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 CVYV and CYSDV in host plant material
Date, reference of the validation report	2014-01-01 - Cousseau P., Gentit P. (2014). Assesment of a detection method for Cucurbitaceae yellow diseases: Cucumber vein yellowing virus (CVYV), Cucurbit yellow stunting disorder virus (CYSDV), Anses, Laboratory of Plant Health, Angers.
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)	Ipomovirus cucumisvenaflavi (CVYV00) Cucurbit yellow stunting disorder virus / Crinivirus cucurbitae (CYSDV0)
Detection / identification	detection
Matrix(ces) tested	Leaves Leaves
Plant species tested	Citrullus lanatus, Cucumis melo, Cucumis sativus, Cucurbita pepo, Solanum lycopersicum
Method(s)	Molecular Extraction DNA RNA Molecular real time RT PCR
Method: Molecular Extraction DNA RNA	
Reference of the test description	
Other information	
Method: Molecular real time 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	Gil-Salas et al., 2007. Development of real-time RT-PCR assays for the detection of Cucumber vein yellowing virus (CVYV) and Cucurbit yellow stunting disorder virus (CYSDV) in the whitefly vector Bemisia tabaci. Journal of Virological Methods 146, pp. 45–51.
Other information	
Are the performance characteristics included in the EPPO diagnostic protocol?	**no**
Performance Criteria :	
Organism 1.:	**Ipomovirus cucumisvenaflavi(CVYV00)**
<u>**Analytical sensitivity**</u>	
What is the smallest amount of target that can be detected reliably?	Not concerned because a virus is not quantifiable
<u>**Diagnostic sensitivity**</u>	
Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98	CVYV: 100%
Standard test(s)	CVYV: 30/30 (3 replicate for each sample)
<u>**Analytical specificity - inclusivity**</u>	
Number of strains/populations of target organisms tested	Target organisms tested : 1.Cucumis melo infected by Cucumber vein yellowing virus (CVYV) 2.Cucumis melo infected by Cucumber vein yellowing virus (CVYV) 3.Cucumis sativus infected by Cucumber vein yellowing virus (CVYV) 4.Cucurbita pepo infected by Cucumber vein yellowing virus (CVYV) 5.Cucumis lanatus infected by Cucumber vein yellowing virus (CVYV) 6. Cucumber vein yellowing virus (CVYV) 7.Cucumber vein yellowing virus (CVYV) 8. Cucumber vein yellowing virus (CVYV) 9.co-infected Cucumber vein yellowing virus (CVYV) and Cucurbit yellow stunting disorder virus (CYSDV) 10-0 : co-infected Cucumber vein yellowing virus (CVYV) and Cucurbit yellow stunting disorder virus (CYSDV)
Specificity value	100%
<u>**Analytical specificity - exclusivity**</u>	
Number of non-target organisms tested	Non-target organisms tested : 11.Cucurbita pepo infected by Squash vein yellowing virus (SqVYV) 12.Solanum lycopersicum infected by Tomato chlorosis virus (ToCV) 13.Cucurbita pepo infected by Zucchini yellow mosaic virus (ZYMV) 14.Cucumis sativus infected by Watermelon mosaic virus (WMV) 15.Cucurbita pepo infected by Papaya ringspot virus (PRSV) 16. healthy Cucumis melo 17. healthy Cucumis sativus 18. healthy Cucurbita pepo 19. healthy Cucumis lanatus
Specificity value	No cross reaction observed
<u>**Diagnostic Specificity**</u>	

Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	CVYV: 100%
Specify the test(s)	CVYV: 30 samples agreement/30 (3 replicate for each sample)
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)	CVYV: 100% - 60 samples agreement/60
Organism 2.:	**Crinivirus cucurbitae(CYSDV0)**
Analytical sensitivity	
What is the 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	CYSDV: 100%
Standard test(s)	CYSDV: 30/30 (3 replicate for each sample)
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	1. Cucurbit yellow stunting disorder virus (CYSDV) 2. Cucurbit yellow stunting disorder virus (CYSDV) 3. Cucurbit yellow stunting disorder virus (CYSDV) 5. co-infected Cucumber vein yellowing virus (CVYV) and Cucurbit yellow stunting disorder virus (CYSDV) 6. Cucurbit yellow stunting disorder virus (CYSDV) 7. co-infected Cucumber vein yellowing virus (CVYV) and Cucurbit yellow stunting disorder virus (CYSDV) 8. Cucurbit yellow stunting disorder virus (CYSDV) 9. Cucumis sativus infected by Cucurbit yellow stunting disorder virus (CYSDV) 10. Cucumis sativus infected by Cucurbit yellow stunting disorder virus (CYSDV)
Specificity value	100%
Analytical specificity - exclusivity	
Number of non-target organisms tested	Non-target organisms tested : 11.Cucurbita pepo infected by Squash vein yellowing virus (SqVYV) 12.Solanum lycopersicum infected by Tomato chlorosis virus (ToCV) 13.Cucurbita pepo infected by Zucchini yellow mosaic virus (ZYMV) 14.Cucumis sativus infected by Watermelon mosaic virus (WMV) 15.Cucurbita pepo infected by Papaya ringspot virus (PRSV) 16. healthy Cucumis melo 17. healthy Cucumis sativus 18. healthy Cucurbita pepo 19. healthy Cucumis lanatus
Specificity value	No cross reaction observed
Diagnostic Specificity	

Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	CYSDV: 100%
Specify the test(s)	CYSDV: 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)	CYSDV: 100% - 60 samples agreement/60
Test performance study	
Test performance study?	no
Other information	
Any other information considered useful	For details, contact lab.

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