

**EUROPEAN AND MEDITERRANEAN PLANT PROTECTION ORGANIZATION**  
**ORGANISATION EUROPEENNE ET MEDITERRANEENNE POUR LA PROTECTION DES PLANTES**  
(11-17239)

**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>Target Organism</b>	Nepovirus	
<b>Short description</b>	Detection of Nepovirus by RT-PCR	
<b>Laboratory contact details</b>	Anses, Laboratoire de la Santé des Végétaux - Unité de bactériologie, virologie OGM 7 rue Jean Dixméras, 49044 Angers, France	
<b>Date and reference of the validation report</b>	2015-11-01 - Rapport de caractérisation et de validation de la méthode d'analyse par RT-PCR pour la détection polyvalente des virus du genre Nepovirus (A Leguay, P Gentit)	
<b>Validation process according to EPPO Standard PM 7/98:</b>	Yes	
<b>Reference of the test description</b>	N/R Wei, T. and G. Clover (2008). "Use of primers with 5' non-complementary sequences in RT-PCR for the detection of nepovirus subgroups A and B." journal of Virological Methods 153(1): 16-21.	
<b>Is the test the same as described in the EPPO DP?</b>		
<b>Is the lab accredited for this test?</b>	No	
<b>Plant species tested (if relevant)</b>	Ajuga spp, Chenopodium quinoa, Cucumis sativa, Nicotiana bentamiana, Nicotiana clevelandii, Prunus avium, Prunus persicae, Solanum lycopersicum, Solanum tuberosum, Vitis vinifera	
<b>Matrices tested (if relevant)</b>	Freeze-dried leaves	
<b>List of methods used</b>		
<b>Method for extraction / isolation / baiting of target organism from matrix</b>	X	RNeasy® Plant mini kit (Qiagen)
<b>Molecular methods, e.g. hybridization, PCR and real time PCR</b>	X	RT-PCR
<b>Serological methods: IF, ELISA, Direct Tissue Blot Immuno Assay</b>		
<b>Plating methods: selective isolation</b>		
<b>Bioassay methods: selective enrichment in host plants, baiting, plant test and grafting.</b>		

<b>Pathogenicity test</b>		
<b>Fingerprint methods: protein profiling, fatty acid profiling &amp; DNA profiling</b>		
<b>Morphological and morphometrical methods intended for identification</b>		
<b>Biochemical methods: e.g. enzyme electrophoresis, protein profiling</b>		
<b>Other</b>		
<b>Analytical sensitivity (= limit of detection)</b>		
<b>What is smallest amount of target that can be detected reliably?</b>	Analytical sensitivity tested with the following target isolates (3 replicates for each isolate) : -ArMV (PC-0045 DSMZ) 1.10 <sup>-5</sup> diluted in RNA from healthy plant material -GFLV (PC-0084 DSMZ) 1.10 <sup>-3</sup> diluted in RNA from healthy plant material -PBRV (PC-0056 DSMZ) 1.10 <sup>-1</sup> diluted in RNA from healthy plant material -RpRSV (139/2014-09 Ctifl) 1.10 <sup>-1</sup> diluted in RNA from healthy plant material	
<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>	$\text{sensitivity} = \text{NA}/(\text{NA} + \text{PD}) = 13/(13 + 5) = 72\%$	
<b>Specify the standard test</b>	Reference material were used for this validation	
<b>Analytical specificity</b>		
<b>Specificity value</b>		
<b>Number of strains/populations of target organisms tested</b>	Analytical specificity tested with the following target isolates (3 replicates for each isolate) : -N°1 ArMV Arabis mosaic virus - Chenopodium quinoa - 15/15 - DSMZ (PC-0045) -N°2 GFLV Grapevine fanleaf virus - Chenopodium quinoa - 15/14 - DSMZ (PC-0084) -N°3 PBRV Potato black ringspot virus- Nicotiana bentamiana - 3891 - DSMZ (PC-0056) -N°4 RpRSV Raspberry ringspot virus - Chenopodium quinoa - 14/328 - DSMZ (PC-0429) -N°5 RpRSV Raspberry ringspot virus - Prunus persicae - 14/373 CTIFL (139/2014-09) -N°6 TRSV - Tobacco ringspot virus - Chenopodium quinoa - 4144 - DSMZ (PC-0235)	
<b>Number of non-target organisms tested</b>	Analytical specificity tested with the following non target isolates (3 replicates for each isolate) : -N°7 - BRSV Beet ringspot virus (B) - Ajuga sp. - 14/414 NPPO -N°9 TBRV Tomato black ring virus (B) - Nicotiana clevelandii - 3894 - DSMZ -N°10 ALRSV Apricot latent ringspot virus (C) - Prunus persicae - 14/374 CTIFL	

	<p>-N°11 AYRSV Artichoke yellow ringspot virus (C) - Chenopodium quinoa - 14/400 INRA 33</p> <p>-N°12 CLRV Cherry leaf roll virus (C) - Chenopodium quinoa - 14/327 - DSMZ</p> <p>-N°17 MyLRSV Myrobalan latent ringspot virus (C) - Prunus persicae - 14/371 CTIFL</p> <p>-N°18 PRMV Peach rosette mosaic virus (C) - Chenopodium quinoa - 14/402 INRA 33</p> <p>-N°19 ToRSV Tomato ringspot virus (C) - Chenopodium quinoa - 3895 - DSMZ</p> <p>-N°22 Sain - Solanum lycopersicum - 08/06/10 LSV</p> <p>-N°23 Sain - Prunus persicae - 14/375 CTIFL</p> <p>-N°24 Sain - Vitis vinifera - 14/431b LSV</p> <p>-N°25 Sain - Solanum tuberosum - 10/452.6 LSV</p> <p>-N°26 Sain - Cucumis sativa - TS23 - LSV</p> <p>-N°27 Sain - Prunus avium - 14/376 CTIFL</p> <p>-N°28 PPV-Rec Plum pox virus - Prunus persicae - 10/102 LSV</p> <p>-N°29 TICV Tomato infectious chlorotic virus &amp; ToCV Tomato chlorosis virus - Solanum lycopersicum - LSV</p> <p>-N°30 PepMV-EU Pepino mosaic virus - Solanum lycopersicum - LSV</p> <p>-N°31 SLRSV Strawberry latent ringspot virus - Prunus persicae - 14/372 CTIFL</p>
<b>Cross reacts with (specify the species)</b>	Cross reaction with one isolate belonging to the subgrupup C : -N°11 AYRSV Artichoke yellow ringspot virus (C) - Chenopodium quinoa - 14/400 INRA 33
<b><u>Diagnostic Specificity</u></b>	
<b>Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test</b>	Specificity = $NA / (NA+PD) = 51 / (51 + 3) = 94\%$
<b>Specify the standard test</b>	Reference material were used for this validation.
<b><u>Reproducibility</u></b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	100% Each test with target and non-target isolates (see above) was performed with 3 replicates for each .
<b><u>Repeatability</u></b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	Not tested
<b><u>Test performance study</u></b>	
<b>Test performance study?</b>	No
<b>Include brief details of the test performance study and its output.It available, provide a link to published article/report</b>	
<b><u>Other information</u></b>	
<b>Any other information considered useful e.g. robustness, ease of performing</b>	

the test, etc.	
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