

EUROPEAN AND MEDITERRANEAN PLANT PROTECTION ORGANIZATION
ORGANISATION EUROPEENNE ET MEDITERRANEEENNE POUR LA PROTECTION DES PLANTES
(11-17239)

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.

Target Organism	Phytophthora ramorum	
Short description	Detection of Phytophthora ramorum by plating infected plant material and morfological evaluation the culture	
Laboratory contact details	ILVO Institute for Agricultural and Fisheries Research Burg. Van Gansberghelaan 96, 9820 Merelbeke, Belgium	
Date and reference of the validation report	2009-03-31 - F16_S08	
Validation process according to EPPO Standard PM 7/98:	No	
Reference of the test description	N/R EPPO Diagnostic Protocol for regulated pests : Phytophthora ramorum. EPPO Bulletin 36, 145-155 (2006)	
Is the test the same as described in the EPPO DP?	Modified slight modification in composition of semi-selective medium	
Is the lab accredited for this test?	Yes	
Plant species tested (if relevant)	Rhododendron “Cunningham’s White”, Viburnum x bodnantense “Dawn”, Camellia japonica	
Matrices tested (if relevant)	Leaves and stems of Rhododendron “Cunningham’s White”, leaves of V. x bodnantense and C. japonica	
List of methods used		
Method for extraction / isolation / baiting of target organism from matrix	X	Pieces of surface sterilized infected material are plated on semi-selective media (P5ARP)
Molecular methods, e.g. hybridization, PCR and real time PCR		
Serological methods: IF, ELISA, Direct Tissue Blot Immuno Assay		
Plating methods: selective isolation		
Bioassay methods: selective enrichment in host plants, baiting, plant test and grafting.		
Pathogenicity test		
Fingerprint methods: protein profiling, fatty acid profiling & DNA profiling		

Morphological and morphometrical methods intended for identification	X	Morphological identification using a microscope and checklist (F03_S03) with most important morphological characters of the fungus.
Biochemical methods: e.g. enzyme electrophoresis, protein profiling		
Other		
<u>Analytical sensitivity (= limit of detection)</u>		
What is smallest amount of target that can be detected reliably?	Two plated pieces of freshly infected leaf material out of 20 plated pieces	
<u>Diagnostic sensitivity</u>		
Proportion of infected/infested samples tested positive compared to results from the standard test , see appendix 2 of PM 7/98	100%. All samples that were also analysed with real-time PCR gave identical results, i.e. there were no false negatives	
Specify the standard test	real-time PCR	
<u>Analytical specificity</u>		
Specificity value		
Number of strains/populations of target organisms tested	One in our own validation scheme but the test gave correct results with several isolates from proficiency tests.	
Number of non-target organisms tested	5 (Phytophthora multivora, P. kernoviae, P. hedraiaandra, P. syringae, P. lateralis)	
Cross reacts with (specify the species)	none known	
<u>Diagnostic Specificity</u>		
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	100%. All samples that were also analysed with real-time PCR gave identical results, i.e. there were no false positives.	
Specify the standard test	real-time PCR	
<u>Reproducibility</u>		
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100 %	
<u>Repeatability</u>		
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100 %	
<u>Test performance study</u>		
Test performance study?	No	
Include brief details of the test performance study and its output. If available, provide a link to published article/report		
<u>Other information</u>		

Any other information considered useful e.g. robustness, ease of performing the test, etc.	Participation in proficiency tests, including a published one (EPPO Bulletin, 38: 191-197, (2008)) Robustness and selectivity have also been established.
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