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	Council for Agricultural Research and Economics–Research Centre for Plant Protection and Certification Via Carlo Giuseppe Bertero, 22, 00156 Rome, Italy
Short description of the test	Identification of Xylella fastidiosa subspecies by molecular real time PCR of Dupas et al., 2019
Date, reference of the validation report	2024-03-11 - Pucci et al. 2023
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	Proteggo 1.4 “DISR-05-0001837-04/01/2022 Ministero delle politiche agricole alimentari e forestali–Consiglio per la ricerca in agricoltura e l’analisi dell’economia Agraria
Description of the test	
Organism(s)	Xylella fastidiosa (XYLEFA)
Detection / identification	identification
Matrix(ces) tested	Leaves Olea europaea, Vitis vinifera, Prunus dulcis, Rosmarinus officinalis, Lavandula sp.
Method(s)	Molecular real time PCR
Method: Molecular real time PCR	
Reference of the test description	
Other information	
Performance Criteria :	
Organism 1.:	**Xylella fastidiosa(XYLEFA)**
Analytical sensitivity	
What is the smallest amount of target that can be detected reliably?	Evaluated on DNA extracted from spiked samples obtained by adding the bacterial suspensions of Xylella fastidiosa subsp. fastidiosa (Xff), Xylella fastidiosa subsp. multiplex (Xfm), and Xylella fastidiosa subsp. pauca (Xfp) from 10^6 to 10 cfu/mL to the plant matrices of Olea europaea, Vitis vinifera, and Prunus dulcis, respectively. Each

	sample was repeated in three biological replicate and each biological replicate was repeated in two technical replicates. ASE (Analytical sensitivity) shows a range of 10^2-10^4 cfu/mL depending on the combination of plant matrix/Xf subspecies/mastermixes used. With Bio-Rad the sensitivity value is lowered to 10^4 cfu/mL except for the detection of Xfp in O. europea with 10^3 cfu/mL. Agilent showed the best ASE value of 10^2 cfu/mL achieved for the detection of Xff in V. vinifera (for both probes of Xf and Xff), 10^3 cfu/mL for Xfm in P. dulcis (for both probes Xf and Xfm), 10^4 cfu/mL and 10^2 cfu/mL for the detection of Xf and Xfp in O. europea, respectively.
Diagnostic sensitivity	
Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98	Evaluated on DNA extracted from 8 positive samples and 4 healthy samples (see Test performance study section for samples details). Percentage values obtained with the tested master mixes: 100% using Applied Biosystems and Agilent for Xf and all the subsp. tested; 87.5% using QIAGEN for Xf, Xff and Xfm and 100% for Xfp; 97.91% using Bio-Rad (annealing T 60 °C) for Xf, 100% for Xff and Xfm, 91.66% for Xfp; 95% using Bio-Rad (annealing T 63 °C) for Xf, 100% for Xff and Xfm, 87.5% for Xfp.
Standard test(s)	Comparison of samples with known status
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	Evaluated on DNA extracted from 8 positive samples and 4 healthy samples (see Test performance study section for samples details). Percentage value obtained with the tested master mixes: 100% using Applied Biosystems, Agilent, and Bio-Rad (annealing T 63°C) for Xf and all the subspecies tested; 100% using Bio-Rad (annealing T 60°C) for Xf, Xff and Xfm, 94.58% for Xfp; 93.70% obtained using Qiagen for Xf, 100% for Xff, Xfm and Xfp.
Specify the test(s)	Comparison of samples with known status
Reproducibility	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	Evaluated on DNA extracted from 7 samples consisted of 3 artificially infected (petioles and leaves of Rosmarinus officinalis, Vitis vinifera and Lavandula spp. spiked with 0.6 pg/µL of Xfm, Xff and Xfm respectively) and 4 healthy samples. Percentage value obtained with the tested master mixes: 100% using Applied Biosystems and Agilent for Xf and all the subsp. tested; 95% using Bio-Rad (annealing T 63 °C) for Xf and 100% for all the subsp. tested; 95.83 % using Bio-Rad (annealing T 60 °C) for Xf and Xfp, 100% for Xff and Xfm; 95% using Qiagen for Xf, 93.75% for Xff, 87.5% for Xfm and 100% for Xfp.
Test performance study	

Test performance study?	yes
Brief details of the test performance study and its output.It available, link to published article/report	The Test Performance Study was performed with the participation of 18 Italian official laboratories (OLs) to assess the influence of different master mixes on the performance of real time PCR of Dupas et al. (2019), primer of Set 2. The samples sent to OLs consisted in 6 spiked samples (petioles and leaves of Lavandula spp. and Vitis vinifera spiked with 0.6 and 6 pg/µL of Xfm and Xff respectively; petioles and leaves of Olea europea and Rosmarinus officinalis spiked with 0.6 pg/µL of Xfp and Xfm respectively), 2 naturally infected samples (petioles and leaves of O. europea and Prunus dulcis) and 4 healthy samples. DNA was extracted using the Gentra Puregene Yeast/Bact. Kit (Qiagen) for bacterial suspensions and using the QuickPick™ SML Plant DNA kit (QRET Technologies Ltd.), associated with the automated platform KingFisher™ mL Purification System (Thermo Fisher), for the healthy plant matrices. Naturally infected samples were processed using the DNeasy Mericon Food Kit (Qiagen). Each master mix employed to perform real time PCR of Dupas et al. (2019) was tested by groups of four/five OLs: Bio-Rad (SsoAdvanced™ Universal Probes Supermix) with annealing temperature of 60°C/63°C; Qiagen (QuantiNovaPathogen + IC kit); Applied Biosystems TM (TaqManTM Fast Universal PCR MasterMix); Agilent (Brilliant Multiplex qPCR Master Mix).
Other information	
Any other information considered useful	An Intra-laboratory study was performed by CREA-DC to evaluate the analytical sensitivity of real time PCR of Dupas et al (2019) carried out with Agilent and Bio-Rad mastermix by testing spiked plant matrices.
The following complementary files are available online:	• Pucci et al., 2023

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