



AmplifyRP® XRT for Xhp
Validation Report
Xanthomonas hortorum pv. pelargonii
Product No. XCS 32503



Test Characteristics

Test Name	Xanthomonas hortorum pv. pelargonii	Test Label	FAM-labeled target probe
Catalog Number	32503	Internal Control	N/A
Acronym	Xhp	Format	XRT
Genus	Xanthomonas	Diluents	AMP1/PD1
Binomial Name	Xanthomonas hortorum pv. pelargonii	Sample Dilution	1:20

Summary

AmplifyRP® XRT for Xhp is a rapid DNA amplification and detection platform designed for testing euphorbia, geraniums, pelargoniums, and poinsettias for Xanthomonas hortorum pv. pelargonii. This kit includes lyophilized reaction pellets containing the necessary reagents to amplify Xhp DNA at a single operating temperature (42 °C).

Diagnostic Sensitivity

True Positives	106
Correct Diagnoses	106
Percent	100%

Analytical Sensitivity

Analytical Sensitivity:	The assay is 81.3% sensitive between 10 ag/μL and 100 ag/μL. (n=24)
Limit of Detection:	The assay has a 100% detection rate at 100 ag/μL with DNA fragments. (n=16)
	The assay has a 62.5% detection rate at 10 fg/μL with DNA fragments. (n=8)

Analytical Specificity

Inclusivity:

This assay was designed to detect all strains and isolates of Xhp. One hundred six distinct samples of Xhp have been experimentally proven to be detected including samples of DSM 1350 (ICPB P 121).

Exclusivity:

Cross-reacts With:

None Known	
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Does Not Cross-react With:

Agrobacterium tumefaciens	Pseudomonas syringae pv. syringae
Ralstonia solanacearum	Xanthomonas albilineans
Xanthomonas arboricola pv. pruni	Xanthomonas axonopodis pv. begoniae
Xanthomonas axonopodis pv. poinsettiiicola	Xanthomonas campestris pv. campestris
Xanthomonas campestris pv. zinniae	Xanthomonas euroxanthea
Xanthomonas euvesicatoria pv. euvesicatoria	Xanthomonas fragariae
Xanthomonas hortorum pv. carotae	Xanthomonas hortorum pv. gardneri
Xanthomonas hortorum pv. hederae	Xanthomonas hortorum pv. vitians
Xanthomonas phaseoli pv. phaseoli	

Diagnostic Specificity

True Negatives 63
Correct Diagnoses 63
Percent 100%

Selectivity:

No Matrix Effect Observed With:			
Cranesbill leaves	Cranesbill petioles	Cranesbill stems	Euphorbia leaves
Euphorbia petioles	Euphorbia roots ¹	Euphorbia stems ¹	Geranium roots ¹
Geranium stems ¹	Pelargonium inquinans leaves	Pelargonium inquinans petioles	Pelargonium inquinans stems
Pelargonium odoratissimum leaves	Pelargonium odoratissimum petioles	Pelargonium odoratissimum stems	Pelargonium peltatum leaves
Pelargonium peltatum petioles	Pelargonium peltatum stems	Pelargonium zonale leaves	Pelargonium zonale petioles
Pelargonium zonale stems	Poinsettia roots	Poinsettia stems	
¹ A 10-fold reduction in sensitivity has been observed due to inhibition.			
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:			
Euphorbia roots ¹	Euphorbia stems ¹	Geranium roots ¹	Geranium stems ¹
¹ A 10-fold reduction in sensitivity has been observed due to inhibition.			

Repeatability

Number of Samples 160
Replicates per Sample 2 - 8
Total Replicates 362
Replicates in Agreement 362
Percent Agreement 100%

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 sensitivity¹:	The percentage of positive samples correctly identified in an experiment with known positive controls.
Diagnostic specificity¹:	The percentage of negative samples correctly identified in an experiment with known negative controls.
Analytical sensitivity³:	The smallest amount of target that can be detected reliably (this is sometimes referred to as the 'limit of detection')
Analytical specificity²:	(comprises inclusivity and exclusivity)
Inclusivity³:	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.
Exclusivity³:	The performance of a test with a range of non-targets (e.g. cross-reaction with closely related organisms, contaminants)
Selectivity²:	The level of effect that matrices and relevant plant parts have on the performance of the assay.
Repeatability²:	The agreement between test replicates of the same sample tested by the same operator.
Reproducibility³:	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.
Stability¹:	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.

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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.

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