



The Growing Standard.



Pathogen Assay Validation

April 13, 2023



Alex Eads

Technical Support Director

Alex Eads is the Technical Support Director at Agdia, Inc. and leads the team that responds to technical customer questions, documentation support, and assay troubleshooting. He maintains the product webpages, validation reports, and instructional documentation. His goal is to help customers reach their diagnostic testing goals by providing excellent, data-driven, product support. He is an alumnus of Purdue University. In his free time, he loves traveling and camping with his wife and two children and playing music at his church.

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Agdia History

- Incorporated September 1981
- Started with Potato virus assays
- Potato virus X
- “Validated” to the customer needs and “requirements” of the day





Customer Trends

- Customers inquiries
- Requests began in 2018
- Testing Laboratories
- ISO 17025





Initial Validation Reports

- Created on-demand by customer request
- What to include?
- EPPO PM 7/98
- ISO/TS 16393

Validation Results

Test Name:Aac
Bacteria: Acidovorax citrulli
Formerly: Acidovorax avenae subsp. Citrulli
Common Name:..... Watermelon fruit blotch
Symptoms: Necrotic spots on cotyledons and leaves
Tissue specificity: Use symptomatic tissue
Detection methods: ELISA, ImmunoStrip
Hosts:Watermelon, melon, squash, pumpkin, muskmelon
Spread by: Infected seed

ELISA:

Sensitivity: 1.6×10^5 - 6.2×10^7 cells/ml CFU/ml range was lowest positive with four pure cultures

Specificity:

No cross reactions were seen with plant pathogens: Acidovorax avenae pv. avenae, Agrobacterium tumefaciens, Agrobacterium radiobacter, Agrobacterium rhizogenes, Agrobacterium vitas, Burkholderia glumae, Clavibacter michiganensis subsp. insidiosus, Clavibacter michiganensis subsp. michiganensis, Clavibacter michiganensis subsp. nebraskensis, Clavibacter michiganensis subsp. sepedonicus, Clavibacter michiganensis subsp. tessellarius, Curtobacterium flaccumfaciens subsp. poinsettiae, Dickeya chrysanthemi, Erwinia amylovora, Erwinia tracheiphila, Pantoea agglomerans, Pantoea stewartii, Pectobacterium atroseptica, Pectobacterium carotovora subsp. carotovora, Pseudomonas fuscovaginae, Pseudomonas savastanoi pv. glycinea, Pseudomonas savastanoi pv. phaseolicola, Pseudomonas syringae pv. syringae, Pseudomonas syringae pv. tomato, Ralstonia solanacearum, Spiroplasma citri, Stenotrophomonas maltophilia, Xanthomonas albilineans, Xanthomonas arboricola pv. celebensis, Xanthomonas arboricola pv. pruni, Xanthomonas axonopodis sp., Xanthomonas axonopodis pv. begoniae, Xanthomonas axonopodis pv. citri, Xanthomonas axonopodis pv. dieffenbachia, Xanthomonas axonopodis pv. phaseoli, Xanthomonas campestris sp., Xanthomonas campestris pv. armoraciae, Xanthomonas campestris pv. campestris, Xanthomonas campestris pv. zinnae, Xanthomonas citri pv. aurantifolii, Xanthomonas citromelo, Xanthomonas fragariae, Xanthomonas hortorum pv. pelargonii, Xanthomonas oryzae pv. oryzae, Xanthomonas translucens pv. translucens, Xanthomonas vesicatoria, Xylella fastidiosa

No cross reactions were seen with non-plant pathogens: Acinetobacter calcoaceticus, Bacillus cereus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Pseudomonas



Initial Validation Reports

- Quantity of data is variable
- Historical data

fluorescens, Pseudomonas putida, Serratia marcescens, Staphylococcus aureus, Streptococcus faecalis

ImmunoStrips:

Sensitivity: 4×10^5 CFU/ml with lowest positive culture

Specificity:

