

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> using barcoding
Date, reference of the validation report	2025-07-21 - Gottsberger, R.A., Bacher E., Reisenzein, 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., Reisenzein, 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> by conventional PCR - Gottsberger, R.A., Bacher E., Reisenzein, 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 LAMP (Kyei-Poku et al. (2020))
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
Method(s)	Molecular Extraction DNA RNA Molecular Sanger seq
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 Sanger seq	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	yes
EPPO Diagnostic Protocol name	PM 7/129 DNA barcoding as an identification tool for a number of regulated pests (version 2)
As or adapted from an IPPC diagnostic protocol	no
Is the test modified compared to the reference test	yes The LCO1490/HCO2198 primer set were tested with 5x HOT FIREPol Blend Master Mix with 15 mM MgCl ₂ (Solis Biodyne), reaction volume (10µl reactions)
Other information	
Other details on the test	EPPO PM 7/129 Appendix 1 - DNA barcoding of arthropods. Two different primer sets, LCO1490/HCO2198 (Folmer et al. 1994) and LepF/LepR (Hajibabaei et al. 2006), were validated. Molecular amplification (simplex)
Are the performance characteristics included in the EPPO diagnostic protocol?	no
Performance Criteria :	
Organism 1.:	Agrilus planipennis(AGRLPL)
Analytical sensitivity	
What is smallest amount of target that can be detected reliably?	Primer set LCO1490/HCO2198: 0.44ng/µl Primer set LepF/LepR: 1.03 ng/µl
Diagnostic sensitivity	
Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98	Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 6 of PM 7/98 Primer set LCO1490/HCO2198: 100% Primer set LepF/LepR: 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	Primer sets LCO1490/HCO2198 and LepF/LepR: 100% (6 specimens of the target organism could be identified out of the 6 tested)
Analytical specificity - exclusivity	
Number of non-target organisms tested	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>Coraeus florentinus</i> , <i>Lamprodila rutilans</i>)
Specificity value	Primer LCO1490/HCO2198: 100% (21 specimens of the non-target organism could be excluded out of the 21 tested). The sample <i>Lamprodila rutilans</i> was excluded for this calculation, because no amplicon could be generated. Primer LepF/LepR: 100% (22 specimens of the non-target organism could be excluded out of the 22 tested).
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	Primer set LCO1490/HCO2198: 100% Primer set LepF/LepR: 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)	Primer set LCO1490/HCO2198: 100% Primer set LepF/LepR: 100% (evaluated with 3 technical 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)	Primer set LCO1490/HCO2198: 100% Primer set LepF/LepR: 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