

**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>	EUPHRESKO PANTOEA STEWARTII , , EUPHRESKO
<b>Short description of the test</b>	Detection of 'Candidatus Phytoplasma mali', 'Ca. P. pyri' and 'Ca. P. prunorum' by real-time PCR
<b>Date, reference of the validation report</b>	2011-05-31 - Not specified
<b>Validation process according to EPPO Standard PM7/98?</b>	yes
<b>Is the lab accredited for this test?</b>	no
<b>Was the validated data generated in the framework of a project?</b>	Euphresco
<b>If yes, please specify</b>	EUPH-PANTOEA
<b>Description of the test</b>	
<b>Organism(s)</b>	Pantoea stewartii subsp. stewartii (ERWIST)
<b>Detection / identification</b>	detection and identification
<b>Matrix(ces) tested</b>	Other IF 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, IF with Linaris antibodies was the most sensitive and the most specific in comparison with Loewe antibodies.
<b>Plant species tested</b>	Zea mays
<b>Method(s)</b>	Serological IF Serological IF (2)
<b>Method: Serological IF</b>	
<b>Reference of the test description</b>	
<b>As or adapted from an EPPO diagnostic protocol</b>	yes
<b>EPPO Diagnostic Protocol name</b>	PM 7/060 Pantoea stewartii (version 2)
<b>Name of the test</b>	Cell staining according to PM7/97
<b>Is the test modified compared to the reference test</b>	no
<b>Kit</b>	
<b>Is a kit used</b>	yes

<b>Manufacturer name</b>	
<b>Specify the kit used</b>	
Kit used following the manufacturer's instructions?	
<b>Other information</b>	
<b>Other details on the test</b>	Commercial Loewe polyclonal antibodies - IF on seed macerates
<b>Method: Serological IF (2)</b>	
<b>Reference of the test description</b>	
<b>As or adapted from an EPPO diagnostic protocol</b>	yes
<b>EPPO Diagnostic Protocol name</b>	PM 7/060 Pantoea stewartii (version 2)
<b>Name of the test</b>	Cell staining according to PM7/97
<b>Kit</b>	
<b>Is a kit used</b>	yes
<b>Manufacturer name</b>	LINARIS
<b>Specify the kit used</b>	Pantoea stewartii:AP (Pstew), Monoclonal, Conjugated Antibody
Kit used following the manufacturer's instructions?	
<b>Other information</b>	
<b>Other details on the test</b>	Linaris monoclonal antibodies
<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 the smallest amount of target that can be detected reliably?</b>	Linaris: $10^3$ to $10^4$ CFU/mL ; Loewe: $10^3$ CFU/mL 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>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 $10^7$ bact./mL) Pantoea stewartii subsp. stewartii: CFBP 3167/ NCPPB 2295/ICMP 257/ATCC 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 Linaris = 100% on 30 target and no-target strains Loewe= 98,6% 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 (bacterial suspension concentration of about $10^7$ bact./mL): Linaris: 100% Loewe: 96.2% Clavibacter michiganensis subsp. michiganensis CFBP 4999 / LNPV 30.31; Clavibacter michiganensis subsp. nebraskensis CFBP 2405 / LNPV 10.17; Curtobacterium flaccumfasciens pv. flaccumfasciens 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 / NCPPB 528; Pantoea stewartii subsp. indologenes CFBP 3614/ ICMP 77 / LMG 2632 / NCPPB 2280; Pseudomonas syringae pv. lapsa CFBP 1731; Pseudomonas corrugata CFBP 2431
<b>Specificity value</b>	
<b>Cross-reacts with</b>	Erwinia amylovora Pseudomonas corrugata
<b>Repeatability</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	100%
<b>Test performance study</b>	
<b>Test performance study?</b>	yes
<b>Brief details of the test performance study and its output. It is 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, EUPHRESCO Final Report 2010-2011 Linaris: Analytical sensitivity: 95% of agreement at $<7 \times 10^3$ CFU/mL Diagnostic sensitivity (10 samples/2 repeats/soak/lab): 75 % Diagnostic specificity (5 samples/2 repeats/soak/lab): 33 % (cross reaction with P st indologenes) Qualitative Repeatability: 97 % Accuracy: 61% Qualitative Reproducibility (common Linaris and Loewe): 65 % Loewe: Analytical sensitivity: 70% of agreement at $<7 \times 10^3$ CFU/mL Diagnostic sensitivity (10 samples/2 repeats/soak/lab): 92,5 % Diagnostic specificity (5 samples/2 repeats/soak/lab): 95 % (cross reaction with P st indologenes) Qualitative Repeatability: 79 % Accuracy: 93%