Validation Report: ELISA

PSA/SRA 20501 • *Impatiens necrotic spot virus* (INSV)

Test Characteristics

Test Name	Impatiens necrotic spot virus	**Capture Antibody**	Monoclonal (Mouse)
Catalog Number	20501	**Detection Antibody**	Monoclonal (Mouse)
Acronym	INSV	**Format**	DAS-ELISA
Genus	Orthotospovirus	**Diluents**	GEB/ECI
Binomial Name	Orthotospovirus impatiensnecromaculae	**Sample Dilution**	1:10

Summary

This ELISA test is a qualitative serological assay for the detection of Impatiens necrotic spot virus (INSV) in fruit, ornamental, and vegetable leaves. INSV is a member of the Orthotospovirus genus known for their enveloped, spherical-shaped virus particles.

Diagnostic Sensitivity

True Positives	59
Correct Diagnoses	58
Percent	98.1%

Analytical Sensitivity

Limit of Detection: 1:100,000 dilution of infected tissue (pathogen titer unknown)

Analytical Specificity

Inclusivity:

This assay was designed to detect all strains and isolates of INSV. Fifty-eight distinct samples of INSV have been experimentally proven to be detected.

Exclusivity:

Cross-reacts With:

None Known	

Does Not Cross-react With:

Bean yellow mosaic virus (BYMV)	Nepovirus nicotianae (Tobacco ringspot virus) (TRSV)
Orthotospovirus arachianuli (Groundnut ringspot virus) (GRSV)	Orthotospovirus citrullomaculosi (Watermelon silver mottle virus) (WSMoV)
Orthotospovirus glycininecrovenae (Soybean vein necrosis virus) (SVNV)	Orthotospovirus iridimaculaflavi (Iris yellow spot virus) (IYSV)
Orthotospovirus tomatoflavi (Tomato chlorotic spot virus) (TCSV)	Orthotospovirus tomatomaculae (Tomato spotted wilt virus) (TSWV)

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Diagnostic Specificity

True Negatives	207
Correct Diagnoses	205
Percent	99.0%

Selectivity:

No Matrix Effect Observed With:			
Angelonia leaves	Basil leaves	Basil petioles	Begonia leaves
Calibrachoa leaves	Calla leaves	Celosia leaves	Coleus leaves
Dahlia leaves	Dahlia petioles	Dianthus leaves	Diascia leaves
Evolvulus leaves	Geranium leaves	Gladiolus leaves	Hosta leaves
Impatiens leaves	Impatiens petioles	Ipomoea leaves	Lantana leaves
Lavender leaves	Lettuce leaves	Limonium leaves	Lisanthus leaves
Lobelia leaves	Nemesia leaves	New Guinea impatiens leaves	New Guinea impatiens petioles
Osteospermum leaves	Osteospermum petioles	Pelargonium leaves	Pepper leaves[1]
Pepper petioles	Petunia leaves	Phlox leaves	Plectranthus leaves
Portulaca leaves	Portulaca petioles	Rosemary leaves	Sage leaves
Salvia leaves	Scaevola leaves	Sedum leaves	Snapdragon leaves
Tomato leaves	Tomato petioles	Verbena leaves	Zinnia leaves
[1]False positive observed in 2 out of 15 samples of Pepper leaves.			

Repeatability

Number of Samples	266
Replicates per Sample	3 - 8
Total Replicates	1215
Replicates in Agreement	1203
Percent Agreement	99.0%

Reproducibility

Number of Samples	31
Replicates per Sample	6
Number of Operators	3
Total Replicates	558
Replicates in Agreement	540
Percent Agreement	96.8%

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 #1	Pass	Monitoring
Negative Sample #2	Pass	Monitoring
Buffer	Pass	Monitoring
Negative Control	Pass	Monitoring

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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)	3.161	2.745	3.861	3.789
Positive Sample #1 (Low)	0.269	0.231	0.702	0.482
Positive Sample #2 (High)	3.882	3.879	3.894	3.891
Positive Sample #2 (Low)	0.670	0.532	1.674	1.165
Negative Sample #1	0.078	0.082	0.083	0.092
Negative Sample #2	0.076	0.080	0.081	0.095
Negative Sample #3	0.081	0.085	0.087	0.090
Negative Sample #4	0.084	0.090	0.085	0.088
Negative Sample #5	0.088	0.086	0.084	0.083
Buffer	0.078	0.081	0.076	0.081

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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