Validation Report: ELISA

PSA/SRA 87601 • *Alfalfa mosaic virus* (AMV)

Test Characteristics

Test Name	Alfalfa mosaic virus	**Capture Antibody**	Monoclonal (Mouse)
Catalog Number	87601	**Detection Antibody**	Monoclonal (Mouse)
Acronym	AMV	**Format**	DAS-ELISA
Genus	Alfamovirus	**Diluents**	GEB/ECI
		Sample Dilution	1:10

Summary

This ELISA test is a qualitative serological assay for the detection of Alfalfa mosaic virus (AMV) in ornamental and vegetable crops. AMV is a member of the Alfamovirus genus known for their bacilliform, with a constant diameter of 18 nm, viral particles.

Diagnostic Sensitivity

True Positives	325
Correct Diagnoses	318
Percent	97.8%

Analytical Sensitivity

Analytical Sensitivity:	The assay is 83.3% sensitive between 1000 ng/mL and 10 ng/mL. (n=18)
Limit of Detection:	The assay has a 100% detection rate at 100 ng/mL with purified virus. (n=6)
	The assay has a 50% detection rate at 10 ng/mL with purified virus. (n=6)

Analytical Specificity

Inclusivity:

Isolates and Geographic Regions Detected:	Analytical Sensitivity
AMV PV-0779 (Germany)	1:1,000,000 dilution of infected tissue (pathogen titer unknown)
AMV PV-1282 (Czech Republic)	1:1,000,000 dilution of infected tissue (pathogen titer unknown)
AMV-425M (ATCC® PV-92™) (WI, USA)	1:10,000,000 dilution of infected tissue (pathogen titer unknown)
AMV-Aman (Argentina)[1]	1:100,000 dilution of infected tissue (pathogen titer unknown)
AMV-Ars2 (Italy)	1:100,000 dilution of infected tissue (pathogen titer unknown)
AMV-ASdE (Argentina)[1]	1:100,000 dilution of infected tissue (pathogen titer unknown)
AMV-BSdE (Argentina)[1]	1:100,000 dilution of infected tissue (pathogen titer unknown)
AMV-Chiba1 (MAFF# 104001) (Japan)	1:1,000,000 dilution of infected tissue (pathogen titer unknown)
AMV-Nevq (Argentina)[1]	1:100,000 dilution of infected tissue (pathogen titer unknown)
AMV-P (Kanagawa PP84-1) (MAFF# 104002) (Japan)	1:1,000,000 dilution of infected tissue (pathogen titer unknown)
AMV-PS (Argentina)[1]	1:100,000 dilution of infected tissue (pathogen titer unknown)
AMV-S40 (ATCC® PV-848™) (Australia)	1:100,000,000 dilution of infected tissue (pathogen titer unknown)
[1]Isolates provided by Instituto de Patología Vegetal (IPAVE)	

Exclusivity:

Cross-reacts With:	
None Known	

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Does Not Cross-react With:

Alternanthera mosaic virus (AlMV)	Apple mosaic virus (ApMV)
Arabis mosaic virus (ArMV)	Bean common mosaic virus (BCMV)
Bean pod mottle virus (BPMV)	Broad bean wilt virus 1 (BBWV-1)
Broad bean wilt virus 2 (BBWV-2)	Brome mosaic virus (BMV)
Cowpea mosaic virus (CPMV)	Cucumber mosaic virus (CMV)
Impatiens necrotic spot virus (INSV)	Papaya mosaic virus (PapMV)
Peanut stunt virus (PSV)	Pelargonium zonate spot virus (PZSV)
Potato yellowing virus (PYV) (PV-0706)[1]	Prunus necrotic ringspot virus (PNRSV)
Ribgrass mosaic virus (RMV)	Southern bean mosaic virus (SBMV)
Soybean mosaic virus (SMV)	Tobacco mosaic virus (TMV)
Tobacco ringspot virus (TRSV)	Tobacco streak virus (TSV)
Tomato aspermy virus (TAV)	Tomato mosaic virus (ToMV)
Tomato ringspot virus (ToRSV)	Tomato spotted wilt virus (TSWV)
[1]Not a regognized specied by ICTV; EPPO classifies PYV in the Ilarvirus genus	

Diagnostic Specificity

True Negatives 498
Correct Diagnoses 497
Percent 99.8%

Selectivity:

No Matrix Effect Observed With:			
African violet leaves	Ajuga leaves	Alfalfa leaves	Alfalfa seeds
Alstroemeria leaves	Angelonia leaves	Anthurium leaves	Bacopa leaves
Basil leaves	Bean leaves	Calendula leaves	Calibrachoa leaves
Cannabis (Hemp) leaves	Cannabis (Hemp) seeds	Cantaloupe leaves	Chenopodium leaves
Chrysanthemum leaves	Clover leaves	Cowpea leaves	Cucumber leaves
Dahlia leaves	Dianthus leaves	Echinacea leaves	Endive leaves
Foxglove leaves	Grape leaves	Heliotrope leaves	Hibiscus leaves
Hydrandgea leaves	Impatiens leaves	Lavender leaves	Lettuce leaves
Lily leaves	Mint leaves	Nicotiana leaves	Osteospermum leaves
Pachysandra leaves	Parsley leaves	Pea leaves	Pelargonium leaves
Peony leaves	Peperomia leaves	Pepper leaves	Pepper seeds
Petunia leaves	Phlox leaves	Poinsettia leaves	Portulaca leaves
Potato leaves	Rosemary leaves	Salvia leaves	Snapdragon leaves
Soybean leaves	Soybean seeds	Squash leaves	Star jasmine leaves
Strawflower leaves	Sweet potato vine leaves	Thyme leaves	Tomato leaves
Tomato seeds	Verbena leaves	Vinca leaves	

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Repeatability

Number of Samples	692
Replicates per Sample	3 - 12
Total Replicates	2322
Replicates in Agreement	2298
Percent Agreement	99.0%

Reproducibility

Number of Samples	92
Replicates per Sample	3
Number of Operators	2 - 3
Total Replicates	738
Replicates in Agreement	729
Percent Agreement	98.8%

Robustness

Planned deviation analysis:

Average OD Values				
	O.N. coating / 2 hour sample	4 hour coating / 2 hour sample	4 hour coating / O.N. sample	O.N. coating / O.N. sample
Positive Sample #1 (High)	3.914	3.890	3.912	3.918
Positive Sample #1 (Low)	2.960	2.994	3.866	3.874
Positive Sample #2 (High)	3.666	2.183	3.239	3.860
Positive Sample #2 (Low)	0.636	0.527	0.917	1.417
Positive Sample #3 (High)	3.825	3.528	3.894	3.908
Negative Sample #1	0.083	0.083	0.083	0.082
Negative Sample #2	0.082	0.082	0.081	0.083
Negative Sample #3	0.081	0.080	0.080	0.081
Negative Sample #4	0.095	0.081	0.080	0.107
Negative Sample #5	0.073	0.075	0.078	0.076
Buffer	0.072	0.079	0.078	0.085

Stability:

	1-year stability (accelerated)	1-year stability (real time)
Positive Sample (High)	Pass	Monitoring
Positive Sample (Medium)	Pass	Monitoring
Positive Sample (Low)	Pass	Monitoring
Negative Sample	Pass	Monitoring
Buffer	Pass	Monitoring
Negative Control	Pass	Monitoring

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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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