Validation Report: ELISA

PSA/SRA 79500 • *Cowpea mild mottle virus* (CPMMV)

Test Characteristics

Test Name	Cowpea mild mottle virus	**Capture Antibody**	Polyclonal (Rabbit)
Catalog Number	79500	**Detection Antibody**	Polyclonal (Rabbit)
Acronym	CPMMV	**Format**	DAS-ELISA
Genus	Carlavirus	**Diluents**	GEB/ECI
		Sample Dilution	1:10

Summary

This ELISA test is a qualitative serological assay for the detection of Cowpea mild mottle virus (CPMMV) in bean and vegetable leaves. CPMMV is a member of the Carlavirus genus known for their flexuous rod-shaped virus particles.

Diagnostic Sensitivity

True Positives	88
Correct Diagnoses	88
Percent	100%

Analytical Sensitivity

Limit of Detection: 1:5,000 dilution of infected tissue (pathogen titer unknown)

Analytical Specificity

Inclusivity:

Isolates and Geographic Regions Detected:

CPMMV Florida/Puerto Rico Isolate	CPMMV PV-0090 (Ghana) (DSMZ)
CPMMV PV-0907 (Sudan) (DSMZ)	

Exclusivity:

Cross-reacts With:

None Known	

Does Not Cross-react With:

Alfalfa mosaic virus (AMV)	Bean common mosaic virus (BCMV)
Bean Yellow Mosaic Virus (BYMV)	Comovirus siliquae (Bean pod mottle virus) (BPMV)
Comovirus vignae (Cowpea mosaic virus) (CPMV)	Cucumber mosaic virus (CMV)
Nepovirus arabis (Arabis mosaic virus) (ArMV)	Nepovirus lycopersici (Tomato ringspot virus) (ToRSV)
Nepovirus nicotianae (Tobacco ringspot virus) (TRSV)	Orthotospovirus arachianuli (Groundnut ringspot virus) (GRSV)
Orthotospovirus glycininecrovenae (Soybean vein necrosis virus) (SVNV)	Orthotospovirus impatiensnecromaculae (Impatiens necrotic spot virus) (INSV)
Orthotospovirus tomatomaculae (Tomato spotted wilt virus) (TSWV)	Pea Streak Virus (PeSV)
Peanut stunt virus (PSV)	Potato virus S (PVS)

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Does Not Cross-react With:

Southern bean mosaic virus (SBMV)	Soybean mosaic virus (SMV)
Tobacco mosaic virus (TMV)	Tobacco streak virus (TSV)
Tomato Aspermy Virus (TAV)	

Diagnostic Specificity

True Negatives 117
Correct Diagnoses 117
Percent 100%

Selectivity:

No Matrix Effect Observed With:			
Alfalfa leaves	Browallia leaves	Chickpea leaves	Common bean leaves
Common bean seeds	Cotton leaves	Cowpea leaves	Cowpea seeds
Eggplant leaves	Faba bean leaves	Mirabilis jalapa leaves	Pea leaves
Peanut leaves	Petunia leaves	Salvia hispanica leaves	Soybean leaves
Soybean seeds	Tomato leaves	Tomato seeds	Vigna subterranea 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 205
Replicates per Sample 3 - 6
Total Replicates 801
Replicates in Agreement 800
Percent Agreement 99.9%

Reproducibility

Number of Samples 32
Replicates per Sample 6
Number of Operators 3
Total Replicates 576
Replicates in Agreement 565
Percent Agreement 98.1%

Robustness

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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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)	4.000	4.000	3.760	3.782
Positive Sample #1 (Med)	2.309	2.331	2.276	2.501
Positive Sample #1 (Low)	0.491	0.495	0.789	0.875
Negative Sample #1	0.099	0.100	0.073	0.081
Negative Sample #2	0.097	0.097	0.074	0.108
Negative Sample #3	0.097	0.098	0.075	0.080
Negative Sample #4	0.102	0.101	0.077	0.082
Negative Sample #5	0.145	0.123	0.107	0.120
Negative Sample #6	0.105	0.099	0.075	0.080
Negative Sample #7	0.097	0.094	0.077	0.079
Buffer	0.096	0.082	0.061	0.083

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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