

**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.

<b>Laboratory contact details</b>	Anses Plant Health Laboratory - Bacteriology, Virology and GMO Unit 7 rue Jean Dixm�ras, 49044 Angers, France
<b>Short description of the test</b>	Detection of Nepovirus by RT-PCR
<b>Date, reference of the validation report</b>	2015-11-01 - Rapport de caract�risation et de validation de la m�thode d'analyse par RT-PCR pour la d�tection polyvalente des virus du genre Nepovirus (A Leguay, P Gentit)
<b>Validation process according to EPPO Standard PM7/98?</b>	yes
<b>Is the lab accredited for this test?</b>	no
<b>Was the validated data generated in the framework of a project?</b>	
<b>If yes, please specify</b>	
<b>Description of the test</b>	
<b>Organism(s)</b>	Nepovirus(1NEPOG)
<b>Detection / identification</b>	
<b>Matrix(ces) tested</b>	Leaves Freeze-dried leaves
<b>Plant species tested</b>	Ajuga sp., Chenopodium quinoa, Cucumis sativus, Nicotiana benthamiana, Nicotiana clevelandii, Prunus avium, Prunus persica, Solanum lycopersicum, Solanum tuberosum, Vitis vinifera
<b>Method(s)</b>	Molecular Extraction DNA RNA Molecular Conventional RT 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>	
<b>New test being considered for inclusion in the next version of the EPPO diagnostic protocol?</b>	
<b>As or adapted from an IPPC diagnostic protocol</b>	
<b>Is the test modified compared to the reference test</b>	

<b>Kit</b>	
<b>Is a kit used</b>	yes
<b>Manufacturer name</b>	QIAGEN
<b>Specify the kit used</b>	RNeasy Plant Mini Kit
Kit used following the manufacturer's instructions?	
<b>Other information</b>	
<b>Other details on the test</b>	
<b>Method: Molecular Conventional RT 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>	
<b>As or adapted from an IPPC diagnostic protocol</b>	no
<b>Reference of the test</b>	Wei, T. and G. Clover (2008). "Use of primers with 5' noncomplementary 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 modified compared to the reference test</b>	
<b>Kit</b>	
<b>Is a kit used</b>	
<b>Other information</b>	
<b>Reaction type</b>	
<b>Other details on the test</b>	
<b>Are the performance characteristics included in the EPPO diagnostic protocol?</b>	no
<b>Performance Criteria :</b>	
<b>Organism 1.:</b>	<b>Nepovirus(1NEPOG)</b>
<b>Analytical sensitivity</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>	sensitivity= NA/(NA+PD) = 13/(13 + 5) = 72%

<b>Standard test(s)</b>	Reference material were used for this validation
<b>Analytical specificity - inclusivity</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>Specificity value</b>	
<b>Analytical specificity - exclusivity</b>	
<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 -N°11 AYRSV Artichoke yellow ringspot virus (C) - Chenopodium quinoa - 14/400 INRA 33 -N°12 CLRV Cherry leaf roll virus (C) - Chenopodium quinoa - 14/327 - DSMZ -N°17 MyLRSV Myrobalan latent ringspot virus (C) - Prunus persicae - 14/371 CTIFL -N°18 PRMV Peach rosette mosaic virus (C) - Chenopodium quinoa - 14/402 INRA 33 -N°19 ToRSV Tomato ringspot virus (C) - Chenopodium quinoa - 3895 - DSMZ -N°22 Sain - Solanum lycopersicum - 08/06/10 LSV -N°23 Sain - Prunus persicae - 14/375 CTIFL -N°24 Sain - Vitis vinifera - 14/431b LSV -N°25 Sain - Solanum tuberosum - 10/452.6 LSV -N°26 Sain - Cucumis sativa - TS23 - LSV -N°27 Sain - Prunus avium - 14/376 CTIFL -N°28 PPV-Rec Plum pox virus - Prunus persicae - 10/102 LSV -N°29 TICV Tomato infectious chlorotic virus & ToCV Tomato chlorosis virus - Solanum lycopersicum - LSV -N°30 PepMV- EU Pepino mosaic virus - Solanum lycopersicum - LSV -N°31 SLRSV Strawberry latent ringspot virus - Prunus persicae - 14/372 CTIFL
<b>Specificity value</b>	Cross reaction with one isolate belonging to the subgropup C : -N°11 AYRSV Artichoke yellow ringspot virus (C) - Chenopodium quinoa - 14/400 INRA 33
<b>Cross reacts with</b>	Artichoke yellow ringspot virus
<b>Diagnostic Specificity</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 test(s)</b>	Reference material were used for this validation.
<b>Reproducibility</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>Repeatability</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	Not tested
<b>Test performance study</b>	
<b>Test performance study?</b>	no
<b>Brief details of the test performance study and its output.It available, link to published article/report</b>	
<b>Other information</b>	
<b>Any other information considered useful</b>	

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