

**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.

<b>Laboratory contact details</b>	Netherlands Institute for Vectors, Invasive plants and Plant health P.O. Box 9102, 6700 HC Wageningen, Netherlands
<b>Short description of the test</b>	Detection of <i>Ralstonia pseudosolanacearum</i> and <i>Ralstonia solanacearum</i> by real-time PCR in various plant matrices
<b>Date, reference of the validation report</b>	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
<b>Validation process according to EPPO Standard PM7/98?</b>	yes
<b>Is the lab accredited for this test?</b>	yes
<b>Was the validated data generated in the framework of a project?</b>	Other_project
<b>If yes, please specify</b>	FB project
<b>Description of the test</b>	
<b>Organism(s)</b>	<i>Ralstonia pseudosolanacearum</i> (RALSPS) <i>Ralstonia solanacearum</i> (RALSSL)
<b>Detection / identification</b>	detection
<b>Method(s)</b>	Molecular real time PCR
<b>Method: Molecular real time PCR</b>	
<b>Reference of the test description</b>	
<b>As or adapted from an EPPO diagnostic protocol</b>	yes
<b>New test being considered for inclusion in the next version of the EPPO diagnostic protocol?</b>	yes
<b>EPPO Diagnostic Protocol name</b>	PM 7/021 <i>Ralstonia solanacearum</i> , <i>R. pseudosolanacearum</i> and <i>R. syzygii</i> (version 2)
<b>Name of the test</b>	Real-time TaqMan PCR test (Weller et al., 2000)
<b>As or adapted from an IPPC diagnostic protocol</b>	no
<b>Is the test modified compared to the reference test</b>	yes Modified according to Vreeburg et al. 2016

<b>Kit</b>	
<b>Is a kit used</b>	yes
<b>Manufacturer name</b>	
<b>Specify the kit used</b>	
Kit used following the manufacturer's instructions?	
<b>Other information</b>	
<b>Reaction type</b>	Simplex - Probe
<b>Other details on the test</b>	- 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).
<b>Performance Criteria :</b>	
<b>Organism 1.:</b>	<b>Ralstonia pseudosolanacearum(RALSPS)</b>
<b>Analytical sensitivity</b>	
<b>What is smallest amount of target that can be detected reliably?</b>	The analytical sensitivity for R. pseudosolanacearum in anthurium, rose, pelargonium and tomato was found to be $1.6 \times 10^4$ cfu/ml, whereas for paprika and eggplant it was $3.2 \times 10^3$ cfu/ml and for begonia $6.4 \times 10^2$ cfu/ml
<b>Organism 2.:</b>	<b>Ralstonia solanacearum(RALSSL)</b>
<b>Analytical sensitivity</b>	
<b>What is smallest amount of target that can be detected reliably?</b>	The analytical sensitivity for R. solanacearum in anthurium and rose was found to be $1.6 \times 10^4$ cfu/ml, whereas for paprika, eggplant, tomato and pelargonium it was $3.2 \times 10^3$ cfu/ml and for begonia $6.4 \times 10^2$ cfu/ml
<b>Test performance study</b>	
<b>Test performance study?</b>	no

Creation date: 2021-05-25 11:01:01 - Last update: 2022-08-30 16:55:03