

**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.

<b>Laboratory contact details</b>	Council for Agricultural Research and Economics– Research Centre for Plant Protection and Certification Via Carlo Giuseppe Bertero, 22, 00156 Rome, Italy
<b>Short description of the test</b>	Detection of <i>Pseudomonas syringae</i> pv. <i>actinidiae</i> by isolation and PCR assays in plant material and bacterial cultures
<b>Date, reference of the validation report</b>	2014-06-05 - Loreti et al., 2014 - Inter-laboratory ring test for the detection of <i>Pseudomonas syringae</i> pv. <i>actinidiae</i> in <i>Actinidia</i> spp.
<b>Validation process according to EPPO Standard PM7/98?</b>	yes
<b>Is the lab accredited for this test?</b>	no
<b>Was the validated data generated in the framework of a project?</b>	Other_project
<b>If yes, please specify</b>	MIPAAF Projects: STRATECO and OIGA-ACTINIDIA, n. 247; and by a Lazio and Emilia-Romagna Regional Project
<b>Description of the test</b>	
<b>Organism(s)</b>	<i>Pseudomonas syringae</i> pv. <i>actinidiae</i> (PSDMAK)
<b>Detection / identification</b>	detection
<b>Method(s)</b>	Extraction Molecular Extraction DNA RNA Molecular Conventional PCR Molecular Conventional PCR (2) Isolation Isolation (2) Fingerprint Fingerprint (2) Fingerprint (3) Morphological
<b>Method: Extraction</b>	
<b>Reference of the test description</b>	
<b>Other information</b>	
<b>Other details on the test</b>	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 <i>Pseudomonas syringae</i> pv. <i>actinidiae</i> , causal agent of bacterial canker of kiwifruit, from symptomless fruits, twigs, and from pollen. <i>Phytopathologia Mediterranea</i> 50, 473-483. Vanneste J.L., D. Giovanardi, J. Yu, D.A. Cornish, C. Kay, F. Spinelli and E. Stefani, 2011. Detection of <i>Pseudomonas syringae</i> pv. <i>actinidiae</i> in pollen samples. <i>New Zealand Plant Protection</i> 64, 246-251.
<b>Method: Molecular Extraction DNA RNA</b>	
<b>Reference of the test description</b>	
<b>Other information</b>	
<b>Other details on the test</b>	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 <i>Pseudomonas syringae</i> pv. <i>actinidiae</i> , causal agent of bacterial canker of kiwifruit, from symptomless fruits, twigs, and from pollen. <i>Phytopathologia Mediterranea</i> 50, 473-483. Vanneste J.L., D. Giovanardi, J. Yu, D.A. Cornish, C. Kay, F. Spinelli and E. Stefani, 2011. Detection of <i>Pseudomonas syringae</i> pv. <i>actinidiae</i> in pollen samples. <i>New Zealand Plant Protection</i> 64, 246-251.
<b>Method: Molecular Conventional PCR</b>	
<b>Reference of the test description</b>	
<b>As or adapted from an EPPO diagnostic protocol</b>	yes
<b>EPPO Diagnostic Protocol name</b>	PM 7/120 <i>Pseudomonas syringae</i> pv. <i>actinidiae</i> (version 1)
<b>Name of the test</b>	Duplex PCR (according to Gallelli et al., 2011a)
<b>As or adapted from an IPPC diagnostic protocol</b>	no
<b>Other information</b>	
<b>Reaction type</b>	Duplex
<b>Method: Molecular Conventional PCR (2)</b>	
<b>Reference of the test description</b>	
<b>As or adapted from an EPPO diagnostic protocol</b>	yes
<b>EPPO Diagnostic Protocol name</b>	PM 7/120 <i>Pseudomonas syringae</i> pv. <i>actinidiae</i> (version 1)
<b>Name of the test</b>	PCR targeting the 16S-23S rDNA ITS regions (according to Rees-George et al., 2010)
<b>As or adapted from an IPPC diagnostic protocol</b>	no
<b>Other information</b>	

