

**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.

<b>Target Organism</b>	Clavibacter sepedonicus	
<b>Short description</b>	Detection of Clavibacter sepedonicus in potato extract by conventional PCR, in a multiplex assay with Ralstonia solanacearum	
<b>Laboratory contact details</b>	Bavarian State Research Center for Agriculture, Institute for Plant Protection - Phytopathology and Diagnosis Lange Point 10, 85354 Freising, Germany	
<b>Date and reference of the validation report</b>	2018-04-16 - n/a	
<b>Validation process according to EPPO Standard PM 7/98:</b>	Yes	
<b>Reference of the test description</b>	PM 7/059 Pastrik K-H (2000) Detection of Clavibacter michiganensis ssp. sepedonicus in potato tubers by multiplex PCR with coamplification of host DNA. Eur. J. Plant Pathol. 106, 155-165.	
<b>Is the test the same as described in the EPPO DP?</b>	Modified - PCR mastermix (Qiagen Multiplex PCR Plus Kit) - DNA extraction: MasterPure Complete DNA Purification kit (Lucigen) - Multiplex setup with primers Rs 1 F/R for Rs (Pastrik et al., 2002) - IPC after White et al., 1990 (primer NS7, NS8)	
<b>Is the lab accredited for this test?</b>	Yes	
<b>Plant species tested (if relevant)</b>	Solanum tuberosum	
<b>Matrices tested (if relevant)</b>	tuber extract	
<b>List of methods used</b>		
<b>Method for extraction / isolation / baiting of target organism from matrix</b>		
<b>Molecular methods, e.g. hybridization, PCR and real time PCR</b>	X	conventional PCR
<b>Serological methods: IF, ELISA, Direct Tissue Blot Immuno Assay</b>		
<b>Plating methods: selective isolation</b>		
<b>Bioassay methods: selective enrichment in host plants, baiting,</b>		

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?	10^3 cells/ ml tuber extract	
<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	not done	
Specify the standard test	n/a	
<u>Analytical specificity</u>		
Specificity value	100%	
Number of strains/populations of target organisms tested	6 LMG 2894 LMG 2889 NCP PB 3898 LMG 6722 NCP PB 2140 LMG 25595	
Number of non-target organisms tested	10 Clavibacter michiganensis subsp. michiganensis LMG 3687 Clavibacter michiganensis subsp. insidiosus LMG 7268 Pseudomonas syringae pv. striaefaciens GSPB 2570 Pectobacterium atrosepticum SCRI 1039 Pectobacterium carotovorum subsp. carotovorum LMG 2401 Pectobacterium wasabiae DSM 18074 Pectobacterium carotovorum subsp. brasiliensis LMG 21371 Pectobacterium carotovorum subsp. odoriferum LMG 6688 Pectobacterium betavascularum LMG 2466 Dickeya solani JKI	
Cross reacts with (specify the species)	none	
<u>Diagnostic Specificity</u>		
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	not done	

<b>Specify the standard test</b>	n/a
<b><u>Reproducibility</u></b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	100% for $10^3$ cells/ ml (a total of 56 PCR reactions containing $10^3$ samples (in 24 PCR runs, two different operators, different days), of which 56 were positive)
<b><u>Repeatability</u></b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	100% for $10^3$ cells/ ml (a total of 28 PCR reactions containing $10^3$ samples, in 12 PCR runs, each repeated once - same day, same operator -, of which 28 with the same result)
<b><u>Test performance study</u></b>	
<b>Test performance study?</b>	No
<b>Include brief details of the test performance study and its output. If available, provide a link to published article/report</b>	
<b><u>Other information</u></b>	
<b>Any other information considered useful e.g. robustness, ease of performing the test, etc.</b>	