

**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 fragariae</i> by TaqMan PCR in strawberry roots
<b>Date, reference of the validation report</b>	2018-09-01 - Validation data for SPN-M041 <i>Phytophthora fragariae</i>
<b>Validation process according to EPPO Standard PM7/98?</b>	yes
<b>Is the lab accredited for this test?</b>	no
<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 fragariae</i> (PHYTFR)
<b>Detection / identification</b>	detection
<b>Matrix(ces) tested</b>	Roots
<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>	Bonants et al., 2004
<b>Is the test modified compared to the reference test</b>	yes A modified version of the forward primer DC1 and reverse primer mp5 were used. DC1: Final base on 3' mismatch corrected (T->C). Flaps added to DC1 and mp5 to improve real-time PCR efficiency (Afonina et al., 2007). Probe was redesigned on the same region. Primer/Probe concentration (0.3 µM F/R primers; 0.2 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 1500 µL of Lysisbuffer PVP (LGC Genomics) 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 fragariae(PHYTFR)</b>
<b>Analytical sensitivity</b>	
<b>What is the smallest amount of target that can be detected reliably?</b>	1 zoospore/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 fragariae isolates: 4x (CBS 209.46, NFC-2822, NFC-2823, NFC-2824). PCR positive Phytophthora fragariae samples confirmed by sequence analysis of the ATP9 gene: 5x (INS-17-25264, INS-17-00966, INS-19-01234, INS-19-25310, INS-24-01881).
<b>Specificity value</b>	100%
<b>Analytical specificity - exclusivity</b>	
<b>Number of non-target organisms tested</b>	Phytophthora cactorum (4x), Phytophthora infestans, Phytophthora citricola, Phytophthora niederhauserii, Phytophthora megasperma, Phytophthora cryptogea (2x), Phytophthora lateralis (2x), Phytophthora kernoviae, Phytophthora palmivora, Phytophthora ramorum

	(4x), <i>Phytophthora plurivora</i> , <i>Phytophthora rubi</i> (8x).
<b>Specificity value</b>	92% (12 non-target species).
<b>Cross-reacts with</b>	<i>Phytophthora rubi</i>
<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%. Eight replicates of sample extract, spiked with <i>P. fragariae</i> positive sample extract to a final Cq value of ~27, were tested under reproducibility conditions in eight separate runs over period of six weeks. Cq mean: 27.2 standard deviation: 0.46
<b>Repeatability</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	100%. Eight replicates of sample extract, spiked with <i>P. fragariae</i> positive sample extract to a final Cq value of ~27, were tested under repeatability conditions (within the same run). Cq mean: 26.9 standard deviation: 0.19
<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 root tips (max. 150 gram). Root tips are homogenized two times in a paintshaker for 5 minutes in 1 liter bottles containing 10 stainless steel balls (20 mm) and 300 mL 0.05M PBS. Root extract is filtered through a BagPage+ 400 mL bagmixer bag (Interscience). 10 mL 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. In silico analysis of primers/probe showed potential reaction with non-host species: <i>Phytophthora alni</i> , <i>Phytophthora colocasiae</i> , <i>P. x heterohybrida</i> and <i>P. x. incrasata</i> .

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