

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	AGES Institute of Sustainable Plant Production Spargelfeldstrasse 191, 1220 Vienna, Austria
Short description of the test	Identification of <i>Agrilus planipennis</i> by conventional PCR
Date, reference of the validation report	2025-07-21 - Gottsberger, R.A., Bacher E., Reizenzein, H., (2024) Validation of molecular diagnostic protocols for identification of <i>Agrilus planipennis</i> (Fairmaire). Validation Report No. 2024/01, Version 02. Austrian Agency for Health and Food Safety, Vienna.
Link to other validation data	- Gottsberger, R.A., Bacher E., Reizenzein, H., (2024) Validation of molecular diagnostic protocols for identification of <i>Agrilus planipennis</i> (Fairmaire). Validation Report No. 2024/01, Version 02. Austrian Agency for Health and Food Safety, Vienna. Identification of <i>Agrilus planipennis</i> using barcoding
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?	EURL
Description of the test	
Organism(s)	<i>Agrilus planipennis</i> (AGRLPL)
Detection / identification	identification
Matrix(ces) tested	Specimen Specimens (larvae and adults) obtained from ANSES-LSV reference collection, ONF, France, Université d'Orléans, France and Austrian Research Centre for Forests (BFW)
Method(s)	Molecular Extraction DNA RNA Molecular Conventional PCR
Method: Molecular Extraction DNA RNA	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	no
As or adapted from an IPPC diagnostic protocol	no

Kit	
Is a kit used	yes
Manufacturer name	QIAGEN
Specify the kit used	DNeasy Blood & Tissue Kits
Kit used following the manufacturer's instructions?	yes DNeasy Blood & Tissue Kit (Qiagen) was used. For adults destructive DNA extraction was used, for larvae non-destructive extraction.
Other information	
Other details on the test	If non-destructive DNA extraction applied, extended incubation was performed.
Method: Molecular Conventional PCR	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	no
As or adapted from an IPPC diagnostic protocol	no
Reference of the test	Based on Kyei-Poku, G., Gauthier, D., & Quan, G. (2020). Development of a loop-mediated isothermal amplification assay as an early-warning tool for detecting emerald ash borer (Coleoptera: Buprestidae) incursions. Journal of Economic Entomology, 113(5), 2480-2494.
Is the test modified compared to the reference test	yes PCR reagents (5x HOT FIREPoI Blend Master Mix with 15 mM MgCl ₂ (Solis Biodyne) used for the test, reaction volume (10µl reactions)
Kit	
Is a kit used	no
Other information	
Reaction type	Simplex
Are the performance characteristics included in the EPPO diagnostic protocol?	no
Performance Criteria :	
Organism 1.:	Agrilus planipennis(AGRLPL)
Analytical sensitivity	
What is the smallest amount of target that can be detected reliably?	4.4pg/µl of DNA
Diagnostic sensitivity	
Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98	100%
Standard test(s)	Comparison of samples with known status (specimens were morphologically identified)
Analytical specificity - inclusivity	

Number of strains/populations of target organisms tested	6 specimens of <i>Agrilus planipennis</i> (adults and larvae) from Canada, USA and Russia
Specificity value	100%
Analytical specificity - exclusivity	
Number of non-target organisms tested	11 adult specimens from the genera <i>Agrilus</i> and <i>Anthaxia</i> were tested (<i>Agrilus anxius</i> , <i>Agrilus roscidus</i> , <i>Agrilus convexicollis</i> , <i>Agrilus graminis</i> , <i>Agrilus hastulifer</i> , <i>Agrilus viridis</i> , <i>Anthaxia caseyi</i> , <i>Anthaxia hungarica</i>), 11 larvae from different genera were tested (<i>Agrilus biguttatus</i> , <i>Agrilus sinuatus</i> , <i>Agrilus hastulifer</i> , <i>Buprestidae</i> sp., <i>Buprestis haemorrhoidalis</i> , <i>Ptosima flavoguttata</i> , <i>Phaenops cyanea</i> , <i>Coraebus florentinus</i> , <i>Lamprodila rutilans</i>)
Specificity value	100%. A reproducible cross-reaction was observed with conventional PCR (EABFOT/EABROT) for the sample <i>Anthaxia hungarica</i> . Further analysis indicated that the sample was contaminated with target DNA. Therefore, it was excluded from the calculation of performance characteristics for conventional PCR and EAB-LAMP (Kyei-Poku et al. 2020).
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	100%
Specify the test(s)	Comparison of samples with known status (specimens were morphologically identified)
Reproducibility	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100% (evaluated with 3 replicates at the limit of detection by 2 operators on 2 different days and with 2 different PCR equipment)
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100% (evaluated with 2 biological replicates in 3 technical repetitions at the limit of detection)
Test performance study	
Test performance study?	no
Other information	
Any other information considered useful	https://eurl-insects-mites.anses.fr/ https://eurl-insects-mites.anses.fr/en/minisite/insects-and-mites/validation-studies

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