EUROPEAN AND MEDITERRANEAN PLANT PROTECTION ORGANIZATION ORGANISATION EUROPEENNE ET MEDITERRANEENNE POUR LA PROTECTION DES PLANTES (11-17239)

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.

Target Organism	Plum pox virus
Short description	Detection of PPV by DAS ELISA and real-time PCR in leaves of Prunus
Laboratory contact details	ILVO Institute for Agricultural and Fisheries Research Burg. Van Gansberghelaan 96, 9820 Merelbeke - Melle, Belgium
Date and reference of the validation report	2007 & 2016 - F16_V03; F16_V04
Validation process according to EPPO Standard PM 7/98:	Yes
Reference of the test description	PM 7/032 screening by DAS-ELISA and one-step real-time PCR according to Schneider et al (2004)
Is the test the same as described in the EPPO DP?	Yes
Is the lab accredited for this test?	Yes
Plant species tested (if relevant)	Prunus spp. (with exception of P. avium and P. cerasus)
Matrices tested (if relevant)	leaves
List of methods used	

Method for extraction / isolation / baiting of target organism from matrix		
Molecular methods, e.g. hybridization, PCR and real time PCR	Х	one-step real-time PCR according to Schneider et al (2004)
Serological methods: IF, ELISA, Direct Tissue Blot Immuno Assay	Х	DAS ELISA, BIOREBA AG (Switzerland); Coating IgG: polyclonal; conjugate: polyclonal/monoclonal
Plating methods: selective isolation		
Bioassay methods: selective enrichment in host plants, baiting, plant test and grafting.		
Pathogenicity test		
Fingerprint methods: protein profiling, fatty acid profiling & DNA		

profiling					
Morphological and morphometrical methods intended for identification					
Biochemical methods: e.g. enzyme electrophoresis, protein profiling					
Other					
Analytical sensitivity (= limit of detection)					
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.				
Diagnostic sensitivity					
Proportion of infected/infested samples tested positive compared to results from the standard test , see appendix 2 of PM 7/98					
Specify the standard test					
Analytical specificity					
Specificity value	Only non	cherry Prunus samples are included in our scope.			
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				
Number of non-target organisms tested	Covered b positive E time PCR)	y the validation of the supplier of the antibodies. All LISA tests are submitted to a confirmation test (real-			
Cross reacts with (specify the species)	-				
Diagnostic Specificity					
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a	100%				

standard test			
Specify the standard test			
Reproducibility			
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100% (at low – medium – high concentration)		
Repeatability			
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		
Include brief details of the test performance study and its output.It available, provide a link to published article/report	2016: organised by NAKTuinbouw.		
Other information			
Any other information considered useful e.g. robustness, ease of performing the test, etc.	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.		