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.

Laboratory contact details	EUPHRESCO PANTOEA STEWARTII , , EUPHRESCO
Short description of the test	Real-time PCR from corn seed macerate
Date, reference of the validation report	2011-05-31 -
Validation process according to EPPO Standard PM7/98?	yes
Is the lab accredited for this test?	yes
Was the validated data generated in the framework of a project?	Euphresco
Description of the test	
Organism(s)	Pantoea stewartii subsp. stewartii (ERWIST)
Detection / identification	detection
Matrix(ces) tested	Other, Pure culture, Seeds Real-time PCR can be performed on the macerates obtained from symptomatic plant parts or after soaking of seeds.
Plant species tested	Zea mays
Method(s)	Molecular real time PCR
Method: Molecular real time PCR	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	yes
New test being considered for inclusion in the next version of the EPPO diagnostic protocol?	no
EPPO Diagnostic Protocol name	PM 7/060 Pantoea stewartii (version 2)
Name of the test	Real -time PCR (Tambong et al., 2008)
As or adapted from an IPPC diagnostic protocol	no
Other information	
Are the performance characteristics included in the EPPO diagnostic protocol?	**yes**
Performance Criteria :	
Organism 1.:	**Pantoea stewartii subsp. stewartii(ERWIST)**

Analytical sensitivity	
What is the smallest amount of target that can be detected reliably?	10^4 CFU/mL (95% of agreement) 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
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	Inclusivity tested with15 target strains: 100% (bacterial suspension concentrations about 10^7 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;
Specificity value	Inclusivity + exclusivity= 96,7% on 30 target and no-target strains
Analytical specificity - exclusivity	
Number of non-target organisms tested	Exclusity tested with 15 non-target strains: 92.9 % (bacterial suspension concentration of about 10^7 bact./mL) Clavibacter michiganensis subsp.michiganensis CFBP 4999 / LNPV 30.31; Clavibacter michiganensis subsp. nebraskensis CFBP 2405 / LNPV 10.17; Curtobacterium flacumfasciens pv flacumfasciens 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;
Specificity value	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
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	1

Test performance study	
Test performance study?	yes
Brief details of the test performance study and its output.It available, link to published article/report	Based on the test performance study with 5 laboratories involved, in the framework of EUPH05 Pantoea stewartii ssp. stewartii, EUPHRESCO Final Report 2010-2011 Analytical sensitivity: 95% of agreement at <7 x10^3 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 %
Other information	
Any other information considered useful	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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