**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.

| | |
|---|---|
| **Laboratory contact details** | Netherlands Institute for Vectors, Invasive plants and Plant health<br>P.O. Box 9102, 6700 HC Wageningen, Netherlands |
| **Short description of the test** | Real-time PCR for detection and identification purposes of Xanthomonas fragariae |
| **Date, reference of the validation report** | 2011-05-17 - 2010.Molbio.028 |
| **Validation process according to EPPO Standard PM7/98?** | yes |
| **Is the lab accredited for this test?** | no |
| **Was the validated data generated in the framework of a project?** | no |
| | |
| **Description of the test** | |
| | |
| **Organism(s)** | Xanthomonas fragariae (XANTFR) |
| **Detection / identification** | detection and identification |
| **Method(s)** | Molecular Extraction DNA RNA<br>Molecular Extraction DNA RNA (2)<br>Molecular Extraction DNA RNA (3)<br>Molecular real time PCR |
| **Method: Molecular Extraction DNA RNA** | |
| ***Reference of the test description*** | |
| **As or adapted from an EPPO diagnostic protocol** | no |
| **As or adapted from an IPPC diagnostic protocol** | no |
| ***Kit*** | |
| **Is a kit used** | yes |
| **Manufacturer name** | BIONOBILE |
| **Specify the kit used** | QuickPick Plant DNA kit |
| Kit used following the manufacturer's instructions? | |
| ***Other information*** | |
| **Method: Molecular Extraction DNA RNA (2)** | |
| ***Reference of the test description*** | |

| | |
|---|---|
| **As or adapted from an EPPO diagnostic protocol** | no |
| **As or adapted from an IPPC diagnostic protocol** | no |
| ***Kit*** | |
| **Is a kit used** | yes |
| **Manufacturer name** | ROCHE |
| **Specify the kit used** | High Pure PCR Template Preparation Kit |
| Kit used following the manufacturer's instructions? | |
| ***Other information*** | |
| **Method: Molecular Extraction DNA RNA (3)** | |
| ***Reference of the test description*** | |
| ***Other information*** | |
| **Other details on the test** | Boiling method |
| **Method: Molecular real time PCR** | |
| ***Reference of the test description*** | |
| **As or adapted from an EPPO diagnostic protocol** | no |
| **As or adapted from an IPPC diagnostic protocol** | no |
| **Reference of the test** | Weller et al., 2007 |
| ***Other information*** | |
| **Are the performance characteristics included in the EPPO diagnostic protocol?** | **no** |
| **Performance Criteria :** | |
| **Organism 1.:** | **Xanthomonas fragariae(XANTFR)** |
| **Analytical sensitivity** | |
| **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% |
| **Standard test(s)** | 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 - inclusivity** | |
| **Number of strains/populations of target organisms tested** | 19 |
| **Specificity value** | 100% |

| | |
|---|---|
| **Analytical specificity - exclusivity** | |
| **Number of non-target organisms tested** | 6 |
| **Specificity value** | 100% no cross reaction |
| **Diagnostic Specificity** | |
| **Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test** | 100% |
| **Specify the test(s)** | 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 |
| **Other information** | |
| **Any other information considered useful** | 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. 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: 1. QuickPick Plant DNA Kit (Bio-Nobile, KingFisher method) 2. High Pure PCR Template preparation kit (Roche) 3. Boiling method Application of High Pure PCR Template preparation kit (Roche) resulted in a more sensitive detection. |
| | |
| The following complementary files are available online: | • Real-time PCR for detection and identification of Xanthomonas fragariae |

*Creation date: 2013-05-28 00:00:00 - Last update: 2021-01-21 18:34:42*