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.

Target Organism	Nepovirus	
Short description	Detection of Nepovirus by RT-PCR	
Laboratory contact details	Anses Plant Health Laboratory - Bacteriology, Virology and GMO Unit 7 rue Jean Dixméras, 49000 Angers, France	
Date and reference of the validation report	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)	
Validation process according to EPPO Standard PM 7/98:	Yes	
Reference of the test description	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.	
Is the test the same as described in the EPPO DP?		
Is the lab accredited for this test?	No	
Plant species tested (if relevant)	Ajuga spp, Chenopodium quinoa, Cucumis sativa, Nicotiana bentamiana, Nicotiana clevelandii, Prunus avium, Prunus persicae, Solanum lycopersicum, Solanum tuberosum, Vitis vinifera	
Matrices tested (if relevant)	Freeze-dried leaves	
List of methods used		
Method for extraction / isolation / baiting of target organism from matrix	X	RNeasy® Plant mini kit (Qiagen)
Molecular methods, e.g. hybridization, PCR and real time PCR	X	RT-PCR
Serological methods: IF, ELISA, Direct Tissue Blot Immuno Assay		
Plating methods: selective isolation		
Bioassay methods: selective enrichment in host plants, baiting, plant test and grafting.		

Pathogenicity test		
Fingerprint methods: protein profiling, fatty acid profiling & DNA profiling		
Morphological and morphometrical methods intended for identification		
Biochemical methods: e.g. enzyme electrophoresis, protein profiling		
Other		
Analytical sensitivity (= limit of detection)		
What is smallest amount of target that can be detected reliably?	Analytical sensitivity tested with the following target isolates (3 replicates for each isolate) : -ArMV (PC-0045 DSMZ) 1.10-5 diluted in RNA from healthy plant material -GFLV (PC-0084 DSMZ) 1.10-3 diluted in RNA from healthy plant material -PBRSV (PC-0056 DSMZ) 1.10-1 diluted in RNA from healthy plant material -RpRSV (139/2014-09 CtifI) 1.10-1 diluted in RNA from healthy plant material	
Diagnostic sensitivity		
Proportion of infected/infested samples tested positive compared to results from the standard test , see appendix 2 of PM 7/98	sensitivity= NA/(NA+PD) = 13/(13 + 5) = 72%	
Specify the standard test	Reference material were used for this validation	
Analytical specificity		
Specificity value		
Number of strains/populations of target organisms tested	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 PBRSV 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)	
Number of non-target organisms tested	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
Cross reacts with (specify the species)	Cross reaction with one isolate belonging to the subgropup C : -N°11 AYRSV Artichoke yellow ringspot virus (C) - Chenopodium quinoa - 14/400 INRA 33
<u>Diagnostic Specificity</u>	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	Specificity = NA / (NA+PD) = 51 / (51 + 3) = 94%
Specify the standard test	Reference material were used for this validation.
<u>Reproducibility</u>	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100% Each test with target and non-target isolates (see above) was performed with 3 replicates for each .
<u>Repeatability</u>	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	Not tested
<u>Test performance study</u>	
Test performance study?	No
Include brief details of the test performance study and its output.It available, provide a link to published article/report	
<u>Other information</u>	
Any other information considered useful e.g. robustness, ease of performing	

the test, etc.	