

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 (Coplin 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 Coplin DL & Majerczak DR (2002) Identification of Pantoea stewartii subsp. stewartii by PCR and strain differentiation by PFGE. Plant Disease 86, 304-311.	
Is the test the same as described in the EPPO DP?	Modified In the Euphresco project, unmodified PCR was used and the results were not good due to mistakes in the original publication	
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 Euphresco study, this PCR was not the most sensitive but the original article of Coplin contained errors.	
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 ⁴ (ES16/IG2) to 10 ⁶ (HRP) CFU/mL according to primers 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		
Number of strains/populations of target organisms tested	<p>Inclusivity tested with 15 target strains: 62% (ES16/IG2); 58,9% (HRP) (bacterial suspension concentrations about 10⁷ bact./mL) Pantoea stewartii subsp. stewartii: CFBP 3167/ NCPPB 2295/ICMP 257ATCC 8199; CFBP1719/ ICPB SS104; CFBP 2502 / NCPPB 449; 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	<p>Exclusivity: 100% Clavibacter michiganensis subsp. michiganensis CFBP 4999 / LNPV 30.31; Clavibacter michiganensis subsp. nebraskensis CFBP 2405 / LNPV 10.17; Curtobacterium flaccumfaciens pv flaccumfaciens CFBP 3456 /LNPV 10.24; Erwinia chrisanthemi pv.zea CFBP 2052; Erwinia amylovora CFBP 1232/ NCPPB 683/ ATCC 15580/ CCM 114; Erwinia carotovora subsp. carotovora CFBP 2046; Erwinia carotovora subsp. atroseptica CFBP 1526; Pantoea agglomerans CFBP 3845/ ATCC 27155/ CIP 5751; Pantoea ananas pv. uredovora CFBP 3171; Pseudomonas syringae pv. syringae CFBP 1392; Pseudomonas viridiflava CFBP 1141 / LNPV 3.40; Xanthomonas campestris pv. campestris CFBP 5251 /NCPBP 528; Pantoea stewartii subsp.indologenes CFBP 3614/ ICMP 77 / LMG 2632 / NCPPB 2280; Pseudomonas syringae pv lapsa CFBP 1731; Pseudomonas corrugata CFBP 2431</p>	
Cross reacts with (specify the species)	No cross reaction identified on 15 non-target strains	
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 agreement for a given level of the pest (see PM 7/98)	100%
Test performance study	
Test performance study?	No
Include brief details of the test performance study and its output. If available, provide a link to published article/report	
Other information	
Any other information considered useful e.g. robustness, ease of performing the test, etc.	Due to the bad results in inclusivity of the primers (false negative results), it was decided do not use Coplin primers and PCR in the Euphresco test performance study. According to information of J Nemeth (Hungary), there were some mistakes in the original paper causing risk of false negative results