EUROPEAN AND MEDITERRANEAN PLANT PROTECTION ORGANIZATION
ORGANISATION EUROPEENNE ET MEDITERRANEENNE POUR LA PROTECTION DES PLANTES
Summary sheet of validation data for a diagnostic test

The EPPO Standard PM 7/98 *Specific requirements for laboratories preparing accreditation for a plant pest diagnostic activity* describes how validation should be conducted. It also includes definitions of performance criteria.

Laboratory contact details	Netherlands Institute for Vectors, Invasive plants and Plant health P.O. Box 9102, 6700 HC Wageningen, Netherlands
Short description of the test	Detection of rose rosette virus by real-time RT-PCR in Rosa spp. leaves
Date, reference of the validation report	2023-08-21 - Comparison and validation of real-time RT-PCRs for the detection of rose rosette virus in Rosa spp.
Validation process according to EPPO Standard PM7/98?	yes
Is the lab accredited for this test?	no
Was the validated data generated in the framework of a project?	EURL
If yes, please specify	EURL-Virology (European Union Reference Laboratory for pest of plants on viruses, viroids and phytoplasmas)
Description of the test	
Organism(s)	Emaravirus rosae(RRV000)
Detection / identification	detection
Method(s)	Extraction Molecular real time RT PCR
Method: Extraction	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	no
New test being considered for inclusion in the next version of the EPPO diagnostic protocol?	yes
As or adapted from an IPPC diagnostic protocol	no
Reference of the test	RNeasy plant mini kit (QIAGEN)
Is the test modified compared to the reference test	yes The equivalent of one gr fresh leaf tissue was ground in 3.5 ml GH+ buffer (6 M guanidine hydrochloride, 0.2 M sodium acetate pH 5.2, 25 mM EDTA, and 2.5% PVP-10). One ml homogenate was incubated in a thermoshaker (850 rpm, 65°C for 10 min). After centrifugation (16,000 x g, 2 min), 500

	µl supernatant was loaded on the QIAshredder spin column and centrifuges (16,000 x g, 2 min). Thereafter the manufacturer’s instructions were followed. RNA was eluted from the spin column with 40 µl RNase-free water
Other information	
Method: Molecular real time RT PCR	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	no
New test being considered for inclusion in the next version of the EPPO diagnostic protocol?	yes
As or adapted from an IPPC diagnostic protocol	no
Reference of the test	Test A: Vazquez-Iglesias I, Scrace J, McGreig S, Pufal H, Robinson R, Clover GRG, Adams IP, Boonham N, Fox A, (2020). First report of Rose spring dwarf-associated virus in Rosa spp. in United Kingdom. New Disease Reports 42: 13. Test B (primers/probe set 2-1), Test C (primers/probe set 2-2, Test D (primers/probe set 3-2), Test E (primers/probe set 3-5): Babu B, Jeyaprakash A, Jones D, Schubert TS, Baker C, Washburn BK, Miller SH, Poduch K, Knox GW, Ochoa-Corona FM, Paret ML (2016). Development of a rapid, sensitive TaqMan real-time RT-PCR assay for the detection of rose rosette virus using multiple gene targets. Journal of Virological Methods 235: 41–50. Test F: Dobhal S, Olson JD, Arif M, Garcia Suarez JA, Ochoa-Corona FM (2016). A simplified strategy for sensitive detection of rose rosette virus compatible with three RT-PCR chemistries. Journal of Virological Methods 232: 47–56. Test G: Vazquez-Iglesias I, Ochoa-Corona FM, Tang J, Robinson R, Clover GRG, Fox A, Boonham N (2020). Facing Rose rosette virus: A risk to European rose cultivation. Plant Pathology 69: 1603–1617
Is the test modified compared to the reference test	yes Real-time PCR kit and primer/probe concentrations
Kit	
Is a kit used	yes
Manufacturer name	Applied Biosystems
Specify the kit used	TaqMan RNA-to-CT 1-Step Kit
Kit used following the manufacturer's instructions?	yes
Other information	
Performance Criteria :	
Organism 1.:	**Emaravirus rosae(RRV000)**
Analytical sensitivity	

What is smallest amount of target that can be detected reliably?	Maximum dilution in healthy Rosa Test A: Not tested (reason: false negative for OK-1 isolate) Test B: Not tested (reason: false negative for OK-1 isolate) Test C: 10^-4 (MD, 'Knock out'), 10^-3 (OK-1) Test D: 10^-4 (MD, 'Knock out'), 10^-2(OK-1) Test E: 10^-4 ('Knock out'), 10^-3 (MD, OK-1) Test F: 10^-3 (MD, 'Knock out', OK-1) Test G: Not tested (reason: very low fluorescence plateau for gBlock2)
Diagnostic sensitivity	
Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98	67% (tests A and B (OK-1 isolate not detected)), 100% (Tests C, D, E, F and G)
Standard test(s)	High-throughput sequencing
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	MD, Ok-1, 'Knock out', gBlock1, gBlock2, gBlock3, gBlock4
Specificity value	57% (test A) 71% (tests A and B), 100% (Tests C, D, E, F and G)
Analytical specificity - exclusivity	
Number of non-target organisms tested	Prunus necrotic ringspot virus (PNRSV0)
Specificity value	100% (Tests C, D, E and F), Not tested for test A, B and G
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	67% (tests A and B (OK-1 isolate not detected)), 100% (Tests C, D, E, F and G)
Specify the test(s)	High-throughput sequencing
Reproducibility	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	Not tested
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	Not tested
Test performance study	
Test performance study?	no
Other information	
Any other information considered useful	Full validation is available on the EURL webpage: https://eurlplanthealth.nl/groups/view/5f6c0e2e-3a3a-4c35-9413-4094af29c30d/virology-public/files/80be3a70-6e90-4b7f-ad4a-6cad1c3ab540

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