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 Thaumatotibia leucotreta using barcoding
Date, reference of the validation report	2023-07-18 - Validation of the morphological and molecular identification protocols for Thaumatotibia leucotreta (Meyrick, 1913). Version N. 01 – 18 July 2023
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)	Thaumatotibia leucotreta (ARGPLE)
Detection / identification	identification
Matrix(ces) tested	Specimen Specimens (larvae and adults) obtained from ANSES-LSV reference collection, AGES reference collection
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 MgCl2 (Solis Biodyne), reaction volume (10µl reactions)
Other information	
Other details on the test	Appendix 1 of PM 7/129 - 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.:	**Thaumatotibia leucotreta(ARGPLE)**
Analytical sensitivity	
What is the smallest amount of target that can be detected reliably?	Primer set LCO1490/HCO2198: 4pg/µl Primer set LepF/LepR: 42pg/µl
Diagnostic sensitivity	
Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 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	10 Thaumatotibia leucotreta specimens (larvae) from Israel, South Africa, Togo and Zimbabwe
Specificity value	Primer set LCO1490/HCO2198: 100% Primer set LepF/LepR: 100% All tested specimens of the target organism could be identified.
Analytical specificity - exclusivity	
Number of non-target organisms tested	27 non-target specimens (larvae) were tested: Anarsia sp. (2), Apomyelois ceratoniae (2), Cacoecimorpha pronubana (1), Cryptoblabes sp. (1), Cryptophlebia peltastica (5), Cydia pomonella (4), Epinotia thapsiana (1), Grapholita funebrana

	(2), Grapholita molesta (2), Gymnandrosoma aurantianum (2), Leucinodes orbonalis (2), Talponia batesi (2), Lobesia botrana (1)
Specificity value	Primer set LCO1490/HCO2198: 100% (26 specimens of the non-target organisms could be excluded out of the 26 tested – one sample (Cryptoblabes sp.) had to be excluded for this calculation, as no amplicon could be generated). Primer set LepF/LepR: 100% (27 specimens of the non-target organisms could be excluded out of the 27 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 3 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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