

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 <i>Xanthomonas citri</i> pv. <i>citri</i> in leaves/ fruits of <i>Citrus limon</i> , <i>citrus sinensis</i>
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)	<i>Xanthomonas citri</i> pv. <i>citri</i> (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 <i>Xanthomonas axonopodis</i> pv. <i>citri</i> (version 1)
As or adapted from an IPPC diagnostic protocol	yes
IPPC diagnostic Protocol name	ISPM 27 Annex 06 DP 06: <i>Xanthomonas citri</i> subsp. <i>Citri</i> (version 2014)

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
<u>Diagnostic Specificity</u>	
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:	<ul style="list-style-type: none"> • 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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