

**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.

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| <b>Laboratory contact details</b>                                      | National Institute of Biology, Department of Biotechnology and Systems Biology<br>Vecna pot 121, 1000 Ljubljana, Slovenia  |
| <b>Short description of the test</b>                                   | Detection of Maize redness phytoplasma by real time PCR  |
| <b>Date, reference of the validation report</b>                        | 2015-06-19 - Validation report on the testing of phytoplasma which cause Maize redness   |
| <b>Validation process according to EPPO Standard PM7/98?</b>           | yes  |
| <b>Is the lab accredited for this test?</b>                            | yes  |
| <b>Was the validated data generated in the framework of a project?</b> |  |
| <b>Description of the test</b>   |  |
| <b>Organism(s)</b>   | 'Candidatus Phytoplasma solani' (PHYPSO)   |
| <b>Detection / identification</b>                                      | detection  |
| <b>Matrix(ces) tested</b>  | Leaves, Roots leaf veins, vascular tissue [phloem] from roots of maize   |
| <b>Plant species tested</b>  | Zea mays   |
| <b>Method(s)</b>   | Molecular Extraction DNA RNA<br>Molecular real time PCR  |
| <b>Method: Molecular Extraction DNA RNA</b>                            |  |
| <b>Reference of the test description</b>                               |  |
| <b>As or adapted from an EPPO diagnostic protocol</b>                  | no   |
| <b>As or adapted from an IPPC diagnostic protocol</b>                  | no   |
| <b>Reference of the test</b>   | 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. |
| <b>Other information</b>   |  |

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| <b>Method: Molecular real time PCR</b>   |  |
| <b>Reference of the test description</b>   |  |
| <b>As or adapted from an EPPO diagnostic protocol</b>                                | no   |
| <b>As or adapted from an IPPC diagnostic protocol</b>                                | no   |
| <b>Reference of the test</b>   | Reference for amplicon BNgen: Hren M, Boben J, Rotter A, Kralj P, Gruden K, Ravnihar 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. |
| <b>Other information</b>   |  |
| <b>Are the performance characteristics included in the EPPO diagnostic protocol?</b> | <b>no</b>  |
| <b>Performance Criteria :</b>  |  |
| <b>Organism 1.:</b>  | <b>'Candidatus Phytoplasma solani'(PHYPSO)</b>   |
| <b>Analytical sensitivity</b>  |  |
| <b>What is the smallest amount of target that can be detected reliably?</b>          | 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.   |
| <b>Analytical specificity - inclusivity</b>  |  |
| <b>Number of strains/populations of target organisms tested</b>                      | 4 (see Validation report on the testing of phytoplasma which cause Maize redness)  |
| <b>Specificity value</b>   | 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 %  |
| <b>Analytical specificity - exclusivity</b>  |  |
| <b>Number of non-target organisms tested</b>   | 26 (see Validation report on the testing of phytoplasma which cause Maize redness)   |
| <b>Specificity value</b>   | No cross reactivity was observed. In silico analysis:  |

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|  | <p>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.</p>   |
| <b>Reproducibility</b>   |  |
| <p><b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b></p>  | <p>No. of samples per 1 amplicon: 1 No. of devices: 2<br/>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.</p>  |
| <b>Repeatability</b>   |  |
| <p><b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b></p>  | <p>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.</p>   |
| <b>Test performance study</b>  |  |
| <p><b>Test performance study?</b></p>  | no   |
| <b>Other information</b>   |  |
| <p><b>Any other information considered useful</b></p>  | <p>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/ origin). 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).</p> |
| <p>The following complementary files are available online:</p>   |  |
| <ul style="list-style-type: none"> <li>• <a href="#">Identification of critical points</a></li> <li>• <a href="#">Validation report on testing of phytoplasma which cause Maize redness</a></li> </ul> |  |

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