

**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.

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| <b>Laboratory contact details</b>   | Council for Agricultural Research and Economics–<br>Research Centre for Plant Protection and<br>Certification<br>Via Carlo Giuseppe Bertero, 22, 00156 Rome, Italy                |
| <b>Short description of the test</b>  | Influence of DNA extraction methods in the<br>detection of <i>Xanthomonas citri</i> pv. <i>citri</i> in leaves/<br>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<br>PORT_Xc_EURL_Bacteria  |
| <b>Validation process according to EPPO<br/>Standard PM7/98?</b>  | yes   |
| <b>Is the lab accredited for this test?</b>   | no  |
| <b>Was the validated data generated in the<br/>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>Method(s)</b>  | Extraction<br>Molecular Extraction DNA RNA<br>Molecular Extraction DNA RNA (2)<br>Molecular Extraction DNA RNA (3)<br>Molecular Extraction DNA RNA (4)<br>Molecular real time PCR |
| <b>Method: Extraction</b>   |   |
| <b>Reference of the test description</b>  |   |
| <b>As or adapted from an EPPO diagnostic<br/>protocol</b>   | yes   |
| <b>New test being considered for inclusion in the<br/>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><br>(version 1)  |
| <b>As or adapted from an IPPC diagnostic<br/>protocol</b>   | yes   |
| <b>IPPC diagnostic Protocol name</b>  | ISPM 27 Annex 06 DP 06: <i>Xanthomonas citri</i> subsp.<br><i>Citri</i> (version 2014)  |

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| Is the test modified compared to the reference test  | no   |
| <b>Other information</b>   |  |
| <b>Method: Molecular Extraction DNA RNA</b>  |  |
| <b>Reference of the test description</b>   |  |
| As or adapted from an EPPO diagnostic protocol   | no   |
| As or adapted from an IPPC diagnostic protocol   | no   |
| <b>Kit</b>   |  |
| Is a kit used  | no   |
| <b>Other information</b>   |  |
| 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. |
| <b>Method: Molecular Extraction DNA RNA (2)</b>  |  |
| <b>Reference of the test description</b>   |  |
| 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   |
| <b>Kit</b>   |  |
| Is a kit used  | yes  |
| Manufacturer name  | QIAGEN   |
| Specify the kit used   | DNeasy Plant Mini Kit  |
| Kit used following the manufacturer's instructions?  | yes  |
| <b>Other information</b>   |  |
| <b>Method: Molecular Extraction DNA RNA (3)</b>  |  |
| <b>Reference of the test description</b>   |  |
| 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   |
| <b>Kit</b>   |  |
| Is a kit used  | yes  |

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| <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 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</b>         | 100% for all DNA extraction test  |

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| <b>standard test, see appendix 2 of PM 7/98</b>   |  |
| <b>Standard test(s)</b>   | Comparison with samples at known status  |
| <b><u>Diagnostic Specificity</u></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  |
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| 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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