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 Tomato brown rugose fruit virus by DAS-ELISA in leaves and seeds of tomato
Date, reference of the validation report	2021-12-01 - Euphresco 2019-A-327 project report
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?	Euphresco
If yes, please specify	Euphresco 2019-A-327
Description of the test	
Organism(s)	Tomato brown rugose fruit virus / Tobamovirus fructirugosum (TOBRFV)
Detection / identification	detection
Method(s)	Extraction Serological DAS-ELISA Serological DAS-ELISA (2) Serological DAS-ELISA (3) Serological DAS-ELISA (4)
Method: Extraction	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	yes
EPPO Diagnostic Protocol name	PM 7/146 Tomato brown rugose fruit virus (version 1)
As or adapted from an IPPC diagnostic protocol	no
Is the test modified compared to the reference test	no
Other information	
Other details on the test	Leaves or seeds were ground in extraction buffer using extraction bags (Bioreba) and a hand-held homogeniser (Bioreba).

Method: Serological DAS-ELISA	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	no
As or adapted from an IPPC diagnostic protocol	no
Kit	
Is a kit used	yes
Manufacturer name	PRIME DIAGNOSTICS
Specify the kit used	DAS-ELISA diagnostic kit for the detection of ToBRFV
Kit used following the manufacturer's instructions?	See report
Other information	
Method: Serological DAS-ELISA (2)	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	no
As or adapted from an IPPC diagnostic protocol	no
Kit	
Is a kit used	yes
Manufacturer name	LOEWE
Specify the kit used	Tomato Brown Rugose Fruit Tobamovirus. TBRFV DAS ELISA Cat No 07175
Kit used following the manufacturer's instructions?	see report
Other information	
Method: Serological DAS-ELISA (3)	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	no
As or adapted from an IPPC diagnostic protocol	no
Kit	
Is a kit used	yes
Manufacturer name	AGDIA
Specify the kit used	ELISA Reagent Set for ToBRFV (SRA 66800)
Kit used following the manufacturer's instructions?	see report
Other information	
Method: Serological DAS-ELISA (4)	
Reference of the test description	

As or adapted from an EPPO diagnostic protocol	no
As or adapted from an IPPC diagnostic protocol	no
Kit	
Is a kit used	yes
Manufacturer name	DSMZ
Specify the kit used	Tomato brown rugose fruit virus (DAS-ELISA) ToBRFV (RT-1236)
Kit used following the manufacturer's instructions?	see report
Other information	
Performance Criteria :	
Organism 1.:	**Tobamovirus fructirugosum(TOBRFV)**
Analytical sensitivity	
What is smallest amount of target that can be detected reliably?	With samples consisting of extract from uninfected tomato leaves, and serial dilutions of virus infected leaf material in uninfected tomato leaf extract: ToBRFV was detected up to a dilution of 10-4 (Loewe, DSMZ, Prime Diagnostics) or 10-5 (Agdia). With samples of infected seeds: The highly infected samples were barely detected by modified DSMZ test and clearly detected by modified Prime Diagnostics test. The medium infected samples were not or only barely detected by both modified tests.
Analytical specificity - exclusivity	
Number of non-target organisms tested	Samples consisting of extract from uninfected tomato leaves, and serial dilutions of virus infected leaf material in uninfected tomato leaf extract: TMV (x2 isolate) ToMV (x1 isolate)
Specificity value	Cross-reactions were observed for Loewe with ToMV (up to 10-1), for Agdia with all tested isolates (generally up to 10-5), for DSMZ with all tested isolates (depending on the isolate ranging from undiluted up to 10-3) and for Prime Diagnostics with TMV isolate 1 (undiluted) and ToMV (up to 10-3).
Test performance study	
Test performance study?	no
Other information	
Any other information considered useful	The report also contains a comparison between extraction buffer from manufacturer and extraction buffer from NVWA
The following complementary files are available online:	• Report_2019-A-327_Euphresco