**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.

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| **Target Organism** | Xylella fastidiosa |
| **Short description** | Detection of Xylella fastidiosa by real-time PCR (Harper et al., 2010, erratum 2013) in plant material |
| **Laboratory contact details** | National Institiute of Biology, Department of Biotechnology and Systems Biology<br>Vecna pot 121, 1000 Ljubljana, Slovenia |
| **Date and reference of the validation report** | 2018-09-17 - Dreo, Tanja, 2018. qPCR for detection of Xylella fastidiosa based on Harper et al., 2010, erratum 2013: Literature review and modification of test (No. D0023/18). National Institute of Biology, Department of Biotechnology and Systems Biology, Ljubljana; Dreo, Tanja and Pirc, Manca, 2018. qPCR for detection of Xylella fastidiosa based on Harper et al., 2010, erratum 2013: Analytical sensitivity – standard curves (No. D0024/18). National Institute of Biology, Department of Biotechnology and Systems Biology, Ljubljana; Dreo, Tanja and Pirc, Manca, 2018 qPCR for detection of Xylella fastidiosa based on Harper et al., 2010, erratum 2013: Diagnostic specificity and sensitivity determined in spiked samples (PKIe) (No. D0025/18). National Institute of Biology, Department of Biotechnology and Systems Biology, Ljubljana; Dreo, Tanja and Pirc, Manca, 2018 qPCR for detection of Xylella fastidiosa 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. |
| **Validation process according to EPPO Standard PM 7/98:** | Yes |
| **Reference of the test description** | 0<br>EPPO PM 7/24 (3) - Appendix 5 - Real time PCR (Harper et al., 2010; erratum 2013) |
| **Is the test the same as described in the EPPO DP?** | Modified<br>For modification see report Dreo, Tanja, 2018. qPCR for detection of Xylella fastidiosa based on Harper et al., 2010, erratum 2013: Literature review and modification of test (No. D0023/18). |
| **Is the lab accredited for this test?** | Yes |
| **Plant species tested (if relevant)** | Genera: Acacia, Acer, Asparagus, Callistemon, Citrus, Coffea, Cytisus, Ficus, Ginko, Grevillea, Hebe, Hedera, Heliotropium, Helichrysum, Hydrangea, Juglans, Laurus, Lavandula, Lonicera, Morus, Myrtus, Origanum, Nerium, Olea, Polygala, Prunus, Quercus, Rhamnus, Rosa, Rosmarinus, Rubus, Spartium, Vaccinium , Vinca, and Vitis |
| **Matrices tested (if relevant)** | Plant material (leaf veins and petioles, vascular tissue [xylem] |

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| | from shoots) | |
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| ***List of methods used*** | | |
| **Method for extraction / isolation / baiting of target organism from matrix** | | |
| **Molecular methods, e.g. hybridization, PCR and real time PCR** | X | DNA extraction from plant material using QuickPick™ SML Plant DNA kit (Bionobile). Modified real-time PCR adapted from Harper, S.J., Ward, L.I., Clover, G.R.G., 2010. Development of LAMP and real-time PCR methods for the rapid detection of Xylella fastidiosa for quarantine and field applications. Phytopathology 100, 1282–1288. https://doi.org/10.1094/PHYTO-06-10-0168 |
| **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?** | DNA: 500 target copies per mL extracted DNA (log 2.7 cps/mL as determined with digital PCR) were reliably (min. 2/3 parallel reactions positive) with real-time PCR when testing NIB Z 1963 (X. fastidiosa subsp. fastidiosa from almond, LMG 15099), 1000 target copies per mL extracted DNA (log 3,0 cps/mL as determined with digital PCR) were reliably (min. 2/3 parallel reactions positive) detected with real time PCR in when testing CoDiRo strain and 5000 target copies per mL extracted DNA (log 3,7 cps/mL as determined with digital PCR) were reliably (min. 2/3 parallel reactions positive) detected with real time PCR when testing NIB Z 1962 (X. fastidiosa subsp. multiplex)<br><br>Standard curves in plant material: Concentrations from 10^4 to down to 10^3 to (target cps/mL) can be reliably detected in samples of olives (10^4), oleander (10^3), rosemary (10^3) and lavender (5x10^3) plants tested for latent infection.<br><br>Spiked PKIe controls: 100 % analytical sensitivity (139 different symptomatic samples of 27 different genera and 77 asymptomatic (latent) samples of 22 different genera were | |

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| | tested). |
| **Diagnostic sensitivity** | |
| **Proportion of infected/infested samples tested positive compared to results from the standard test , see appendix 2 of PM 7/98** | No data available. |
| **Specify the standard test** | |
| **Analytical specificity** | |
| **Specificity value** | 100% |
| **Number of strains/populations of target organisms tested** | 3 |
| **Number of non-target organisms tested** | 90 |
| **Cross reacts with (specify the species)** | No cross reactivity. |
| **Diagnostic Specificity** | |
| **Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test** | No data available. |
| **Specify the standard test** | |
| **Reproducibility** | |
| **Provide the calculated % of agreement for a given level of the pest (see PM 7/98)** | 100% |
| **Repeatability** | |
| **Provide the calculated % of agreement for a given level of the pest (see PM 7/98)** | 100% |
| **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**<br>**e.g. robustness, ease of performing the test, etc.** | |
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| The following complementary files are available online: | • qPCR for detection of Xylella fastidiosa based on Harper et al., 2010, erratum 2013: Analytical sensitivity – standard curves (No. D0024/18)<br>• qPCR for detection of Xylella fastidiosa based on |

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| | Harper et al., 2010, erratum 2013: Diagnostic specificity and sensitivity determined in spiked samples (PKIe) (No. D0025/18)<br>• qPCR for detection of Xylella fastidiosa based on Harper et al., 2010, erratum 2013: Literature review and modification of test (No. D0023/18)<br>• qPCR for detection of Xylella fastidiosa based on Schaad et al. (2000), Francis et al. (2006), Harper et al., 2010, erratum 2013: Analytical specificity (No. D0027/18) |