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	Institute for Sustainable Plant Protection via Amendola, 122/D, 70126 Bari, Italy
Short description of the test	detection of Xylella fastidiosa in olive samples by Molecular real time PCR using the automatized DNA extraction protocol by Promega (Maxwell® RSC PureFood GMO and Authentication Kit AS1600)
Date, reference of the validation report	2019-10-28 - Implementation and validation of rapid diagnostic procedures for Xylella fastidsa. In: Book of Abstract, 3RD JOINT ANNUAL MEETING POnTE-XF-AXTORS, AJACCIO (FRANCE), 28–30 OCTOBER 2019.
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	XF-ACTORS
Description of the test	
Organism(s)	Xylella fastidiosa (XYLEFA)
Detection / identification	detection
Method(s)	Extraction Molecular Extraction DNA RNA Molecular real time PCR
Method: Extraction	
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	Loconsole et al., 2019
Is the test modified compared to the reference test	no
Other information	

Method: Molecular Extraction DNA RNA	
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	Loconsole et al., 2019
Is the test modified compared to the reference test	no
Kit	
Is a kit used	yes
Manufacturer name	PROMEGA
Specify the kit used	Maxwell® RSC PureFood GMO and Authentication Kit
Kit used following the manufacturer's instructions?	yes the amount of starting plant material is increased: at least 0.5 gr are homogenized with 5 ml of CTAB buffer (Promega) (1:10 w/v)
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 - simplex (Harper et al., 2010; erratum 2013)
As or adapted from an IPPC diagnostic protocol	no
Is the test modified compared to the reference test	yes BSA was not included in the amplification MIX
Kit	
Is a kit used	no
Other information	
Reaction type	Simplex
Performance Criteria :	
Organism 1.:	**Xylella fastidiosa(XYLEFA)**
Analytical sensitivity	
What is smallest amount of target that can be detected reliably?	10^2 cfu/ml (cfu/ml)

Diagnostic sensitivity	
Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98	100% (on 3 replicates for each 10-fold serial dilutions from 10^7 to 10 CFU/ml of bacterial suspension spiked in plant sap from healthy plant and 75 positive field samples)
Standard test(s)	CTAB-based extraction protocol and Modified DNeasy® Mericon™ Food Standard Protocol (Qiagen), followed by Real time PCR Harper et al., 2010
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	3 different Sequence type, ST53, ST72, ST87, ST6
Specificity value	100%
Analytical specificity - exclusivity	
Number of non-target organisms tested	Paraburkholderia phytofirmans PsJN
Specificity value	100%
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	100% (3 replicates of plant sap from healthy plant and 25 field negative samples)
Specify the test(s)	Modified DNeasy® Mericon™ Food Standard Protocol (Qiagen), followed by real time PCR Harper et al., 2010
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100% (on 3 replicates for each 10-fold serial dilutions from 10^7 to 10 CFU/ml of bacterial suspension spiked in plant sap from healthy plant; 3 replicates of plant sap from healthy plant; on 100 field olive trees: - 75 positive samples - 25 negative sample)
Test performance study	
Test performance study?	no
The following complementary files are available online:	• poster Loconsole

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