Validation Report: ELISA

SRA 37502 • *Pepper mild mottle virus* (PMMoV)

Test Characteristics

Test Name	Pepper mild mottle virus	**Capture Antibody**	Polyclonal (Rabbit)
Catalog Number	37502	**Detection Antibody**	Polyclonal (Rabbit)
Acronym	PMMoV	**Format**	DAS-ELISA
Genus	Tobamovirus	**Diluents**	GEB/RUB6
Binomial Name	Tobamovirus capsici	**Sample Dilution**	1:10

Summary

This ELISA test is a qualitative serological assay for the detection of Pepper mild mottle virus (PMMoV) in ornamental leaves and vegetable leaves and seed. PMMoV is a member of the Tobamovirus genus known for their rod-shaped virus particles. It was able to consistently detect 1 PMMoV-infected seed in a subsample of 249 PMMoV-free pepper seeds.

Diagnostic Sensitivity

True Positives	29
Correct Diagnoses	29
Percent	100%

Analytical Sensitivity

Limit of Detection: 1:102,400 dilution of infected tissue (pathogen titer unknown)

Analytical Specificity

Inclusivity:

Isolates and Geographic Regions Detected:

PMMoV Canada isolate	PMMoV-CNU-1 (South Korea)[3]
PMMoV-GG1 (South Korea)[1]	PMMoV-Is (Israel)
PMMoV-J (Japan)	PMMoV-JHD (South Korea)[4]
PMMoV-JI1 (South Korea)[2]	PMMoV Southern Europe isolate
[1]PMMoV-GG1 has been externally reported to be detected.	
[2]PMMoV-JI1 has been externally reported to be detected.	
[3]PMMoV-CNU-1 has been externally reported to be detected.	
[4]PMMoV-JHD has been externally reported to be detected.	

Exclusivity:

Cross-reacts With:

Virus Name	Species Name
African eggplant-associated virus (AEaV)[2]	N/A
Bell pepper mottle virus (BPeMV)[1]	Tobamovirus maculacapsici
Chili pepper mild mottle virus (CPMMoV)[3]	N/A
Odontoglossum ringspot virus (ORSV)[1]	Tobamovirus odontoglossi
Rehmannia mosaic virus (ReMV)	Tobamovirus rehmanniae

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Cross-reacts With:

Virus Name	Species Name
Scopolia mild mottle virus (SMMoV)[4]	N/A
Tobacco mild green mosaic virus (TMGMV)[1]	Tobamovirus mititessellati
Tomato mosaic virus (ToMV)[1]	Tobamovirus tomatotessellati
[1]Mild cross-reactivity was observed.	
[2]African eggplant-associated virus (AEaV) has been reported to be a possible novel Tobamovirus.	
[3]Chili pepper mild mottle virus (CPMMoV) has been reported to be a possible novel Tobamovirus.	
[4]Scopolia mild mottle virus (SMMoV) has been reported to be a possible novel Tobamovirus.	

Does Not Cross-react With:

