ImmunoStrip® Validation Report

On-site Plant Pathogen Testing

Orchid fleck virus (OFV)

ISK/STX 52400

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Test Characteristics

Test Name	Orchid fleck virus	**Capture Antibody**	Polyclonal (Rabbit)
Catalog Number	52400	**Detection Antibody**	Polyclonal (Rabbit)
Acronym	OFV	**Format**	Lateral Flow Device
Genus	Dichorhavirus	**Diluents**	SEB1
Binomial Name	Dichorhavirus orchidaceae	**Sample Dilution**	1:20

Summary

The Orchid fleck virus (OFV) ImmunoStrip is used to detect the presence of OFV in citrus, orchid, and ornamental crops. ImmunoStrips are the perfect screening tool for use in the field, greenhouse, and the lab. OFV is a member of the Dichorhavirus genus known for their non-enveloped, short bacilliform-shaped virus particles.

Diagnostic Sensitivity

True Positives	29
Correct Diagnoses	29
Percent	100%

Analytical Sensitivity

Limit of Detection: 1:256 dilution of infected tissue (pathogen titer unknown)

Analytical Specificity

Inclusivity:

This assay was designed to detect all strains and isolates of OFV. Twenty-nine distinct samples of OFV have been experimentally proven to be detected including samples of Australian isolates and Florida, USA isolates.

Exclusivity:

Cross-reacts With:

Virus Name	Species Name
None Known	

Does Not Cross-react With:

Virus Name	Species Name
Calibrachoa mottle virus (CbMV)	Alphacarmovirus calibrachoae
Capsicum chlorosis virus (CaCV)	Orthotospovirus capsiciflavi
Carnation mottle virus (CarMV)	Alphacarmovirus dianthi
Cucumber green mottle mosaic virus (CGMMV)	Tobamovirus viridimaculae
Cucumber mosaic virus (CMV)	Cucumovirus CMV
Cymbidium mosaic virus (CymMV)	Potexvirus cymbidii
Cymbidium ringspot virus (CymRSV)	Tombusvirus cymbidii
Hosta virus X (HVX)	Potexvirus ecshostae
Impatiens necrotic spot virus (INSV)	Orthotospovirus impatiensnecromaculae
Odontoglossum ringspot virus (ORSV)	Tobamovirus odontoglossi

Does Not Cross-react With:

Virus Name	Species Name
Pepino mosaic virus (PepMV)	Potexvirus pepini
Piper chlorosis virus (PChV)[1]	N/A
Ribgrass mosaic virus (RMV)	Tobamovirus plantagonis
Tobacco mosaic virus (TMV)	Tobamovirus tabaci
Tomato mosaic virus (ToMV)	Tobamovirus tomatotessellati
Tomato ringspot virus (ToRSV)	Nepovirus lycopersici
Tomato spotted wilt virus (TSWV)	Orthotospovirus tomatomaculae
[1]Piper chlorosis virus (PChV) has been reported to be a possible novel Tobamovirus.	

Diagnostic Specificity

True Negatives 100
Correct Diagnoses 100
Percent 100%

Selectivity:

No Matrix Effect Observed With:			
Cast Iron Plant leaves	Cattleya leaves	Cymbidium bulbs	Cymbidium leaves
Cymbidium petioles	Cymbidium pseudobulbs	Cymbidium spikes	Cymbidium stems
Dendrobium leaves	Grapefruit leaves	Grapefruit petioles	Lemon leaves
Lemon petioles	Lilyturf leaves	Lime leaves	Lime petioles
Miltonia leaves	Odontoglossum leaves	Oncidium leaves	Orange leaves
Orange petioles	Paphiopedilum leaves	Phalaenopsis leaves	Phragmipedium leaves
Spathoglottis leaves	Ti leaves	Vandas 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 127
Replicates per Sample 2 - 3
Total Replicates 370
Replicates in Agreement 370
Percent Agreement 100%

Reproducibility

Number of Samples 32
Replicates per Sample 3
Number of Operators 4
Total Replicates 384
Replicates in Agreement 384
Percent Agreement 100%

Robustness

Planned deviation analysis:
No deviations from the user guide protocol were validated.

Stability:

	1-year stability (accelerated)	Real-time Stability Verification
Positive Sample (High)	Pass	Monitoring
Positive Sample (High)	Pass	Monitoring
Positive Sample (Low)	Pass	Monitoring
Positive Sample (Low)	Pass	Monitoring
Positive Sample (Low)	Pass	Monitoring
Negative Sample	Pass	Monitoring
Negative Sample	Pass	Monitoring
Negative Sample	Pass	Monitoring

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.