

**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.

<b>Laboratory contact details</b>	EUPHRESCO PANTOEA STEWARTII , , EUPHRESCO
<b>Short description of the test</b>	Real-time PCR from corn seed macerate
<b>Date, reference of the validation report</b>	2011-05-31 -
<b>Validation process according to EPPO Standard PM7/98?</b>	yes
<b>Is the lab accredited for this test?</b>	yes
<b>Was the validated data generated in the framework of a project?</b>	Euphresco
<b>If yes, please specify</b>	
<b>Description of the test</b>	
<b>Organism(s)</b>	Pantoea stewartii subsp. stewartii(ERWIST)
<b>Detection / identification</b>	detection
<b>Matrix(ces) tested</b>	Other, Pure culture, Seeds Real-time PCR can be performed on the macerates obtained from symptomatic plant parts or after soaking of seeds.
<b>Plant species tested</b>	Zea mays
<b>Method(s)</b>	Molecular real time PCR
<b>Method: Molecular real time PCR</b>	
<b>Reference of the test description</b>	
<b>As or adapted from an EPPO diagnostic protocol</b>	yes
<b>New test being considered for inclusion in the next version of the EPPO diagnostic protocol?</b>	no
<b>EPPO Diagnostic Protocol name</b>	PM 7/060 Pantoea stewartii (version 2)
<b>Name of the test</b>	Real -time PCR (Tambong et al., 2008)
<b>As or adapted from an IPPC diagnostic protocol</b>	no
<b>Is the test modified compared to the reference test</b>	
<b>Kit</b>	
<b>Is a kit used</b>	

<b>Other information</b>	
<b>Reaction type</b>	
<b>Other details on the test</b>	
<b>Are the performance characteristics included in the EPPO diagnostic protocol?</b>	<b>yes</b>
<b>Performance Criteria :</b>	
<b>Organism 1.:</b>	<b>Pantoea stewartii subsp. stewartii(ERWIST)</b>
<b>Analytical sensitivity</b>	
<b>What is smallest amount of target that can be detected reliably?</b>	10 <sup>4</sup> CFU/mL (95% of agreement) on pure culture
<b>Diagnostic sensitivity</b>	
<b>Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98</b>	Not tested
<b>Standard test(s)</b>	
<b>Analytical specificity - inclusivity</b>	
<b>Number of strains/populations of target organisms tested</b>	Inclusivity tested with 15 target strains: 100% (bacterial suspension concentrations about 10 <sup>7</sup> bact./mL) <i>Pantoea stewartii</i> subsp. <i>stewartii</i> : 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;
<b>Specificity value</b>	Inclusivity + exclusivity= 96,7% on 30 target and no-target strains
<b>Analytical specificity - exclusivity</b>	
<b>Number of non-target organisms tested</b>	Exclusivity tested with 15 non-target strains: 92.9 % (bacterial suspension concentration of about 10 <sup>7</sup> bact./mL) <i>Clavibacter michiganensis</i> subsp. <i>michiganensis</i> CFBP 4999 / LNPV 30.31; <i>Clavibacter michiganensis</i> subsp. <i>nebraskensis</i> CFBP 2405 / LNPV 10.17; <i>Curtobacterium flaccumfasciens</i> pv <i>flaccumfasciens</i> CFBP 3456 /LNPV 10.24; <i>Erwinia chrisanthemi</i> pv. <i>zea</i> CFBP 2052; <i>Erwinia amylovora</i> CFBP 1232/ NCPPB 683/ ATCC 15580/ CCM 114; <i>Erwinia carotovora</i> subsp. <i>carotovora</i> CFBP 2046; <i>Erwinia carotovora</i> subsp. <i>atroseptica</i> CFBP 1526; <i>Pantoea agglomerans</i> CFBP 3845/ ATCC 27155/ CIP 5751; <i>Pantoea ananas</i> pv. <i>uredovora</i> CFBP 3171; <i>Pseudomonas syringae</i> pv. <i>syringae</i> CFBP 1392; <i>Pseudomonas viridiflava</i> CFBP 1141 / LNPV 3.40; <i>Xanthomonas campestris</i> pv. <i>campestris</i> CFBP 5251 /NCPPB 528; <i>Pantoea stewartii</i> subsp. <i>indologenes</i> CFBP 3614/ ICMP 77 / LMG 2632 / NCPPB 2280; <i>Pseudomonas syringae</i> pv <i>lapsa</i> CFBP 1731; <i>Pseudomonas corrugata</i> CFBP

	2431;
<b>Specificity value</b>	Yes with Pantoea stewartii subsp.indologenes
<b>Cross reacts with</b>	
<b>Diagnostic Specificity</b>	
<b>Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test</b>	Not tested
<b>Specify the test(s)</b>	
<b>Reproducibility</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	
<b>Repeatability</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	1
<b>Test performance study</b>	
<b>Test performance study?</b>	yes
<b>Brief details of the test performance study and its output.It available, link to published article/report</b>	Based on the test performance study with 5 laboratories involved, in the framework of EUPH05 Pantoea stewartii ssp. stewartii, EUPHRESKO Final Report 2010-2011 Analytical sensitivity: 95% of agreement at $<7 \times 10^3$ CFU/mL Diagnostic sensitivity (10 samples/2repeats/soak/lab): 96 % Diagnostic specificity (5 samples/2repeats/soak/lab): 78 % (cross reaction with P st indologenes) Qualitative Repeatability: 97 % Accuracy: 90% Qualitative Reproducibility: 87 %
<b>Other information</b>	
<b>Any other information considered useful</b>	No DNA extraction is required according to Tambong publication; during the Euphresco test performance study, no extraction was used and the data of performance got by this PCR was good in comparison with other methods.

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