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	Bursaphelenchus xylophilus	
Short description	detection of Bursaphelenchus xylophilus by real-time PCR	
Laboratory contact details	National Reference Centre, National Plant Protection Organization P.O. Box 9102, 6700 HC Wageningen, Netherlands	
Date and reference of the validation report	2014-07-21 - F-315-000-004	
Validation process according to EPPO Standard PM 7/98:	Yes	
Reference of the test description	PM 7/004	
Is the test the same as described in the EPPO DP?	Modified Use of SYBR green real-time PCR from ClearDetections	
Is the lab accredited for this test?	No	
Plant species tested (if relevant)		
Matrices tested (if relevant)	nematode suspension from pine bark	
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	Three different isolation methods were first compared before the lab validation was carried out further. The three methods tested from the EPPO protocol were the QIAamp DNA mini kit Qiagen, FES protocol Agowa sbeadex plant mini kit using the KingFisher and ClearDetections Nematode DNA extraction & purification kit. Taqman real-time PCR from the EPPO protocol PM7/4(3) and SYBR green real-time PCR from ClearDetections
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		
<u>Analytical sensitivity (= limit of detection)</u>		
What is smallest amount of target that can be detected reliably?	The detection limit for the Taqman real-time PCR, for the Sbeadex extraction method, is 10 B. xylophilus per 2 ml of nematode suspension from wood (St=0). The detection limit for SYBR green real-time PCR is 1 B. xylophilus per 2 mL of nematode suspension from wood for both the Sbeadex and the ClearDetections extraction method.	
<u>Diagnostic sensitivity</u>		
Proportion of infected/infested samples tested positive compared to results from the standard test , see appendix 2 of PM 7/98	Not evaluated	
Specify the standard test		
<u>Analytical specificity</u>		
Specificity value		
Number of strains/populations of target organisms tested	4 populations of target organisms were tested: FR-04-415-1 Bursaphelenchus xylophilus Frankrijk, collectie LNPV Botrytis cinerea E4757-1 B. xylophilus Canada Hout, Pinus E4757-2 B. xylophilus Canada Hout, Pinus F0426 B. xylophilus Portugal verpakkingshout	
Number of non-target organisms tested	10 F0881 B. mucronatus China emballagehout E9674-1 B. mucronatus Rusland Larix F0335-1 B. fungivorus Portugal Schors van Pinus F0444 B. minutus Portugal Schors van Pinus E9674-2 B. mucronatus China meubels F0228 A. blastophtorus Nederland Crocus E9805 A. saprophilus Nederland Pinus E9597 A. subtenuis Nederland Crocus E9192 A. besseyi Turkije Oryza sativa E7072-2 Aphelenchoides sp. Portugal verpakkingshout	
Cross reacts with (specify the species)	These 10 non-targets were negative in the Tagman real-time PCR. In the SYBER green real-time PCR, 1 of the 4 biological samples from Bursaphelenchus minutes (F0444) and 1 of the four biological samples from Bursaphelenchus sp. F0881 gave a false positive result. It is possible that the samples are contaminated, but it is no longer possible to determine where this happened. The reliability of the samples is called into	

	question and not the final assessment.
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)	
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	
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 results show that the Sbeadex and ClearDetections extraction methods perform better than those of QiaAmp extraction methods. The differences between the Sbeadex and ClearDetections extraction methods are small. No additional wood species have been included for selectivity.
The following complementary files are available online:	• Short validation and implementation of real-time PCR detection B xylophilus in nematode suspension of wood bark final