Validation Report: ELISA

PSA/SRA 57400 • *Tobacco mosaic virus* (TMV)

Test Characteristics

Test Name	Tobacco mosaic virus	**Capture Antibody**	Polyclonal (Rabbit)
Catalog Number	57400	**Detection Antibody**	Polyclonal (Rabbit)
Acronym	TMV	**Format**	Compound-ELISA
Genus	Tobamovirus	**Diluents**	GEB/ECI
Binomial Name	Tobamovirus tabaci	**Sample Dilution**	1:10

Summary

This ELISA test is a qualitative serological assay for the detection of Tobacco mosaic virus (TMV), the causal agent of Tobacco mosaic disease, in crops including vegetables, fruits, and ornamentals. TMV is a member of the Tobamovirus genus known for their rod-shaped virus particles. This ELISA has a wide cross-reactivity with other Tobamoviruses.

Diagnostic Sensitivity

True Positives	36
Correct Diagnoses	36
Percent	100%

Analytical Sensitivity

Limit of Detection: 100-1000 pg/mL of purified virus

Analytical Specificity

Inclusivity:
This assay was designed to detect all strains and isolates of TMV. Thirty-six distinct samples of TMV have been experimentally proven to be detected.

Exclusivity:

Cross-reacts With: (Virus Name) (Species Name)	**Analytical Sensitivity:**
African eggplant-associated virus (AEaV)[2] Proposed Tobamovirus	Unknown
Bell pepper mottle virus (BPeMV) Tobamovirus maculacapsici	1:50,000 dilution of infected tissue (pathogen titer unknown)
Brugmansia latent virus (BrLV)[4] Proposed Tobamovirus	Unknown
Chili pepper mild mottle virus (CPMMoV)[5] Proposed Tobamovirus	Unknown
Cucumber green mottle mosaic virus (CGMMV) Tobamovirus viridimaculae	10-1000 ng/mL of purified virus

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Cross-reacts With: (Virus Name) (Species Name)	Analytical Sensitivity:
Cucumber mottle virus (CMoV) Tobamovirus cucumeris	Unknown
Frangipani mosaic virus (FrMV) Tobamovirus frangipani	Unknown
Kyuri green mottle mosaic virus (KGMMV) Tobamovirus kyuri	1:500,000 to 1:1,000,000 dilution of infected tissue (pathogen titer unknown)
Obuda pepper virus (ObPV) Tobamovirus obudae	Unknown
Odontoglossum ringspot virus (ORSV) Tobamovirus odontoglossi	1:100,000 dilution of infected tissue (pathogen titer unknown)
Paprika mild mottle virus (PaMMV) Tobamovirus paprikae	Unknown
Pepper mild mottle virus (PMMoV) Tobamovirus capsici	10-100 ng/mL of purified virus
Piper chlorosis virus (PChV)[1] Proposed Tobamovirus	Unknown
Rehmannia mosaic virus (ReMV) Tobamovirus rehmanniae	Unknown
Ribgrass mosaic virus (RMV) Tobamovirus plantagonis	1:5,000,000 dilution of infected tissue (pathogen titer unknown)
Scopolia mild mottle virus (SMMoV)[6] Proposed Tobamovirus	Unknown
Streptocarpus flower break virus (SFBV) Tobamovirus streptocarpi	Unknown
Sunn-hemp mosaic virus (SHMV) Tobamovirus crotalariae	1:100,000 to 1:500,000 dilution of infected tissue (pathogen titer unknown)
Tobacco mild green mosaic virus (TMGMV) Tobamovirus mititessellati	1:500,000 dilution of infected tissue (pathogen titer unknown)
Tomato brown rugose fruit virus (ToBRFV) Tobamovirus fructirugosum	10-100 pg/mL of purified virus
Tomato mosaic virus (ToMV) Tobamovirus tomatotessellati	1-10 ng/mL of purified virus
Tomato mottle mosaic virus (ToMMV) Tobamovirus maculatessellati	1:50,000 to 1:100,000 dilution of infected tissue (pathogen titer unknown)
Turnip vein-clearing virus (TVCV) Tobamovirus rapae	1:5,000,000 dilution of infected tissue (pathogen titer unknown)
Wasabi mottle virus (WMoV) Tobamovirus wasabi	Unknown

