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 - Bacteriology, Virology and GMO Unit 7 rue Jean Dixméras, 49044 Angers, France
Short description of the test	Detection of Xylella fastidiosa by real-time PCR Harper et al., 2010 on composite sample of Helichrysum italicum
Date, reference of the validation report	2020-12-24 - Detection of Xylella fastidiosa by real-time PCR Harper et al., 2010 on composite sample of Helichrysum italicum version 1
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?	no
Description of the test	
Organism(s)	Xylella fastidiosa (XYLEFA)
Detection / identification	detection
Method(s)	Molecular Extraction DNA RNA Molecular real time PCR
Method: Molecular Extraction DNA RNA	
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?	yes
EPPO Diagnostic Protocol name	PM 7/024 Xylella fastidiosa (version 4)
As or adapted from an IPPC diagnostic protocol	yes
IPPC diagnostic Protocol name	ISPM 27 Annex 25 DP 25: Xylella fastidiosa (version 2018)
Name of the test	QuickPick SML Plant DNA kit (Bio-Nobile)
Is the test modified compared to the reference test	yes Crushing into sterile demineralized water rather than PBS Centrifugation speeds and durations

Kit	
Is a kit used	yes
Manufacturer name	BIONOBILE
Specify the kit used	QuickPick™ SML Plant DNA
Kit used following the manufacturer's instructions?	yes
Other information	
Other details on the test	Pool of 50 plants 2 pieces of stem of 2-3 cm per plant 2 steps of bacterial concentration centrifugation A sonication step is applied
Method: Molecular real time 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?	no
EPPO Diagnostic Protocol name	PM 7/024 Xylella fastidiosa (version 4)
Name of the test	Real-time PCR - simplex (Harper et al., 2010; erratum 2013)
As or adapted from an IPPC diagnostic protocol	yes
IPPC diagnostic Protocol name	ISPM 27 Annex 25 DP 25: Xylella fastidiosa (version 2018)
Name of the test	Real-time PCR -simplex (Harper et al., 2010;errtum 2013)
Is the test modified compared to the reference test	no
Kit	
Is a kit used	no
Other information	
Reaction type	Simplex - Probe
Other details on the test	A cut-off of 38 is applied
Performance Criteria :	
Organism 1.:	**Xylella fastidiosa(XYLEFA)**
Analytical sensitivity	
What is smallest amount of target that can be detected reliably?	10^4 cells/ with a detetection rate of 100%
Diagnostic sensitivity	
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)	QuickPick SML plant DNA kit + Harper et al., 2010 applied on a single naturally infected sample in

	comparison with the evaluated test on the same sample pooled with 49 healthy plants
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	55 target strains Cf. Validation data MA039 v5
Specificity value	100%
Analytical specificity - exclusivity	
Number of non-target organisms tested	18 non target strains Cf. Validation data MA039 v5
Specificity value	100%
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	100%
Specify the test(s)	QuickPick SML plant DNA kit + Harper et al., 2010 applied on a single naturally infected sample
Reproducibility	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	Pool of 50 plants Bacterial concentration (Cells/mL) Reproducibility 10^3 92,6% 10^4 100% 10^5 100% Evaluated with3 replicates per concentration by operators on 3 different days
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	Pool of 50 plants Bacterial concentration (Cells/mL) Repeatability 10^3 93,4% 10^4 100% 10^5 100% Evaluated with3 replicates per concentration by 1operators on 3 different days
Test performance study	
Test performance study?	no
The following complementary files are available online:	• Rapport de validation Pool Helichrysum v1

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