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
Matrix(ces) tested	Fruits, Leaves
Plant species tested	Citrus x aurantiifolia, Citrus x aurantium var. sinensis, Citrus x limon
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.
Performance Criteria :	
Organism 1.:	**Xanthomonas citri pv. aurantifolii(XANTAU)**
Diagnostic sensitivity	
Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98	- cPCR (Mavrodieva et al., 2004): 100%; - real-time PCR (Mavrodieva et al., 2004): 99%.
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 (Mavrodieva et al., 2004): 98%; - real-time PCR (Mavrodieva et al., 2004): 96%.
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)	- cPCR (Mavrodieva et al., 2004): 99%; - real-time PCR (Mavrodieva et al., 2004): 98%.

Organism 2.:	**Xanthomonas citri pv. citri(XANTCI)**
Diagnostic sensitivity	
Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98	- cPCR (Robène et al., 2020): 100% - real-time PCR (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)	- cPCR (Robène et al., 2020): 100% - real-time PCR (Robène et al., 2020): 94% - real-time PCR (Cubero and Graham, 2005): 89%
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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