit used	yes
Manufacturer name	LGC
Specify the kit used	Sbeadex maxi plant
Kit used following the manufacturer's instructions?	no Lysismix consists of 400 µL of Lysisbuffer PVP (LGC Genomics), 44 µL Protease solution (LGC Genomics) and 16 µL 5M DTT per sample and is added to the pellet with two steal ball bearings (2,778mm Ø). The pellet is resuspended by putting the tubes for 3 minutes at 1500rpm in a Geno/Grinder and incubated for at 55°C for 1 hour (homogenized by shaking a couple of times). The sample is cooled on ice and centrifuged at 3400g for 20 minutes. DNA extraction is performed with the Kingfisher platform using the Sbeadex maxi plant kit (LGC Genomics) by adding 400 µL supernatant from the sample to 500 µL bindingbuffer PN and 20 µL magnetic beads. Four wash steps are performed with 600 µL wash buffers (2x PN1, 1x PN2, 1x ultrapure water). DNA is eluted in 100 µL elution buffer PN
Other information	
Reaction type	Duplex
Performance Criteria :	
Organism 1.:	**Phytophthora ramorum(PHYTRA)**
Analytical sensitivity	
What is smallest amount of target that can be detected reliably?	Rhododendron: 2.7 zoospores/mL sample extract could be detected with a reliability of 95%. Viburnum: 1.2 zoospores/mL sample extract could be detected with a reliability of 95%.
Diagnostic sensitivity	
Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98	n/a
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	Phytophthora ramorum (PD 03/01391728), Phytophthora ramorum (PD 20024500), Phytophthora ramorum (93/56), Phytophthora ramorum (94/844), Phytophthora ramorum (INS--22-08550) PCR Positive Phytophthora ramorum samples (not isolated but confirmed by Sequence analysis of the ATP9 gene): 18x (INS-18-09214, INS-18-14172, INS-18-17652, INS-18-17659, INS-18-19293, INS-19-24496, INS-19-27395, INS-20-04553, INS-20-05556, INS-20-13482, INS-20-20692, INS-20-22887, INS-20-25752, INS-21-02609, INS-21-10274, INS-21-19485, INS-21-21794, INS-22-15013).
Specificity value	100%

Analytical specificity - exclusivity	
Number of non-target organisms tested	Phytophthora cactorum (2x), Phytophthora infestans, Phytophthora citricola, Phytophthora fragariae (4x), Phytophthora niederhauserii (6x), Phytophthora megasperma, Phytophthora cryptogea (3x), Phytophthora lateralis (2x), Phytophthora kernoviae, Phytophthora palmivora (2x), Phytophthora hedraiandra, Phytophthora cinnamomi (2x).
Specificity value	83.3%
Cross reacts with	Phytophthora niederhauseri Phytophthora lateralis
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	n/a
Reproducibility	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100% for Rhododendron: Eight replicates of sample extract spiked with zoospores to a final concentration of 10 zoospores/mL were tested under reproducibility conditions in eight separate runs over a period of 60 days. 100% for Viburnum: Eight replicates of sample extract spiked with zoospores to a final concentration of 10 zoospores/mL were tested under reproducibility conditions in eight separate runs over a period of 60 days.
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100% for Rhododendron: Eight replicates of sample extract spiked with zoospores to a final concentration of 10 zoospores/mL were tested under repeatability conditions (within the same run). 100% for Viburnum: Eight replicates of sample extract spiked with zoospores to a final concentration of 10 zoospores/mL were tested under repeatability conditions (within the same run).
Test performance study	
Test performance study?	no
Other information	
Any other information considered useful	Test samples consist of 20 leaves. Samples are incubated overnight in 100 mL 0.05M PBS with Tween (final concentration 0.02% Tween-20). After incubation the sample is homogenized two times at maximum setting in an Interscience BagMixer for 2 minutes. Plant extract is transferred to a 50 ml centrifuge tube and allowed to settle (on ice) for 10 minutes. 10 ml of the upper part of the extract is transferred to a 15 ml centrifuge tube and spiked with an internal isolation control. The sample is

	placed in a swing-out centrifuge at 3400g and 4°C for 20 minutes. Subsequently the supernatant is removed. The pellet is used for DNA isolation.
The following complementary files are available online:	• Validation report P.ramorum Naktuinbouw

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