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	Plant Health Laboratory Department Of Agriculture, Food and the Marine, W23X3PH Backweston Agri-laboratories, Young's Cross, Celbridge, Co. Kildare, Ireland
Short description of the test	Detection of Phytophthora ramorum by Real time PCR in Leaf material
Date, reference of the validation report	2018-02-05 - Ram3
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	yes
IPPC diagnostic Protocol name	ISPM 27 Annex 23 DP 23: Phytophthora ramorum (version 2017)
Name of the test	Real-time PCR of Schena et al. (2006) targeting P. ramorum
Is the test modified compared to the reference test	yes Primer/Probe concentration (0.9uM F primer; 0.9uM R primer; 0.2uM probe); Qiagen QuantiNova Probe PCR Kit; annealing temperature 62.5 degrees; final volume of the reaction 20ul; volume of DNA template 2ul
Kit	

Is a kit used	no
Other information	
Reaction type	Simplex
Other details on the test	For sensitivity, the LOD was determined as number of copies of DNA by digital PCR. This data was subsequently complemented with the number of zoospores from which a detectable amount of DNA can be obtained in line with PM7/98.
Performance Criteria :	
Organism 1.:	**Phytophthora ramorum(PHYTRA)**
Analytical sensitivity	
What is smallest amount of target that can be detected reliably?	1 zoospore per microliter (corresponding to 1 copy of DNA per microliter), obtained from a 10 point serial dilution of spore suspension (10 fold and 5-fold), spiked into plant lysate. 5 copies per microliter could be detected by digital PCR (16/16 reps yielded 100% amplification) from a serial dilution of DNA.
Diagnostic sensitivity	
Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98	100% or 28 out of 28 infected samples.
Standard test(s)	Microbiological isolation (EPPO PM7/66 (1)) and real time PCR method by Hughes et al., 2005 (EPPO PM7/66 (1) Appendix 5 method A).
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	Phytophthora ramorum, EU1 15x isolates Phytophthora ramorum, EU2 1x isolate Phytophthora ramorum, NA2 1x isolate
Specificity value	100%
Analytical specificity - exclusivity	
Number of non-target organisms tested	Phytophthora gonapodyides, Phytophthora alni, Phytophthora cactorum, Phytophthora cryptogea, Phytophthora hibernalis, Phytophthora kernoviae (x2 isolates), Phytophthora lateralis (x3 isolates), Phytophthora plurivora, Phytophthora pseudosyringae, Phytophthora syringae
Specificity value	100% (13 non-target species/isolates could be excluded out of 13 tested).
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	100% or 42 out of 42 infected samples.
Specify the test(s)	Microbiological isolation (EPPO PM7/66 (1)) and real time PCR method by Hughes et al., 2005 (EPPO PM7/66 (1) Appendix 5 method A).
Reproducibility	

Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	Reproducibility was evaluated at different mycelium DNA concentrations quantified by digital PCR (6 replicates at 5 copies DNA/reaction by 3 operators on 3 different days and with 3 different real time thermocyclers) and different concentration of zoospore extracts (evaluated on at least 6 replicates at 360 zoospores/mL of plant lysate by 3 operators on 3 different days and with 2 different real time thermocyclers); in both cases 100% reproducibility was achieved. Data for more than one concentration level can be provided.
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	Repeatability was evaluated at different mycelium DNA concentrations quantified by digital PCR (6 replicates at 5 copies DNA/reaction) and different concentration of zoospore extracts (at least 6 replicates at 360 zoospores/mL of plant lysate, corresponding to 1 zoospore/µl of DNA); in both cases 100% repeatability was achieved. Data for more than one concentration level can be provided.
Test performance study	
Test performance study?	no
Brief details of the test performance study and its output.It available, link to published article/report	n/a
Other information	
Any other information considered useful	For DNA extractions the following procedure was used: 3x 0.6mm diameter leaf disks homogenised in extraction buffer (1ml 2% CTAB with Proteinase K and RNase A), incubated at 65 degrees for 20 minutes and purified using magnetic bead purification kit on Maxwell RSC Cell Purification kit and elution in 200ul elution buffer. Additional data can be provided upon request.

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