EUROPEAN AND MEDITERRANEAN PLANT PROTECTION ORGANIZATION
ORGANISATION EUROPEENNE ET MEDITERRANEENNE POUR LA PROTECTION DES PLANTES
(11-17239)
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.

Target Organism	Cucumber vein yellowing virus Cucurbit yellow stunting disorder virus	
Short description	Detection of CVYV and CYSDV in host plant material	
Laboratory contact details	Anses Plant Health Laboratory - Bacteriology, Virology and GMO Unit 7 rue Jean Dixméras, 49044 Angers, France	
Date and reference of the validation report	2014 - 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 PM 7/98:	Yes	
Reference of the test description	0 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 the same as described in the EPPO DP?	No 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 lab accredited for this test?	No	
Plant species tested (if relevant)	Cucumis melo, Cucumis sativus, Cucumis lanatus, Cucurbita pepo, Solanum lycopersicum	
Matrices tested (if relevant)	Leaves	
List of methods used		
Method for extraction / isolation / baiting of target organism from matrix		
Molecular methods, e.g. hybridization, PCR and real time PCR	X	real-time RT-PCR - Gil-Salas (2007)
Serological methods: IF, ELISA, Direct Tissue Blot Immuno Assay		
Plating methods: selective isolation		

Bioassay methods: selective enrichment in host plants, baiting, plant test and grafting.		
Pathogenicity test		
Fingerprint methods: protein profiling, fatty acid profiling & DNA profiling		
Morphological and morphometrical methods intended for identification		
Biochemical methods: e.g. enzyme electrophoresis, protein profiling		
Other		
Analytical sensitivity (= limit of detection)		
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% CYSDV: 100%	
Specify the standard test	CVYV: 30/30 (3 replicate for each sample) CYSDV: 30/30 (3 replicate for each sample)	
Analytical specificity		
Specificity value	100% for each virus	
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) 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)
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
Cross reacts with (specify the species)	No cross reaction observed
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	CVYV: 100% CYSDV: 100%
Specify the standard test	CVYV: 30 samples agreement/30 (3 replicate for each sample) 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)	CVYV: 100% - 60 samples agreement/60 CYSDV: 100% - 60 samples agreement/60
Test performance study	
Test performance study?	No
Include brief details of the test performance study and its output.It available, provide a link to published article/report	
Other information	
Any other information considered useful **e.g. robustness, ease of performing the test, etc.**	For details, contact lab.