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	Anses Plant Health Laboratory - Bacteriology, Virology and GMO Unit 7 rue Jean Dixméras, 49000 Angers, France
Short description of the test	Detection of Nepovirus by RT-PCR
Date, 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 PM7/98?	yes
Is the lab accredited for this test?	no
Was the validated data generated in the framework of a project?	
Description of the test	
Organism(s)	Nepovirus (1NEPOG)
Detection / identification	detection
Matrix(ces) tested	Leaves Freeze-dried leaves
Plant species tested	Ajuga sp., Chenopodium quinoa, Cucumis sativus, Nicotiana benthamiana, Nicotiana clevelandii, Prunus avium, Prunus persica, Solanum lycopersicum, Solanum tuberosum, Vitis vinifera
Method(s)	Molecular Extraction DNA RNA Molecular Conventional RT PCR
Method: Molecular Extraction DNA RNA	
Reference of the test description	
Kit	
Is a kit used	yes
Manufacturer name	QIAGEN
Specify the kit used	RNeasy Plant Mini Kit
Kit used following the manufacturer's instructions?	
Other information	
Method: Molecular Conventional RT PCR	
Reference of the test description	

As or adapted from an EPPO diagnostic protocol	no
As or adapted from an IPPC diagnostic protocol	no
Reference of the test	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.
Other information	
Are the performance characteristics included in the EPPO diagnostic protocol?	**no**
Performance Criteria :	
Organism 1.:	**Nepovirus(1NEPOG)**
Analytical sensitivity	
What is the 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 Ctifl) 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%
Standard test(s)	Reference material were used for this validation
Analytical specificity - inclusivity	
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)
Specificity value	
Analytical specificity - exclusivity	
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
Specificity value	Cross reaction with one isolate belonging to the subgropup C : -N°11 AYRSV Artichoke yellow ringspot virus (C) - Chenopodium quinoa - 14/400 INRA 33
Cross-reacts with	Artichoke yellow ringspot virus
Diagnostic Specificity	
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 test(s)	Reference material were used for this validation.
Reproducibility	
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 .
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	Not tested
Test performance study	
Test performance study?	no

Creation date: 2016-12-13 00:00:00 - Last update: 2025-02-26 15:20:52