**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.

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| **Laboratory contact details** | National Instituite of Biology, Department of Biotechnology and Systems Biology<br>Vecna pot 121, 1000 Ljubljana, Slovenia |
| **Short description of the test** | Validation report on PCR tests for begomoviruses that can infect Solanum tuberosum |
| **Date, reference of the validation report** | 2025-09-23 - Validation report on PCR tests for begomoviruses that can infect Solanum tuberosum |
| **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) |
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| **Description of the test** | |
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| **Organism(s)** | Begomovirus (1BEGOG) |
| **Detection / identification** | detection |
| **Method(s)** | Molecular Extraction DNA RNA<br>Molecular Conventional PCR<br>Molecular Conventional PCR (2)<br>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 |

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| **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 |

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| **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 µL) -Li et al. (2004): 10^-5 (4.88 x 104 copy of target DNA/ 1 µL) -Saison and Gentit (2015): 10^-8 (48.8 copy of target DNA/ 1 µL) |
| **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 |

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| **Specificity value** | For each of 3 PCRs by itself: 100%. |
| **Reproducibility** | |
| **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. |
| **Repeatability** | |
| **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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