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	Screening TAS-ELISA test for Tomato yellow leaf curl virus - Antibody set ADGEN/NEOGEN 1072-25
Date, reference of the validation report	2018-02-12 - F16_V01; F16_V06
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)	Tomato yellow leaf curl virus / Begomovirus coheni (TYLCV0)
Detection / identification	detection
Method(s)	Molecular real time PCR Serological DASI-ELISA
Method: Molecular real time PCR	
Reference of the test description	
Other information	
Other details on the test	method of fera, UK; EUPHRESCO final report project "Validation of diagnostic methods for the detection and identification of whitefly transmitted viruses of regulatory or quarantine concern to the EU."
Method: Serological DASI-ELISA	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	yes
EPPO Diagnostic Protocol name	PM 7/050 Tomato yellow leaf curl and Tomato mottle begomoviruses (version 1)
Name of the test	TAS-ELISA (based on Thomas, 1986)
Is the test modified compared to the reference test	no

Other information	
Other details on the test	the ELISA is used as a screening test. Positive results are submitted to one-step real-time PCR procedure TAS-ELISA screening (AB set 1072-25, Adgen/neogen phytodiagnostics)
Performance Criteria :	
Organism 1.:	**Begomovirus coheni(TYLCV0)**
Analytical sensitivity	
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	N/A standard test used
Standard test(s)	not relevant
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	18 Tomato yellow leaf curl virus - mild (TYLCV) G. Anfoka – origine: Jordanië RefV_TYLCV_01 Tomato yellow leaf curl Sardinia virus (TYLCSV) G. Anfoka – origine: Jordanië RefV_TYLCV_02 Tomato yellow leaf curl virus - mild (TYLCV) DSMZ – PV-0560 – origine Israël RefV_TYLCV_03 Tomato yellow leaf curl Sardinia virus (TYLCSV) DSMZ – PV-0596 – origine Spanje RefV_TYLCV_04 Tomato yellow leaf curl Sardinia virus (TYLCSV) G. Anfoka – origine: Jordanië RefV_TYLCV_05 Tomato yellow leaf curl virus – mild+Israël (TYLCV) G. Anfoka – origine: Jordanië RefV_TYLCV_06 Tomato yellow leaf curl virus (TYLCV) Euphresco THREE – MAF-NZ – non NZ origin RefV_TYLCV_07 Tomato yellow leaf curl virus (TYLCV) Euphresco EIGHT – ARI-CY – Cyprus RefV_TYLCV_08 Tomato yellow leaf curl virus (TYLCV) Euphresco B – FERA UK – Spanje RefV_TYLCV_09 Tomato yellow leaf curl virus (TYLCV) Euphresco FOURTEEN – PPS-Nl -CY – Nederland RefV_TYLCV_10 Tomato yellow leaf curl virus (TYLCV) Euphresco E -FERA UK – Spanje RefV_TYLCV_11 Tomato yellow leaf curl virus Israël (TYLCV) Murad Ghanim, Israël RefV_TYLCV_12 Tomato yellow leaf curl virus - mild (TYLCV) DSMZ – PV-0588 RefV_TYLCV_13 Tomato yellow leaf curl virus - mild (TYLCV) DSMZ – PV-0561 RefV_TYLCV_14 Tomato yellow leaf curl virus - mild (TYLCV) BIOREBA - 223897 RefV_TYLCV_15 Tomato yellow leaf curl virus (TYLCV) Accotto – Italie – 02/02/10 RefV_TYLCV_16 Tomato yellow leaf curl virus (TYLCV) Accotto – Italie - 02/02/10 RefV_TYLCV_17 Tomato yellow leaf curl virus (TYLCV) Verlodt – Tunesie – 02/08/10 RefV_TYLCV_18 Tomato yellow leaf curl virus

	(TYLCV) DSMZ – PV - 0595 RefV_TYLCV_19
Specificity value	
Analytical specificity - exclusivity	
Number of non-target organisms tested	8 Pepino mosaic virus (PepMV) Tomato apical stunt viroid (TASVd) Potato spindle tuber viroid (PSTVd) Tomato spotted wilt virus (TSWV) Cucumber vein yellowing virus (CVYV) Curcubit yellow stunt disorder virus (CYSDV) Tomato infectious chlorosis virus (TiCV) Tomato chlorosis virus (ToCV)
Specificity value	ELISA: possible cross reaction with other begomoviruses qPCR: no cross reaction known
Diagnostic Specificity	
Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test	100%
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
Brief details of the test performance study and its output.It available, link to published article/report	qPCR: EUPHRESCO project report ""Validation of diagnostic methods for the detection and identification of whitefly transmitted viruses of regulatory or quarantine concern to the EU.""
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
The following complementary files are available online:	• Validation report real-time PCR • Validation report TYLCV ELISA

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