

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	Conventional PCR (AGES primers) from corn seed macerate	
Laboratory contact details	EUPHRESCO PANTOEA STEWARTII , , EUPHRESCO	
Date and reference of the validation report	2011-05-31 -	
Validation process according to EPPO Standard PM 7/98:	Yes	
Reference of the test description	PM 7/060 Unpublished test	
Is the test the same as described in the EPPO DP?	Yes	
Is the lab accredited for this test?	No	
Plant species tested (if relevant)	Zea mays	
Matrices tested (if relevant)	PCR can be performed for identification of isolates/strains and symptomatic plant parts; for asymptomatic part or seed detection, in the framework of the test performance study, this PCR was not the most sensitive but the evaluation of this PCR was performed with DNA extraction.	
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	Conventional PCR
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?	10 ² CFU/mL on pure culture	
Diagnostic sensitivity		
Proportion of infected/infested samples tested positive compared to results from the standard test , see appendix 2 of PM 7/98	Not tested	
Specify the standard test		
Analytical specificity		
Specificity value	Inclusivity + exclusivity= 96,7% on 30 target and no-target strains	
Number of strains/populations of target organisms tested	<p>Inclusivity tested with 15 target strains: 100% (bacterial suspension concentrations about 10⁷ bact./mL)</p> <p>Pantoea stewartii subsp. stewartii: CFBP 3167/ NCPPB 2295/ICMP 257ATCC 8199; CFBP1719/ ICPB SS104; CFBP 2502 / NCPPB 449</p> <p>CFBP 3157/ NCPPB 1553; CFBP 3166 / ICMP 5930; CFBP 3393/ LMG 2716/ PDDCC 270; CFBP 3394/ LMG 2717/ PDDCC 722; CFBP 3395/ LMG 2718/ ATCC 8200; CFBP 3396/ LMG 2719/ PDDCC 5929; CFBP 3445/ NCPPB 3379; CFBP 3517; CFBP 3168; CFBP 3165; CFBP 3169; NCPPB 3253</p>	
Number of non-target organisms tested	Exclusivity was performed by one lab on a list of 100 non-target strains and obtained 99% with the cross reaction of P indologenes	
Cross reacts with (specify the species)	Yes with Pantoea stewartii subsp. indologenes	
Diagnostic Specificity		
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	Not tested	
Specify the standard test		
Reproducibility		
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)		
Repeatability		
Provide the calculated % of	100%	

agreement for a given level of the pest (see PM 7/98)	
Test performance study	
Test performance study?	Yes
Include brief details of the test performance study and its output. If available, provide a link to published article/report	<p>Based on the test performance study with 5 laboratories involved, in the framework of EUPH05 Pantoea stewartii ssp. stewartii, EUPHRESKO Final Report 2010-2011</p> <p>Analytical sensitivity: 70% of agreement at 7×10^4 CFU/mL Diagnostic sensitivity (10 samples/2repeats/soak/lab): 70 % Diagnostic specificity (5 samples/2repeats/soak/lab): 76 % (cross reaction with Pantoea stewartii subsp. indologenes) Qualitative Repeatability: 95 % Accuracy: <90% Qualitative Reproducibility: 58 %</p>
Other information	
Any other information considered useful e.g. robustness, ease of performing the test, etc.	