

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

Method: Molecular Conventional PCR	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	yes
EPPO Diagnostic Protocol name	PM 7/023 Xanthomonas axonopodis pv. dieffenbachiae (version 2)
Name of the test	Nested PCR (Robene-Soustrade et al., 2006)
Other information	
Reaction type	Nested
Other details on the test	Nested-PCR as in Appendix 4 of PM7/23(2)
Method: Molecular PCR-RFLP	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	yes
EPPO Diagnostic Protocol name	PM 7/023 Xanthomonas axonopodis pv. dieffenbachiae (version 2)
Name of the test	Nested PCR (Robene-Soustrade et al., 2006) + RFLP
Other information	
Reaction type	Nested
Are the performance characteristics included in the EPPO diagnostic protocol?	no
Performance Criteria :	
Organism 1.:	Xanthomonas axonopodis pv. dieffenbachiae(XANTDF)
Analytical sensitivity	
What is the smallest amount of target that can be detected reliably?	1x10 ⁴ CFU.mL-1
Diagnostic sensitivity	
Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98	Comparative study : 100% ; Collaborative study : 97.5%
Standard test(s)	Isolation + AGDIA Indirect-ELISA on pure culture (OEPP PM7/23)
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	50 (see attached downloadable file Appendix 1)
Specificity value	1
Analytical specificity - exclusivity	
Number of non-target organisms tested	53 (see attached downloadable file Appendix 2)
Specificity value	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.
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	Comparative study : 96% ; Collaborative study : 95%
Specify the test(s)	Isolation + AGDIA Indirect-ELISA on pure culture (OEPP PM7/23)
Reproducibility	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	0,93
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	0,94
Test performance study	
Test performance study?	yes
Brief details of the test performance study and its output. It available, link to published article/report	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.
Other information	
Any other information considered useful	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"> • Appendix 1-List target strains-2 • Appendix 2-List non target strains-2 • EILVReport-V02 01.03.2012 correction

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