

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 <i>Xylella fastidiosa</i> by Molecular real time PCR in insect vectors 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 <i>Xylella fastidiosa</i>
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)	<i>Xylella fastidiosa</i> (XYLEFA)
Detection / identification	detection
Matrix(ces) tested	Other insects: <i>Philaenus spumarius</i>
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 <i>Xylella fastidiosa</i> , 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 A single insect head, should be homogenized in a 2 mL tube with one or two 5-mm tungsten carbide beads (for a maximum of 15–20 s at a frequency of 24 cycles s ⁻¹ , in Mill300 mixer/Tissue Lyser II (Qiagen) or similar equipment). Five hundred microlitres of CTAB buffer (Promega), 10µl of RNase A Solution (to eliminate RNA) and 20µl of Proteinase K (PK) Solution are added to each microcentrifuge tubes. The tubes are tapped, inverted and vigorously vortexed until the sample is resuspended. Tubes are placed in a heat block at 65°C for 30 minutes. During the incubation, cartridges are prepared according to manufacturer instructions. After incubation, tubes with the lysate are inverted or vortexed to mix thoroughly. Tubes are centrifuged at ≥16,000 g for 10 minutes to separate any oils and solids. 300 µl of clear lysate are transferred into well #1 of the reagent cartridge by avoiding pipetting any solid material from the bottom of the tube or on the surface of the liquid and avoiding putting oil on the surface (these materials may inhibit downstream tests). If necessary, transfer the cleared lysate to a new tube and centrifuge again to avoid oils and solids. Purify on the Maxwell® Instrument according to the manufacturer's instructions. For elution, 50 µl of Elution Buffer are used.
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	98.75% (on 3 replicates for each 10-fold serial dilutions from 10 ⁶ to 10 CFU/ml of bacterial suspension spiked in Xylella-free P. spumarius homogenate)
Standard test(s)	CTAB-based extraction protocol (diagnostic sensitivity 95.11%)
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	97.92% (on 3 replicates of Xylella-free P. spumarius)
Specify the test(s)	CTAB-based extraction protocol (diagnostic sensitivity 88.89%, repeatability 96.30%, reproducibility 94.07 %)
Reproducibility	
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 Xylella-free P. spumarius homogenate; on 3 replicates of Xylella-free P. spumarius; tested in 16 different laboratories)
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	97.22% (on 3 replicates for each 10-fold serial dilutions from 10 ⁶ to 10 CFU/ml of bacterial

	suspension spiked in Xylella-free P. spumarius homogenate; on 3 replicates of Xylella-free P. spumarius)
Test performance study	
Test performance study?	no
Brief details of the test performance study and its output. It available, link to published article/report	TPS organized within the interlaboratory 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-XFIC-2020-03-Report-V2-1.pdf
The following complementary files are available online:	
	<ul style="list-style-type: none"> • Report IC

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