

**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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| <b>Laboratory contact details</b>                                                                       | Walloon Agricultural Research Centre (CRA-W)<br>Département Sciences du Vivant Unité Santé des<br>plantes et forêts Bâtiment Marchal Rue de Liroux,<br>4, 5030 Gembloux, Belgium                                                                       |
| <b>Short description of the test</b>                                                                    | Detection and identification of Polerovirus by<br>Molecular Conventional RT PCR in Leaves                                                                                                                                                              |
| <b>Date, reference of the validation report</b>                                                         | 2025-11-13 - Generic detection and identification of<br>Polerovirus                                                                                                                                                                                    |
| <b>Validation process according to EPPO<br/>Standard PM7/98?</b>                                        | yes                                                                                                                                                                                                                                                    |
| <b>Is the lab accredited for this test?</b>                                                             | no                                                                                                                                                                                                                                                     |
| <b>Was the validated data generated in the<br/>framework of a project?</b>                              | Other_project                                                                                                                                                                                                                                          |
| <b>If yes, please specify</b>                                                                           | VIROBETT project (PRW 204/3, grant -D65-1429)                                                                                                                                                                                                          |
| <b>Description of the test</b>                                                                          |                                                                                                                                                                                                                                                        |
| <b>Organism(s)</b>                                                                                      | Polerovirus (1POLVG)                                                                                                                                                                                                                                   |
| <b>Detection / identification</b>                                                                       | detection and identification                                                                                                                                                                                                                           |
| <b>Method(s)</b>                                                                                        | Molecular Conventional RT PCR                                                                                                                                                                                                                          |
| <b>Method: Molecular Conventional RT PCR</b>                                                            |                                                                                                                                                                                                                                                        |
| <b>Reference of the test description</b>                                                                |                                                                                                                                                                                                                                                        |
| <b>As or adapted from an EPPO diagnostic<br/>protocol</b>                                               | no                                                                                                                                                                                                                                                     |
| <b>New test being considered for inclusion in the<br/>next version of the EPPO diagnostic protocol?</b> | yes                                                                                                                                                                                                                                                    |
| <b>As or adapted from an IPPC diagnostic<br/>protocol</b>                                               | no                                                                                                                                                                                                                                                     |
| <b>Reference of the test</b>                                                                            | Knierim, D., Tsai, W.S., Kenyon, L., 2013. Analysis<br>of sequences from field samples reveals the<br>presence of the recently described Pepper vein<br>yellows virus (genus Polerovirus) in six additional<br>countries. Arch. Virol. 158, 1337-1341. |
| <b>Is the test modified compared to the<br/>reference test</b>                                          | yes Primers Gen1 and Gen2 were used for RT-PCR<br>using Titan One Tube RT-PCR System                                                                                                                                                                   |
| <b>Kit</b>                                                                                              |                                                                                                                                                                                                                                                        |

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| Is a kit used                                                                                                                 | no                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
| <b>Other information</b>                                                                                                      |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
| Reaction type                                                                                                                 | Simplex                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
| Other details on the test                                                                                                     | RT-PCR using the generic primers Gen1/2 (Knierim et al., 2013) was performed using the Titan One Tube RT-PCR System (Roche) in 20 µL reaction containing Titan reaction buffer 5X, 500 nM of each primers, dNTPs at 10 nM each, and 0.4 µL of enzyme mix. Thermocycling conditions consisted of a first cycle at 50°C for 30 min for the RT followed by 2 min at 94°C and then 40 cycles of 94°C for 15 s, 54°C for 30 s and 72°C for 45 s. A final elongation step of 5 min at 72°C ended the run. |
| <b>Performance Criteria :</b>                                                                                                 |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
| Organism 1.:                                                                                                                  | <b>Polerovirus(1POLVG)</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
| <b>Analytical sensitivity</b>                                                                                                 |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
| What is smallest amount of target that can be detected reliably?                                                              | Up to 10 <sup>3</sup> -fold dilution of the starting material infected with BWYV isolate (P6-6D-W11_BWYV)                                                                                                                                                                                                                                                                                                                                                                                           |
| <b>Diagnostic sensitivity</b>                                                                                                 |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
| Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98 | 100% for the polerovirus (BWYV, BChV, BMYV and TuYV) that were compared with ELISA                                                                                                                                                                                                                                                                                                                                                                                                                  |
| Standard test(s)                                                                                                              | BWYV TAS-ELISA (RT-0049) kit from DSMZ                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
| <b>Analytical specificity - inclusivity</b>                                                                                   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
| Number of strains/populations of target organisms tested                                                                      | BWYV, BChV, BMYV, TuYV, PLRV, CYDV-RPV, PeVYV, CABYV, UPoIV, BLYV                                                                                                                                                                                                                                                                                                                                                                                                                                   |
| Specificity value                                                                                                             | 100%                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
| <b>Analytical specificity - exclusivity</b>                                                                                   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
| Number of non-target organisms tested                                                                                         | BYV, BtMV                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |
| Specificity value                                                                                                             | 100%                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
| Cross reacts with                                                                                                             | Polerovirus                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
| <b>Diagnostic Specificity</b>                                                                                                 |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
| Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test        | 100%                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
| Specify the test(s)                                                                                                           | BWYV TAS-ELISA (RT-0049) kit from DSMZ                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
| <b>Reproducibility</b>                                                                                                        |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
| Provide the calculated % of agreement for a given level of the pest (see PM 7/98)                                             | 100%                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
| <b>Repeatability</b>                                                                                                          |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
| Provide the calculated % of agreement for a given level of the pest (see PM 7/98)                                             | 100%                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |

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| <b>Test performance study</b>  |    |
| <b>Test performance study?</b> | no |

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