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	Grapevine flavescence dorée phytoplasma	
Short description	Detection of flavescence doree phytoplasma by LAMP in grapevine	
Laboratory contact details	National Institiute of Biology, Department of Biotechnology and Systems Biology Vecna pot 121, 1000 Ljubljana, Slovenia	
Date and reference of the validation report	2015-11-26 - validation data included in Kogovšek P, Hodgetts J, Hall J, Prezelj N, Nikolić P, Mehle N, Lenarčič R, Rotter A, Dickinson M, Boonham N, Dermastia M, Ravnikar M (2015) LAMP assay and rapid sample preparation method for on-site detection of flavescence dorée phytoplasma in grapevine. Plant Pathology, 64, 286-296. Result of the test performance study: Euphresco GRAFDEPI2, WP4: Test performance study of the LAMP assays for the detection of BNp and FDp (Final report).	
Validation process according to EPPO Standard PM 7/98:	Yes	
Reference of the test description	0 Kogovšek P, Hodgetts J, Hall J, Prezelj N, Nikolić P, Mehle N, Lenarčič R, Rotter A, Dickinson M, Boonham N, Dermastia M, Ravnikar M (2015) LAMP assay and rapid sample preparation method for on-site detection of flavescence dorée phytoplasma in grapevine. Plant Pathology, 64, 286-296.	
Is the test the same as described in the EPPO DP?	No In current version of PM 7/79 LAMP is not included.	
Is the lab accredited for this test?	No	
Plant species tested (if relevant)	Vitis vinifera	
Matrices tested (if relevant)	leaf vein, flower, berry	
List of methods used		
Method for extraction / isolation / baiting of target organism from matrix	X	Two options: - with DNA extraction (Mehle, N., Nikolić, P., Rupar, M., Boben, J., Ravnikar, M., Dermastia, M. 2013. Automated DNA extraction for large numbers of plant samples. In: Dickinson, M. (ed.), Hodgetts, J. (ed.). Phytoplasma: methods and protocols, (Methods in Molecular Biology, vol. 938), (Springer Protocols). New York: Humana Press: 139-145.)

		- without DNA extraction = direct testing of crude homogenates (Kogovšek P, Hodgetts J, Hall J, Prezelj N, Nikolić P, Mehle N, Lenarčič R, Rotter A, Dickinson M, Boonham N, Dermastia M, Ravnikar M (2015) LAMP assay and rapid sample preparation method for on-site detection of flavescence dorée phytoplasma in grapevine. Plant Pathology, 64, 286-296)
Molecular methods, e.g. hybridization, PCR and real time PCR	X	LAMP (loop-mediated isothermal amplification)
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?	3 experiments with 8 serial dilutions of DNA were performed: maximum dilution of FDp DNA that was detected was 1:270, which corresponding to 9-27 copies of FDp DNA (maximum dilution of FDp DNA that was detected using real-time PCR (Hren et al., 2007) was 1:2430, which corresponding to 1-3 copies of FDp DNA). 3 experiments with at least 6 serial dilutions of plant homogenate were performed: maximum dilution of FDp infected homogenate that was detected was 1:81, which corresponding to 9-27 copies of FDp DNA (maximum dilution of FDp infected homogenate that was detected using real-time PCR (Hren et al., 2007) after DNA extraction (Mehle et al., 2013) was 1:729, which corresponding to 1-3 copies of FDp DNA).	
<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	Two options for LAMP testing was compared: testing of isolated DNA:100% (no. of targets analysed: 52 FDp infected samples (38 grapevine leaf vein, 8 Clematis vitalba, 3 Alnus glutinosa, 1 Scaphoideus titanus and 2 Orientus ishidae samples))	

