## 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	Molecular detection of Xanthomonas citri pv. citri (lemon leaves and orange fruits) and Xanthomonas citri pv. aurantifolii (lime fruits) by cPCR and real- time PCR	
Date, reference of the validation report	2022-09-28 - Report_EURL-PT-2021-01-Xc_IV	
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?	EURL	
Description of the test		
Organism(s)	Xanthomonas citri pv. aurantifolii (XANTAU) Xanthomonas citri pv. citri (XANTCI)	
Detection / identification	detection	
Method(s)	Molecular Conventional PCR Molecular Conventional PCR (2) Molecular real time PCR Molecular real time PCR (2) Molecular real time PCR (3)	
Method: Molecular Conventional PCR		
Reference of the test description		
As or adapted from an EPPO diagnostic protocol	no	
New test being considered for inclusion in the next version of the EPPO diagnostic protocol?	yes	
As or adapted from an IPPC diagnostic protocol	no	
Reference of the test	Mavrodieva et al., 2004	
Kit		
Is a kit used	no	
Other information		

Reaction type	Simplex	
Other details on the test	The targets of this test are both Xanthomonas citri pv. citri and Xanthomonas citri pv. aurantifolii.	
Method: Molecular Conventional PCR (2)		
Reference of the test description		
As or adapted from an EPPO diagnostic protocol	no	
New test being considered for inclusion in the next version of the EPPO diagnostic protocol?	yes	
As or adapted from an IPPC diagnostic protocol	no	
Reference of the test	Robène et al., 2020	
Kit		
Is a kit used	no	
Other information		
Reaction type	Simplex	
Other details on the test	The target of this test is Xanthomonas citri pv. citri.	
Method: Molecular real time PCR		
Reference of the test description		
As or adapted from an EPPO diagnostic protocol	no	
New test being considered for inclusion in the next version of the EPPO diagnostic protocol?	yes	
As or adapted from an IPPC diagnostic protocol	yes	
IPPC diagnostic Protocol name	(version )	
Name of the test	Cubero and Graham, 2005	
Kit		
Is a kit used	no	
Other information		
Reaction type	Simplex - Probe	
Other details on the test	TaqMan. The target of this test is Xanthomonas citri pv. citri.	
Method: Molecular real time PCR (2)		
Reference of the test description		
As or adapted from an EPPO diagnostic protocol	no	
New test being considered for inclusion in the next version of the EPPO diagnostic protocol?	yes	
As or adapted from an IPPC diagnostic protocol	no	

