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 Xanthomonas axonopodis pv. dieffenbachiae by nested-PCR in leaves and pure culture
Date, reference of the validation report	2012-03-01 - Inter-laboratory ring test : Xanthomonas axonopodis pv. dieffenbachiae 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?	
If yes, please specify	
Description of the test	
Organism(s)	Xanthomonas axonopodis pv. dieffenbachiae(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
New test being considered for inclusion in the next version of the EPPO diagnostic protocol?	
EPPO Diagnostic Protocol name	PM 7/023 Xanthomonas axonopodis pv. dieffenbachiae (version 2)
Name of the test	Extraction from symptomatic plant material in PBS buffer (Appendix 1.1)

As or adapted from an IPPC diagnostic protocol	
Is the test modified compared to the reference test	
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
New test being considered for inclusion in the next version of the EPPO diagnostic protocol?	
EPPO Diagnostic Protocol name	PM 7/023 Xanthomonas axonopodis pv. dieffenbachiae (version 2)
Name of the test	Nested PCR (Robene-Soustrade et al., 2006)
As or adapted from an IPPC diagnostic protocol	
Is the test modified compared to the reference test	
Kit	
Is a kit used	
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
New test being considered for inclusion in the next version of the EPPO diagnostic protocol?	
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
As or adapted from an IPPC diagnostic protocol	
Is the test modified compared to the reference test	
Kit	
Is a kit used	
Other information	
Reaction type	Nested

Other details on the test	
Are the performance characteristics included in the EPPO diagnostic protocol?	**no**
Performance Criteria :	
Organism 1.:	**Xanthomonas axonopodis pv. dieffenbachiae(XANTDF)**
Analytical sensitivity	
What is 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.
Cross reacts with	
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 (>= 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:	• Appendix 1-List target strains-2 • Appendix 2-List non target strains-2 • EILVReport-V02 01.03.2012 correction

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