

**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.

<b>Laboratory contact details</b>	AGES Institute of Sustainable Plant Production Spargelfeldstrasse 191, 1220 Vienna, Austria
<b>Short description of the test</b>	Identification of <i>Agrilus planipennis</i> using barcoding
<b>Date, reference of the validation report</b>	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.
<b>Link to other validation data</b>	- 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))
<b>Validation process according to EPPO Standard PM7/98?</b>	yes
<b>Is the lab accredited for this test?</b>	yes
<b>Was the validated data generated in the framework of a project?</b>	EURL
<b>Description of the test</b>	
<b>Organism(s)</b>	<i>Agrilus planipennis</i> (AGRLPL)
<b>Detection / identification</b>	identification
<b>Matrix(ces) tested</b>	Specimen Specimens (larvae and adults) obtained from ANSES-LSV reference collection, ONF, France, Université d'Orléans, France and Austrian Research Centre for Forests (BFW)
<b>Method(s)</b>	Molecular Extraction DNA RNA Molecular Sanger seq
<b>Method: Molecular Extraction DNA RNA</b>	

<b>Reference of the test description</b>	
<b>As or adapted from an EPPO diagnostic protocol</b>	no
<b>As or adapted from an IPPC diagnostic protocol</b>	no
<b>Kit</b>	
<b>Is a kit used</b>	yes
<b>Manufacturer name</b>	QIAGEN
<b>Specify the kit used</b>	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.
<b>Other information</b>	
<b>Other details on the test</b>	If non-destructive DNA extraction applied, extended incubation was performed.
<b>Method: Molecular Sanger seq</b>	
<b>Reference of the test description</b>	
<b>As or adapted from an EPPO diagnostic protocol</b>	yes
<b>EPPO Diagnostic Protocol name</b>	PM 7/129 DNA barcoding as an identification tool for a number of regulated pests (version 2)
<b>As or adapted from an IPPC diagnostic protocol</b>	no
<b>Is the test modified compared to the reference test</b>	yes The LCO1490/HCO2198 primer set were tested with 5x HOT FIREPol Blend Master Mix with 15 mM MgCl <sub>2</sub> (Solis Biodyne), reaction volume (10µl reactions)
<b>Other information</b>	
<b>Other details on the test</b>	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)
<b>Are the performance characteristics included in the EPPO diagnostic protocol?</b>	<b>no</b>
<b>Performance Criteria :</b>	
<b>Organism 1.:</b>	<b>Agrilus planipennis(AGRLPL)</b>
<b>Analytical sensitivity</b>	
<b>What is the smallest amount of target that can be detected reliably?</b>	Primer set LCO1490/HCO2198: 0.44ng/µl Primer set LepF/LepR: 1.03 ng/µl
<b>Diagnostic sensitivity</b>	
<b>Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98</b>	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%
<b>Standard test(s)</b>	Comparison of samples with known status (specimens were morphologically identified)
<b>Analytical specificity - inclusivity</b>	
<b>Number of strains/populations of target organisms tested</b>	6 specimens of Agrilus planipennis (adults and larvae) from Canada, USA and Russia
<b>Specificity value</b>	Primer sets LCO1490/HCO2198 and LepF/LepR: 100% (6 specimens of the target organism could be identified out of the 6 tested)
<b>Analytical specificity - exclusivity</b>	
<b>Number of non-target organisms tested</b>	Non-target organisms tested 11 adult specimens from the genera Agrilus and Anthaxia were tested (Agrilus anxius, Agrilus roscidus, Agrilus convexicollis, Agrilus graminis, Agrilus hastulifer, Agrilus viridis, Anthaxia caseyi, Anthaxia hungarica), 11 larvae from different genera were tested (Agrilus biguttatus, Agrilus sinuatus, Agrilus hastulifer, Buprestidae sp., Buprestis haemorrhoidalis, Ptosima flavoguttata, Phaenops cyanea, Coraebus florentinus, Lamprodila rutilans)
<b>Specificity value</b>	Primer LCO1490/HCO2198: 100% (21 specimens of the non-target organism could be excluded out of the 21 tested). The sample Lamprodila rutilans 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).
<b>Diagnostic Specificity</b>	
<b>Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test</b>	Primer set LCO1490/HCO2198: 100% Primer set LepF/LepR: 100%
<b>Specify the test(s)</b>	Comparison of samples with known status (specimens were morphologically identified)
<b>Reproducibility</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	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)
<b>Repeatability</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	Primer set LCO1490/HCO2198: 100% Primer set LepF/LepR: 100% (evaluated with 2 biological replicates in 3 technical repetitions at the limit of detection)
<b>Test performance study</b>	
<b>Test performance study?</b>	no
<b>Other information</b>	

**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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