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 Xylella fastidiosa by Molecular real time PCR in Specimen
Date, reference of the validation report	2020-03-04 - MA065 version 01 report version 1
Validation process according to EPPO Standard PM7/98?	yes
Is the lab accredited for this test?	yes
Was the validated data generated in the framework of a project?	Euphresco
If yes, please specify	Euphresco project 09/2016-08/2018 “Harmonized protocol for monitoring and detection of Xylella fastidiosa in its host plants and its vectors” AND H2020 Ponte project 2016-2020
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	no
Is the test modified compared to the reference test	yes Analyse on single specimen and on composite sample pooling till 10 specimens
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	
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 - duplex (Harper et al., 2010; erratum 2013)
As or adapted from an IPPC diagnostic protocol	no
Is the test modified compared to the reference test	no
Kit	
Is a kit used	no
Other information	
Reaction type	Duplex - Probe
Other details on the test	A cut-off value of 38 is applied
Performance Criteria :	
Organism 1.:	**Xylella fastidiosa(XYLEFA)**
Analytical sensitivity	
What is smallest amount of target that can be detected reliably?	1.10^3 cells in a single insect and in composite sample grouping till 10 insects with a detection rate of 100% and with a cut-off value of 38 Ct
Diagnostic sensitivity	
Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98	Not evaluated
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	14 target strains Cf. attached file "Rapport de validation MA065 v1"
Specificity value	100%
Analytical specificity - exclusivity	
Number of non-target organisms tested	40 non target organisms Cf. attached file "Rapport de validation MA065 v1"
Specificity value	100%
Diagnostic Specificity	

Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	Not evaluated
Reproducibility	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100% at 10^3 cells/head Single sample (one head) : Evaluated with 3 DNA extract replicates per concentration and 3 amplification replicates par DNA extracts by 1 operators on 3 different days. 3 bacterial concentration tested : 10^2 cells/mL; 10^3 cells/mL, 10^4 cells/mL and healthy samples. Composite sample (10 heads) : Evaluated with 3 DNA extract replicates per concentration and 3 amplification replicates par DNA extracts by 1 operators on 2 different days. 3 bacterial concentration tested : 10^3 cells/mL; 10^4 cells/mL, 10^5 cells/mL and healthy samples. Bacterial concentrations evaluated by ddPCR.
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100% at 10^3 cells/head Single sample (one head) : Evaluated with 3 DNA extract replicates per concentration and 3 amplification replicates par DNA extracts by 1 operators on 3 different days. 3 bacterial concentration tested : 10^2 cells/mL; 10^3 cells/mL, 10^4 cells/mL and healthy samples. Composite sample (10 heads) : Evaluated with 3 DNA extract replicates per concentration and 3 amplification replicates par DNA extracts by 1 operators on 2 different days. 3 bacterial concentration tested : 10^3 cells/mL; 10^4 cells/mL, 10^5 cells/mL and healthy samples. Bacterial concentrations evaluated by ddPCR.
Test performance study	
Test performance study?	yes
Brief details of the test performance study and its output.It available, link to published article/report	Reference: 17-XFAST-EU (Euphresco / H2020 Ponte project) TPS organized in 2017 by Anses. For the present method, 5 laboratories participated. Samples were constituted by Phileanus spumarius head (one head : sample) spiked with a Xf bacterial suspension in order to get final concentrations of 5.10^3 to 5.10^5 cells/head. Performance criteria Results Sensitivity 100% Specificity 100% Accuracy 100% Repeatability 99% Reproducibility 100% Limit of detection (with detection rate of 100%) 5.10^3 cells/head of insect
Other information	
Any other information considered useful	Bibliography : Cunty C., Legendre B., De Jerphanion P., Juteau V., Forveille A., Germain J-F., Ramel J-M., Reynaud P., Olivier V. et Poliakoff F., (2020), Xylella fastidiosa subspecies and sequence types detected in Philaenus spumarius and in infected plants in France share the same locations, Plant Pathology, 00 : 1-14.

The following complementary files are available online:	• Rapport de validation MA065 v1

Creation date: 2021-09-16 14:43:45 - Last update: 2021-09-20 09:58:43