

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.

Target Organism	Xanthomonas fragariae	
Short description	Real-time PCR for detection and identification purposes of Xanthomonas fragariae	
Laboratory contact details	Netherlands Institute for Vectors, Invasive plants and Plant health 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 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	
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		

Morphological and morphometrical methods intended for identification		
Biochemical methods: e.g. enzyme electrophoresis, protein profiling		
Other		
<u>Analytical sensitivity (= limit of detection)</u>		
What is smallest amount of target that can be detected reliably?	8,0x10 ⁴ cfu/ml for detection in Fragaria extract and 7,8x10 ⁴ cfu/ml for pure cultures.	
<u>Diagnostic sensitivity</u>		
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).	
<u>Analytical specificity</u>		
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	
<u>Diagnostic Specificity</u>		
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).	
<u>Reproducibility</u>		
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100%	
<u>Repeatability</u>		
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100%	
<u>Test performance study</u>		
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 e.g. robustness, ease of performing the test, etc.	<p>1. Selectivity: tested with 5 isolates of <i>X. fragariae</i> in 4 different matrices (leaf and rhizome of <i>Fragaria</i> 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 $8,0 \times 10^4$ 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.</p> <p>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:</p> <ol style="list-style-type: none"> 1. QuickPick Plant DNA Kit (Bio-Nobile, KingFisher method) 2. High Pure PCR Template preparation kit (Roche) 3. Boiling method <p>Application of High Pure PCR Template preparation kit (Roche) resulted in a more sensitive detection.</p>
The following complementary files are available online:	<ul style="list-style-type: none"> • Real-time PCR for detection and identification of <i>Xanthomonas fragariae</i>