

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.

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| 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 <i>Chalara fraxinea</i> 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 <i>Chalara fraxinea</i> |
| 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 <i>Chalara fraxinea</i> 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 <i>Chalara fraxinea</i> ." EPPO Bulletin 41(1): 21-26. |
| Is the test modified compared to the reference test | no |

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| 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 loos 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 loos 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 $4.8 \cdot 10^4$ copies of the target DNA; 1.63% for a target concentration of $4.8 \cdot 10^3$ copies of the target DNA; 3.32% for a target concentration of $4.8 \cdot 10^2$ 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 $4.8 \cdot 10^4$ copies of the target DNA 1.70% for a target concentration of $4.8 \cdot 10^3$ copies of the target DNA; 2.19% for a target concentration of $4.8 \cdot 10^2$ 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 |

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| | Ct. (see loos et al. 2009 and loos et al. 2011 attached) |
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| The following complementary files are available online: | <ul style="list-style-type: none">• Rapport_évaluation_C_fraxinea• loos_EPPO_2011• loos_EJPP_2009 |

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