Cross-reacts With: (Virus Name) (Species Name)	Analytical Sensitivity:
Youcai mosaic virus (YoMV) Tobamovirus youcai	Unknown
Zucchini green mottle mosaic virus (ZGMMV)[3] Tobamovirus cucurbitae	Unknown
[1]Piper chlorosis virus (PChV) has been reported to be a possible novel Tobamovirus.	
[2]African eggplant-associated virus (AEaV) has been reported to be a possible novel Tobamovirus.	
[3]1 out of 2 isolates of Zucchini green mottle mosaic virus (ZGMMV) were detected.	
[4]Brugmansia latent virus (BrLV) has been reported to be a possible novel Tobamovirus.	
[5]Chili pepper mild mottle virus (CPMMoV) has been reported to be a possible novel Tobamovirus.	
[6]Scopolia mild mottle virus (SMMoV) has been reported to be a possible novel Tobamovirus.	

Does Not Cross-react With:

Virus Name	Species Name
Alfalfa mosaic virus (AMV)	Alfamovirus AMV
Cucumber mosaic virus (CMV)	Cucumovirus CMV
Impatiens necrotic spot virus (INSV)	Orthotospovirus impatiensnecromaculae
Maracuja mosaic virus (MarMV)	Tobamovirus maracujae
Papaya mosaic virus (PapMV)	Potexvirus papayae
Pepino mosaic virus (PepMV)	Potexvirus pepini
Potato virus X (PVX)	Potexvirus ecspotati
Potato virus Y (PVY)	Potyvirus yituberosi
Tobacco ringspot virus (TRSV)	Nepovirus nicotianae
Tobacco streak virus (TSV)	Ilarvirus TSV
Tomato aspermy virus (TAV)	Cucumovirus TAV
Tomato ringspot virus (ToRSV)	Nepovirus lycopersici
Tomato spotted wilt virus (TSWV)	Orthotospovirus tomatomaculae
Zucchini green mottle mosaic virus (ZGMMV)[1]	Tobamovirus cucurbitae
[1]1 out of 2 isolates of Zucchini green mottle mosaic virus (ZGMMV) were detected.	

Diagnostic Specificity

True Negatives 189
Correct Diagnoses 189
Percent 100%

Selectivity:

No Matrix Effect Observed With:			
African Violet leaves	Angelonia leaves	Aster leaves	Begonia leaves
Blueberry leaves	Browalia leaves	Calendula leaves	Calibrachoa leaves
Cannabis (Hemp) leaves	Catharanthus leaves	Coleus leaves	Convolvulus leaves
Corn leaves	Cucumber leaves	Cucurbit seeds	Daisy leaves

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No Matrix Effect Observed With:			
Datura leaves	Dorotheanthus leaves	Draecaena leaves	Eggplant leaves
English Lavender leaves	Gaillardia leaves	Garlic leaves	Geranium leaves
Gladiolus leaves	Guara leaves	Hellobore leaves	Heuchera leaves
Hibiscus leaves	Impatiens leaves	Kalancho leaves	Lantana leaves
Leek leaves	Limonium leaves	Lobelia leaves	Marjoram leaves
Melon leaves	Melon seeds	Nasturtium leaves	Nemesia leaves
Nicotiana leaves	Onion leaves	Orchid leaves	Oregano leaves
Penstemon leaves	Peperomia leaves	Pepper leaves	Pepper seeds
Petunia leaves	Phlox leaves	Potato leaves	Purslane leaves
Rose leaves	Salvia leaves	Soybean leaves	Squash leaves
Sweet Potato leaves	Tomato leaves	Tomato seeds	Tradescantia leaves
Tulip leaves	Watermelon 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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