AmplifyRP® XRT for *Xf*
Validation Report
Xylella fastidiosa
Product No. XCS 34503

Test Characteristics

Test Name	Xylella fastidiosa	**Test Label**	FAM-labeled target probe
Catalog Number	34503	**Internal Control**	N/A
Acronym	Xf	**Format**	XRT
Genus	Xylella	**Diluents**	AMP1/PD1
		Sample Dilution	1:20

Summary

AmplifyRP XRT for Xf is a rapid DNA amplification and detection platform designed for testing almond, blueberry, citrus, grapevine, and olive for Xylella fastidiosa. This kit includes lyophilized reaction pellets containing the necessary reagents to amplify Xf DNA at a single operating temperature (42 °C).

Diagnostic Sensitivity

True Positives	146
Correct Diagnoses	145
Percent	99.3%

Analytical Sensitivity

Analytical Sensitivity:	The assay is 66.7% sensitive between 100 ag/µL and 10 ag/µL. (n=12)
Limit of Detection:	The assay has a 100% detection rate at 100 ag/µL with DNA fragments. (n=6)
	The assay has a 33.3% detection rate at 10 ag/µL with DNA fragments. (n=6)

Analytical Specificity

Inclusivity:

Isolates and Geographic Regions Detected:

Xf subsp. fastidiosa	Xf subsp. multiplex
Xf subsp. pauca	Xf subsp. sandyi
Xf subsp. temecula	

Exclusivity:

Cross-reacts With:

None Known	

Does Not Cross-react With:

Curtobacterium flaccumfaciens[1]	Erwinia amylovora (Ea)[1]
Erwinia carotovora[1]	Erwinia herbicola
Erwinia tracheiphila[1]	Pseudomonas syringae
Xanthomonas albilineans (Xalb)	Xanthomonas arboricola
Xanthomonas axonopodis	Xanthomonas campestris
Xanthomonas citri	Xanthomonas euvesicatoria
Xanthomonas fragariae	Xanthomonas gardneri
Xanthomonas perforans	Xanthomonas vesicatoria

[1]Based on *in silico* analysis

Diagnostic Specificity

True Negatives 91
Correct Diagnoses 91
Percent 100%

Selectivity:

No Matrix Effect Observed With:			
Almond midribs	Almond petioles	Blueberry midribs	Blueberry petioles
Blueberry roots	Blueberry stems	Citrus midribs	Citrus petioles
Citrus roots	Citrus stems	Coffee midribs	Coffee petioles
Grapevine canes	Grapevine midribs	Grapevine petioles	Grapevine roots
Grapevine stems	Lavender midribs	Maple midribs	Maple petioles
Oak midribs	Oak petioles	Olive midribs	Olive petioles
Peach midribs	Peach petioles	Peach stems	
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 236
Replicates per Sample 2 - 8
Total Replicates 530
Replicates in Agreement 526
Percent Agreement 99.2%

Reproducibility

Number of Samples 24
Replicates per Sample 3
Number of Operators 4
Total Replicates 288
Replicates in Agreement 288
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
Positive Sample (Low)	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.

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