

**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.

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

<b>Kit</b>	
<b>Is a kit used</b>	yes
<b>Manufacturer name</b>	
<b>Specify the kit used</b>	
Kit used following the manufacturer's instructions?	
<b>Other information</b>	
<b>Other details on the test</b>	DAS ELISA, BIOREBA AG (Switzerland); Coating IgG: polyclonal; conjugate: polyclonal/monoclonal
<b>Are the performance characteristics included in the EPPO diagnostic protocol?</b>	no
<b>Performance Criteria :</b>	
<b>Organism 1.:</b>	<b>Potyvirus plumpoxi(PPV000)</b>
<b><u>Analytical sensitivity</u></b>	
<b>What is smallest amount of target that can be detected reliably?</b>	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.
<b><u>Analytical specificity - inclusivity</u></b>	
<b>Number of strains/populations of target organisms tested</b>	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
<b>Specificity value</b>	Only non cherry Prunus samples are included in our scope.
<b><u>Analytical specificity - exclusivity</u></b>	
<b>Number of non-target organisms tested</b>	Covered by the validation of the supplier of the antibodies. All positive ELISA tests are submitted to a confirmation test (real-time PCR)
<b>Specificity value</b>	-
<b><u>Diagnostic Specificity</u></b>	
<b>Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test</b>	1
<b><u>Reproducibility</u></b>	

<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	100% (at low – medium – high concentration)
<b><u>Repeatability</u></b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	100% (at low – medium – high concentration)
<b>Test performance study</b>	
<b>Test performance study?</b>	yes
<b>Brief details of the test performance study and its output. It available, link to published article/report</b>	2016: organised by NAKTuinbouw.
<b>Other information</b>	
<b>Any other information considered useful</b>	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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