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.

	National Institiute of Biology, Department of Biotechnology and Systems Biology Vecna pot 121, 1000 Ljubljana, Slovenia	
	Validation report on the testing of begomoviruses capable of infecting tomatoes and plants of the family Cucurbitaceae by PCR.	
	2023-09-29 - Testing of begomoviruses capable of infecting tomatoes and plants of the family Cucurbitaceae by PCR	
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?	EURL	
	EURL-Virology (European Union Reference Laboratory for pests of plants on viruses, viroids and phytoplasmas)	
Description of the test		
Organism(s)	Begomovirus (1BEGOG)	
Detection / identification	detection	
	Molecular Extraction DNA RNA Molecular Conventional PCR Molecular Conventional PCR (2) Molecular Conventional PCR (3)	
Method: Molecular Extraction DNA RNA		
Reference of the test description		
As or adapted from an EPPO diagnostic protocol	yes	
EPPO Diagnostic Protocol name	PM 7/152 Begomoviruses (version 1)	
As or adapted from an IPPC diagnostic protocol	no	
Is the test modified compared to the reference test	no	
Kit		
Is a kit used	yes	

Manufacturer name	BIONOBILE	
Specify the kit used	QuickPick™ SML Plant DNA	
Kit used following the manufacturer's instructions?	no Plant material (~200 mg) is homogenized in 1 mL of lysis buffer (from a QuickPick™ SML Plant DNA kit, Bio-Nobile) using a tissue homogenizer (FastPrep®-24, MP Biochemicals).	
Other information		
Method: Molecular Conventional PCR		
Reference of the test description		
As or adapted from an EPPO diagnostic protocol	yes	
EPPO Diagnostic Protocol name	PM 7/152 Begomoviruses (version 1)	
Name of the test	Conventional PCR Wyatt and Brown (1996)	
As or adapted from an IPPC diagnostic protocol	no	
Is the test modified compared to the reference test	no	
Kit		
Is a kit used	yes	
Manufacturer name	INVITROGEN	
Specify the kit used	Platinum™ Taq DNA Polymerase	
Kit used following the manufacturer's instructions?	yes	
Other information		
Reaction type	Simplex	
Method: Molecular Conventional PCR (2)		
Reference of the test description		
As or adapted from an EPPO diagnostic protocol	yes	
New test being considered for inclusion in the next version of the EPPO diagnostic protocol?	no	
EPPO Diagnostic Protocol name	PM 7/152 Begomoviruses (version 1)	
Name of the test	Conventional PCR Li et al. (2004)	
As or adapted from an IPPC diagnostic protocol	no	
Is the test modified compared to the reference test	no	
Kit		
Is a kit used	yes	
Manufacturer name	INVITROGEN	
Specify the kit used	Platinum™ Taq DNA Polymerase	

Simplex			
Reaction type Method: Molecular Conventional PCR (3) Reference of the test description As or adapted from an EPPO diagnostic protocol? EPPO Diagnostic Protocol name Name of the test Conventional PCR Saison and Gentit (2015) As or adapted from an IPPC diagnostic protocol? EPPO Diagnostic Protocol name PM 7/152 Begomoviruses (version 1) Name of the test Conventional PCR Saison and Gentit (2015) As or adapted from an IPPC diagnostic protocol no Is the test modified compared to the reference test Kit Is a kit used Yes Manufacturer name INVITROGEN Specify the kit used Platinum Taq DNA Polymerase yes Other information Reaction type Simplex Performance Criteria: Organism 1: Begomovirus(1BEGOG) Analytical sensitivity What is smallest amount of target that can be detected reliably? What is smallest amount of target that can be detected reliably? Analytical Sensitivity Tested concentrations: dilutions of ChaYMV (chayote yellow mosaic virus), TYLCTHV (tomato yellow defoct) 10^-2 est Saison and Gentit (2015): unclituted test Wyatt and Brown (1996): 10^-3 - TYLCTHV (set test L) at al. (2004): 10^-2 test Saison and Gentit (2015): 10^-1 test Wyatt and Brown (1996): 10^-3 - TYLCTHV (set test Saison and Gentit (2015): 10^-1 test Wyatt and Brown (1996): 10^-3 - TYLCTHV (set test Saison and Gentit (2015): 10^-1 test Wyatt and Brown (1996): 10^-3 - TYLCTHV (set Saison and Gentit (2015): 10^-1 test Saison and Gentit (2015): 10^-1 test Wyatt and Brown (1996): 10^-3 - TYLCTHV (set Saison and Gentit (2015): 10^-1 test Saison and Gentit (2015): 10^-1 test Wyatt and Brown (1996): 10^-3 - TYLCTHV (set Saison and Gentit (2015): 10^-1 test Wyatt and Brown (1996): 10^-3 - TYLCTHV (set Saison and Gentit (2015): 10^-1 test Wyatt and Brown (1996): 10^-3 - TYLCTHV (set Saison and Gentit (2015): 10^-1 test Wyatt and Brown (1996): 10^-3 - TYLCTHV (set Saison and Gentit (2015): 10^-1 test Wyatt and Brown (1996): 10^-3 - TYLCTHV (set Saison and Gentit (2015): 10^-1 test Wyatt and Brown (1996): 10^-3 - TYLCTHV (set Sa	Kit used following the manufacturer's instructions?	yes	
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•		No of targets tested: 36	
	Specificity value		

Analytical specificity - exclusivity		
Number of non-target organisms tested	No of non-targets tested (healthy plant material and other viruses): 69	
Specificity value	test Li et al. (2004): 100% test Saison and Gentit (2015): 100% test Wyatt and Brown (1996): 100%	
Reproducibility		
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	Percentage of identical results is 100% for all three PCRs. No. of target samples tested: 5 No. of nontarget samples tested: 2 No. of operators: up to 4 No. of PCR instruments: up to 5 No. of different days: up to 9	
Repeatability		
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	Repeatability for all three PCRs is 100% in both tested samples (ChaYMV and TYLCTHV at the limit of detection).	
Test performance study		
Test performance study?	no	
Other information		
Any other information considered useful	Full validation report is available on the EURL webpage: https://eurlplanthealth.nl/groups/view/5f6 c0e2e-3a3a-4c35-9413-4094af29c30d/virology-publ ic/files/0d7e84a7-39d0-467f-9033-8a4a1e93c997	

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