

**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.

<b>Laboratory contact details</b>	Naktuinbouw Sotaweg 22, 2371 GD Roelofarendsveen, Netherlands
<b>Short description of the test</b>	Detection of <i>Phytophthora ramorum</i> by Molecular real time PCR in leaf material.
<b>Date, reference of the validation report</b>	2023-02-27 - Validation report <i>Phytophthora ramorum</i> v3.1
<b>Validation process according to EPPO Standard PM7/98?</b>	yes
<b>Is the lab accredited for this test?</b>	yes
<b>Was the validated data generated in the framework of a project?</b>	no
<b>Description of the test</b>	
<b>Organism(s)</b>	<i>Phytophthora ramorum</i> (PHYTRA)
<b>Detection / identification</b>	detection
<b>Method(s)</b>	Molecular real time PCR
<b>Method: Molecular real time PCR</b>	
<b>Reference of the test description</b>	
<b>As or adapted from an EPPO diagnostic protocol</b>	no
<b>New test being considered for inclusion in the next version of the EPPO diagnostic protocol?</b>	yes
<b>As or adapted from an IPPC diagnostic protocol</b>	no
<b>Reference of the test</b>	Hayden et al., 2006
<b>Is the test modified compared to the reference test</b>	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.

<b>Kit</b>	
<b>Is a kit used</b>	yes
<b>Manufacturer name</b>	LGC
<b>Specify the kit used</b>	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 steel 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
<b>Other information</b>	
<b>Reaction type</b>	Duplex
<b>Performance Criteria :</b>	
<b>Organism 1.:</b>	<b>Phytophthora ramorum(PHYTRA)</b>
<b>Analytical sensitivity</b>	
<b>What is smallest amount of target that can be detected reliably?</b>	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%.
<b>Diagnostic sensitivity</b>	
<b>Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98</b>	n/a
<b>Analytical specificity - inclusivity</b>	
<b>Number of strains/populations of target organisms tested</b>	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).
<b>Specificity value</b>	100%

<b>Analytical specificity - exclusivity</b>	
<b>Number of non-target organisms tested</b>	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).
<b>Specificity value</b>	83.3%
<b>Cross reacts with</b>	Phytophthora niederhauseri Phytophthora lateralis
<b>Diagnostic Specificity</b>	
<b>Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test</b>	n/a
<b>Reproducibility</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	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.
<b>Repeatability</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	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).
<b>Test performance study</b>	
<b>Test performance study?</b>	no
<b>Other information</b>	
<b>Any other information considered useful</b>	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:	<ul style="list-style-type: none"><li>• <a href="#">Validation report P.ramorum Naktuinbouw</a></li></ul>



*Creation date: 2023-10-04 15:51:37 - Last update: 2024-07-02 10:44:38*