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

<b>Name of the test</b>	Rep-PCR REP according to PM7/100
<b>Other information</b>	
<b>Other details on the test</b>	Repetitive-PCR fingerprinting (rep-PCR), using the BOX, REP, ERIC primers according to Louws et al. (1994) and following Ferrante and Scortichini (2009; 2010).
<b>Method: Fingerprint (3)</b>	
<b>Reference of the test description</b>	
<b>As or adapted from an EPPO diagnostic protocol</b>	yes
<b>EPPO Diagnostic Protocol name</b>	PM 7/120 <i>Pseudomonas syringae</i> pv. <i>actinidiae</i> (version 1)
<b>Name of the test</b>	Rep-PCR ERIC according to PM7/100
<b>Other information</b>	
<b>Other details on the test</b>	Repetitive-PCR fingerprinting (rep-PCR), using the BOX, REP, ERIC primers according to Louws et al. (1994) and following Ferrante and Scortichini (2009; 2010).
<b>Method: Morphological</b>	
<b>Reference of the test description</b>	
<b>As or adapted from an EPPO diagnostic protocol</b>	yes
<b>EPPO Diagnostic Protocol name</b>	PM 7/120 <i>Pseudomonas syringae</i> pv. <i>actinidiae</i> (version 1)
<b>Name of the test</b>	Morphological identification
<b>Other information</b>	
<b>Other details on the test</b>	Levan positive morphology on NSA medium, absence of fluorescence on KB medium
<b>Are the performance characteristics included in the EPPO diagnostic protocol?</b>	<b>yes</b>
<b>Performance Criteria :</b>	
<b>Organism 1.:</b>	<b><i>Pseudomonas syringae</i> pv. <i>actinidiae</i>(PSDMAK)</b>
<b>Analytical sensitivity</b>	
<b>What is smallest amount of target that can be detected reliably?</b>	Isolation on modified NSA: 10 <sup>3</sup> CFU/ml pollen ( <i>A. chinensis</i> ) Duplex PCR and simplex-PCR (primerF1/R2) of DNA purified from pollen (following Gallelli et al., 2011a) : 10 <sup>3</sup> CFU/ml (source: Gallelli et al., 2011a) Duplex-PCR of <i>Psa</i> bacterial suspension: 2x10 CFU/PCR reaction Duplex-PCR of genomic DNA: 0.5 pg/PCR reaction (source: Gallelli et al., 2011a)
<b>Diagnostic sensitivity</b>	
<b>Proportion of infected/infested samples tested positive compared to results from the</b>	Isolation on modified NSA semi-selective media: 79% Isolation on modified KB semi-selective media:

<b>standard test, see appendix 2 of PM 7/98</b>	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%
<b>Standard test(s)</b>	Isolation + duplex-PCR (Gallelli et al., 2011a) + simplex PCR (Rees-George et al., 2010) + rep-PCR (Louws et al., 1994)
<b>Analytical specificity - inclusivity</b>	
<b>Number of strains/populations of target organisms tested</b>	Four Psa bacterial strains tested from seven laboratoty partecipating to the ITL (see Loreti et al., 2014. Phytopathologia Mediterranea 53, 1, 159-167)
<b>Specificity value</b>	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
<b>Analytical specificity - exclusivity</b>	
<b>Number of non-target organisms tested</b>	Nine non target bacterial strains tested from seven laboratoty partecipating to the ITL (see Loreti et al., 2014. Phytopathologia Mediterranea 53, 1, 159-167)
<b>Specificity value</b>	duplex-PCR (Gallelli et al., 2011a): no cross reaction. simplex-PCR (Rees-George et al., 2010): cross reacts with Pseudomonas syringae pv. tomato, Pseudomonas syringae pv. theae, P. avellanae. rep-PCR (primer ERIC): cross reacts 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
<b>Diagnostic Specificity</b>	
<b>Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test</b>	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%
<b>Specify the test(s)</b>	Isolation + duplex-PCR (Gallelli et al., 2011a) + simplex PCR (Rees-George et al., 2010) + rep-PCR (Louws et al., 1994)
<b>Reproducibility</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	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 %
<b>Test performance study</b>	
<b>Test performance study?</b>	yes
<b>Brief details of the test performance study and its output.It available, link to published article/report</b>	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 <i>Pseudomonas syringae</i> pv. <i>actinidiae</i> . <i>Phytopathologia Mediterranea</i> 53, 1, 159-167
The following complementary files are available online:	
	<ul style="list-style-type: none"> <li>• <a href="#">The Italian inter-laboratory study on the detection of <i>Pseudomonas syringae</i> pv. <i>actinidiae</i></a></li> </ul>

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