EUROPEAN AND MEDITERRANEAN PLANT PROTECTION ORGANIZATION
ORGANISATION EUROPEENNE ET MEDITERRANEENNE POUR LA PROTECTION DES PLANTES
(11-17239)
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.

Target Organism	Xylella fastidiosa	
Short description	Validation of the real-time PCR for the detection of Xylella fastidiosa (Ouyang et al., 2013) in comparison with the real time PCR of Harper et al. (2010; erratum 2013).	
Laboratory contact details	Netherlands Institute for Vectors, Invasive plants and Plant health P.O. Box 9102, 6700 HC Wageningen, Netherlands	
Date and reference of the validation report	2019-09-02 - 2018.molbio.006 Validatie Real-time PCR voor de detectie en identificatie van Xylella fastidiosa (Ouyang et al., 2013) en een vergelijking met de Harper et al. (2010) real-time PCR.	
Validation process according to EPPO Standard PM 7/98:	Yes	
Reference of the test description	PM 7/024 Ouyang et al., (2013) Harper et al. (2010; erratum 2013)	
Is the test the same as described in the EPPO DP?	Yes	
Is the lab accredited for this test?	Yes	
Plant species tested (if relevant)	Coffea arabica, Nerium oleander, Polygala myrtifolia, Olea europaea, Lavandula stoechas and Prunus avium	
Matrices tested (if relevant)	petioles and/or stems of Coffea arabica, Nerium oleander, Polygala myrtifolia, Olea europaea, Lavandula stoechas and Prunus avium	
List of methods used		
Method for extraction / isolation / baiting of target organism from matrix	X	DNA isolation with the QuickPick™ SML Plant DNA kit (Bio-Nobile) in combination with the KingFisher Flex isolation robot
Molecular methods, e.g. hybridization, PCR and real time PCR	X	Real-time PCR performed with Premix Ex Taq (TaKaRa)
Serological methods: IF, ELISA, Direct Tissue Blot Immuno Assay		
Plating methods: selective isolation		
Bioassay methods: selective enrichment in host plants, baiting, plant test and grafting.		
Pathogenicity test		

Fingerprint methods: protein profiling, fatty acid profiling & DNA profiling		
Morphological and morphometrical methods intended for identification		
Biochemical methods: e.g. enzyme electrophoresis, protein profiling		
Other		
<u>Analytical sensitivity (= limit of detection)</u>		
What is smallest amount of target that can be detected reliably?	The number of Xylella fastidiosa genomic copies that can be reliably detected (LOD average per matrix + 3 SD) for both the real-time PCR's of Ouyang et al., (2013) and Harper et al., (2010;erratum 2013) is <60 genomic copies when all plant species are considered. The average number of Xylella fastidiosa genome copies that was consistently detected was significantly lower (t-test, $p<0.001$) in the Harper et al., (2010; erratum 2013) test (3 copies) as compared to the Ouyang et al., (2013) test (6 copies). Although this significant difference was observed, both real-time PCR tests are very sensitive.	
<u>Diagnostic sensitivity</u>		
Proportion of infected/infested samples tested positive compared to results from the standard test , see appendix 2 of PM 7/98	100%, all 39 of the Xylella fastidiosa infected samples tested positive by the real time PCR of Ouyang et al., (2013) as compared to the real time PCR of Harper et al. (2010; erratum 2013)	
Specify the standard test	Ouyang et al., (2013) Harper et al. (2010; erratum 2013)	
<u>Analytical specificity</u>		
Specificity value	100%, intotal 134 isolates were included for the specificity and tested by both real time PCR's of Ouyang et al., (2013) and Harper et al., (2010; erratum 2013). All non-targets, including the Xylella taiwanensis and the endophytes isolated from a Xylella fastidiosa naturally infected Coffea arabica plant, gave a negative result with both real-time PCR's (Table 6 in validatie report).	
Number of strains/populations of target organisms tested	Inclusivity = 100% for both real-time PCR's of Ouyang et al., (2013) and Harper et al., (2010; erratum 2013), based on 39 Xylella fastidiosa strains (Table 6 in validatie report). X. f. fastidosa (n=13; ST=1, 2, 72, 75, 76) X. f. morus (n=1; ST=29) X. f. mulitiplex (n=13; ST=6, 7, 10, 26, 27, 41, 42, 51) X. f. pauca (n=13; ST=53, 68, 78, 74) X. f. sandyi (n=2; ST=5)	
Number of non-target organisms tested	Exclusivity = 100% for both real-time PCR's of Ouyang et al., (2013) and Harper et al., (2010; erratum 2013), based on 95 bacterial isolates, other than Xylella fastidiosa (Table 6 in validatie report).	
Cross reacts with (specify the species)	No cross reactions were found, either in the real time PCR of Ouyang et al., (2013) or the real time PCR of Harper et al.	

	(2010; erratum 2013).
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	100%, all 95 bacterial isolates included, other than Xylella fastidiosa were tested negative (true negatives) by the real time PCR of Ouyang et al., (2013) as compared to the real time PCR of Harper et al. (2010; erratum 2013)
Specify the standard test	Ouyang et al., (2013) Harper et al. (2010; erratum 2013)
Reproducibility	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100%
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100%
Test performance study	
Test performance study?	No
Include brief details of the test performance study and its output.It available, provide a link to published article/report	
Other information	
Any other information considered useful **e.g. robustness, ease of performing the test, etc.**	Useful information on the reaction mix: We compared two different reaction mixes, namely: 1) Premix Ex Taq (perfect real-time) (TaKaRa), 2) PerfeCTa qPCR ToughMix (Quanta Biosciences). The acquired results were comparable (see Figure 1 and Figure 2 and & 4.1 of validation report), therefore, it was decided to perform the validation of the real time PCR of Ouyang et al., (2013) based on the Premix Ex Taq (perfect real-time) (TaKaRa), that is already used at our lab for the real time PCR of Harper et al. (2010; erratum 2013). Useful information on selectivity: Lavendula stoaches gives inhibition in both real-time PCRs, Ouyang et al., (2013) and Harper et al. (2010; erratum 2013). In diagnostic samples the following matrices gave frequently inhibition in the Harper et al. (2010; erratum 2013) real-time PCR: Lavendula spp., Prunus spp., Rosmarinus spp., and Rubus spp.
The following complementary files are available online:	• 2018.molbio.006