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	Clavibacter michiganensis subsp. michiganensis	
Short description	Identification of Clavibacter michiganensis subsp. michiganensis by real-time PCR	
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	2011-03-28 - Validation report of Clavibacter michiganensis subsp. michiganensis PTSSK primers and probe, Rijk Zwaan	
Validation process according to EPPO Standard PM 7/98:	Yes	
Reference of the test description	0 Osterhof J. and Berendsen S, 2011. The development of a specific Real-Time TaqMan for the detection of Clavibacter michiganensis supsp. michiganensis (Abstr.) Phytopathology 101:S133.	
Is the test the same as described in the EPPO DP?	No may be used for revision of EPPO PM 7/042	
Is the lab accredited for this test?	No	
Plant species tested (if relevant)		
Matrices tested (if relevant)		
List of methods used		
Method for extraction / isolation / baiting of target organism from matrix		
Molecular methods, e.g. hybridization, PCR and real time PCR	X	Real-time PCR for identification of cmm isolates based on the PTSSK putative two-component system sensor kinase using sequence data acquired from cmm strain NCPPB 382
Serological methods: IF, ELISA, Direct Tissue Blot Immuno Assay		
Plating methods: selective isolation		
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		
Analytical sensitivity (= limit of detection)		
What is smallest amount of target that can be detected reliably?	2x10^3 cfu*ml-1	
Diagnostic sensitivity		
Proportion of infected/infested samples tested positive compared to results from the standard test , see appendix 2 of PM 7/98	97.6%	
Specify the standard test	Pastrik & Rainey (1999)	
Analytical specificity		
Specificity value		
Number of strains/populations of target organisms tested	41 cmm strains covering different geographical origins, which were all positive in pathogenicity on tomato (see details in the full validation report)	
Number of non-target organisms tested	26 related strains (look-a-likes and others) which were all negative in pathogenicity on tomato (see details in the full validation report)	
Cross reacts with (specify the species)	Cross reaction was observed with one look-a-like isolate (see details in the full validation report)	
Diagnostic Specificity		
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	96.15%	
Specify the standard test	Pastrik & Rainey (1999)	
Reproducibility		
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100%	
Repeatability		
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100%	
Test performance study		
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.**	Results from this PCR correlate very well with the pathogenicity results after inoculation on tomato plants
The following complementary files are available online:	• Poster: The development of a specific Real-Time TaqMan for the detection of Clavibacter michiganensis subsp. michiganensis • Validation report of Clavibacter michiganensis subsp. michiganensis PTSSK primers and probe, Rijk Zwaan