

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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| Laboratory contact details | Anses Plant Health Laboratory - Bacteriology, Virology and GMO Unit 7 rue Jean Dixm ras, 49044 Angers, France |
| Short description of the test | Detection of grapevine phytoplasmas of the 16SrV and 16SrXII-A groups |
| Date, reference of the validation report | 2021-05-18 - Pelletier et al., 2009. Triplex real-time PCR assay for sensitive and simultaneous detection of grapevine phytoplasmas. <i>Vitis</i> 48(2), 87-95. |
| 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? | |
| Description of the test | |
| Organism(s) | Grapevine flavescence dor e phytoplasma (PHYP64) 'Candidatus Phytoplasma solani' (PHYPSO) |
| Detection / identification | detection |
| Method(s) | Molecular real time PCR |
| Method: Molecular real time PCR | |
| Reference of the test description | |
| As or adapted from an EPPO diagnostic protocol | yes |
| New test being considered for inclusion in the next version of the EPPO diagnostic protocol? | yes |
| EPPO Diagnostic Protocol name | PM 7/079 Grapevine flavescence dor e phytoplasma (version 2) |
| Name of the test | Multiplex real-time PCR according to Pelletier et al. (2009) |
| As or adapted from an IPPC diagnostic protocol | no |
| Other information | |
| Reaction type | Triplex - Probe |
| Are the performance characteristics included | yes |

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| in the EPPO diagnostic protocol? | |
| Performance Criteria : | |
| Organism 1.: | Grapevine flavescence dorée phytoplasma(PHYP64) |
| Analytical sensitivity | |
| What is smallest amount of target that can be detected reliably? | In our condition, FD: to a dilution of 5 ⁷ of a FD infected sample in water (100 times more sensitive than nested PCR) |
| Diagnostic sensitivity | |
| Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98 | 100% for each target |
| Standard test(s) | For FD: 4 samples agreement/4 |
| Analytical specificity - inclusivity | |
| Number of strains/populations of target organisms tested | samples for FD: FD (CAM-05) type FD1/V. faba (Gironde, France) FD (PEY-05) type FD2/V. faba (Gironde, France) FD (VI04-Lig2) type FD3/V. vinifera (Veneto, Italy) FD (VI04-C28) type FD3/V. vinifera (Veneto, Italy) |
| Specificity value | 100% for each target |
| Analytical specificity - exclusivity | |
| Number of non-target organisms tested | Healthy C. roseus Healthy V. faba Healthy V. vinifera cv Pinot noir Healthy V. vinifera cv Gewurztraminer Healthy V. vinifera cv Chardonnay Healthy V. vinifera cv Riesling Healthy V. vinifera cv Cabernet Franc Healthy V. vinifera cv Cabernet sauvignon 16SrI- Aster yellow (AY Whitcomb)/ C. roseus (USA) 16SrI - Clover phyllody (KVF)/C. roseus (France) 16SrII - Tomato big bud (TBB)/C. roseus (Australia) 16SrII - Whitches' broom disease of lime (WBDL)/C. roseus (Oman Sultanate) 16SrIII - Peach western X (Peach WX)/C. roseus (USA) 16SrVI - Brinjal little leaf (BLL)/C. roseus (India) 16SrVII - Ash yellows (Ash 12)/C. roseus (USA) 16SrX - Apple proliferation (AP-15)/C. roseus (Italy) 16SrX - European stone fruit yellows (ESFY)/C. roseus (Italy) 16SrX - Pear decline (PD)/C. roseus (Germany) |
| Specificity value | other phytoplasmas of the 16SrV group can be detected: PGY (PGYA et PGYC), GY (V04-11-1), AldY (ALY), RS, Spa W |
| Diagnostic Specificity | |
| Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test | 100% for each target |
| Specify the test(s) | For FD: 29 samples agreement/29 |
| Reproducibility | |
| Provide the calculated % of agreement for a given level of the pest (see PM 7/98) | For FD: 98.72% |

