

**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>	Molecular detection of <i>Xanthomonas citri</i> pv. <i>citri</i> (lemon leaves and orange fruits) and <i>Xanthomonas citri</i> pv. <i>aurantifolii</i> (lime fruits) by cPCR and real-time PCR
<b>Date, reference of the validation report</b>	2022-09-28 - Report_EURL-PT-2021-01-Xc_IV
<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>Description of the test</b>	
<b>Organism(s)</b>	<i>Xanthomonas citri</i> pv. <i>aurantifolii</i> (XANTAU) <i>Xanthomonas citri</i> pv. <i>citri</i> (XANTCI)
<b>Detection / identification</b>	detection
<b>Method(s)</b>	Molecular Conventional PCR Molecular Conventional PCR (2) Molecular real time PCR Molecular real time PCR (2) Molecular real time PCR (3)
<b>Method: Molecular Conventional PCR</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>Reference of the test</b>	Mavrodieva et al., 2004
<b>Kit</b>	
<b>Is a kit used</b>	no
<b>Other information</b>	

<b>Reaction type</b>	Simplex
<b>Other details on the test</b>	The targets of this test are both <i>Xanthomonas citri</i> pv. <i>citri</i> and <i>Xanthomonas citri</i> pv. <i>aurantifolii</i> .
<b>Method: Molecular Conventional PCR (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>Reference of the test</b>	Robène et al., 2020
<b>Kit</b>	
<b>Is a kit used</b>	no
<b>Other information</b>	
<b>Reaction type</b>	Simplex
<b>Other details on the test</b>	The target of this test is <i>Xanthomonas citri</i> pv. <i>citri</i> .
<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>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>	yes
<b>IPPC diagnostic Protocol name</b>	(version )
<b>Name of the test</b>	Cubero and Graham, 2005
<b>Kit</b>	
<b>Is a kit used</b>	no
<b>Other information</b>	
<b>Reaction type</b>	Simplex - Probe
<b>Other details on the test</b>	TaqMan. The target of this test is <i>Xanthomonas citri</i> pv. <i>citri</i> .
<b>Method: Molecular real time PCR (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>Reference of the test</b>	Mavrodieva et al., 2004
<b>Kit</b>	
<b>Is a kit used</b>	no
<b>Other information</b>	
<b>Reaction type</b>	Simplex
<b>Other details on the test</b>	SYBR Green. The targets of this test are both <i>Xanthomonas citri</i> pv. <i>citri</i> and <i>Xanthomonas citri</i> pv. <i>aurantifolii</i> .
<b>Method: Molecular real time PCR (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>Reference of the test</b>	Robène et al., 2020
<b>Kit</b>	
<b>Is a kit used</b>	no
<b>Other information</b>	
<b>Reaction type</b>	Duplex - Probe
<b>Other details on the test</b>	TaqMan. The target of this test is <i>Xanthomonas citri</i> pv. <i>citri</i> .
<b>Performance Criteria :</b>	
<b>Organism 1.:</b>	<b><i>Xanthomonas citri</i> pv. <i>aurantifolii</i>(XANTAU)</b>
<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>	- cPCR (Mavrodieva et al., 2004): 100%; - real-time PCR (Mavrodieva et al., 2004): 99%.
<b>Standard test(s)</b>	Comparison with samples of known status.
<b>Diagnostic Specificity</b>	
<b>Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test</b>	- cPCR (Mavrodieva et al., 2004): 98%; - real-time PCR (Mavrodieva et al., 2004): 96%.
<b>Specify the test(s)</b>	Comparison with samples of known status.
<b>Reproducibility</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	- cPCR (Mavrodieva et al., 2004): 99%; - real-time PCR (Mavrodieva et al., 2004): 98%.
<b>Organism 2.:</b>	<b><i>Xanthomonas citri</i> pv. <i>citri</i>(XANTCI)</b>
<b>Diagnostic sensitivity</b>	
<b>Proportion of infected/infested samples</b>	- cPCR (Robène et al., 2020): 100% - real-time PCR

<b>tested positive compared to results from the standard test, see appendix 2 of PM 7/98</b>	(Robène et al., 2020): 100% - real-time PCR (Cubero and Graham, 2005): 98%
<b>Standard test(s)</b>	Comparison with samples of known status.
<b>Diagnostic Specificity</b>	
<b>Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test</b>	- cPCR (Robène et al., 2020): 100% - real-time PCR (Robène et al., 2020): 86% - real-time PCR (Cubero and Graham, 2005): 81%
<b>Specify the test(s)</b>	Comparison with samples of known status.
<b>Reproducibility</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	- cPCR (Robène et al., 2020): 100% - real-time PCR (Robène et al., 2020): 94% - real-time PCR (Cubero and Graham, 2005): 89%
<b>Test performance study</b>	
<b>Test performance study?</b>	no
<b>Brief details of the test performance study and its output. It available, link to published article/report</b>	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;
<b>Other information</b>	
<b>Any other information considered useful</b>	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).
<b>The following complementary files are available online:</b>	<ul style="list-style-type: none"> <li>• <a href="#">EURL_presentation Xcc_Xca</a></li> </ul>

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