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	Pseudomonas syringae pv. actinidiae
Short description	Detection of Pseudomonas syringae pv. actinidiae by isolation and PCR assays in plant material and bacterial cultures
Laboratory contact details	Council for Agricultural Research and Economics– Research Centre for Plant Protection and Certification Via Carlo Giuseppe Bertero, 22, 00156 Rome, Italy
Date and reference of the validation report	2014- 06-05 - Loreti et al., 2014 - Inter-laboratory ring test for the detection of Pseudomonas syringae pv. actinidiae in Actinidia spp.
Validation process according to EPPO Standard PM 7/98:	Yes
Reference of the test description	0 Loreti S., Pucci N., Gallelli A., Minardi P., Ardizzi S., Balestra G.M., Mazzaglia A., Taratufolo M.C. 2014. Experience from the Italian inter-Laboratory study on the detection of Pseudomonas syringae pv. actinidiae. Phytopathologia Mediterranea 53, 1, 159−167 Gallelli A., A. L'Aurora and S. Loreti, 2011a. Gene sequence analysis for the molecular detection of Pseudomonas syringae pv. actinidiae: developing diagnostic protocols. Journal of Plant Pathology 93, 425-435. Gallelli A., S. Talocci, A. L'Aurora and S. Loreti, 2011b. Detection of Pseudomonas syringae pv. actinidiae, causal agent of bacterial canker of kiwifruit, from symptomless fruits, twigs, and from pollen. Phytopathologia Mediterranea 50, 473-483. Louws F.J., D.W. Fullbright, C.T. Stephens and F.J. De Bruijn, 1994. Specific genomic fingerprinting of phytopathogenic Xanthomonas and Pseudomonas pathovars and strains generated with repetitive sequence and PCR. Applied and Environmental Microbiology 60, 2286-2295. Rees-George J., J. Vanneste, D.A. Cornish, I.P.S. Pushparajah, J. Yu, M.D. Templeton and K.R. Everett, 2010. Detection of Pseudomonas syringae pv. actinidiae using polymerase chain reaction (PCR) primers based on the 16S-23S r DNA intertrascribed spacer region and comparison with PCR primers based on other gene regions. Plant Pathology 59, 453-464. Vanneste J.L., D. Giovanardi, J. Yu, D.A. Cornish, C. Kay, F. Spinelli and E. Stefani, 2011. Detection of Pseudomonas syringae pv. actinidiae in pollen samples. New Zealand Plant Protection 64, 246-251.
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)	Actinidia chinensis

Matrices tested (if relevant)	leaves, pollen, bark, fruits, bacterial cultures	
List of methods used		
Method for extraction / isolation / baiting of target organism from matrix	X	Procedures for bacterial extraction from vegetal matrices/ DNA purification from plant extract and isolation by dilution plating (Gallelli et al., 2011b; Vanneste et al., 2011)
Molecular methods, e.g. hybridization, PCR and real time PCR	X	Single-PCR (Rees-George et al., 2010) Duplex-PCR (Gallelli et al., 2011)
Serological methods: IF, ELISA, Direct Tissue Blot Immuno Assay		
Plating methods: selective isolation	X	Semi-selective media : Nutrient Sucrose Agar, (Crosse, 1959)and King's medium B (King et al., 1954), modified by adding antibiotics according Mohan and Schaad (1987)
Bioassay methods: selective enrichment in host plants, baiting, plant test and grafting.		
Pathogenicity test		
Fingerprint methods: protein profiling, fatty acid profiling & DNA profiling	X	Repetitive-PCR fingerprinting (rep-PCR), using the BOX, REP, ERIC primers according to Louws et al. (1994) and following Ferrante and Scortichini (2009; 2010).
Morphological and morphometrical methods intended for identification	X	Levan positive morphology on NSA medium, absence of fluorescence on KB medium
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?	Isolation on modified NSA: 103^CFU/ml pollen (A. chinensis) Duplex PCR and simplex-PCR (primerF1/R2) of DNA purified from pollen (following Gallelli et al., 2011a) : 10^3 CFU/ml (source: Gallelli et al., 2011a) Duplex-PCR of Psa bacterial suspension: 2x10 CFU/PCR reaction Duplex-PCR of genomic DNA: 0.5 pg/PCR reaction (source: Gallelli et al., 2011a)	
<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	Isolation on modified NSA semi-selective media: 79% Isolation on modified KB semi-selective media: 86% Isolation on generic media NSA or KB: 71% Duplex-PCR from plant extract: 95% Simplex-PCR from plant extract: 95% Duplex-PCR from bacterial cultures: 93% Simplex-PCR from bacterial cultures: 96% Rep-PCR (primer ERIC): 89%	

Specify the standard test	Isolation + duplex-PCR (Gallelli et al., 2011a) + simplex PCR (Rees-George et al., 2010) + rep-PCR (Louws et al., 1994)
Analytical specificity	
Specificity value	See Loreti S., Pucci N., Gallelli A., Minardi P., Ardizzi S., Balestra G.M., Mazzaglia A., Taratufolo M.C. 2014. Experience from the Italian inter-Laboratory study on the detection of Pseudomonas syringae pv. actinidiae. Phytopathologia Mediterranea 53, 1, 159−167
Number of strains/populations of target organisms tested	Four Psa bacterial strains tested from seven laboratoty partecipating to the ITL (see Loreti et al., 2014. Phytopathologia Mediterranea 53, 1, 159−167)
Number of non-target organisms tested	Nine non target bacterial strains tested from seven laboratoty partecipating to the ITL (see Loreti et al., 2014. Phytopathologia Mediterranea 53, 1, 159−167)
Cross reacts with (specify the species)	duplex-PCR (Gallelli et al., 2011a): no cross reaction. simplex-PCR (Rees-George et al., 2010): cross reacs with Pseudomonas syringae pv. tomato, Pseudomonas syringae pv. theae,P. avellanae. rep-PCR (primer ERIC): cross reacs with Pseudomonas syringae pv. theae,P. avellanae.
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	Isolation on modified NSA semi-selective media:100 % Isolation on modified KB semi-selective media: 100% Isolation on generic media NSA or KB: 100% Duplex-PCR from plant extract: 100% Simplex-PCR from plant extract: 100% Duplex-PCR from bacterial cultures: 100% Simplex-PCR from bacterial cultures: 74% Rep-PCR (primer ERIC): 97%
Specify the standard test	Isolation + duplex-PCR (Gallelli et al., 2011a) + simplex PCR (Rees-George et al., 2010) + rep-PCR (Louws et al., 1994)
Reproducibility	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	Isolation on modified NSA semi-selective media: 89% Isolation on modified KB semi-selective media: 93% Isolation on generic media NSA or KB: 89% Duplex-PCR from plant extract: 98% Simplex-PCR from plant extract: 98% Duplex-PCR from bacterial cultures: 95.5% Simplex-PCR from bacterial cultures: 94% Rep-PCR (primer ERIC):95 %
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	

Test performance study	
Test performance study?	Yes
Include brief details of the test performance study and its output.It available, provide a link to published article/report	Loreti S., Pucci N., Gallelli A., Minardi P., Ardizzi S., Balestra G.M., Mazzaglia A., Taratufolo M.C. 2014. Experience from the Italian inter-Laboratory study on the detection of Pseudomonas syringae pv. actinidiae. Phytopathologia Mediterranea 53, 1, 159−167
Other information	
Any other information considered useful **e.g. robustness, ease of performing the test, etc.**	
The following complementary files are available online:	• Experience from the Italian inter-Laboratory study on the detection of Pseudomonas syringae pv. actinidiae