

**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.

<b>Laboratory contact details</b>	Anses Plant Health Laboratory - Bacteriology, Virology and GMO Unit 7 rue Jean Dixm�ras, 49044 Angers, France
<b>Short description of the test</b>	Detection of CVYV and CYSDV in host plant material
<b>Date, reference of the validation report</b>	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.
<b>Validation process according to EPPO Standard PM7/98?</b>	yes
<b>Is the lab accredited for this test?</b>	no
<b>Was the validated data generated in the framework of a project?</b>	
<b>Description of the test</b>	
<b>Organism(s)</b>	Ipomovirus cucumisvenafavi(CVYV00) Crinivirus cucurbitae(CYSDV0)
<b>Detection / identification</b>	detection
<b>Method(s)</b>	Molecular Extraction DNA RNA Molecular real time RT PCR
<b>Method: Molecular Extraction DNA RNA</b>	
<b>Reference of the test description</b>	
<b>Other information</b>	
<b>Method: Molecular real time RT PCR</b>	
<b>Reference of the test description</b>	
<b>As or adapted from an EPPO diagnostic protocol</b>	no
<b>As or adapted from an IPPC diagnostic protocol</b>	no
<b>Reference of the test</b>	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.

<b>Other information</b>	
<b>Are the performance characteristics included in the EPPO diagnostic protocol?</b>	<b>no</b>
<b>Performance Criteria :</b>	
<b>Organism 1.:</b>	<b>Ipomovirus cucumisvenafavi(CVYV00)</b>
<b>Analytical sensitivity</b>	
<b>What is smallest amount of target that can be detected reliably?</b>	Not concerned because a virus is not quantifiable
<b>Diagnostic sensitivity</b>	
<b>Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98</b>	CVYV: 100%
<b>Standard test(s)</b>	CVYV: 30/30 (3 replicate for each sample)
<b>Analytical specificity - inclusivity</b>	
<b>Number of strains/populations of target organisms tested</b>	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)
<b>Specificity value</b>	100%
<b>Analytical specificity - exclusivity</b>	
<b>Number of non-target organisms tested</b>	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
<b>Specificity value</b>	No cross reaction observed
<b>Diagnostic Specificity</b>	
<b>Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test</b>	CVYV: 100%
<b>Specify the test(s)</b>	CVYV: 30 samples agreement/30 (3 replicate for each sample)

<b>Reproducibility</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	Not tested
<b>Repeatability</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	CVYV: 100% - 60 samples agreement/60
<b>Organism 2.:</b>	<b>Crinivirus cucurbitae(CYSDV0)</b>
<b>Analytical sensitivity</b>	
<b>What is smallest amount of target that can be detected reliably?</b>	Not concerned because a virus is not quantifiable
<b>Diagnostic sensitivity</b>	
<b>Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98</b>	CYSDV: 100%
<b>Standard test(s)</b>	CYSDV: 30/30 (3 replicate for each sample)
<b>Analytical specificity - inclusivity</b>	
<b>Number of strains/populations of target organisms tested</b>	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)
<b>Specificity value</b>	100%
<b>Analytical specificity - exclusivity</b>	
<b>Number of non-target organisms tested</b>	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
<b>Specificity value</b>	No cross reaction observed
<b>Diagnostic Specificity</b>	
<b>Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test</b>	CYSDV: 100%
<b>Specify the test(s)</b>	CYSDV: 30 samples agreement/30 (3 replicate for each sample)

<b>Reproducibility</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	Not tested
<b>Repeatability</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	CYSDV: 100% - 60 samples agreement/60
<b>Test performance study</b>	
<b>Test performance study?</b>	no
<b>Other information</b>	
<b>Any other information considered useful</b>	For details, contact lab.

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