

**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.

<b>Laboratory contact details</b>	Council for Agricultural Research and Economics- Research Centre for Plant Protection and Certification Via Carlo Giuseppe Bertero, 22, 00156 Rome, Italy
<b>Short description of the test</b>	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>
<b>Date, reference of the validation report</b>	2021-05-25 - 2019_2020_ANNEXES_TECHNICAL_RE PORT_Xc_EURL_Bacteria
<b>Validation process according to EPPO Standard PM7/98?</b>	yes
<b>Is the lab accredited for this test?</b>	no
<b>Was the validated data generated in the framework of a project?</b>	EURL
<b>If yes, please specify</b>	EURL activity 2019-2020
<b>Description of the test</b>	
<b>Organism(s)</b>	<i>Xanthomonas citri</i> pv. <i>citri</i> (XANTCI)
<b>Detection / identification</b>	detection
<b>Matrix(ces) tested</b>	Fruits, Leaves Orange fruit, lemon fruit and leaf
<b>Plant species tested</b>	<i>Citrus x aurantium</i> var. <i>sinensis</i> , <i>Citrus x limon</i>
<b>Method(s)</b>	Extraction Molecular Extraction DNA RNA Molecular Extraction DNA RNA (2) Molecular Extraction DNA RNA (3) Molecular Extraction DNA RNA (4) Molecular real time PCR
<b>Method: Extraction</b>	
<b>Reference of the test description</b>	
<b>As or adapted from an EPPO diagnostic protocol</b>	yes
<b>New test being considered for inclusion in the next version of the EPPO diagnostic protocol?</b>	yes
<b>EPPO Diagnostic Protocol name</b>	PM 7/044 <i>Xanthomonas axonopodis</i> pv. <i>citri</i> (version 1)
<b>As or adapted from an IPPC diagnostic</b>	yes

<b>protocol</b>	
<b>IPPC diagnostic Protocol name</b>	ISPM 27 Annex 06 DP 06: Xanthomonas citri subsp. Citri (version 2014)
<b>Is the test modified compared to the reference test</b>	no
<b>Other information</b>	
<b>Method: Molecular Extraction DNA RNA</b>	
<b>Reference of the test description</b>	
<b>As or adapted from an EPPO diagnostic protocol</b>	no
<b>As or adapted from an IPPC diagnostic protocol</b>	no
<b>Kit</b>	
<b>Is a kit used</b>	no
<b>Other information</b>	
<b>Other details on the test</b>	C-TAB -based protocol (following the procedure described in PM 7/24 (4) Xylella fastidiosa). Final DNA resuspension in 130 ul.
<b>Method: Molecular Extraction DNA RNA (2)</b>	
<b>Reference of the test description</b>	
<b>As or adapted from an EPPO diagnostic protocol</b>	no
<b>New test being considered for inclusion in the next version of the EPPO diagnostic protocol?</b>	yes
<b>As or adapted from an IPPC diagnostic protocol</b>	no
<b>Is the test modified compared to the reference test</b>	no
<b>Kit</b>	
<b>Is a kit used</b>	yes
<b>Manufacturer name</b>	QIAGEN
<b>Specify the kit used</b>	DNeasy Plant Mini Kit
<b>Kit used following the manufacturer's instructions?</b>	yes
<b>Other information</b>	
<b>Method: Molecular Extraction DNA RNA (3)</b>	
<b>Reference of the test description</b>	
<b>As or adapted from an EPPO diagnostic protocol</b>	no
<b>New test being considered for inclusion in the next version of the EPPO diagnostic protocol?</b>	yes
<b>As or adapted from an IPPC diagnostic protocol</b>	no

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

<b>Diagnostic sensitivity</b>	
<b>Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98</b>	100% for all DNA extraction test
<b>Standard test(s)</b>	Comparison with samples at known status
<b>Diagnostic Specificity</b>	
<b>Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test</b>	100% for all DNA extraction test
<b>Specify the test(s)</b>	Comparison with samples at known status
<b>Test performance study</b>	
<b>Test performance study?</b>	yes
<b>Brief details of the test performance study and its output. It available, link to published article/report</b>	Test performance study performed as intralaboratory activity within EURL-BAC activity
The following complementary files are available online:	<ul style="list-style-type: none"> <li>• <a href="#">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</a></li> </ul>

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