No cross reactions were seen with plant pathogens: Clavibacter michiganensis subsp. insidiosus, Clavibacter michiganensis subsp. michiganensis, Clavibacter michiganensis subsp. nebraskensis, Clavibacter michiganensis subsp. sepedonicus, Clavibacter michiganensis subsp. tessellarius, Curtobacterium flaccumfaciens subsp. poinsettia, Erwinia carotovora, Pseudomonas syringae subsp. syringae, Pseudomonas syringae subsp. tomato, Ralstonia solanacearum, Xanthomonas campestris subsp. armoraciae, Xanthomonas axonopodis pv. dieffenbachiae, Xanthomonas vesicatoria

Growth Conditions

Media	Colony Morphology
LB	yellowish, shiny
KB	butyrous, cream colored
Lima	cream, shiny
NBY	butyrous, cream color, v. small, pigment diffuses into agar
PDA	N/A
SPA	lt. yellow, flat, shiny
TSA*	beige, satiny best for coming out of water culture
TTC	red, shiny
Tween	N/A
TZC	N/A
YDC	beige, satiny

*preferred plating medium



Report Improvements

- Create reports for all assays
- Identified categories to include

Validation Report: ELISA

SRA 62000 • *Kalanchoe latent virus (KLV)*



Test Characteristics

Test Name	Kalanchoe latent virus	Capture Antibody	Polyclonal (rabbit)
Catalog Number	62000	Detection Antibody	Polyclonal (rabbit)
Acronym	KLV	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 *Kalanchoe latent virus (KLV)* in kalanchoes. KLV is a member of the *Carlavirus* genus known for their flexuous rod-shaped virus particles.

Diagnostic Sensitivity

True Positives	15
Correct Diagnoses	15
Percent	100%

Analytical Sensitivity

Limit of Detection: 1:405 dilution of infected tissue (pathogen titer unknown)

Analytical Specificity

Inclusivity:

This assay was designed to detect all strains and isolates of KLV. Fifteen distinct samples of KLV have been experimentally proven to be detected including samples of KaV-1 and KaV-2.

Exclusivity:

Cross-reacts With:

None known	
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Diagnostic Specificity

True Negatives	13
Correct Diagnoses	13
Percent	100%

Selectivity:

No Matrix Effect Observed With:			
Kalanchoe leaves			



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Validation Reports Today

- More data collected during development
- Direct focus on validation requirements
- Filling the gaps where no plant pathogen regulatory requirements exist.

Test Characteristics

Test Name	Phytophthora ramorum	Test Label	FAM-labeled target probe
Catalog Number	93400	Internal Control	ROX-labeled control probe (endogenous)
Acronym	Pram	Format	XRT
Genus	Phytophthora	Diluents	GEB2/PD1
		Sample Dilution	1:20

Summary

AmplifyRP XRT for Pram is a rapid DNA amplification and detection platform designed for testing ornamental plants for *Phytophthora ramorum*. This kit includes lyophilized reaction pellets containing the necessary reagents to amplify Pram DNA and an endogenous DNA control at a single operating temperature (42 °C).

Diagnostic Sensitivity

True Positives	131
Correct Diagnoses	128
Percent	97.7%

Analytical Sensitivity

Limit of Detection:	Approximately 1 fg/μL of plasmid DNA fragments
Limit of Detection:	Approximately 100 fg/μL of whole genomic DNA fragments

Analytical Specificity

Inclusivity:

Isolates and Geographic Regions Detected:

Pram-EU1	Pram-EU2
Pram-NA1	Pram-NA2

Exclusivity:

Cross-reacts With:

None Known	
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Does Not Cross-react With:

Botrytis cinerea (Bcin)	Botrytis galanthina
Colletotrichum acutatum	Colletotrichum fioriniae
Colletotrichum gloeosporioides	Colletotrichum karsti
Fusarium proliferatum	Fusarium solani
Fusarium sporotrichioides	Penicillium olsonii
Phyllostica capitalensis	Phyllostica concentrica
Phyllostica cryptomeriae	Phytophthora hibernalis
Phytophthora cactorum	Phytophthora cambivora
Phytophthora capsici	Phytophthora chlamydospora
Phytophthora cinnamomi	Phytophthora citricola
Phytophthora citrophthora	Phytophthora cryptogea



