

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	National Reference Centre, National Plant Protection Organization P.O. Box 9102, 6700 HC Wageningen, Netherlands
Short description of the test	Detection of <i>Ralstonia pseudosolanacearum</i> and <i>Ralstonia solanacearum</i> by real-time PCR in various plant matrices
Date, reference of the validation report	2019-09-12 - 2018.molbio-013 Aantoonbaarheidsgrens bepalen van de real-time PCR voor de detectie van <i>Ralstonia solanacearum</i> en <i>Ralstonia pseudosolanacearum</i> in plantmateriaal anders dan aardappelknollen
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?	Other_project
If yes, please specify	FB project
Description of the test	
Organism(s)	<i>Ralstonia pseudosolanacearum</i> (RALSPS) <i>Ralstonia solanacearum</i> (RALSSL)
Detection / identification	detection
Matrix(ces) tested	Herbaceous cuttings, Shoots, Woody cuttings
Plant species tested	Anthurium, Begonia, <i>Capsicum annum</i> , <i>Pelargonium</i> , <i>Rosa</i> , <i>Solanum lycopersicum</i> , <i>Solanum melongena</i>
Method(s)	Molecular real time PCR
Method: Molecular real time PCR	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	yes
New test being considered for inclusion in the next version of the EPPO diagnostic protocol?	yes
EPPO Diagnostic Protocol name	PM 7/021 <i>Ralstonia solanacearum</i> , <i>R. pseudosolanacearum</i> and <i>R. syzygii</i> (version 2)
Name of the test	Real-time TaqMan PCR test (Weller et al., 2000)

As or adapted from an IPPC diagnostic protocol	no
Is the test modified compared to the reference test	yes Modified according to Vreeburg et al. 2016
Kit	
Is a kit used	no
Other information	
Reaction type	Simplex - Probe
Other details on the test	- TaqMan™ Universal PCR Master Mix was used. - For DNA extraction the following kit was used: QuickPick™ SML Plant DNA Kit (Bio-Nobile). Deviation from the protocol from the manufacturer: The DNA extraction was automated using the KingFisher Flex (ThermoFisher, MA, USA) instead of the QuicPick MultiEight (ThermoFisher, MA, USA).
Are the performance characteristics included in the EPPO diagnostic protocol?	
Performance Criteria :	
Organism 1.:	Ralstonia pseudosolanacearum(RALSPS)
Analytical sensitivity	
What is smallest amount of target that can be detected reliably?	The analytical sensitivity for R. pseudosolanacearum in anthurium, rose, pelargonium and tomato was found to be 1.6×10^4 cfu/ml, whereas for paprika and eggplant it was 3.2×10^3 cfu/ml and for begonia 6.4×10^2 cfu/ml
Diagnostic sensitivity	
Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98	
Standard test(s)	
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	
Specificity value	
Analytical specificity - exclusivity	
Number of non-target organisms tested	
Specificity value	
Cross reacts with	
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	
Specify the test(s)	

Reproducibility	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	
Organism 2.:	Ralstonia solanacearum(RALSSL)
Analytical sensitivity	
What is smallest amount of target that can be detected reliably?	The analytical sensitivity for R. solanacearum in anthurium and rose was found to be 1.6×10^4 cfu/ml, whereas for paprika, eggplant, tomato and pelargonium it was 3.2×10^3 cfu/ml and for begonia 6.4×10^2 cfu/ml
Diagnostic sensitivity	
Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98	
Standard test(s)	
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	
Specificity value	
Analytical specificity - exclusivity	
Number of non-target organisms tested	
Specificity value	
Cross reacts with	
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	
Specify the test(s)	
Reproducibility	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	
Test performance study	
Test performance study?	no
Brief details of the test performance study and its output.It available, link to published article/report	

Other information	
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Any other information considered useful	
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