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	National Instituite of Biology, Department of Biotechnology and Systems Biology Vecna pot 111, 1000 Ljubljana, Slovenia
Short description of the test	Detection of Maize redness phytoplasma by real time PCR
Date, 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 PM7/98?	yes
Is the lab accredited for this test?	yes
Was the validated data generated in the framework of a project?	
If yes, please specify	
Description of the test	
Organism(s)	'Candidatus Phytoplasma solani'(PHYPSO)
Detection / identification	detection
Matrix(ces) tested	Leaves, Roots leaf veins, vascular tissue [phloem] from roots of maize
Plant species tested	Zea mays
Method(s)	Molecular Extraction DNA RNA Molecular real time PCR
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?	
As or adapted from an IPPC diagnostic protocol	no
Reference of the test	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.
Is the test modified compared to the reference test	
Kit	
Is a kit used	
Other information	
Other details on the test	
Method: Molecular real time PCR	
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?	
As or adapted from an IPPC diagnostic protocol	no
Reference of the test	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 modified compared to the reference test	
Kit	
Is a kit used	
Other information	
Reaction type	
Other details on the test	
Are the performance characteristics included in the EPPO diagnostic protocol?	**no**
Performance Criteria :	
Organism 1.:	**'Candidatus Phytoplasma solani'(PHYPSO)**
Analytical sensitivity	
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	
Standard test(s)	
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	4 (see Validation report on the testing of phytoplasma which cause Maize redness)
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 %
Analytical specificity - exclusivity	
Number of non-target organisms tested	26 (see Validation report on the testing of phytoplasma which cause Maize redness)
Specificity value	No cross reactivity was observed. In silico analysis: Amplicon BNgen: none of publically available sequences of Maize redness isolates have a sequence of Stol11 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.
Cross reacts with	
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	
Specify the test(s)	
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	Repeatability for both amplicons is 100% in all

given level of the pest (see PM 7/98)	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
Brief details of the test performance study and its output.It available, link to published article/report	
Other information	
Any other information considered useful	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

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