

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 Institute of Biology, Department of Biotechnology and Systems Biology Vecna pot 121, 1000 Ljubljana, Slovenia
Short description of the test	Validation report on PCR tests for begomoviruses that can infect <i>Solanum tuberosum</i>
Date, reference of the validation report	2025-09-23 - Validation report on PCR tests for begomoviruses that can infect <i>Solanum tuberosum</i>
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 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
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
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 gBlock KC706617 (tomato severe rugose virus; ToSRV) in a homogenate of potato leaves. Maximum dilution of target DNA detected: -Wyatt and Brown (1996): 10^{-8} (48.8 copy of target DNA/ 1 μ L) -Li et al. (2004): 10^{-5} (4.88 x 10 ⁴ copy of target DNA/ 1 μ L) -Saison and Gentit (2015): 10^{-8} (48.8 copy of target DNA/ 1 μ L)
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	No. of targets tested: 3 virus species (ChiLCV, PYMV and four isolates of ToLCNDV) + 1 gBlock (ToSRV) + 30 isolates/ corresponding gBlocks of begomoviruses capable of infecting other host plants. The in-silico analysis showed that there is at least one PCR for all begomoviruses examined that has no mismatches or only one mismatch between primer and targeted sequences, similar to ToRSV of which a gBlock was successfully detected by all three PCRs -> all begomoviruses that can infect potato are likely to be detectable by at least one of the PCRs.
Specificity value	For 3 PCRs together: 100% (each isolate would be detected by at least one of the PCRs).
Analytical specificity - exclusivity	
Number of non-target organisms tested	No. of non-target viruses tested: 5

Specificity value	For each of 3 PCRs by itself: 100%.
<u>Reproducibility</u>	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	No. of samples tested: 2 different dilutions of qBlock of ToRSV. No. of operators: 2. No. of PCR instruments: 3 (2 different per each PCR). No. of different days: 3. Percentage of identical results (positive replicates) is 100% for all three PCRs.
<u>Repeatability</u>	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	No. of samples tested: 2 different dilutions of qBlock of ToRSV. No. of replicates tested: 3. Percentage of identical results (positive replicates) is 100% for all three PCRs.
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/files/view/99e9ba7f-320a-434e-9cba-e8462892afb5/20250923_begomoviruses-of-potato_conventional-pcrs_validation-report_nib.pdf

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