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| Repeatability | |
| Provide the calculated % of agreement for a given level of the pest (see PM 7/98) | For FD: 99 to 100% |
| Organism 2.: | 'Candidatus Phytoplasma solani'(PHYPSO) |
| Analytical sensitivity | |
| What is smallest amount of target that can be detected reliably? | In our condition, BN: to a dilution of 5 ⁴ of a BN infected sample in water (5 times more sensitive than nested PCR) |
| Diagnostic sensitivity | |
| Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98 | 100% for each target |
| Standard test(s) | For BN: 11 samples agreement/11 |
| Analytical specificity - inclusivity | |
| Number of strains/populations of target organisms tested | Samples for BN: Stolbur (P7)/C. roseus (Lebanon) Stolbur (Moliere)/C. roseus (France) Stolbur (Charente-1)/C. roseus (Charente, France) Stolbur (Charente-2)/C. roseus (Charente, France) Stolbur (LG)/C. roseus (Lot et Garonne, France) Stolbur (C)/C. roseus (France) Stolbur (PO)/C. roseus (Pyrénées Orientales, France) Stolbur (Red-Pepper)/C. roseus (Serbia) VK (GGY)C. roseus (Pfalz, Germany) VK (19-25)/C. roseus (Pfalz, Germany) BN (CH1)C. roseus (Italy) |
| Specificity value | 100% for each target |
| Analytical specificity - exclusivity | |
| Number of non-target organisms tested | Healthy C. roseus Healthy V. faba Healthy V. vinifera cv Pinot noir Healthy V. vinifera cv Gewurztraiminer Healthy V. vinifera cv Chardonnay Healthy V. vinifera cv Riesling Healthy V. vinifera cv Cabernet Franc Healthy V. vinifera cv Cabernet sauvignon 16Srl- Aster yellow (AY Whitcomb)/ C. roseus (USA) 16Srl - Clover phyllody (KVF)/C. roseus (France) 16SrII - Tomato big bud (TBB)/C. roseus (Australia) 16SrII - Whitches' broom disease of lime (WBDL)/C. roseus (Oman Sultanate) 16SrIII - Peach western X (Peach WX)/C. roseus (USA) 16SrVI - Brinjal little leaf (BLL)/C. roseus (India) 16SrVII - Ash yellows (Ash 12)/C. roseus (USA) 16SrX - Apple proliferation (AP-15)/C. roseus (Italy) 16SrX - European stone fruit yellows (ESFY)/C. roseus (Italy) 16SrX - Pear decline (PD)/C. roseus (Germany) |
| Specificity value | other phytoplasmas of the 16SrV group can be detected: PGY (PGYA et PGYC), GY (V04-11-1), AldY (ALY), RS, Spa W |
| Diagnostic Specificity | |
| Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test | 100% for each target |

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| Specify the test(s) | For BN: 30 samples agreement/30 |
| Reproducibility | |
| Provide the calculated % of agreement for a given level of the pest (see PM 7/98) | For BN: 94.87% |
| Repeatability | |
| Provide the calculated % of agreement for a given level of the pest (see PM 7/98) | For BN: 92.31 to 100% |
| Test performance study | |
| Test performance study? | yes |
| Brief details of the test performance study and its output. It available, link to published article/report | Ring-tested during the GRAFDEPI (Euphresco projet), 6 laboratories tested this method on a total of 15 participants. Results obtained for FD detection: - Accuracy: 96.27% - Diagnostic sensitivity: 97.75% - Diagnostic specificity: 93.33% - Repeatability: 94.93% - Reproducibility: 93.27% Loiseau, M. (2015). European interlaboratory comparison of detection methods for “flavescence dorée” phytoplasma: preliminary results. Phytopathogenic Mollicutes, 5(1s), S35-S37. |
| Other information | |
| Any other information considered useful | other validation data available on request at the Plant Health Laboratory of ANSES (ANSES-LSV, France) |

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