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	Julius Kuehn-Institute, Federal Research Centre for Cultivated Plants (JKI), Institute for Epidemiology and Pathogen Diagnostics Messeweg 11-12, 38104 Braunschweig, Germany
Short description of the test	Detection and identification of pea necrotic yellow dwarf virus by molecular conventional PCR in leaves
Date, reference of the validation report	2021-03-02 - F0_09_00_01-EPV_A42_03_24 PNYDV
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?	no
Description of the test	
Organism(s)	Nanovirus necropisi (PNYDV0)
Detection / identification	detection and identification
Matrix(ces) tested	Leaves Fresh plant material from own virus collection and maintained under greenhouse conditions. For exclusivity tests, calcium chloride dried leaf material was used for DNA extraction for some viruses.
Plant species tested	Lupinus, Pisum sativum, Vicia faba
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
New test being considered for inclusion in the next version of the EPPO diagnostic protocol?	yes
As or adapted from an IPPC diagnostic protocol	no
Reference of the test	Edwards et al. (1991) Nucleic Acids Res.; 19(6): 1349. doi: 10.1093/nar/19.6.1349
Is the test modified compared to the	yes see SOP A42_03_07

reference test	
Kit	
Is a kit used	no
Other information	
Method: Molecular Conventional PCR	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	no
New test being considered for inclusion in the next version of the EPPO diagnostic protocol?	no
As or adapted from an IPPC diagnostic protocol	no
Reference of the test	Gaafar et al., New Disease Reports 35 (2017), 23, http://dx.doi.org/10.5197/j.2044-0588.2017.035.02
Is the test modified compared to the reference test	yes inclusion of IPC in duplex reaction
Kit	
Is a kit used	no
Other information	
Reaction type	Duplex
Other details on the test	PCR test for detection and identification of pea necrotic yellow dwarf virus including IPC Use of One Taq Quick-Load 2X Master Mix with Standard Buffer from New England Biolabs (NEB)
Performance Criteria :	
Organism 1.:	**Nanovirus necropisi(PNYDV0)**
Analytical sensitivity	
What is the smallest amount of target that can be detected reliably?	In serial dilutions of DNA extracts PNYDV was detected in dilutions of 10-3. A PCR inhibition was observed when using undiluted DNA extracts
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	100%
Specificity value	This test detects PNYDV isolates DE15, Holtsee, AT, NL, Denmark
Analytical specificity - exclusivity	
Number of non-target organisms tested	The test does not detect other nanoviruses (FBNSV, FBNYV, PYSV, BMLRV, SCSV, MVCDV (isolate G55). In addition, PEMV, BLRV and BYMV were not detected.
Specificity value	100%
Reproducibility	

Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100 %
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100 %
Test performance study	
Test performance study?	no
Other information	
Any other information considered useful	Inhibition of undiluted DNA extracts were also observed by the co-authors of the original publication. We suggest to use undiluted, 1:10 and 1:100 diluted DNA extracts in each test.

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