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	Pantoea stewartii subsp. stewartii	
Short description	detection of Pantoea stewartii subsp.stewartii using a FLASH PCR detection Kit	
Laboratory contact details	Federal State Organization "All-Russian Plant Quarantine Center" Pogranichnaya str.32, Ramensky region, Moscow obl., 140150 Bykovo, Russia	
Date and reference of the validation report	2012 - Validation of FLASH-PCR method for detection of bacterial wilt Pantoea stewartii subsp. stewartii (Smith) Mergaert et al. in seed extract	
Validation process according to EPPO Standard PM 7/98:	Yes	
Reference of the test description	PM 7/060	
Is the test the same as described in the EPPO DP?	Modified use of DNA extraction of "PREP-GS" kit and FLASH PCR detection Kit from the company OOO AgroDiagnostiKa (Moscow)	
Is the lab accredited for this test?	Yes	
Plant species tested (if relevant)	Zea mays	
Matrices tested (if relevant)	Seed	
List of methods used		
Method for extraction / isolation / baiting of target organism from matrix	X	Plant extracts were obtained according to EPPO diagnostic protocol PM 7/60 (1) (OEPP/EPPO Bulletin, 2006) and STO VNIIKR 4.002-2010.
Molecular methods, e.g. hybridization, PCR and real time PCR	X	DNA extraction of "PREP-GS" kit from the company OOO AgroDiagnostiKa (Moscow) according to manufacturer instructions. FLASH PCR detection Kit (for fluorescence end-point detection) for detection and identification of P. stewartii subsp. stewartii from samples of plant and seeds from the company OOO AgroDiagnostiKa (Moscow) according to manufacturer instructions
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		
Analytical sensitivity (= limit of detection)		
What is smallest amount of target that can be detected reliably?	The analytical sensitivity of the method was 10^2- 10^3 CFU/ml	
Diagnostic sensitivity		
Proportion of infected/infested samples tested positive compared to results from the standard test , see appendix 2 of PM 7/98	Not evaluated	
Specify the standard test		
Analytical specificity		
Specificity value		
Number of strains/populations of target organisms tested	4 the strain P. s. ssp. stewartii SW4 and DNA preparations of 3 strains of this type: CFBP1719, CFBP 3167, DSM3017660, purchased from bacteriological collections of Europe	
Number of non-target organisms tested	43 strains and DNA preparations of the following species from of the VNIIKR FGBU collection: Clavibacter michiganensis subsp. michiganensis (Smith) Devis et al.; Clavibacter michiganensis subsp. sepedonicus (Spieckermann & Kotthoff) Davis et al.; Erwinia amylovora (Burrill) Winslow et al.; Dickeya chrysanthemi bv. parthenii (Starr) Hauben et al.; Dickeya chrysanthemi pv. chrysanthemi (Burkholder et al.) Brenner et al. Pantoea agglomerans (Beijerinck, 1888) Gavini et al.; Pantoea dispersa Gavini et al.; Pantoea stewartii subsp. indologenes Mergaert et al.; Pantoea stewartii subsp. stewartii (Smith) Mergaert et al.; Pectobacterium atrosepticum (Gardan et al.); Pectobacterium carotovorum subsp. carotovorum (Jones) Waldee et al.; Pseudomonas syringae pv. syringae van Hall; Ralstonia solanacearum (Smith) Yabuuchi et al.; Xanthomonas campestris pv. campestris; Xanthomonas fragariae Kennedy and King; Xanthomonas oryzae (ex Ishiyama) Swings et al. pv. oryzae (Ishiyama) Swings et al.;	

	Xanthomonas vesicatoria (ex Doidge) Vauterin et al.
Cross reacts with (specify the species)	A nonspecific reaction was observed with one strain (out of 10) of Pantoea agglomerans (Pa 68) DNA, obtained from the bacteriological laboratory in Vienna
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	
Specify the standard test	
Reproducibility	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	Tests were performed by different operators on different equipment and at different time. Reproducibility of test results was absolute (100%) for samples with 10^3 and 10^4 CFU/ml contamination and low (56%) for samples with an infection rate of 10^2 CFU/ml.
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	Repeatability of test results was absolute (100%) for samples with 10^3 CFU/ml contamination and low (50%) for samples with an infection rate of 10^2 CFU/ml.
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 e.g. robustness, ease of performing the test, etc.	The effect of host plant extracts was assessed using 5 varieties of treated or untreated seeds. No impact of the substrate on the analysis was identified at the 10^3 cfu/ml contamination level.
The following complementary files are available online:	• О ВНИИКР 02-2012_оф.копия