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 fragariae by TaqMan PCR in strawberry roots
Date, reference of the validation report	2018-09-01 - Validation data for SPN-M041 Phytophthora fragariae
Validation process according to EPPO Standard PM7/98?	yes
Is the lab accredited for this test?	no
Was the validated data generated in the framework of a project?	no
Description of the test	
Organism(s)	Phytophthora fragariae (PHYTFR)
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	Bonants et al., 2004
Is the test modified compared to the reference test	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.
Kit	
Is a kit used	yes
Manufacturer name	LGC
Specify the kit used	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 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 fragariae(PHYTFR)**
Analytical sensitivity	
What is smallest amount of target that can be detected reliably?	1 zoospore/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 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).
Specificity value	100%
Analytical specificity - exclusivity	
Number of non-target organisms tested	Phytophthora cactorum (4x), Phytophthora infestans, Phytophthora citricola, Phytophthora niederhauserii, Phytophthora megasperma, Phytophthora cryptogea (2x), Phytophthora lateralis (2x), Phytophthora kernoviae, Phytophthora palmivora, Phytophthora ramorum (4x), Phytophthora plurivora, Phytophthora rubi

	(8x).
Specificity value	92% (12 non-target species).
Cross reacts with	Phytophthora rubi
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%. Eight replicates of sample extract,spiked with P. fragariae 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
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100%. Eight replicates of sample extract, spiked with P. fragariae 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
Test performance study	
Test performance study?	no
Other information	
Any other information considered useful	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: Phytophthora alni, Phytophthora colocasiae, P. x heterohybrida and P. x. incrassata.

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