Reference of the test	Mavrodieva et al., 2004	
Kit		
Is a kit used	no	
Other information		
Reaction type	Simplex	
Other details on the test	SYBR Green. The targets of this test are both Xanthomonas citri pv. citri and Xanthomonas citri pv. aurantifolii.	
Method: Molecular real time PCR (3)		
Reference of the test description		
As or adapted from an EPPO diagnostic protocol	no	
New test being considered for inclusion in the next version of the EPPO diagnostic protocol?	yes	
As or adapted from an IPPC diagnostic protocol	no	
Reference of the test	Robène et al., 2020	
Kit		
Is a kit used	no	
Other information		
Reaction type	Duplex - Probe	
Other details on the test	TaqMan. The target of this test is Xanthomonas citri pv. citri.	
Other details on the test Performance Criteria :	TaqMan. The target of this test is Xanthomonas citri pv. citri.	
Other details on the test Performance Criteria : Organism 1.:	TaqMan. The target of this test is Xanthomonas citri pv. citri. Xanthomonas citri pv. aurantifolii(XANTAU)	
Other details on the test Performance Criteria : Organism 1.: Diagnostic sensitivity	TaqMan. The target of this test is Xanthomonas citri pv. citri. Xanthomonas citri pv. aurantifolii(XANTAU)	
Other details on the test Performance Criteria : Organism 1.: Diagnostic sensitivity Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98	TaqMan. The target of this test is Xanthomonas citri pv. citri. Xanthomonas citri pv. aurantifolii(XANTAU) - cPCR (Mavrodieva et al., 2004): 100%; - real-time PCR (Mavrodieva et al., 2004): 99%.	
Other details on the test Performance Criteria : Organism 1.: 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)	TaqMan. The target of this test is Xanthomonas citri pv. citri. Xanthomonas citri pv. aurantifolii(XANTAU) - cPCR (Mavrodieva et al., 2004): 100%; - real-time PCR (Mavrodieva et al., 2004): 99%. Comparison with samples of known status.	
Other details on the test Performance Criteria : Organism 1.: 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) Diagnostic Specificity	TaqMan. The target of this test is Xanthomonas citri pv. citri. Xanthomonas citri pv. aurantifolii(XANTAU) - cPCR (Mavrodieva et al., 2004): 100%; - real-time PCR (Mavrodieva et al., 2004): 99%. Comparison with samples of known status.	
Other details on the testPerformance Criteria :Organism 1.:Diagnostic sensitivityProportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98Standard test(s)Diagnostic SpecificityProportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	TaqMan. The target of this test is Xanthomonas citri pv. citri. Xanthomonas citri pv. aurantifolii(XANTAU) - cPCR (Mavrodieva et al., 2004): 100%; - real-time PCR (Mavrodieva et al., 2004): 99%. Comparison with samples of known status. - cPCR (Mavrodieva et al., 2004): 98%; - real-time PCR (Mavrodieva et al., 2004): 98%; - real-time PCR (Mavrodieva et al., 2004): 98%; - real-time	
Other details on the testPerformance Criteria :Organism 1.:Diagnostic sensitivityProportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98Standard test(s)Diagnostic SpecificityProportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard testSpecify the test(s)	TaqMan. The target of this test is Xanthomonas citri pv. citri.Xanthomonas citri pv. aurantifolii(XANTAU)CPCR (Mavrodieva et al., 2004): 100%; - real-time PCR (Mavrodieva et al., 2004): 99%.Comparison with samples of known status cPCR (Mavrodieva et al., 2004): 98%; - real-time PCR (Mavrodieva et al., 2004): 98%; - real-time PCR (Mavrodieva et al., 2004): 98%; - real-time PCR (Mavrodieva et al., 2004): 96%.	
Other details on the testPerformance Criteria :Organism 1.:Diagnostic sensitivityProportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98Standard test(s)Diagnostic SpecificityProportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard testSpecify the test(s)Reproducibility	TaqMan. The target of this test is Xanthomonas citri pv. citri. Xanthomonas citri pv. aurantifolii(XANTAU) - cPCR (Mavrodieva et al., 2004): 100%; - real-time PCR (Mavrodieva et al., 2004): 99%. Comparison with samples of known status. - cPCR (Mavrodieva et al., 2004): 98%; - real-time PCR (Mavrodieva et al., 2004): 98%; - real-time PCR (Mavrodieva et al., 2004): 98%; - real-time PCR (Mavrodieva et al., 2004): 98%; - real-time	
Other details on the testPerformance Criteria :Organism 1.:Diagnostic sensitivityProportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98Standard test(s)Diagnostic SpecificityProportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard testSpecify the test(s)ReproducibilityProvide the calculated % of agreement for a given level of the pest (see PM 7/98)	TaqMan. The target of this test is Xanthomonas citri pv. citri. Xanthomonas citri pv. aurantifolii(XANTAU) - cPCR (Mavrodieva et al., 2004): 100%; - real-time PCR (Mavrodieva et al., 2004): 99%. Comparison with samples of known status. - cPCR (Mavrodieva et al., 2004): 98%; - real-time PCR (Mavrodieva et al., 2004): 98%; - real-time PCR (Mavrodieva et al., 2004): 98%.	
Other details on the testPerformance Criteria :Organism 1.:Diagnostic sensitivityProportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98Standard test(s)Diagnostic SpecificityProportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard testSpecify the test(s)ReproducibilityProvide the calculated % of agreement for a given level of the pest (see PM 7/98)Organism 2.:	TaqMan. The target of this test is Xanthomonas citri pv. citri.Xanthomonas citri pv. aurantifolii(XANTAU)Xanthomonas citri pv. aurantifolii(XANTAU)- cPCR (Mavrodieva et al., 2004): 100%; - real-time PCR (Mavrodieva et al., 2004): 99%.Comparison with samples of known status cPCR (Mavrodieva et al., 2004): 98%; - real-time PCR (Mavrodieva et al., 2004): 96%.Comparison with samples of known status cPCR (Mavrodieva et al., 2004): 96%.Comparison with samples of known status.Comparison with samples of known status.Comparison with samples of known status.Canthomonas citri pv. citri(XANTCI)	
Other details on the testPerformance Criteria :Organism 1.:Diagnostic sensitivityProportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98Standard test(s)Diagnostic SpecificityProportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard testSpecify the test(s)ReproducibilityProvide the calculated % of agreement for a given level of the pest (see PM 7/98)Organism 2.:Diagnostic sensitivity	TaqMan. The target of this test is Xanthomonas citri pv. citri. Xanthomonas citri pv. aurantifolii(XANTAU) - cPCR (Mavrodieva et al., 2004): 100%; - real-time PCR (Mavrodieva et al., 2004): 99%. Comparison with samples of known status. - cPCR (Mavrodieva et al., 2004): 98%; - real-time PCR (Mavrodieva et al., 2004): 98%; - real-time PCR (Mavrodieva et al., 2004): 96%. Comparison with samples of known status.	

tested positive compared to results from the standard test, see appendix 2 of PM 7/98	(Robène et al., 2020): 100% - real-time PCR (Cubero and Graham, 2005): 98%	
Standard test(s)	Comparison with samples of known status.	
Diagnostic Specificity		
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	- cPCR (Robène et al., 2020): 100% - real-time PCR (Robène et al., 2020): 86% - real-time PCR (Cubero and Graham, 2005): 81%	
Specify the test(s)	Comparison with samples of known status.	
Reproducibility		
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	<ul> <li>- cPCR (Robène et al., 2020): 100% - real-time PCR</li> <li>(Robène et al., 2020): 94% - real-time PCR (Cubero and Graham, 2005): 89%</li> </ul>	
Test performance study		
Test performance study?	no	
Brief details of the test performance study and its output.It available, link to published article/report	Proficiency test organised in the framework of EURL activity. This activity allowed to obtain informations on the proficiency of tests used by the participants. Number of participants for each test: - cPCR Robène et al., 2020: 17 participants; - cPCR Mavrodieva et al., 2004: 14 participants; - real-time PCR Mavrodieva et al., 2004: 16 participants; - real- time PCR Robène et al., 2020: 15 participants; - real-time PCR Cubero and Graham, 2005: 14 participants;	
Other information		
Any other information considered useful	Each participant received a panel samples represented by: DNA extracts of three plant matrices added with bacterial DNA at known concentration following the scheme reported in the attached file (see test items panel).	
The following complementary files are available online:	EURL_presentation Xcc_Xca	

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