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	Anses Plant Health Laboratory - Mycology Unit Mycology Unit Domaine de Pixérécourt, Bât. E, 54220 Malzéville, France
Short description of the test	Detection of Chalara fraxinea by duplex real-time PCR test in planta
Date, reference of the validation report	2009-10-01 - LNPV 2009 Developement, évaluation et validation d'une méthode de détection de Chalara fraxinea
Validation process according to EPPO Standard PM7/98?	no
Is the lab accredited for this test?	yes
Was the validated data generated in the framework of a project?	no
Description of the test	
Organism(s)	Hymenoscyphus fraxineus (CHAAFR)
Detection / identification	detection
Method(s)	Molecular real time PCR
Method: Molecular real time PCR	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	no
New test being considered for inclusion in the next version of the EPPO diagnostic protocol?	no
As or adapted from an IPPC diagnostic protocol	no
Reference of the test	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 modified compared to the reference test	no

Other information	
Reaction type	Duplex
Are the performance characteristics included in the EPPO diagnostic protocol?	**no**
Performance Criteria :	
Organism 1.:	**Hymenoscyphus fraxineus(CHAAFR)**
Analytical sensitivity	
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)
Standard test(s)	No standard test
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	20 (see Table 1 in Ioos et al., 2009, in separated file)
Specificity value	100%
Analytical specificity - exclusivity	
Number of non-target organisms tested	34 fungal taxa isolated form ash tissue (see Table 1 in Ioos et al., 2009, in separated file)
Specificity value	No cross reaction observed
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
Other information	
Any other information considered useful	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 loos et al. 2009 and loos et al. 2011 attached)
The following complementary files are available online:	• Rapport_évaluation_C_fraxinea • loos_EPPO_2011 • loos_EJPP_2009

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