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 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
Matrix(ces) tested	Leaves, Pure culture, Roots leaves and rhizomes from different cultivars of Fragaria spp. and bacterial colony material
Plant species tested	Fragaria sp.
Method(s)	Molecular Extraction DNA RNA Molecular Extraction DNA RNA (2) Molecular Extraction DNA RNA (3) 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 the 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

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