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	Chalara fraxinea	
Short description	Detection of Chalara fraxinea by duplex real-time PCR test in planta	
Laboratory contact details	Anses Plant Health Laboratory - Mycology Unit Mycology Unit Domaine de Pixérécourt, Bât. E, 54220 Malzéville, France	
Date and reference of the validation report	2009-10 - LNPV 2009 Developement, évaluation et validation d'une méthode de détection de Chalara fraxinea	
Validation process according to EPPO Standard PM 7/98:	No	
Reference of the test description	0 Ioos R, Kowalski T, Husson C, Holdenrieder O: Rapid in planta detection of Chalara fraxinea by a real-time PCR assay using a dual-labelled probe. Eur J Plant Pathol 2009, 125(2):329-335. Ioos, R. and C. Fourrier (2011). "Validation and accreditation of a duplex real-time PCR test for reliable in planta detection of Chalara fraxinea." EPPO Bulletin 41(1): 21-26.	
Is the test the same as described in the EPPO DP?	No No EPPO DP available	
Is the lab accredited for this test?	Yes	
Plant species tested (if relevant)	Fraxinus spp.	
Matrices tested (if relevant)	Twigs, buds, stems, leaf rachis	
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	Duplex qPCR
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?	20 fg of target DNA in a background of Fraxinus DNA	
Diagnostic sensitivity		
Proportion of infected/infested samples tested positive compared to results from the standard test , see appendix 2 of PM 7/98	The novel qPCR and agar plating were compared separately on a set of naturally infested samples. A chi-2 test was carried out for each of the method, and showed that the qPCR test yielded significantly more positive results than agar plating (chi2=15.7, p<0.05)	
Specify the standard test	No standard test	
Analytical specificity		
Specificity value		
Number of strains/populations of target organisms tested	20 (see Table 1 in Ioos et al., 2009, in separated file)	
Number of non-target organisms tested	34 fungal taxa isolated form ash tissue (see Table 1 in Ioos et al., 2009, in separated file)	
Cross reacts with (specify the species)	No cross reaction observed	
Diagnostic Specificity		
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test		
Specify the standard test		
Reproducibility		
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	1.08% for a target concentration of 4.8 10^4 copies of the target DNA; 1.63% for a target concentration of 4.8 10^3 copies of the target DNA; 3.32% for a target concentration of 4.8 10^2 copies (LOD) of the target DNA; 2.56% for a naturally infested ash sample	
Repeatability		
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	0.96% for a target concentration of 4.8 10^4 copies of the target DNA 1.70% for a target concentration of 4.8 10^3 copies of the target DNA; 2.19% for a target concentration of 4.8 10^2 copies (LOD) of the target DNA; 0.89% for a naturally infested ash sample	
Test performance study		

Test performance study?	No
Include brief details of the test performance study and its output.It available, provide a link to published article/report	
Other information	
Any other information considered useful **e.g. robustness, ease of performing the test, etc.**	The robustness of the test was evaluated by assessing the effect of template DNA volume variation and PCR reaction volume variation on the Ct. (see Ioos et al. 2009 and Ioos et al. 2011 attached)
The following complementary files are available online:	• Ioos et al., 2009 • Ioos et al., 2011 • LNPV 2009 Rapport de validation