

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	ILVO Institute for Agricultural and Fisheries Research Burg. Van Gansberghelaan 96, 9820 Merelbeke - Melle, Belgium
Short description of the test	Detection of PPV by DAS ELISA and real-time PCR in leaves of Prunus
Date, reference of the validation report	2007-01-01 - 2007 & 2016 - F16_V03; F16_V04
Validation process according to EPPO Standard PM7/98?	yes
Is the lab accredited for this test?	yes
Was the validated data generated in the framework of a project?	
Description of the test	
Organism(s)	Plum pox virus / Potyvirus plumpoxi (PPV000)
Detection / identification	detection
Method(s)	Molecular real time RT PCR Serological DAS-ELISA
Method: Molecular real time RT PCR	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	no
As or adapted from an IPPC diagnostic protocol	yes
IPPC diagnostic Protocol name	ISPM 27 Annex 02 DP 02: Plum pox virus (version 2018)
Name of the test	One-step real-time PCR according to Schneider et al (2004)
Other information	
Method: Serological DAS-ELISA	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	yes
EPPO Diagnostic Protocol name	PM 7/032 Plum pox potyvirus (version 1)

Kit	
Is a kit used	yes
Manufacturer name	
Specify the kit used	
Kit used following the manufacturer's instructions?	
Other information	
Other details on the test	DAS ELISA, BIOREBA AG (Switzerland); Coating IgG: polyclonal; conjugate: polyclonal/monoclonal
Are the performance characteristics included in the EPPO diagnostic protocol?	no
Performance Criteria :	
Organism 1.:	Potyvirus plumpoxi(PPV000)
<u>Analytical sensitivity</u>	
What is smallest amount of target that can be detected reliably?	Because the concentration of viruses, viroids and phytoplasmas is never known, determine the maximum dilution of RNA /DNA detected. Therefore, the sensitivity determined here is not an absolute sensitivity but a relative sensitivity.
<u>Analytical specificity - inclusivity</u>	
Number of strains/populations of target organisms tested	Plum pox virus (PPV) D-strain SRC/PPV/Kominek_PPV-D RefV_PPV-02 Plum pox virus (PPV) M-strain SRC/PPV/Kominek_PPV-M RefV_PPV-03 Plum pox virus (PPV) recombinant strain SRC/PPV/Kominek_PPV-rec RefV_PPV-04 Plum pox virus (PPV) - on plum SRC/PPV/Bobev_pruim RefV_PPV-05 Plum pox virus (PPV)- on apricot SRC/PPV/Bobev_abrikoos RefV_PPV-06 Plum pox virus (PPV) D-strain SRC/PPV/Cambra_PPV-D RefV_PPV-07 Plum pox virus (PPV) M-strain SRC/PPV/Cambra_PPV-M RefV_PPV-08 Plum pox virus (PPV) SRC/PPV/DSMZ_PV-0430 RefV_PPV-09 Plum pox virus (PPV) SRC/PPV/DSMZ_PV-001 RefV_PPV-10 Plum pox virus (PPV) SRC/PPV/BIOREBA_PPV ELISA CONTROL ref. 150553 RefV_PPV-11
Specificity value	Only non cherry Prunus samples are included in our scope.
<u>Analytical specificity - exclusivity</u>	
Number of non-target organisms tested	Covered by the validation of the supplier of the antibodies. All positive ELISA tests are submitted to a confirmation test (real-time PCR)
Specificity value	-
<u>Diagnostic Specificity</u>	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	1
<u>Reproducibility</u>	

Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100% (at low – medium – high concentration)
<u>Repeatability</u>	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100% (at low – medium – high concentration)
Test performance study	
Test performance study?	yes
Brief details of the test performance study and its output. It available, link to published article/report	2016: organised by NAKTuinbouw.
Other information	
Any other information considered useful	Robustness – also tested are: Influence of sub sampling (different plant parts) Influence of the place in the ELISA plate Buffer and incubation temperature (sample, AB) Dilution of the controls Comparison between the IVIA magic DAS-ELISA and the BIOREBA DAS-ELISA reagents.

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