Validation Report: ELISA

PSA/SRA 57601 • *Ribgrass mosaic virus* (RMV), *Turnip vein-clearing virus* (TVCV), *Wasabi mottle virus* (WMoV), & *Youcai mosaic virus* (YoMV)

Test Characteristics

Test Name	RMV, TVCV, WMoV & YoMV
Catalog Number	57601
Acronym	RMV/TVCV/WMoV/YoMV
Genus	Tobamovirus
Binomial Name	RMV: Tobamovirus plantagonis TVCV: Tobamovirus rapae WMoV: Tobamovirus wasabi YoMV: Tobamovirus youcai
Capture Antibody	Polyclonal (Rabbit)
Detection Antibody	Polyclonal (Rabbit)
Format	DAS-ELISA
Diluents	GEB/ECI
Sample Dilution	1:10

Summary

This ELISA test is a qualitative serological assay for the detection of Ribgrass mosaic virus (RMV), Turnip vein-clearing virus (TVCV), Wasabi mottle virus (WMoV), and Youcai mosaic virus (YoMV) in crops including vegetables and ornamentals. This test will detect RMV, TVCV, WMoV, and YoMV but will not differentiate between them. RMV, TVCV, WMoV, and YoMV are members of the Tobamovirus genus known for their rod-shaped virus particles.

Diagnostic Sensitivity

True Positives	66
Correct Diagnoses	66
Percent	100%

Analytical Sensitivity

Limit of Detection: 1:291,600 dilution of infected tissue (pathogen titer unknown)

Analytical Specificity

Inclusivity:

Isolates and Geographic Regions Detected:

RMV-PV-0145	RMV-Plantago (ATCC® PV-229™) (KS, USA)
TVCV-HR (ATCC® PV-46™) (NJ, USA)	TVCV-PV-0147 (Yugoslavia)
TVCV-PV-0148	TVCV-PV-0361 (USA)
TVCV-PV-1341 (Germany)	TVCV-PV-1366 (Germany)
WMoV-Tochigi 1 (Japan)	YoMV-722 (Japan)
YoMV-C (Japan)	YoMV-NA23 (Japan)
YoMV-PV-0527 (Germany)	YoMV-YoM-Toy2 (Japan)
YoMV-YoM-Toy5a (Japan)	

Agdia, Inc.
52642 County Road 1
Elkhart, IN 46514
574-264-2014 / 800-622-4342
www.agdia.com / info@agdia.com

Exclusivity:

Cross-reacts With:

Virus Name	Species Name
Brugmansia latent virus (BrLV)[2]	N/A
Cucumber mottle virus (CMoV)	Tobamovirus cucumeris
Paprika mild mottle virus (PaMMV)	Tobamovirus paprikae
Piper chlorosis virus (PChV)[1]	N/A
Rehmannia mosaic virus (ReMV)	Tobamovirus rehmanniae
Streptocarpus flower break virus (SFBV)	Tobamovirus streptocarpi
Tobacco mosaic virus (TMV)	Tobamovirus tabaci
Tomato mosaic virus (ToMV)	Tobamovirus tomatotessellati
[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.	

Does Not Cross-react With:

Virus Name	Species Name
African eggplant-associated virus (AEaV)[2]	N/A
Beet western yellows virus (BWYV)	Polerovirus BWYV
Bell pepper mottle virus (BPeMV)	Tobamovirus maculacapsici
Cauliflower mosaic virus (CaMV)	Caulimovirus tessellobrassicae
Chili pepper mild mottle virus (CPMMoV)[1,4]	N/A
N/A	Clavibacter michiganensis subsp. michiganensis (Cmm)
Cucumber mosaic virus (CMV)	Cucumovirus CMV
Cucumber green mottle mosaic virus (CGMMV)	Tobamovirus viridimaculae
Frangipani mosaic virus (FrMV)	Tobamovirus frangipani
Impatiens necrotic spot virus (INSV)	Orthotospovirus impatiensnecromaculae
Kyuri green mottle mosaic virus (KGMMV)	Tobamovirus kyuri
Lettuce mosaic virus (LMV)	Potyvirus lactucae
Maracuja mosaic virus (MarMV)	Tobamovirus maracujae
Obuda pepper virus (ObPV)	Tobamovirus obudae
Odontoglossum ringspot virus (ORSV)	Tobamovirus odontoglossi
Peanut stunt virus (PSV)	Cucumovirus PSV
N/A	Pectobacterium atrosepticum (Patro)
Pepino mosaic virus (PepMV)	Potexvirus pepini
Pepper mild mottle virus (PMMoV)	Tobamovirus capsici
Potato virus X (PVX)	Potexvirus ecspotati
Potato virus Y (PVY)	Potyvirus yituberosi
Scopolia mild mottle virus (SMMoV)[3]	N/A
Sunn-hemp mosaic virus (SHMV)	Tobamovirus crotalariae
Tobacco etch virus (TEV)	Potyvirus nicotianainsculpentis
Tobacco mild green mosaic virus (TMGMV)	Tobamovirus mititessellati
Tobacco ringspot virus (TRSV)	Nepovirus nicotianae
Tobacco streak virus (TSV)	Ilarvirus TSV

Does Not Cross-react With:

Virus Name	Species Name
Tobacco vein mottling virus (TVMV)	Potyvirus nicotianavenamaculae
Tomato aspermy virus (TAV)	Cucumovirus TAV
Tomato brown rugose fruit virus (ToBRFV)	Tobamovirus fructirugosum
Tomato mottle mosaic virus (ToMMV)	Tobamovirus maculatessellati
Tomato ringspot virus (ToRSV)	Nepovirus lycopersici
Tomato spotted wilt virus (TSWV)	Orthotospovirus tomatomaculae
Turnip mosaic virus (TuMV)	Potyvirus rapae
Zucchini green mottle mosaic virus (ZGMMV)	Tobamovirus cucurbitae
[1]2 out of 16 samples produced a low-level positive response	
[2]African eggplant-associated virus (AEaV) has been reported to be a possible novel Tobamovirus.	
[3]Scopolia mild mottle virus (SMMoV) has been reported to be a possible novel Tobamovirus.	
[4]Chili pepper mild mottle virus (CPMMoV) has been reported to be a possible novel Tobamovirus.	

Diagnostic Specificity

True Negatives 111
Correct Diagnoses 111
Percent 100%

Selectivity:

No Matrix Effect Observed With:			
Alstroemeria leaves	Bean leaves	Broccoli flower	Cabbage leaves
Collard leaves	Double Impatiens leaves	Hosta leaves	Lettuce leaves
Nemesia leaves	Petunia leaves	Tobacco leaves	Tomato leaves
Verbena 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			

Repeatability

Number of Samples 72
Replicates per Sample 2 - 3
Total Replicates 212
Replicates in Agreement 212
Percent Agreement 100%

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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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