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	National Instituite of Biology, Department of Biotechnology and Systems Biology Vecna pot 121, 1000 Ljubljana, Slovenia
Short description of the test	Validation report on the testing of begomoviruses capable of infecting tomatoes and plants of the family Cucurbitaceae by PCR.
Date, reference of the validation report	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
If yes, please specify	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
Method(s)	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

Kit used following the manufacturer's instructions?	yes
Other information	
Reaction type	Simplex
Method: Molecular Conventional PCR (3)	
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 Saison and Gentit (2015)
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
Performance Criteria :	
Organism 1.:	**Begomovirus(1BEGOG)**
Analytical sensitivity	
What is smallest amount of target that can be detected reliably?	Tested concentrations: dilutions of ChaYMV (chayote yellow mosaic virus), TYLCTHV (tomato yellow leaf curl Thailand virus) and WmCSV (watermelon chlorotic stunt virus) in tomato or zucchini leaf material. Maximum dilution of target DNA detected: -ChaYMV test Li et al. (2004): 10^-2 test Saison and Gentit (2015): undiluted test Wyatt and Brown (1996): 10^-3 -TYLCTHV test Li et al. (2004): 10^-2 test Saison and Gentit (2015): 10^-1 test Wyatt and Brown (1996): 10^-3 -WmCSV test Li et al. (2004): no PCR product test Saison and Gentit (2015): 10^-1 test Wyatt and Brown (1996): undiluted
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	No of targets tested: 36
Specificity value	test Li et al. (2004): 66.7% test Saison and Gentit (2015): 94.4% test Wyatt and Brown (1996): 88.9%

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/5f6c0e2e-3a3a-4c35-9413-4094af29c30d/virology-public/files/0d7e84a7-39d0-467f-9033-8a4a1e93c997

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