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	Netherlands Institute for Vectors, Invasive plants and Plant health P.O. Box 9102, 6700 HC Wageningen, Netherlands
Short description of the test	Detection of Ralstonia pseudosolanacearum and Ralstonia solanacearum 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 Ralstonia solanacearum en Ralstonia pseudosolanacearum 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)	Ralstonia pseudosolanacearum (RALSPS) Ralstonia solanacearum (RALSSL)
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	yes
New test being considered for inclusion in the next version of the EPPO diagnostic protocol?	yes
EPPO Diagnostic Protocol name	PM 7/021 Ralstonia solanacearum, R. pseudosolanacearum and R. syzygii (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	yes
Manufacturer name	
Specify the kit used	
Kit used following the manufacturer's instructions?	
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).
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
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
Test performance study	
Test performance study?	no

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