

**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.

|  |   |
|--|---|
| <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 <i>Xylella fastidiosa</i> by real-time PCR (Francis et al., 2006) in plant material  |
| <b>Date, reference of the validation report</b>                        | 2018-06-14 - Dreo, Tanja, 2018. qPCR for detection of <i>Xylella fastidiosa</i> based on Francis et al., 2006, EJPP 115, 203-213: Review of existing validation data, modification of test and in silico analysis (No. D0013/18). National Institute of Biology, Department of Biotechnology and Systems Biology, Ljubljana; Dreo, Tanja and Pirc, Manca, 2018. qPCR for detection of <i>Xylella fastidiosa</i> based on Francis et al., 2006, EJPP 115, 203-213: Diagnostic specificity and sensitivity determined in spiked samples (PKIe) (No. D0014/18). National Institute of Biology, Department of Biotechnology and Systems Biology, Ljubljana; Dreo, Tanja and Pirc, Manca, 2018. qPCR for detection of <i>Xylella fastidiosa</i> based on Francis et al., 2006, EJPP 115, 203-213: Analytical sensitivity - standard curves (No. D0015/18). National Institute of Biology, Department of Biotechnology and Systems Biology, Ljubljana; Dreo, Tanja and Pirc, Manca, 2018 qPCR for detection of <i>Xylella fastidiosa</i> based on Schaad et al. (2002), Francis et al. (2006), Harper et al., 2010, erratum 2013: Analytical specificity (No. D0027/18). National Institute of Biology, Department of Biotechnology and Systems Biology, Ljubljana. |
| <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> | no  |
| <b>Description of the test</b>   |   |
| <b>Organism(s)</b>   | <i>Xylella fastidiosa</i> (XYLEFA)  |
| <b>Detection / identification</b>                                      | detection   |
| <b>Matrix(ces) tested</b>  | Leaves, Shoots Plant material (leaf veins and petioles, vascular tissue [xylem] from shoots)<br>Myrtus, Origanum, Nerium, Olea, Polygala, Prunus, Quercus, Rhamnus, Rosa, Rosmarinus, Rubus,  |

|  |  |
|--|--|
|  | Spartium, Vinca, and Vitis   |
| <b>Plant species tested</b>  | Acacia sp., Acer sp., Asparagus sp., Callistemon sp., Citrus sp., Coffea sp., Cytisus sp., Ficus sp., Ginkgo sp., Grevillea sp., Hedera sp., Heliotropium sp., Hydrangea sp., Juglans sp., Laurus sp., Lavandula sp., Lonicera sp., Morus sp., Myrtus sp., Nerium sp., Olea sp., Origanum sp., Polygala sp., Prunus sp., Quercus sp., Rhamnus sp., Rosa sp., Rubus sp., Salvia, Spartium sp., Vinca sp., Vitis sp. |
| <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>                                | yes  |
| <b>EPPO Diagnostic Protocol name</b>   | PM 7/024 Xylella fastidiosa (version 3)  |
| <b>Kit</b>   |  |
| <b>Is a kit used</b>   | yes  |
| <b>Manufacturer name</b>   | BIONOBILE  |
| <b>Specify the kit used</b>  | QuickPick™ SML Plant DNA   |
| Kit used following the manufacturer's instructions?                                  |  |
| <b>Other information</b>   |  |
| <b>Method: Molecular real time PCR</b>   |  |
| <b>Reference of the test description</b>   |  |
| <b>As or adapted from an EPPO diagnostic protocol</b>                                | yes  |
| <b>EPPO Diagnostic Protocol name</b>   | PM 7/024 Xylella fastidiosa (version 3)  |
| <b>Name of the test</b>  | Taqman real-time PCR tests (based on Francis et al., 2006)   |
| <b>Is the test modified compared to the reference test</b>                           | yes See attached document  |
| <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>Xylella fastidiosa(XYLEFA)</b>  |
| <b>Analytical sensitivity</b>  |  |
| <b>What is the smallest amount of target that can be detected reliably?</b>          | DNA: In total 1000 target copies per mL extracted DNA (log 3 cps/mL as determined with digital PCR) were reliably detected in several X. fastidiosa strains, NIB Z 1962 (X. fastidiosa subsp. multiplex, LMG 9063), NIB Z 1963 (X. fastidiosa subsp. fastidiosa from almond, LMG 15099) and CoDiRo strain. Standard curves in plant material:  |

|  |   |
|--|---|
|  | Concentrations from $5 \times 10^4$ to down to $5 \times 10^3$ to (target cps/mL) can be reliably detected in samples of olives ( $10^4$ ), oleander ( $5 \times 10^3$ ), rosemary ( $10^4$ ) and lavender ( $5 \times 10^4$ ) plants tested for latent infection. Spiked PKIe controls: 98 % analytical sensitivity for symptomatic samples (111 different samples of 27 different genera were tested) and 100% analytical sensitivity for asymptomatic samples (66 different samples of 20 different genera were tested). |
| <b>Diagnostic sensitivity</b>  |   |
| <b>Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98</b> | No data available.  |
| <b>Analytical specificity - inclusivity</b>  |   |
| <b>Number of strains/populations of target organisms tested</b>  | 3   |
| <b>Specificity value</b>   | 100%  |
| <b>Analytical specificity - exclusivity</b>  |   |
| <b>Number of non-target organisms tested</b>   | 90  |
| <b>Specificity value</b>   | No cross reactivity.  |
| <b>Diagnostic Specificity</b>  |   |
| <b>Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test</b>        | No data available.  |
| <b>Reproducibility</b>   |   |
| <b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>   | 100%  |
| <b>Repeatability</b>   |   |
| <b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>   | 100%  |
| <b>Test performance study</b>  |   |
| <b>Test performance study?</b>   | no  |
| The following complementary files are available online:  |   |
|  | <ul style="list-style-type: none"> <li>• <a href="#">D0013_18_qPCR_Xyf_Francis_2006_ModificationInSilico</a></li> <li>• <a href="#">D0014_18_qPCR_Xyf_Francis_2006_DiagnosticSensitivityPKIe</a></li> <li>• <a href="#">D0015_18_qPCR_Xyf_Francis_2006_AnalyticalSensitivity_SCs</a></li> <li>• <a href="#">D0027_qPCR_Xyf_HarperSchaadFrancis_AnalyticalSpecificity</a></li> </ul>   |

Creation date: 2018-10-05 00:00:00 - Last update: 2021-01-22 10:01:05