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	National Reference Centre, National Plant Protection Organization P.O. Box 9102, 6700 HC Wageningen, Netherlands
Short description of the test	Duplex-PCR tests for the identification X. euvesicatoria, X. vesicatoria, X. gardneri and X. perforans.
Date, reference of the validation report	2012-04-17 - MOVA nummer: 2011.molbio.004
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?	
If yes, please specify	
Description of the test	
Organism(s)	Xanthomonas hortorum pv. gardneri(XANTGA) Xanthomonas euvesicatoria pv. perforans(XANTPF) Xanthomonas vesicatoria(XANTVE) Xanthomonas euvesicatoria pv. euvesicatoria(XANTEU)
Detection / identification	identification
Matrix(ces) tested	Pure culture bacterial colony material
Plant species tested	
Method(s)	Molecular Conventional PCR
Method: Molecular Conventional PCR	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	yes
New test being considered for inclusion in the next version of the EPPO diagnostic protocol?	
EPPO Diagnostic Protocol name	PM 7/110 Xanthomonas spp. (Xanthomonas euvesicatoria, Xanthomonas gardneri, Xanthomonas perforans, Xanthomonas vesicatoria) causing bacterial spot of tomato and sweet pepper (version 1)
Name of the test	Conventional PCR (according to Koenraadt et al.,

	2009)
As or adapted from an IPPC diagnostic protocol	
Is the test modified compared to the reference test	
Kit	
Is a kit used	
Other information	
Reaction type	Duplex
Other details on the test	Two conventional duplex-PCR tests
Are the performance characteristics included in the EPPO diagnostic protocol?	**no**
Performance Criteria :	
Organism 1.:	**Xanthomonas hortorum pv. gardneri(XANTGA)**
Analytical sensitivity	
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
Diagnostic sensitivity	
Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98	
Standard test(s)	Sequence analysis of the AvrBs2 gene that has been used on reference material on basis of the Q-BOL protocol.
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	53 isolates of Xcv
Specificity value	
Analytical specificity - exclusivity	
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
Specificity value	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.
Cross reacts with	Pseudomonas syringae pv. tomato Pseudomonas corrugata
Diagnostic Specificity	

Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	
Specify the test(s)	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 agreement for a given level of the pest (see PM 7/98)	93%
Organism 2.:	**Xanthomonas euvesicatoria pv. perforans(XANTPF)**
Analytical sensitivity	
What is smallest amount of target that can be detected reliably?	for X. perforans 1,9x10^7 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	
Standard test(s)	Sequence analysis of the AvrBs2 gene that has been used on reference material on basis of the Q-BOL protocol.
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	53 isolates of Xcv
Specificity value	
Analytical specificity - exclusivity	
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
Specificity value	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.
Cross reacts with	Pseudomonas syringae pv. tomato Pseudomonas corrugata
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	

Specify the test(s)	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 agreement for a given level of the pest (see PM 7/98)	93%
Organism 3.:	**Xanthomonas vesicatoria(XANTVE)**
Analytical sensitivity	
What is smallest amount of target that can be detected reliably?	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	
Standard test(s)	Sequence analysis of the AvrBs2 gene that has been used on reference material on basis of the Q-BOL protocol.
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	53 isolates of Xcv
Specificity value	
Analytical specificity - exclusivity	
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
Specificity value	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.
Cross reacts with	Pseudomonas syringae pv. tomato Pseudomonas corrugata
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	
Specify the test(s)	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 agreement for a given level of the pest (see PM 7/98)	93%
Organism 4.:	**Xanthomonas euvesicatoria pv. euvesicatoria(XANTEU)**
Analytical sensitivity	
What is smallest amount of target that can be detected reliably?	for X. euvesicatoria 5,5x10^5 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	
Standard test(s)	Sequence analysis of the AvrBs2 gene that has been used on reference material on basis of the Q-BOL protocol.
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	53 isolates of Xcv
Specificity value	
Analytical specificity - exclusivity	
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
Specificity value	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.
Cross reacts with	Pseudomonas syringae pv. tomato Pseudomonas corrugata
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	
Specify the test(s)	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	100%

given level of the pest (see PM 7/98)	
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	93%
Test performance study	
Test performance study?	no
Brief details of the test performance study and its output.It available, link to published article/report	
Other information	
Any other information considered useful	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:	• 2011.molbio.004 full validation report

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