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	Maize redness phytoplasma	
Short description	Detection of Maize redness phytoplasma by real time PCR	
Laboratory contact details	National Instituite of Biology, Department of Biotechnology and Systems Biology Vecna pot 121, 1000 Ljubljana, Slovenia	
Date and reference of the validation report	2015-06-19 - Validation report on the testing of phytoplasma which cause Maize redness	
Validation process according to EPPO Standard PM 7/98:	Yes	
Reference of the test description	0 Reference for amplicon BNgen: Hren M, Boben J, Rotter A, Kralj P, Gruden K, Ravnikar M. 2007. Real-time PCR detection systems for Flavescence dorée and Bois noir phytoplasma in grapevine: a comparison with the conventional PCR detection system and their application in diagnostics. Plant Pathol, 56: 785-796. Reference for amplicon Christen: Christensen NM, Nicolaisen M, Hansen M, Schulz A. 2004. Distribution of phytoplasmas in infected plants as revealed by real-time PCR and bioimaging. Mol Plant Microbe Interact 17: 1175–1184.	
Is the test the same as described in the EPPO DP?	No Real time PCR	
Is the lab accredited for this test?	Yes	
Plant species tested (if relevant)	Zea mays	
Matrices tested (if relevant)	leaf veins, vascular tissue [phloem] from roots of maize	
List of methods used		
Method for extraction / isolation / baiting of target organism from matrix	X	MEHLE, Nataša, NIKOLIĆ, Petra, RUPAR, Matevž, BOBEN, Jana, RAVNIKAR, Maja, DERMASTIA, Marina. Automated DNA extraction for large numbers of plant samples. V: DICKINSON, Matthew (ur.), HODGETTS, Jennifer (ur.). Phytoplasma : methods and protocols, (Methods in Molecular Biology, ISSN 1064-3745, vol. 938), (Springer Protocols). New York: Humana Press, 2013, str. 139-145.
Molecular methods, e.g. hybridization, PCR and real time PCR	X	real time PCR
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		
Analytical sensitivity (= limit of detection)		
What is smallest amount of target that can be detected reliably?	Not applicable: only for comparison between Christen and BNgen amplicons 3-fold serial dilutions of DNA samples of 'Ca. P. solani' isolates from maize roots (NIB F 97) in extract of healthy maize roots were carried out in three experiments. No relevant differences between three experiments were observed. 100% probability of detection for the amplicon BNgen was up to dilution 27x in all three experiments, and for the amplicon Christen at least up to dilution 81x.	
Diagnostic sensitivity		
Proportion of infected/infested samples tested positive compared to results from the standard test , see appendix 2 of PM 7/98		
Specify the standard test		
Analytical specificity		
Specificity value	specificity of amplicon Christen: phytoplasmas; 16S rRNA specificity of amplicon BNgen: Stolbur group, 16SrXII; Stol11 genome sequence Percentage of accurate results: Christen amplicon: 100 % BNgen amplicon: 100 % Percentage of false positives: Christen amplicon: 0 % BNgen amplicon: 0 % Percentage of false negatives: Christen amplicon: 0 % BNgen amplicon: 0 %	
Number of strains/populations of target organisms tested	4 (see Validation report on the testing of phytoplasma which cause Maize redness)	

Number of non-target organisms tested	26 (see Validation report on the testing of phytoplasma which cause Maize redness)
Cross reacts with (specify the species)	No cross reactivity was observed. In silico analysis: Amplicon BNgen: none of publically available sequences of Maize redness isolates have a sequence of StolI1 gene. Amplicon Christen: the alignment of all of the publically available sequences of 16S rRNA for 'Ca. P. solani' isolates from maize revealed one mismatch (at 5' end) with reverse primer. However, the Christen amplicon has not been tested yet with these isolates, and thus its ability to recognize these phytoplasma cannot be ruled out completely.
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	
Specify the standard test	
Reproducibility	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	No. of samples per 1 amplicon: 1 No. of devices: 2 No. of real-time PCR runs: 2 Percentage of identical results is 100% in samples with estimated low phytoplasma amount. For details see Validation report on the testing of phytoplasma which cause Maize redness.
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	Repeatability for both amplicons is 100% in all samples with estimated medium and low phytoplasma concentration. For details see Validation report on the testing of phytoplasma which cause Maize redness.
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.**	Selectivity: Number of different spiked samples tested: 8 (roots of different healthy maize cultivars which were spiked with positive DNA sample were from different fields/ orign). There was no observed impact of maize cultivars or origin of the samples on the test results.

	Full validation report is added - see Validation report on the testing of phytoplasma which cause Maize redness. Possible sources and components of uncertainty in real-time PCR testing for Maize redness phytoplasma, their impact and the measures applied to reduce uncertainty were identified - see Identification of critical points. Additional validation data for amplicon BNgen: see Grapevine bois noir phytoplasma, Detection of FD and BN by real time PCR, NIB-FITO (LabID).
The following complementary files are available online:	• identification of critical points • Validation report on testing of phytoplasma which cause Maize redness