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 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?	
If yes, please specify	
Description of the test	
Organism(s)	Cucumber vein yellowing virus(CVYV00) Cucurbit yellow stunting disorder virus(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	
As or adapted from an EPPO diagnostic protocol	
New test being considered for inclusion in the next version of the EPPO diagnostic protocol?	
As or adapted from an IPPC diagnostic protocol	
Is the test modified compared to the reference test	

Kit	
Is a kit used	
Other information	
Other details on the test	
Method: Molecular real time RT PCR	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	no
New test being considered for inclusion in the next version of the EPPO diagnostic protocol?	
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.
Is the test modified compared to the reference test	
Kit	
Is a kit used	
Other information	
Reaction type	
Other details on the test	
Are the performance characteristics included in the EPPO diagnostic protocol?	**no**
Performance Criteria :	
Organism 1.:	**Cucumber vein yellowing virus(CVYV00)**
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	CVYV: 100%
Standard test(s)	CVYV: 30/30 (3 replicate for each sample)
Analytical specificity - inclusivity	
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%
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
Cross reacts with	
Diagnostic Specificity	
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)
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)	CVYV: 100% - 60 samples agreement/60
Organism 2.:	**Cucurbit yellow stunting disorder virus(CYSDV0)**
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	CYSDV: 100%
Standard test(s)	CYSDV: 30/30 (3 replicate for each sample)
Analytical specificity - inclusivity	
Number of strains/populations of target	1. Cucurbit yellow stunting disorder virus (CYSDV)

organisms tested	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
Cross reacts with	
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
Brief details of the test performance study and its output.It available, link to published article/report	
Other information	
Any other information considered useful	For details, contact lab.

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