**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** | Xanthomonas fragariae | |
| **Short description** | Real-time PCR for detection and identification purposes of Xanthomonas fragariae | |
| **Laboratory contact details** | National Reference Centre, National Plant Protection Organization<br>P.O. Box 9102, 6700 HC Wageningen, Netherlands | |
| **Date and reference of the validation report** | 2011-05-17 - 2010.Molbio.028 | |
| **Validation process according to EPPO Standard PM 7/98:** | Yes | |
| **Reference of the test description** | N/R | |
| **Is the test the same as described in the EPPO DP?** | No<br>No, may be used for revision of EPPO PM 7/042 | |
| **Is the lab accredited for this test?** | No | |
| **Plant species tested (if relevant)** | Fragaria spp | |
| **Matrices tested (if relevant)** | leaves and rhizomes from different cultivars of Fragaria spp. and bacterial colony material | |
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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 | Real-time PCR (Weller et al., 2007) |
| **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** | | |

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| **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?** | 8,0x104 cfu/ml for detection in Fragaria extract and 7,8x104 cfu/ml for pure cultures. | |
| **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** | Isolates were identified according the EPPO standard PM 7/65, by using Fatty acid analysis, IF and the conventional PCR based on (Pooler et al., 1996). | |
| **Analytical specificity** | | |
| **Specificity value** | 100% | |
| **Number of strains/populations of target organisms tested** | 19 | |
| **Number of non-target organisms tested** | 6 | |
| **Cross reacts with (specify the species)** | none | |
| **Diagnostic Specificity** | | |
| **Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test** | 100% | |
| **Specify the standard test** | Isolates were identified according the EPPO standard PM 7/65, by using Fatty acid analysis, IF and the conventional PCR based on (Pooler et al., 1996). | |
| **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.** | 1. Selectivity: tested with 5 isolates of X. fragariae in 4 different matrices (leaf and rhizome of Fragaria variety Elsanta and Selva). The selectivity was valid when the samples diluted in the different matrices gave a positive signal inside the defined detection limit of 8,0x104 cfu/ml. This was true for all used matrices. However, the detection limit of the real-time PCR in leaf and rhizome extract was found to be a factor 1,3 and 3,4 lower for variety Selva than for variety Elsanta.<br><br>2. Robustness: This real-time PCR, for both detection in leaves and rhizomes and identification purposes, has been found robust for variations in the DNA extraction method. Three different DNA extraction methodologies have been performed and scored equally well:<br>1. QuickPick Plant DNA Kit (Bio-Nobile, KingFisher method)<br>2. High Pure PCR Template preparation kit (Roche)<br>3. Boiling method<br>Application of High Pure PCR Template preparation kit (Roche) resulted in a more sensitive detection. |
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| The following complementary files are available online: | • Real-time PCR for detection and identification of Xanthomonas fragariae |