

**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.

<b>Laboratory contact details</b>	National Institute of Biology, Department of Biotechnology and Systems Biology Vecna pot 121, 1000 Ljubljana, Slovenia
<b>Short description of the test</b>	Validation report on the testing of begomoviruses capable of infecting tomatoes and plants of the family Cucurbitaceae by PCR.
<b>Date, reference of the validation report</b>	2023-09-29 - Testing of begomoviruses capable of infecting tomatoes and plants of the family Cucurbitaceae by PCR
<b>Validation process according to EPPO Standard PM7/98?</b>	yes
<b>Is the lab accredited for this test?</b>	yes
<b>Was the validated data generated in the framework of a project?</b>	EURL
<b>If yes, please specify</b>	EURL-Virology (European Union Reference Laboratory for pests of plants on viruses, viroids and phytoplasmas)
<b>Description of the test</b>	
<b>Organism(s)</b>	Begomovirus (1BEGOG)
<b>Detection / identification</b>	detection
<b>Method(s)</b>	Molecular Extraction DNA RNA Molecular Conventional PCR Molecular Conventional PCR (2) Molecular Conventional PCR (3)
<b>Method: Molecular Extraction DNA RNA</b>	
<b>Reference of the test description</b>	
<b>As or adapted from an EPPO diagnostic protocol</b>	yes
<b>EPPO Diagnostic Protocol name</b>	PM 7/152 Begomoviruses (version 1)
<b>As or adapted from an IPPC diagnostic protocol</b>	no
<b>Is the test modified compared to the reference test</b>	no
<b>Kit</b>	
<b>Is a kit used</b>	yes

<b>Manufacturer name</b>	BIONOBILE
<b>Specify the kit used</b>	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).
<b>Other information</b>	
<b>Method: Molecular Conventional PCR</b>	
<b>Reference of the test description</b>	
<b>As or adapted from an EPPO diagnostic protocol</b>	yes
<b>EPPO Diagnostic Protocol name</b>	PM 7/152 Begomoviruses (version 1)
<b>Name of the test</b>	Conventional PCR Wyatt and Brown (1996)
<b>As or adapted from an IPPC diagnostic protocol</b>	no
<b>Is the test modified compared to the reference test</b>	no
<b>Kit</b>	
<b>Is a kit used</b>	yes
<b>Manufacturer name</b>	INVITROGEN
<b>Specify the kit used</b>	Platinum™ Taq DNA Polymerase
Kit used following the manufacturer's instructions?	yes
<b>Other information</b>	
<b>Reaction type</b>	Simplex
<b>Method: Molecular Conventional PCR (2)</b>	
<b>Reference of the test description</b>	
<b>As or adapted from an EPPO diagnostic protocol</b>	yes
<b>New test being considered for inclusion in the next version of the EPPO diagnostic protocol?</b>	no
<b>EPPO Diagnostic Protocol name</b>	PM 7/152 Begomoviruses (version 1)
<b>Name of the test</b>	Conventional PCR Li et al. (2004)
<b>As or adapted from an IPPC diagnostic protocol</b>	no
<b>Is the test modified compared to the reference test</b>	no
<b>Kit</b>	
<b>Is a kit used</b>	yes
<b>Manufacturer name</b>	INVITROGEN
<b>Specify the kit used</b>	Platinum™ Taq DNA Polymerase

Kit used following the manufacturer's instructions?	yes
<b>Other information</b>	
Reaction type	Simplex
<b>Method: Molecular Conventional PCR (3)</b>	
<b>Reference of the test description</b>	
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
<b>Kit</b>	
Is a kit used	yes
Manufacturer name	INVITROGEN
Specify the kit used	Platinum™ Taq DNA Polymerase
Kit used following the manufacturer's instructions?	yes
<b>Other information</b>	
Reaction type	Simplex
<b>Performance Criteria :</b>	
Organism 1.:	<b>Begomovirus(1BEGOG)</b>
<b>Analytical sensitivity</b>	
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 <sup>-2</sup> test Saison and Gentit (2015): undiluted test Wyatt and Brown (1996): 10 <sup>-3</sup> -TYLCTHV test Li et al. (2004): 10 <sup>-2</sup> test Saison and Gentit (2015): 10 <sup>-1</sup> test Wyatt and Brown (1996): 10 <sup>-3</sup> -WmCSV test Li et al. (2004): no PCR product test Saison and Gentit (2015): 10 <sup>-1</sup> test Wyatt and Brown (1996): undiluted
<b>Analytical specificity - inclusivity</b>	
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%

<b>Analytical specificity - exclusivity</b>	
<b>Number of non-target organisms tested</b>	No of non-targets tested (healthy plant material and other viruses): 69
<b>Specificity value</b>	test Li et al. (2004): 100% test Saison and Gentit (2015): 100% test Wyatt and Brown (1996): 100%
<b><u>Reproducibility</u></b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	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
<b><u>Repeatability</u></b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	Repeatability for all three PCRs is 100% in both tested samples (ChaYMV and TYLCTHV at the limit of detection).
<b>Test performance study</b>	
<b>Test performance study?</b>	no
<b>Other information</b>	
<b>Any other information considered useful</b>	Full validation report is available on the EURL webpage: <a href="https://eurlplanthealth.nl/groups/view/5f6c0e2e-3a3a-4c35-9413-4094af29c30d/virology-public/files/0d7e84a7-39d0-467f-9033-8a4a1e93c997">https://eurlplanthealth.nl/groups/view/5f6c0e2e-3a3a-4c35-9413-4094af29c30d/virology-public/files/0d7e84a7-39d0-467f-9033-8a4a1e93c997</a>

Creation date: 2023-11-21 11:26:58 - Last update: 2023-11-21 16:10:14