

EUROPEAN AND MEDITERRANEAN PLANT PROTECTION ORGANIZATION
ORGANISATION EUROPEENNE ET MEDITERRANEENNE 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	Ralstonia solanacearum
Short description	Isolation of Ralstonia solanacearum on SMSA
Laboratory contact details	Netherlands Institute for Vectors, Invasive plants and Plant health P.O. Box 9102, 6700 HC Wageningen, Netherlands
Date and reference of the validation report	September 2009 and May 2013 - BAC-2009-01, BAC-2010-05 and BAC 2016-04 (Rosa)
Validation process according to EPPO Standard PM 7/98:	Yes
Reference of the test description	0 isolation on semi-selective medium SMSA
Is the test the same as described in the EPPO DP?	Yes
Is the lab accredited for this test?	No
Plant species tested (if relevant)	Solanum tuberosum, Pelargonium sp; Rosa sp.
Matrices tested (if relevant)	Potato tuber, pelargonium petioles, Rosa sp. and water samples

List of methods used

Method for extraction / isolation / baiting of target organism from matrix		
Molecular methods, e.g. hybridization, PCR and real time PCR		
Serological methods: IF, ELISA, Direct Tissue Blot Immuno Assay		
Plating methods: selective isolation	X	Isolation on semi-selective SMSA
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		
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?	From potato tuber 3×10^2 cfu/ml, From pelargonium petioles $6,5 \times 10^2$ cfu/ml From Rosa $8,6 \times 10^2$ cfu/ml . From water samples 3×10^1 cfu/ml,	
<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%	
Specify the standard test	Real time PCR for identification of Ralstonia solanacearum (Weller et al., 2000)	
<u>Analytical specificity</u>		
Specificity value	100%	
Number of strains/populations of target organisms tested	24 strains of Ralstonia solanacearum (different race/biovar combinations)	
Number of non-target organisms tested	37 strains of non-target organisms that can be found on potato tubers, water and ornamental plants	
Cross reacts with (specify the species)	Some growth on SMSA by Pseudomonas andropogonis, Burkholderia cepacia, Ralstonia pickettii and Pseudomonas syzygii, however colony morphology is not typical for Ralstonia solanacearum (negative results)	
<u>Diagnostic Specificity</u>		
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	100%	
Specify the standard test	Real time PCR for identification of Ralstonia solanacearum (Weller et al., 2000)	
<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.It		

available, provide a link to published article/report	
Other information	
Any other information considered useful e.g. robustness, ease of performing the test, etc.	
The following complementary files are available online:	<ul style="list-style-type: none"> • BAC-1010-005 methodevalidatie kweek Ralstonia solanacearum in Pelargonium en water v2 • BAC-2016-04 verslag labvalidatie kweek Rsol Fylotype L vanuit Rosa sp. • Validation of the isolation of Ralstonia solanacearum from potato tuber extract