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 Polygala myrtifolia 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	2021-01-30 - INTERLABORATORY COMPARISON EU-XF-IC-2020-03. Evaluation of molecular methods for the detection of Xylella fastidiosa
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
Matrix(ces) tested	Leaves Leaf petioles
Plant species tested	Polygala myrtifolia
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	INTERLABORATORY COMPARISON EU-XF-IC-2020-03 Evaluation of molecular methods for the detection of Xylella fastidiosa, January 20201

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	INTERLABORATORY COMPARISON EU-XF-IC-2020-03 Evaluation of molecular methods for the detection of Xylella fastidiosa, January 20201
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?	no 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	yes
IPPC diagnostic Protocol name	(version)
Is the test modified compared to the reference test	no
Kit	
Is a kit used	no
Other information	

Reaction type	Simplex
Performance Criteria :	
Organism 1.:	**Xylella fastidiosa(XYLEFA)**
Analytical sensitivity	
What is the smallest amount of target that can be detected reliably?	10^2 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	99.17% (on 3 replicates for each 10-fold serial dilutions from 10^6 to 10 CFU/ml of bacterial suspension spiked in plant sap from healthy Poligala myrtifolia)
Standard test(s)	CTAB-based extraction protocol (diagnostic sensitivity 98.67%) and Modified DNeasy® Mericon™ Food Standard Protocol (Qiagen) (diagnostic sensitivity 99.56%)
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	ST53
Specificity value	
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	95.83 % (on 3 replicates of plant sap from healthy P. myrtifolia) considering the results of all 16 laboratories 100% considering the results of 15 laboratories. Only 1 laboratory produced false positive samples.
Specify the test(s)	CTAB-based extraction protocol (diagnostic specificity and repeatability 97.78%, reproducibility 98.52%) and Modified DNeasy® Mericon™ Food Standard Protocol (Qiagen) (diagnostic specificity 97.78%, repeatability 98.52%, reproducibility 99.30%)
Reproducibility	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	98.68% (on 3 replicates for each 10-fold serial dilutions from 10^6 to 10 CFU/ml of bacterial suspension spiked in plant sap from healthy Poligala myrtifolia; 3 replicates of plant sap from healthy P. myrtifolia; all samples tested in 16 different laboratories)
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	98.61 % (on 3 replicates for each 10-fold serial dilutions from 10^6 to 10 CFU/ml of bacterial suspension spiked in plant sap from healthy Poligala myrtifolia; - 3 replicates of plant sap from healthy P. myrtifolia)
Test performance study	
Test performance study?	yes
Brief details of the test performance study	TPS organized within the interlaboratory

and its output.It available, link to published article/report	comparison EU-XF-IC-2020-03), in the framework of the activities related to the experimental plan foreseen in WP4/WP9 of the Horizon 2020 project “XF-ACTORS – 727987”, and follows the previous European proficiency testing EU-XF-PT-2017-02 carried out in 2017. https://www.xfactorsproject.eu/wp-content/uploads/2021/01/EU-XF-IC-2020-03-Report-V2-1.pdf
The following complementary files are available online:	• Report IC

Creation date: 2021-12-14 11:56:28 - Last update: 2022-01-25 14:46:42