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	Xanthomonas vesicatoria Xanthomonas euvesicatoria Xanthomonas gardneri	
Short description	Duplex-PCR tests for the identification X. euvesicatoria, X. vesicatoria, X. gardneri and X. perforans.	
Laboratory contact details	National Reference Centre, National Plant Protection Organization P.O. Box 9102, 6700 HC Wageningen, Netherlands	
Date and reference of the validation report	2012-04-17 - MOVA nummer: 2011.molbio.004	
Validation process according to EPPO Standard PM 7/98:	Yes	
Reference of the test description	N/R Draft diagnostic protocol in preparation Koenraadt, H., Betteray, B., Germain, R., Hiddink, G., Jones, J.B., Oosterhof, J., Rijlaarsdam, A., Roorda, P., and Woudt., B (2009). Development of specific primers for the molecular detection of bacterial spot of pepper and tomato. In: Proceedings of the 2nd international Symposium on Tomato Diseases. Acta Horticulturae 808: 99-102.	
Is the test the same as described in the EPPO DP?	No The test is included in a draft EPPO Standard	
Is the lab accredited for this test?	No	
Plant species tested (if relevant)		
Matrices tested (if relevant)	bacterial colony material	
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	Two conventional duplex-PCR tests
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?	For the X. gardneri it is found to be 2,5x10^6 cfu/ml, for X. perforans 1,9x10^7 cfu/ml, for X. euvesicatoria 5,5x10^5 cfu/ml and for X. vesicatoria 1,6x10^6 cfu/ml.	
Diagnostic sensitivity		
Proportion of infected/infested samples tested positive compared to results from the standard test , see appendix 2 of PM 7/98		
Specify the standard test	Sequence analysis of the AvrBs2 gene that has been used on reference material on basis of the Q-BOL protocol.	
Analytical specificity		
Specificity value		
Number of strains/populations of target organisms tested	53 isolates of Xcv	
Number of non-target organisms tested	6 non-target organisms (in total 21 isolates): Clavibacter michiganensis subsp. michiganensis, Ralstonia solanacearum, Pseudomonas syringae pv. tomato, Pseudomonas syringae pv. syringae, Agrobacterium tumefaciens, Pseudomonas corrugata	
Cross reacts with (specify the species)	Five of the 21 related isolates gave weak (non-specific) amplicon(s). It refers to 2 P.syringae pv tomato isolates and 3 Pseudomonas corrugata isolates. This underlines the risk for wrong identifications for Xg or Xp.	
Diagnostic Specificity		
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test		
Specify the standard test	Sequence analysis of the AvrBs2 gene that has been used on reference material on basis of the Q-BOL protocol.	
Reproducibility		
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100%	
Repeatability		
Provide the calculated % of	93%	

agreement for a given level of the pest (see PM 7/98)	
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.**	This PCR test has been found robust for vatiations in the DNA extraction method. Three different DNA extraction methodologies have been performed and scored equally well: 1. QuickPick Plant DNA Kit (Bio-Nobile, KingFisher method) 2. High Pure PCR Template preparation kit (Roche) 3. Boiling method
The following complementary files are available online:	• Full validation report