

**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.

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<b>Short description of the test</b>	Detection and identification of pea necrotic yellow dwarf virus by molecular conventional PCR in leaves
<b>Date, reference of the validation report</b>	2021-03-02 - F0_09_00_01-EPV_A42_03_24 PNYDV
<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>	no
<b>If yes, please specify</b>	
<b>Description of the test</b>	
<b>Organism(s)</b>	Pea necrotic yellow dwarf virus(PNYDV0)
<b>Detection / identification</b>	detection and identification
<b>Matrix(ces) tested</b>	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.
<b>Plant species tested</b>	Lupinus, Pisum sativum, Vicia faba
<b>Method(s)</b>	Molecular Extraction DNA RNA Molecular Conventional PCR
<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>New test being considered for inclusion in the next version of the EPPO diagnostic protocol?</b>	yes
<b>As or adapted from an IPPC diagnostic protocol</b>	no
<b>Reference of the test</b>	Edwards et al. (1991) Nucleic Acids Res.; 19(6): 1349. doi: 10.1093/nar/19.6.1349

<b>Is the test modified compared to the reference test</b>	yes see SOP A42_03_07
<b>Kit</b>	
<b>Is a kit used</b>	no
<b>Other information</b>	
<b>Other details on the test</b>	
<b>Method: Molecular Conventional PCR</b>	
<b>Reference of the test description</b>	
<b>As or adapted from an EPPO diagnostic protocol</b>	no
<b>New test being considered for inclusion in the next version of the EPPO diagnostic protocol?</b>	no
<b>As or adapted from an IPPC diagnostic protocol</b>	no
<b>Reference of the test</b>	Gaafar et al., New Disease Reports 35 (2017), 23, <a href="http://dx.doi.org/10.5197/j.2044-0588.2017.035.02">http://dx.doi.org/10.5197/j.2044-0588.2017.035.02</a>
<b>Is the test modified compared to the reference test</b>	yes inclusion of IPC in duplex reaction
<b>Kit</b>	
<b>Is a kit used</b>	yes
<b>Manufacturer name</b>	New England Biolabs
<b>Specify the kit used</b>	One Taq Quick-Load 2X Master Mix with Standard Buffer
Kit used following the manufacturer's instructions?	no Reaction conditions adapted to primers and duplex protocol
<b>Other information</b>	
<b>Reaction type</b>	Duplex
<b>Other details on the test</b>	PCR test for detection and identification of pea necrotic yellow dwarf virus including IPC
<b>Are the performance characteristics included in the EPPO diagnostic protocol?</b>	
<b>Performance Criteria :</b>	
<b>Organism 1.:</b>	<b>Pea necrotic yellow dwarf virus(PNYDVO)</b>
<b>Analytical sensitivity</b>	
<b>What is smallest amount of target that can be detected reliably?</b>	In serial dilutions of DNA extracts PNYDV was detected in dilutions of 10 <sup>-3</sup> . A PCR inhibition was observed when using undiluted DNA extracts
<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>	
<b>Standard test(s)</b>	

<b>Analytical specificity - inclusivity</b>	
<b>Number of strains/populations of target organisms tested</b>	100%
<b>Specificity value</b>	This test detects PNYDV isolates DE15, Holtsee, AT, NL, Denmark
<b>Analytical specificity - exclusivity</b>	
<b>Number of non-target organisms tested</b>	The test does not detect other nanoviruses (FBNSV, FBNYV, PYSV, BMLRV, SCSV, MVCDV (isolate G55)). In addition, PEMV, BLRV and BYMV were not detected.
<b>Specificity value</b>	100%
<b>Cross reacts with</b>	
<b>Diagnostic Specificity</b>	
<b>Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test</b>	
<b>Specify the test(s)</b>	
<b>Reproducibility</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	100 %
<b>Repeatability</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	100 %
<b>Test performance study</b>	
<b>Test performance study?</b>	no
<b>Brief details of the test performance study and its output. If available, link to published article/report</b>	
<b>Other information</b>	
<b>Any other information considered useful</b>	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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