

**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>	Anses Plant Health Laboratory - Pests and Tropical Pathogens Unit Pôle de Protection des Plantes, 7 Chemin de l'IRAT, 97410 Saint Pierre, France
<b>Short description of the test</b>	Detection of <i>Xanthomonas axonopodis</i> pv. <i>dieffenbachiae</i> by nested-PCR in leaves and pure culture
<b>Date, reference of the validation report</b>	2012-03-01 - Inter-laboratory ring test : <i>Xanthomonas axonopodis</i> pv. <i>dieffenbachiae</i> in Anthurium (Report Xad01-version 2)
<b>Validation process according to EPPO Standard PM7/98?</b>	no
<b>Is the lab accredited for this test?</b>	no
<b>Was the validated data generated in the framework of a project?</b>	
<b>Description of the test</b>	
<b>Organism(s)</b>	<i>Xanthomonas axonopodis</i> pv. <i>dieffenbachiae</i> (XANTDF)
<b>Detection / identification</b>	detection
<b>Method(s)</b>	Extraction Molecular Conventional PCR Molecular PCR-RFLP
<b>Method: Extraction</b>	
<b>Reference of the test description</b>	
<b>As or adapted from an EPPO diagnostic protocol</b>	yes
<b>EPPO Diagnostic Protocol name</b>	PM 7/023 <i>Xanthomonas axonopodis</i> pv. <i>dieffenbachiae</i> (version 2)
<b>Name of the test</b>	Extraction from symptomatic plant material in PBS buffer (Appendix 1.1)
<b>Other information</b>	
<b>Other details on the test</b>	Extraction as in Appendix 1 of PM7/23(2)
<b>Method: Molecular Conventional PCR</b>	
<b>Reference of the test description</b>	

<b>As or adapted from an EPPO diagnostic protocol</b>	yes
<b>EPPO Diagnostic Protocol name</b>	PM 7/023 Xanthomonas axonopodis pv. dieffenbachiae (version 2)
<b>Name of the test</b>	Nested PCR (Robene-Soustrade et al., 2006)
<b>Other information</b>	
<b>Reaction type</b>	Nested
<b>Other details on the test</b>	Nested-PCR as in Appendix 4 of PM7/23(2)
<b>Method: Molecular PCR-RFLP</b>	
<b>Reference of the test description</b>	
<b>As or adapted from an EPPO diagnostic protocol</b>	yes
<b>EPPO Diagnostic Protocol name</b>	PM 7/023 Xanthomonas axonopodis pv. dieffenbachiae (version 2)
<b>Name of the test</b>	Nested PCR (Robene-Soustrade et al., 2006) + RFLP
<b>Other information</b>	
<b>Reaction type</b>	Nested
<b>Are the performance characteristics included in the EPPO diagnostic protocol?</b>	<b>no</b>
<b>Performance Criteria :</b>	
<b>Organism 1.:</b>	<b>Xanthomonas axonopodis pv. dieffenbachiae(XANTDF)</b>
<b>Analytical sensitivity</b>	
<b>What is smallest amount of target that can be detected reliably?</b>	1x10 <sup>4</sup> CFU.mL-1
<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>	Comparative study : 100% ; Collaborative study : 97.5%
<b>Standard test(s)</b>	Isolation + AGDIA Indirect-ELISA on pure culture (OEPP PM7/23)
<b>Analytical specificity - inclusivity</b>	
<b>Number of strains/populations of target organisms tested</b>	50 (see attached downloadable file Appendix 1)
<b>Specificity value</b>	1
<b>Analytical specificity - exclusivity</b>	
<b>Number of non-target organisms tested</b>	53 (see attached downloadable file Appendix 2)
<b>Specificity value</b>	The restriction step performed after the N-PCR enables to exclude all the tested strains. Without the restriction step, a Xanthomonas axonopodis pv. allii strain and some Xanthomonas campestris pv. syngonii strains can not be excluded.

<b>Diagnostic Specificity</b>	
<b>Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test</b>	Comparative study : 96% ; Collaborative study : 95%
<b>Specify the test(s)</b>	Isolation + AGDIA Indirect-ELISA on pure culture (OEPP PM7/23)
<b>Reproducibility</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	0,93
<b>Repeatability</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	0,94
<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>	Results obtained with the N-PCR are excellent for all criteria ( $\geq 90\%$ ) and not statistically different from results obtained with the standard test. The most important difference between the N-PCR and the standard test concerns the analytical sensitivity. Therefore, we proposed maintaining a step of pathogen isolation in the revised EPPO detection scheme.
<b>Other information</b>	
<b>Any other information considered useful</b>	When other criteria besides technical performance are considered, the N-PCR has advantages compared to the other methods tested : -It produces results more quickly (2-3 days) than the reference method for approximately the same cost. -It is easily transferable in comparison to isolation and the IF test, which require experience for recognising the typical bacteria.
The following complementary files are available online:	<ul style="list-style-type: none"> <li>• <a href="#">Appendix 1-List target strains-2</a></li> <li>• <a href="#">Appendix 2-List non target strains-2</a></li> <li>• <a href="#">EILVReport-V02 01.03.2012 correction</a></li> </ul>

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