

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	Fera Sand Hutton, YO41 1LZ York, United Kingdom
Short description of the test	Subspecies identification of <i>Xylella fastidiosa</i> by molecular real time PCR
Date, reference of the validation report	2018-08-14 - Fera_Hodgetts_Xf_subspecies
Validation process according to EPPO Standard PM7/98?	yes
Is the lab accredited for this test?	no
Was the validated data generated in the framework of a project?	Other_project
If yes, please specify	Defra Future Proofing Plant Health
Description of the test	
Organism(s)	<i>Xylella fastidiosa</i> (XYLEFA)
Detection / identification	identification
Method(s)	Molecular real time PCR
Method: Molecular real time PCR	
Reference of the test description	
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
Reference of the test	Hodgetts, J., Glover, R., Cole, J., Hall, J. and Boonham, N. (2021), Genomics informed design of a suite of real-time PCR assays for the specific detection of each <i>Xylella fastidiosa</i> subspecies. J Appl Microbiol, 131: 855-872.
Is the test modified compared to the reference test	yes The final primer concentration was 375 nM (instead of 300 nM)
Kit	
Is a kit used	no
Other information	

Reaction type	Simplex
Other details on the test	Taqman Fast Universal PCR Master Mix, no AmpErase UNG, Ultrapure bovine serum albumin
Performance Criteria :	
Organism 1.:	Xylella fastidiosa(XYLEFA)
<u>Analytical sensitivity</u>	
What is smallest amount of target that can be detected reliably?	X. f. subsp. fastidiosa = 124 fg X. f. subsp. multiplex = 182 fg X. f. subsp. pauca = 84.2 fg X. f. subsp. morus = 59.2 fg X. f. subsp. sandyi = 908 fg
<u>Diagnostic sensitivity</u>	
Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98	100%
Standard test(s)	Real-time PCR Harper et al., 2010
<u>Analytical specificity - inclusivity</u>	
Number of strains/populations of target organisms tested	8 target strains: X. f. subsp. fastidiosa (LMG 17159, LMG 15099, NCPPB 4605), X. f. subsp. multiplex (LMG 9063, NCPPB 4604), X. f. subsp. pauca (NCPBP 4595), X. f. subsp. morus (NCPBP 4589), X. f. subsp. sandyi (NCPBP 4606)
Specificity value	100%
<u>Analytical specificity - exclusivity</u>	
Number of non-target organisms tested	50 strains
Specificity value	100%
<u>Diagnostic Specificity</u>	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	100%
Specify the test(s)	Real-time PCR Harper et al., 2010
<u>Reproducibility</u>	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	High bacterial DNA concentration (5.92-18.2 ng DNA per reaction): 100%; Low bacterial DNA concentration (592-1820 fg DNA per reaction): 92% - 100%; Very low DNA concentration (59.2-182 fg DNA per reaction): test dependent (8% - 63%)
<u>Repeatability</u>	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	High bacterial DNA concentration (5.92-18.2 ng DNA per reaction): 100%; Low bacterial DNA concentration (592-1820 fg DNA per reaction): 100%; Very low bacterial DNA concentration (59.2-182 fg DNA per reaction): test dependent (0% - 62.5%)
Test performance study	
Test performance study?	no

The following complementary files are available online:	• Specificity- List of bacterial species

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