Validation Report: ELISA

PSA/SRA 36600 • *Angelonia flower break virus* (AnFBV)

Test Characteristics

Test Name	Angelonia flower break virus	**Capture Antibody**	Polyclonal (Rabbit)
Catalog Number	36600	**Detection Antibody**	Polyclonal (Rabbit)
Acronym	AnFBV	**Format**	DAS-ELISA
Genus	Alphacarmovirus	**Diluents**	GEB/RUB6
Binomial Name	Alphacarmovirus angeloniae	**Sample Dilution**	1:10

Summary

This ELISA test is a qualitative serological assay for the detection of Angelonia flower break virus (AnFBV) in ornamental samples. AnFBV is a member of the Alphacarmovirus genus known for their non-enveloped, spherical, icosahedral-shaped virus particles.

Diagnostic Sensitivity

True Positives	73
Correct Diagnoses	70
Percent	95.9%

Analytical Sensitivity

Limit of Detection: 1:24,300 dilution of infected tissue (pathogen titer unknown)

Analytical Specificity

Inclusivity:

Isolates and Geographic Regions Detected:

AnFBV Israel isolate	AnFBV PV-0858 (Germany)
AnFBV USA isolate	

Exclusivity:

Cross-reacts With:

None Known	

Does Not Cross-react With:

Alfalfa mosaic virus (AMV)	Alphacarmovirus calibrachoae (Calibrachoa mottle virus) (CbMV)
Alphacarmovirus dianthi (Carnation mottle virus) (CarMV)	Alphacarmovirus pelargonii (Pelargonium flower break virus) (PFBV)
Alternanthera mosaic virus (AltMV)	Cucumber mosaic virus (CMV)
Fabavirus alphaviciae (Broad bean wilt virus 1) (BBWV-1)	Fabavirus betaviciae (Broad bean wilt virus 2) (BBWV-2)
Nepovirus arabis (Arabis mosaic virus) (ArMV)	Nepovirus lycopersici (Tomato ringspot virus) (ToRSV)

Does Not Cross-react With:

Nepovirus nicotianae (Tobacco ringspot virus) (TRSV)	Orthotospovirus arachianuli (Groundnut ringspot virus) (GRSV)
Orthotospovirus impatiensnecromaculae (Impatiens necrotic spot virus) (INSV)	Orthotospovirus tomatoflavi (Tomato chlorotic spot virus) (TCSV)
Orthotospovirus tomatomaculae (Tomato spotted wilt virus) (TSWV)	Papaya mosaic virus (PapMV)
Plantago asiatica mosaic virus (PlAMV)	Prunus necrotic ringspot virus (PNRSV)
Ribgrass mosaic virus (RMV)	Tobacco mosaic virus (TMV)
Tobacco rattle virus (TRV)	Tobacco streak virus (TSV)
Tomato aspermy virus (TAV)	Tomato mosaic virus (ToMV)
Tymovirus nemesiae (Nemesia ring necrosis virus) (NeRNV)	

Diagnostic Specificity

True Negatives	121
Correct Diagnoses	120
Percent	99.2%

Selectivity:

No Matrix Effect Observed With:			
Angelonia leaves	Angelonia petioles	Begonia leaves	Begonia petioles
Calibrachoa leaves	Calibrachoa petioles	Canna leaves	Canna petioles
Celosia leaves	Celosia petioles	Coleus leaves	Coleus petioles
Cosmos leaves	Cosmos petioles	Dahlia leaves	Dahlia petioles
Dianthus leaves	Dianthus petioles	Evolvulus leaves	Evolvulus petioles
Impatiens leaves	Impatiens petioles	Ipomea leaves	Ipomea petioles
Lantana leaves	Lantana petioles	Lavender leaves	Lavender petioles
Lobelia leaves	Lobelia petioles	Marigold leaves	Marigold petioles
Nemesia leaves	Nemesia petioles	Pachysandra leaves	Pachysandra petioles
Petunia leaves	Petunia petioles	Phlox leaves	Phlox petioles
Portulaca leaves	Portulaca petioles	Salvia leaves	Salvia petioles
Scaevola leaves	Scaevola petioles	Sedum leaves	Sedum petioles
Snapdragon leaves	Snapdragon petioles	Verbena leaves	Verbena petioles
Vinca leaves	Vinca petioles		
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	194
Replicates per Sample	3 - 6
Total Replicates	897
Replicates in Agreement	883
Percent Agreement	98.4%

Reproducibility

Number of Samples	31
Replicates per Sample	6
Number of Operators	3
Total Replicates	558
Replicates in Agreement	555
Percent Agreement	99.5%

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)	3.002	3.079	3.382	3.514
Positive Sample #1 (Low)	0.385	0.396	0.787	0.858
Positive Sample #2 (High)	2.970	3.083	3.316	3.493
Positive Sample #2 (Low)	1.019	1.073	1.742	2.014
Positive Sample #3 (High)	2.136	2.188	2.936	3.189
Negative Sample #1	0.128	0.145	0.109	0.111
Negative Sample #2	0.088	0.084	0.079	0.080
Negative Sample #3	0.089	0.086	0.089	0.084
Negative Sample #4	0.099	0.101	0.089	0.092
Buffer	0.090	0.093	0.093	0.090

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.