Post-release Validations

- New detections and new hosts
- Taxonomic changes
- Stability

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
Positive Sample (Low)	Pass	Monitoring
Negative Sample	Pass	Monitoring
Negative Sample	Pass	Monitoring

Glossary

Diagnostic sensitivity: The percentage of positive samples correctly identified in an experiment with known positive controls.

Diagnostic specificity: The percentage of negative samples correctly identified in an experiment with known negative controls.

Analytical sensitivity:² The smallest amount of target that can be detected reliably (this is sometimes referred to as the 'limit of detection')

Analytical specificity:² (comprises inclusivity and exclusivity)

Inclusivity:² 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:² 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:² 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: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: The performance of test reagents or controls over time.

References:

¹Groth-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*.

²Eads, 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*.

³EPPO (2018) PM 7/76 (5) Use of EPPO Diagnostic Standards, EPPO Bulletin 48, 373– 377.

Questions or Technical Support:

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AmplifyRP Test Kits employ recombinase polymerase amplification (RPA) technology, developed by TwistDx Limited, U.K. Use of the RPA process and probe technologies are protected by US patents 7,270,981 B2, 7,399,590 B2, 7,435,561 B2, 7,485,428 B2 and foreign equivalents in addition to pending patents.

AmplifyRP® is a registered trademark of Agdia, Inc.

Limitations

- Reference materials
- Time of the year
- Foreign regulations
- Cannabis
- Lack of US Standardization





External Validations

- Multi-laboratory Ring Trial
- Agdia as a Participating Laboratory
- DAVN





APS DAVN

- National Institute of Food and Agriculture (NIFA) Funded
- Diagnostic development and validation
- Tools for assay developers
- Prepare diagnostic assays for multi-laboratory ring trials

Diagnostic Assay Validation Network (DAVN)

Development • Validation • Application



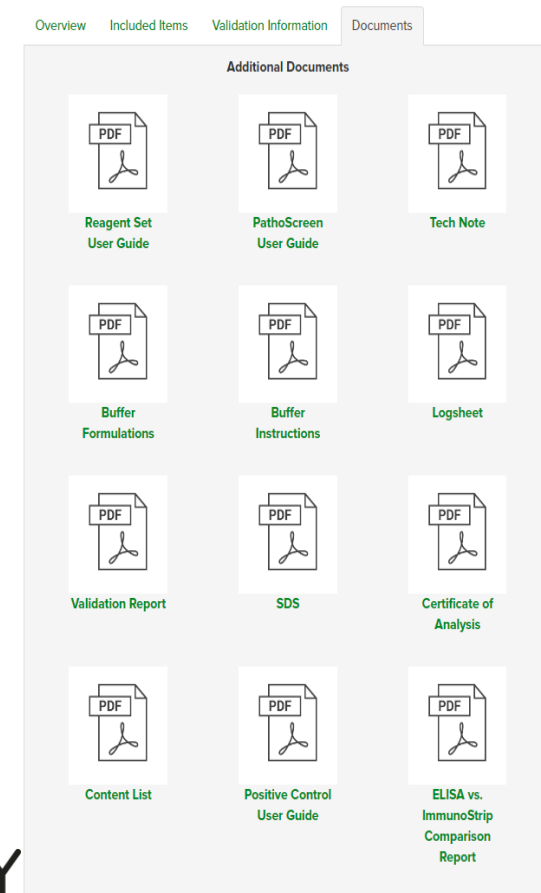


Customer Benefits

- More data
- Transparency
- Available documents and expectations
- Confidence



TRANSPARENCY



CONFIDENCE



Agdia Benefits

- Open dialog with customers
- Customer needs and wants
- Newly emerging pathogens and/or isolates





Thank you!

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