	direct testing of crude homogenates: 100% (no. of targets analysed: 27 FDp infected grapevine samples) Additionally, in 2015 all official Slovenian grapevine samples were tested by LAMP (direct testing of crude homogenates) and by real-time PCR. Number of samples: 286 Number of FDp positive samples (samples positive for FDp with real-time PCR): 28 Diagnostic sensitivity: 100% (27 samples were clear positive with LAMP, while one sample was positive with LAMP only in one out of three parallels)
Specify the standard test	real time PCR (Hren et al., 2007) on DNA isolated according Mehle et al. (2013)
Analytical specificity	
Specificity value	LAMP is specific to 16SrV phytoplasmas (e.g. FD, EY). Percentage of accurate results: 99% (fals negative results: 0%; fals positive results: 1.5%*) Additionally, In silico analysis shown high specificity to 16SrV phytoplasmas including FD. *One healthy grapevine sample and a DNA sample of Ca. P. fraxini (16SrVII; isolate: ASHY 2; origin: USA) from the test performance study (Euphresco: Grafdepi) were positive with LAMP.
Number of strains/populations of target organisms tested	65 FD isolates/ infected samples (FD70, FD-C, FD-D) and 2 EY-phytoplasma isolates (for details see Table 1 in Kogovšek et al., 2014) Additionally, 15 samples with targets (11 samples positive for FD phytoplasma, and 4 samples positive for phytoplasmas of 16SrV group) from the test performance study (Euphresco: Grafdepi) were analysed.
Number of non-target organisms tested	123 (phytoplasma DNA from other 16Sr groups, bacterial and fungal isolates and healthy hosts; for details see Table 1 in Kogovšek et al., 2015) Additionally, 9 samples with non-targets from the test performance study (Euphresco: Grafdepi) were analysed.
Cross reacts with (specify the species)	Ca. P. fraxini (16SrVII)
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	100% (no. of non-targets analysed: 53 FDp non-infected samples (48 grapevines, 2 C. vitalba, 1 A. glutinosa, 1 O. ishidae and 1 S. titanus)) Additionally, in 2015 all official Slovenian grapevine samples were tested by LAMP (direct testing of crude homogenates) and by real-time PCR. Number of samples: 286 Number of FDp negative samples (samples negative for FDp

	with real-time PCR): 258 (FD and BN negative: 50; FD negative, BN positive: 208) Diagnostic specificity: 100%
Specify the standard test	real time PCR (Hren et al., 2007) on DNA isolated according Mehle et al. (2013)
Reproducibility	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	High FDp conc. (3 samples with more than 729 copies of FDp DNA): 100% (3 pos/ 3 repeats) Medium FDp conc. (2 samples with 81-729 copies of FDp DNA): 100% (2 pos/ 2 repeats) Low FDp conc. (8 samples with less than 81 copies of FDp DNA): 100% (8 pos/ 8 repeats) No. of operators: 2 No. of devices: 2 No. of days: 2-9
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	High FDp conc. (5 samples with more than 729 copies of FDp DNA): 100% (29 pos/ 29 repeats) Medium FDp conc. (4 samples with 81-729 copies of FDp DNA): 100% (12 pos/ 12 repeats) Low FDp conc. (8 samples with less than 81 copies of FDp DNA): 81% (22 pos/ 27 repeats)
Test performance study	
Test performance study?	Yes
Include brief details of the test performance study and its output.It available, provide a link to published article/report	A test performance study (TPS) has been performed to validate this LAMP protocol in the frame of WP4 of Euphresco Grafdepi2 project. Note: in this TPS also LAMP assay for BNp detection (which was developed in the frame of WP 1 of Euphresco Grafdepi2 project and is not published yet) was included. Ten laboratories from the research and plant protection area from Europe and Australia participated in this TPS. Samples that were subject of this TPS: DNA samples (1 from FD-infected clematis, 3 from FD-infected grapevine plants, 4 from BN-infected grapevine plants, 1 from FD and BN infected grapevine plants, 6 from healthy grapevine plants, 1 sample of fungi DNA, 1 sample of bacterial DNA, 1 sterile nuclease free water) Results: No. of labs taking in account for evaluation: 10 No. of results: 179 (number of samples with a positive assigned value: 49; number of samples with a negative assigned value: 130) Rate of true positives: 100% Rate of true negatives: 97,7% Accuracy: 98,3% Additionally, LAMP FDp assay was compared with a Qualiplante/Hyris isothermal amplification assay for FD (code: IsoA.FD/80) by three laboratories.

	Results for Qualiplante/Hyris isothermal amplification assay: No. of labs taking in account for evaluation: 3 No. of results: 54 (number of samples with a positive assigned value: 15; number of samples with a negative assigned value: 39) Rate of true positives: 100% Rate of true negatives: 97,4% Accuracy: 98,1% For details see final report (file is attached).
Other information	
Any other information considered useful **e.g. robustness, ease of performing the test, etc.**	Selectivity: There was no impact observed of different hosts, grapevine cultivars or tissues on the test results (FD was confirmed using LAMP in 12 different grapevine cultivars, either in berries or leaf veins, and also in C. vitalba, A. glutinosa, O. ishidae and S. titanus).
The following complementary files are available online:	• Euphresco GRAFDEPI2, WP4: Test performance study of the LAMP assays for the detection of BNp and FDp (Final report) • Kogovšek et al., 2014