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	Influence of DNA extraction methods in the detection of Xanthomonas citri pv. citri in leaves/ fruits of Citrus limon, citrus sinensis
Date, reference of the validation report	2021-05-25 - 2019_2020_ANNEXES_TECHNICAL_RE PORT_Xc_EURL_Bacteria
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
If yes, please specify	EURL activity 2019-2020
Description of the test	
Organism(s)	Xanthomonas citri pv. citri (XANTCI)
Detection / identification	detection
Method(s)	Extraction Molecular Extraction DNA RNA Molecular Extraction DNA RNA (2) Molecular Extraction DNA RNA (3) Molecular Extraction DNA RNA (4) Molecular real time PCR
Method: Extraction	
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?	yes
EPPO Diagnostic Protocol name	PM 7/044 Xanthomonas axonopodis pv. citri (version 1)
As or adapted from an IPPC diagnostic	yes

ISPM 27 Annex 06 DP 06: Xanthomonas citri subsp.

Citri (version 2014)

protocol

IPPC diagnostic Protocol name

Is the test modified compared to the reference test	no	
Other information		
Method: Molecular Extraction DNA RNA		
Reference of the test description		
As or adapted from an EPPO diagnostic protocol	no	
As or adapted from an IPPC diagnostic protocol	no	
Kit		
Is a kit used	no	
Other information		
Other details on the test	C-TAB -based protocol (following the procedure described in PM 7/24 (4) Xylella fastidiosa). Final DNA resuspension in 130 ul.	
Method: Molecular Extraction DNA RNA (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	
Is the test modified compared to the reference test	no	
Kit		
Is a kit used	yes	
Manufacturer name	QIAGEN	
Specify the kit used	DNeasy Plant Mini Kit	
Kit used following the manufacturer's instructions?	yes	
Other information		
Method: Molecular Extraction DNA RNA (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	
Kit		
Is a kit used	yes	

Manufacturer name	QIAGEN	
Specify the kit used	DNeasy mericon Food Kit	
Kit used following the manufacturer's instructions?	yes final DNA elution in 70 ul	
Other information		
Method: Molecular Extraction DNA RNA (4)		
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	
Kit		
Is a kit used	yes	
Manufacturer name	BIONOBILE	
Specify the kit used	QuickPick [™] SML Plant DNA	
Kit used following the manufacturer's instructions?	yes manual device	
Other information		
Method: Molecular real time PCR		
Reference of the test description		
As or adapted from an EPPO diagnostic protocol	no	
As or adapted from an IPPC diagnostic protocol	yes	
IPPC diagnostic Protocol name	ISPM 27 Annex 06 DP 06: Xanthomonas citri subsp. Citri (version 2014)	
Name of the test	Cubero and Graham, 2005	
Is the test modified compared to the reference test	no	
Other information		
Reaction type	Simplex - Probe	
Other details on the test	real-time PCR, TaqMan system	
Performance Criteria :		
Organism 1.:	Xanthomonas citri pv. citri(XANTCI)	
Analytical sensitivity		
What is smallest amount of target that can be detected reliably?	10 ² -10 ³ cfu/ml depending on the combination of DNA extraction Kit/plant matrices (see attached file, Table 6).	
Diagnostic sensitivity		
Proportion of infected/infested samples tested positive compared to results from the	100% for all DNA extraction test	

standard test, see appendix 2 of PM 7/98		
Standard test(s)	Comparison with samples at known status	
Diagnostic Specificity		
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	100% for all DNA extraction test	
Specify the test(s)	Comparison with samples at known status	
Test performance study		
Test performance study?	yes	
Brief details of the test performance study and its output.It available, link to published article/report	Test performance study performed as intralaboratory activity within EURL-BAC activity	
The following complementary files are available online:	 Intra-Laboratory Evaluation of DNA Extraction Methods and Assessment of a Droplet Digital PCR for the Detection of Xanthomonas citri pv. citri on Different Citrus Species 	

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