Virus Name	Species Name
Broad bean wilt virus 1 (BBWV-1)	Fabavirus alphaviciae
Broad bean wilt virus 2 (BBWV-2)	Fabavirus betaviciae
Brugmansia latent virus (BrLV)[2]	N/A
Cucumber green mottle mosaic virus (CGMMV)	Tobamovirus viridimaculae
Cucumber mosaic virus (CMV)	Cucumovirus CMV
Cucumber mottle virus (CMoV)	Tobamovirus cucumeris
Frangipani mosaic virus (FrMV)	Tobamovirus frangipani
Kyuri green mottle mosaic virus (KGMMV)	Tobamovirus kyuri
Maracuja mosaic virus (MarMV)	Tobamovirus maracujae
Obuda pepper virus (ObPV)	Tobamovirus obudae
Paprika mild mottle virus (PaMMV)	Tobamovirus paprikae
Piper chlorosis virus (PChV)[1]	N/A
Ribgrass mosaic virus (RMV)	Tobamovirus plantagonis
Streptocarpus flower break virus (SFBV)	Tobamovirus streptocarpi
Sunn-hemp mosaic virus (SHMV)	Tobamovirus crotalariae
Tobacco etch virus (TEV)	Potyvirus nicotianainsculpentis
Tobacco mosaic virus (TMV)	Tobamovirus tabaci
Tomato brown rugose fruit virus (ToBRFV)	Tobamovirus fructirugosum
Tomato mottle mosaic virus (ToMMV)	Tobamovirus maculatessellati
Tomato spotted wilt virus (TSWV)	Orthotospovirus tomatomaculae
Turnip vein-clearing virus (TVCV)	Tobamovirus rapae
Wasabi mottle virus (WMoV)	Tobamovirus wasabi
Youcai mosaic virus (YoMV)	Tobamovirus youcai
Zucchini green mottle mosaic virus (ZGMMV)	Tobamovirus cucurbitae
[1]Piper chlorosis virus (PChV) has been reported to be a possible novel Tobamovirus.	
[2]Brugmansia latent virus (BrLV) has been reported to be a possible novel Tobamovirus.	

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Diagnostic Specificity

True Negatives	52
Correct Diagnoses	52
Percent	100%

Selectivity:

No Matrix Effect Observed With:			
Hosta leaves	Lavender leaves	New Zealand Spinach leaves	Nicotiana leaves
Oriental Lily leaves	Pepper leaves	Pepper seeds	Primrose leaves
Primrose leaves	Sedum leaves	Tomato leaves	
The hosts on the above list have been chosen to represent those which historically cause a range of matrix effects, in addition to those expected to be screened for this pathogen. Not all plant species susceptible to this pathogen have been screened, but may still be used with this assay unless otherwise noted below. As with all diagnostic tools, Agdia recommends confirming all results with a secondary detection method before making any economic decisions (ex: discarding plants due to positive test results, etc.).			

Matrix Effect Observed With:			
None Known			

Glossary

Diagnostic sensitivity[1]: The percentage of positive samples correctly identified in an experiment with known positive controls.

Diagnostic specificity[1]: The percentage of negative samples correctly identified in an experiment with known negative controls.

Analytical sensitivity[3]: The smallest amount of target that can be detected reliably (this is sometimes referred to as the 'limit of detection')

Analytical specificity[3]: (comprises inclusivity and exclusivity)

Inclusivity[3]: The performance of a test with a range of target isolates covering genetic diversity, different geographical origin and/or hosts associated with the target organism.

Exclusivity[3]: The performance of a test with a range of non-targets (e.g. cross-reaction with closely related organisms, contaminants)

Selectivity[2]: The level of effect that matrices and relevant plant parts have on the performance of the assay.

Repeatability[2]: The agreement between test replicates of the same sample tested by the same operator.

Reproducibility[3]: The ability of a test to provide consistent results when applied to aliquots of the same sample tested under different conditions (e.g. time, users, equipment, location)

Robustness[1,3]: The extent to which varying test conditions (e.g. temperature, volume, change of buffers) affect the established test performance values. May also be referred to as planned deviation analysis.

Stability[1]: The performance of test reagents or controls over time.

References:

[1]Groth-Helms, D., Rivera, Y., Martin, F. N., Arif, M., Sharma, P., Castlebury, L. A. (in press). Terminology and Guidelines for Diagnostic Assay Development and Validation: Best Practices for Molecular Tests. PhytoFrontiers.
[2]Eads, A., Groth-Helms, D., Davenport, B., Cha, X., Li, R., Walsh, C., Schuetz, K., (in press). The Commercial Validation of Three Tomato Brown Rugose Fruit Virus Assays. PhytoFrontiers.
[3]EPPO (2018) PM 7/76 (5) Use of EPPO Diagnostic Standards, EPPO Bulletin 48, 373– 377.

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