EUROPEAN AND MEDITERRANEAN PLANT PROTECTION ORGANIZATION
ORGANISATION EUROPEENNE ET MEDITERRANEENNE POUR LA PROTECTION DES PLANTES
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.

Laboratory contact details	Council for Agricultural Research and Economics–Research Centre for Plant Protection and Certification Via Carlo Giuseppe Bertero, 22, 00156 Rome, Italy
Short description of the test	Detection of Pseudomonas syringae pv. actinidiae by isolation and PCR assays in plant material and bacterial cultures
Date, 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 PM7/98?	yes
Is the lab accredited for this test?	no
Was the validated data generated in the framework of a project?	Other_project
If yes, please specify	MIPAAF Projects: STRATECO and OIGA-ACTINIDIA, n. 247; and by a Lazio and Emilia-Romagna Regional Project
Description of the test	
Organism(s)	Pseudomonas syringae pv. actinidiae (PSDMAK)
Detection / identification	detection
Method(s)	Extraction Molecular Extraction DNA RNA Molecular Conventional PCR Molecular Conventional PCR (2) Isolation Isolation (2) Fingerprint Fingerprint (2) Fingerprint (3) Morphological
Method: Extraction	
Reference of the test description	
Other information	
Other details on the test	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) 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. 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.
Method: Molecular Extraction DNA RNA	
Reference of the test description	
Other information	
Other details on the test	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) 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. 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.
Method: Molecular Conventional PCR	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	yes
EPPO Diagnostic Protocol name	PM 7/120 Pseudomonas syringae pv. actinidiae (version 1)
Name of the test	Duplex PCR (according to Gallelli et al., 2011a)
As or adapted from an IPPC diagnostic protocol	no
Other information	
Reaction type	Duplex
Method: Molecular Conventional PCR (2)	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	yes
EPPO Diagnostic Protocol name	PM 7/120 Pseudomonas syringae pv. actinidiae (version 1)
Name of the test	PCR targeting the 16S-23S rDNA ITS regions (according to Rees-George et al., 2010)
As or adapted from an IPPC diagnostic protocol	no
Other information	

Reaction type	Simplex
Method: Isolation	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	yes
EPPO Diagnostic Protocol name	PM 7/120 Pseudomonas syringae pv. actinidiae (version 1)
Name of the test	Isolation on modified Nutrient Sucrose Agar (NSA)
Other information	
Other details on the test	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)
Method: Isolation (2)	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	yes
EPPO Diagnostic Protocol name	PM 7/120 Pseudomonas syringae pv. actinidiae (version 1)
Name of the test	Isolation on modified King’s B medium (KB)
Other information	
Other details on the test	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)
Method: Fingerprint	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	yes
EPPO Diagnostic Protocol name	PM 7/120 Pseudomonas syringae pv. actinidiae (version 1)
Name of the test	Rep-PCR BOX according to PM7/100
Other information	
Other details on the test	Repetitive-PCR fingerprinting (rep-PCR), using the BOX, REP, ERIC primers according to Louws et al. (1994) and following Ferrante and Scortichini (2009; 2010).
Method: Fingerprint (2)	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	yes
EPPO Diagnostic Protocol name	PM 7/120 Pseudomonas syringae pv. actinidiae (version 1)

Name of the test	Rep-PCR REP according to PM7/100
Other information	
Other details on the test	Repetitive-PCR fingerprinting (rep-PCR), using the BOX, REP, ERIC primers according to Louws et al. (1994) and following Ferrante and Scortichini (2009; 2010).
Method: Fingerprint (3)	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	yes
EPPO Diagnostic Protocol name	PM 7/120 Pseudomonas syringae pv. actinidiae (version 1)
Name of the test	Rep-PCR ERIC according to PM7/100
Other information	
Other details on the test	Repetitive-PCR fingerprinting (rep-PCR), using the BOX, REP, ERIC primers according to Louws et al. (1994) and following Ferrante and Scortichini (2009; 2010).
Method: Morphological	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	yes
EPPO Diagnostic Protocol name	PM 7/120 Pseudomonas syringae pv. actinidiae (version 1)
Name of the test	Morphological identification
Other information	
Other details on the test	Levan positive morphology on NSA medium, absence of fluorescence on KB medium
Are the performance characteristics included in the EPPO diagnostic protocol?	**yes**
Performance Criteria :	
Organism 1.:	**Pseudomonas syringae pv. actinidiae(PSDMAK)**
Analytical sensitivity	
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)
Diagnostic sensitivity	
Proportion of infected/infested samples tested positive compared to results from the	Isolation on modified NSA semi-selective media: 79% Isolation on modified KB semi-selective media:

standard test, see appendix 2 of PM 7/98	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%
Standard test(s)	Isolation + duplex-PCR (Gallelli et al., 2011a) + simplex PCR (Rees-George et al., 2010) + rep-PCR (Louws et al., 1994)
Analytical specificity - inclusivity	
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)
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
Analytical specificity - exclusivity	
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)
Specificity value	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. 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
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 test(s)	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 %
Test performance study	
Test performance study?	yes
Brief details of the test performance study and its output.It available, 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
The following complementary files are available online:	• The Italian inter-laboratory study on the detection of Pseudomonas syringae pv. actinidiae

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