

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 in perennial host species by Real time PCR | |
| Laboratory contact details | Institute for Sustainable Plant Protection via Amendola, 122/D, 70126 Bari, Italy | |
| Date and reference of the validation report | 2014-09 and 2015-07 - Maria Saponari, Giuliana Loconsole, Oriana Potere, Donato Boscia, 2014 and 2015. DETECTION OF XYLELLA FASTIDIOSA, INTERLABORATORY VALIDATION - MOLECULAR AND SEROLOGICAL METHODS | |
| Validation process according to EPPO Standard PM 7/98: | No | |
| Reference of the test description | N/R - Loconsole, G., Potere, O., Boscia, D., Altamura, G., Djelouah, K., Elbeaino, T., Frasheri, D., Lorusso, D., Palmisano, F., Pollastro, P., Silletti, M. R., Trisciuzzi, N., Valentini, F., Savino V. & Saponari, M. (2014). Detection of Xylella fastidiosa in olive trees by serological and molecular methods. Journal of Plant Pathology, 96, 7-14. - 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. | |
| Is the test the same as described in the EPPO DP? | No | |
| Is the lab accredited for this test? | Yes | |
| Plant species tested (if relevant) | Olea Europaea L., Prunus avium, Prunus dulcis, Nerium Oleander, Polygala myrtifolia, Acacia saligna, Quercus ilex, Citrus spp, Vitis spp | |
| Matrices tested (if relevant) | leaf petiols | |
| List of methods used | | |
| Method for extraction / isolation / baiting of target organism from matrix | X | CTAB-based protocol for total DNA extraction Loconsole, G., Potere, O., Boscia, D., Altamura, G., Djelouah, K., Elbeaino, T., Frasheri, D., Lorusso, D., Palmisano, F., Pollastro, P., Silletti, M. R., Trisciuzzi, N., Valentini, F., Savino V. & Saponari, M. (2014a). Detection of Xylella fastidiosa in olive trees by serological and molecular methods. Journal of Plant Pathology, 96, 7-14. |
| Molecular methods, e.g. | X | Real time PCR with Taqman probe |

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| hybridization, PCR and real time PCR | | Harper S.J., Ward L.I., Clover G.R.G., 2010. Development of LAMP and real-time PCR methods for the rapid detection of <i>Xylella fastidiosa</i> for quarantine and field applications. <i>Phytopathology</i> 100: 1282-1288. |
| 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? | | up to 10^2 cfu/ml (corresponding to 7 cfu/reaction) using dilutions ranging from 10^7 to 10^8 CFU/ml, prepared by spiking the inactivated bacterial culture in total nucleic acids recovered from olive reference sources known to be not infected by <i>Xylella fastidiosa</i> . |
| Diagnostic sensitivity | | |
| Proportion of infected/infested samples tested positive compared to results from the standard test , see appendix 2 of PM 7/98 | | 100% |
| Specify the standard test | | 108 obtained positive samples/ 108 expected positive samples |
| Analytical specificity | | |
| Specificity value | | |
| Number of strains/populations of target organisms tested | | |
| Number of non-target organisms tested | | |
| Cross reacts with (specify the species) | | |
| Diagnostic Specificity | | |
| Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test | | 100% |

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| Specify the standard test | 90 obtained negative samples/ 90 expected negative samples |
| 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. If available, provide a link to published article/report | |
| Other information | |
| Any other information considered useful e.g. robustness, ease of performing the test, etc. | <p>Validation of the Real time PCR assay was carried out by the Laboratories listed below, under the supervision of the reference laboratory CNR-UNIBA.</p> <ul style="list-style-type: none"> • IPSP-CNR: Istituto per la Protezione Sostenibile delle Piante CNR, UOS Bari, (Italy); • DiSSPA-UNIBA: Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti, Università degli Studi Aldo Moro, Bari (Italy); • CRSFA: Centro di Ricerca, Sperimentazione e Formazione in Agricoltura Basile Caramia, Locorotondo (BA) (Italy); • IAMB: Istituto Agronomico Mediterraneo, Valenzano (BA) (Italy); • Dipartimento di Scienze Agroambientali, Chimica e Difesa Vegetale - Università degli Studi di Foggia, (Italy). <p>A panel of blind samples was distributed.</p> |
| The following complementary files are available online: | <ul style="list-style-type: none"> • protocols for diagnosis of Xylella fastidiosa • report interlaboratory validation 2014 • report interlaboratory validation 2015 |