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, 49044 Angers, France
Short description of the test	Detection of Tospoviruses by RT-PCR in plant material
Date, reference of the validation report	2015-01-01 - Leguay A., Gentit P. (2015) Evaluation de méthodes de RT-PCR pour la détection polyvalente des virus du genre Tospovirus - Laboratoire de la santé des végétaux - Angers (France)
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)	Chrysanthemum stem necrosis virus / Orthotospovirus chrysanthinecrocaulis (CSNV00) Impatiens necrotic spot virus / Orthotospovirus impatiensnecromaculae (INSV00) Tomato spotted wilt virus / Orthotospovirus tomatomaculae (TSWV00)
Detection / identification	detection
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	Chen T.C., Li J.T., Lin Y.P., Yeh Y.C., Kang Y.C., Huang L.H., Yeh S.D. (2012) Genomic characterization of Calla lily chlorotic spot virus and design of broad-spectrum primers for detection of tospoviruses. Plant Pathology 61:183-194.
Other information	
Are the performance characteristics included in the EPPO diagnostic protocol?	**no**
Performance Criteria :	
Organism 1.:	**Orthotospovirus chrysanthinecrocaulis(CSNV00)**
Analytical sensitivity	
What is smallest amount of target that can be detected reliably?	Not concerned because a virus is not quantifiable
Diagnostic sensitivity	
Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98	1
Standard test(s)	30 / 30 (3 replicate for each sample)
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	Target organisms tested 1. ANSV Alstroemeria necrotic streak virus) 2. CaCV Capsicum chlorosis virus 3. CSNV Chrysanthemum stem necrosis virus 4. GRSV Groundnut ringspot virus 5 INSV Impatiens necrotic spot virus 6. IYSV Iris yellow spot virus 7. TCSV Tomato chlorotic spot virus 8. TYRV Tomato yellow (fruit) ring virus 9. TSWV Tomato spotted wilt virus 10. WSMoV Watermelon silver mottle virus
Specificity value	1
Analytical specificity - exclusivity	
Number of non-target organisms tested	Non-target organisms tested 1. healthy Allium 2. healthy Chrysanthemum 3. healthy Cineraria 4. healthy Allium 5. healthy Allium 6. healthy Solanum lycopersicum 7. healthy Capsicum 8. Solanum Tomato infected by virus (TICV); Tomato chlorosis virus (ToCV); Tomato yellow leaf curl virus (TYLCV) 9. Solanum Tomato infected by Pepino mosaic virus (PepMV) 10. Solanum Tomato infected by Tomato torrado virus (ToTV).
Specificity value	No cross reaction observed
Diagnostic Specificity	

Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	1
Specify the test(s)	30 samples agreement / 30 (3 replicate for each sample)
Reproducibility	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	Not tested
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100% - 60 samples agreement /60 (3 replicate for each sample)
Organism 2.:	**Orthotospovirus impatiensnecromaculae(INSV00)**
Analytical sensitivity	
What is smallest amount of target that can be detected reliably?	Not concerned because a virus is not quantifiable
Diagnostic sensitivity	
Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98	1
Standard test(s)	30 / 30 (3 replicate for each sample)
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	Target organisms tested 1. ANSV Alstroemeria necrotic streak virus) 2. CaCV Capsicum chlorosis virus 3. CSNV Chrysanthemum stem necrosis virus 4. GRSV Groundnut ringspot virus 5 INSV Impatiens necrotic spot virus 6. IYSV Iris yellow spot virus 7. TCSV Tomato chlorotic spot virus 8. TYRV Tomato yellow (fruit) ring virus 9. TSWV Tomato spotted wilt virus 10. WSMoV Watermelon silver mottle virus
Specificity value	1
Analytical specificity - exclusivity	
Number of non-target organisms tested	Non-target organisms tested 1. healthy Allium 2. healthy Chrysanthemum 3. healthy Cineraria 4. healthy Allium 5. healthy Allium 6. healthy Solanum lycopersicum 7. healthy Capsicum 8. Solanum Tomato infected by virus (TICV); Tomato chlorosis virus (ToCV); Tomato yellow leaf curl virus (TYLCV) 9. Solanum Tomato infected by Pepino mosaic virus (PepMV) 10. Solanum Tomato infected by Tomato torrado virus (ToTV).
Specificity value	No cross reaction observed
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	1

Specify the test(s)	30 samples agreement / 30 (3 replicate for each sample)
Reproducibility	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	Not tested
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100% - 60 samples agreement /60 (3 replicate for each sample)
Organism 3.:	**Orthotospovirus tomatomaculae(TSWV00)**
Analytical sensitivity	
What is smallest amount of target that can be detected reliably?	Not concerned because a virus is not quantifiable
Diagnostic sensitivity	
Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98	1
Standard test(s)	30 / 30 (3 replicate for each sample)
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	Target organisms tested 1. ANSV Alstroemeria necrotic streak virus) 2. CaCV Capsicum chlorosis virus 3. CSNV Chrysanthemum stem necrosis virus 4. GRSV Groundnut ringspot virus 5 INSV Impatiens necrotic spot virus 6. IYSV Iris yellow spot virus 7. TCSV Tomato chlorotic spot virus 8. TYRV Tomato yellow (fruit) ring virus 9. TSWV Tomato spotted wilt virus 10. WSMoV Watermelon silver mottle virus
Specificity value	1
Analytical specificity - exclusivity	
Number of non-target organisms tested	Non-target organisms tested 1. healthy Allium 2. healthy Chrysanthemum 3. healthy Cineraria 4. healthy Allium 5. healthy Allium 6. healthy Solanum lycopersicum 7. healthy Capsicum 8. Solanum Tomato infected by virus (TICV); Tomato chlorosis virus (ToCV); Tomato yellow leaf curl virus (TYLCV) 9. Solanum Tomato infected by Pepino mosaic virus (PepMV) 10. Solanum Tomato infected by Tomato torrado virus (ToTV).
Specificity value	No cross reaction observed
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	1
Specify the test(s)	30 samples agreement / 30 (3 replicate for each sample)
Reproducibility	

Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	Not tested
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100% - 60 samples agreement /60 (3 replicate for each sample)
Test performance study	
Test performance study?	no

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