

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 LAMP (Kyei-Poku et al. (2020))
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</i> <i>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> 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
Method(s)	Molecular Extraction DNA RNA Molecular LAMP
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 LAMP	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	yes
EPPO Diagnostic Protocol name	PM 7/154 Agrilus planipennis (version 1)
Name of the test	LAMP test Kyei-Poku et al. (2020)
Is the test modified compared to the reference test	yes The analysis of the LAMP test was modified, as a real time cyler was used to detect the signals. Furthermore, a melting curve analysis was performed.
Kit	
Is a kit used	no
Other information	
Other details on the test	Reaction type : Loop-mediated Isothermal Amplification (LAMP)
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?	103pg/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	Strains/populations/specimen of target organisms tested 6 specimens of Agrilus planipennis (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 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)
Specificity value	100%
<u>Diagnostic Specificity</u>	
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)
<u>Reproducibility</u>	
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)
<u>Repeatability</u>	
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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