Validation Report: ELISA

PSA/SRA 20001 • *Potato virus Y* (PVY)

Test Characteristics

Test Name	Potato virus Y	**Capture Antibody**	Polyclonal (Rabbit)
Catalog Number	20001	**Detection Antibody**	Monoclonal (Mouse)
Acronym	PVY	**Format**	Compound-ELISA
Genus	Potyvirus	**Diluents**	GEB/ECI
Binomial Name	Potyvirus yituberosi	**Sample Dilution**	1:10

Summary

This ELISA test is a qualitative serological assay for the detection of Potato virus Y (PVY) in potato leaf, sprouts, and tubers as well as other solanaceous crops. PVY is a member of the Potyvirus genus known for their non-enveloped, flexuous, filamentous virus particles.

Diagnostic Sensitivity

True Positives	156
Correct Diagnoses	153
Percent	98.1%

Analytical Sensitivity

Limit of Detection: 1:10,000 dilution of infected tissue (pathogen titer unknown)

Analytical Specificity

Inclusivity:

Isolates and Geographic Regions Detected:

PVY^{NE-11}	PVY-Alt (PVY$^{N:O}$)
PVY-HI-14 (PVY^{C})	PVY-HR1 (PVY^{Z}) (PVY^{NTN})
PVY-ID269 (PVY^{O-O5})	PVY-Mont (PVY^{N})
PVY-N1 (PVY$^{N–Wi}$)	PVY-Oz (PVY^{O})
PVY-Poha6 (PVY^{C}) (PVY^{C-Poha})	PVY-Pondo4 (PVY$^{261–4}$) (PVY^{O})
PVY-PVY-AGA (PVY^{E}) (PVY$^{N/AST}$)	PVY-Tam17 (SA-N) (N serotype)[1]
[1]Isolate unable to systemically spread in potato (Green et. al.)	

Isolates and Geographic Regions Not Detected:

PVY-Tam15 (SA-N) (no serotype)[1]	
[1]Isolate unable to systemically spread in potato (Green et. al.)	

Exclusivity:

Cross-reacts With:

None Known	

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Does Not Cross-react With:

Alfamovirus AMV (Alfalfa mosaic virus) (AMV)	Carlavirus latensolani (Potato latent virus) (PotLV)
Carlavirus misolani (Potato virus M) (PVM)	Carlavirus sigmasolani (Potato virus S) (PVS)
Clavibacter michiganenses subsp. michiganensis (Cms)	Comovirus andesense (Andean potato mottle virus) (APMoV)
Cucumovirus CMV (Cucumber mosaic virus) (CMV)	Ilarvirus TSV (Tobacco streak virus) (TSV)
Orthotospovirus arachinecrosis (Groundnut bud necrosis virus) (GBNV)	Orthotospovirus impatiensnecromaculae (Impatiens necrotic spot virus) (INSV)
Orthotospovirus tomatomaculae (Tomato spotted wilt virus) (TSWV)	Pectobacterium atroseptica (Patro)
Pectobacterium carotovorum (Pc)	Phytophthora infestans
Polerovirus PLRV (Potato leafroll virus) (PLRV)	Pomovirus solani (Potato mop-top virus) (PMTV)
Potexvirus ecspotati (Potato virus X) (PVX)	Potexvirus marmoraucuba (Potato aucuba mosaic virus) (PAMV)
Potexvirus pepini (Pepino mosaic virus) (PepMV)	Potyvirus atuberosi (Potato virus A) (PVA)
Potyvirus nicotianainsculpentis (Tobacco etch virus) (TEV)	Potyvirus vetuberosi (Potato virus V) (PVV)
Ralstonia solancearum (Rs)	Tepovirus tafsolani (Potato virus T) (PVT)
Tobamovirus capsici (Pepper mild mottle virus)	Tobamovirus tabaci (Tobacco mosaic virus) (TMV)
Tobamovirus tomatotessellati (Tomato mosaic virus) (ToMV)	Tobravirus tabaci (Tobacco rattle virus) (TRV)
Tymovirus latandigenum (Andean potato latent virus) (APLV)	

Diagnostic Specificity

True Negatives 292
Correct Diagnoses 292
Percent 100%

Selectivity:

No Matrix Effect Observed With:			
Bean leaves	Beet leaves	Cucumber leaves	Dahlia leaves
Eggplant leaves	Eggplant petioles	Garlic scapes	Goosefoot leaves
Impatiens leaves	Kale leaves	Lettuce leaves	Onion leaves
Pepper leaves	Pepper petioles	Potato leaves	Potato petioles
Potato sprouts	Potato tubers	Sugar beet leaves	Tobacco leaves
Tobacco petioles	Tomato leaves	Tomato petioles	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:			
Lantana leaves	Petunia leaves	Petunia petioles	Salvia leaves

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Repeatability

Number of Samples	448
Replicates per Sample	3 - 4
Total Replicates	1404
Replicates in Agreement	1395
Percent Agreement	99.4%

Reproducibility

Number of Samples	56
Replicates per Sample	3
Number of Operators	3
Total Replicates	504
Replicates in Agreement	482
Percent Agreement	95.6%

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)	3.571	3.479	3.120	3.819
Positive Sample #1 (Low)	0.344	0.333	0.821	0.827
Positive Sample #2 (High)	2.486	2.415	3.509	3.387
Positive Sample #2 (Low)	0.278	0.274	0.619	0.562
Positive Sample #3 (High)	2.549	2.460	3.713	3.618
Negative Sample #1	0.087	0.078	0.100	0.080
Negative Sample #2	0.091	0.079	0.099	0.080
Negative Sample #3	0.091	0.083	0.093	0.081
Negative Sample #4	0.093	0.080	0.102	0.081
Negative Sample #5	0.089	0.082	0.097	0.081
Buffer	0.086	0.079	0.093	0.084

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

References

Green KJ, Funke CN, Chojnacky J, Alvarez-Quinto RA, Ochoa JB, Quito-Avila DF, Karasev AV. Potato Virus Y (PVY) Isolates from Solanum betaceum Represent Three Novel Recombinants Within the PVYN Strain Group and Are Unable to Systemically Spread in Potato. Phytopathology. 2020 Sep;110(9):1588-1596. doi: 10.1094/PHYTO-04-20-0111-R. Epub 2020 Jul 29. PMID: 32370660.

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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