

**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>                                      | Netherlands Institute for Vectors, Invasive plants and Plant health<br>P.O. Box 9102, 6700 HC Wageningen, Netherlands                                              |
| <b>Short description of the test</b>                                   | Validation of a conventional RT-PCR test for detection and identification of PSTVd, TCDVd, MPVd and TPMVd using primers 2H1/3H1 described by Shamoul et al. (1997) |
| <b>Date, reference of the validation report</b>                        | 2013-09-17 - NRC-ref: 2011.molbio.006                                                                                                                              |
| <b>Validation process according to EPPO Standard PM7/98?</b>           | yes                                                                                                                                                                |
| <b>Is the lab accredited for this test?</b>                            | no                                                                                                                                                                 |
| <b>Was the validated data generated in the framework of a project?</b> |                                                                                                                                                                    |
| <b>Description of the test</b>                                         |                                                                                                                                                                    |
|                                                                        |                                                                                                                                                                    |
| <b>Organism(s)</b>                                                     | Tomato chlorotic dwarf viroid / Pospiviroid chloronani (TCDVD0)<br>Potato spindle tuber viroid / Pospiviroid fustuberis (PSTVD0)                                   |
| <b>Detection / identification</b>                                      | detection and identification                                                                                                                                       |
| <b>Method(s)</b>                                                       | Molecular Extraction DNA RNA<br>Molecular Conventional RT PCR<br>Molecular other                                                                                   |
| <b>Method: Molecular Extraction DNA RNA</b>                            |                                                                                                                                                                    |
| <b>Reference of the test description</b>                               |                                                                                                                                                                    |
| <b>Kit</b>                                                             |                                                                                                                                                                    |
| <b>Is a kit used</b>                                                   | yes                                                                                                                                                                |
| <b>Manufacturer name</b>                                               | QIAGEN                                                                                                                                                             |
| <b>Specify the kit used</b>                                            | RNeasy Plant Mini Kit                                                                                                                                              |
| Kit used following the manufacturer's instructions?                    |                                                                                                                                                                    |
| <b>Other information</b>                                               |                                                                                                                                                                    |
| <b>Method: Molecular Conventional RT PCR</b>                           |                                                                                                                                                                    |
| <b>Reference of the test description</b>                               |                                                                                                                                                                    |
| <b>As or adapted from an IPPC diagnostic protocol</b>                  | yes                                                                                                                                                                |

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| <b>IPPC diagnostic Protocol name</b>                                                                                                 | ISPM 27 Annex 07 DP 07: Potato spindle tuber viroid (version 2006)                                                                                                                                                                                                                                                                                                         |
| <b>Name of the test</b>                                                                                                              | Conventional RT-PCR using the primers of Shamloul et al. (1997)                                                                                                                                                                                                                                                                                                            |
| <b>Other information</b>                                                                                                             |                                                                                                                                                                                                                                                                                                                                                                            |
| <b>Other details on the test</b>                                                                                                     | section 3.3.4.1 Conventional RT-PCR using the primers of Shamloul et al. (1997) Shamloul AM, Hadidi A, Zhu SF, Singh RP, Sagredo B (1997) Sensitive detection of potato spindle tuber viroid using RT-PCR and identification of a viroid variant naturally infecting pepino plants. Can. J. Pl. Pathol. 19: 89-96 RT-PCR by 2H1/3H1 primers and sequencing of the amplicon |
| <b>Method: Molecular other</b>                                                                                                       |                                                                                                                                                                                                                                                                                                                                                                            |
| <b>Reference of the test description</b>                                                                                             |                                                                                                                                                                                                                                                                                                                                                                            |
| <b>Other information</b>                                                                                                             |                                                                                                                                                                                                                                                                                                                                                                            |
| <b>Other details on the test</b>                                                                                                     | Sequencing                                                                                                                                                                                                                                                                                                                                                                 |
| <b>Are the performance characteristics included in the EPPO diagnostic protocol?</b>                                                 | no                                                                                                                                                                                                                                                                                                                                                                         |
| <b>Performance Criteria :</b>                                                                                                        |                                                                                                                                                                                                                                                                                                                                                                            |
| <b>Organism 1.:</b>                                                                                                                  | <b>Pospiviroid chloronani(TCDVD0)</b>                                                                                                                                                                                                                                                                                                                                      |
| <b>Analytical sensitivity</b>                                                                                                        |                                                                                                                                                                                                                                                                                                                                                                            |
| <b>What is smallest amount of target that can be detected reliably?</b>                                                              | Undiluted leaf sap was considered 100% infected. Starting with undiluted plant sap from PSTVd and TCDVd infected hosts up to 10 <sup>7</sup> times dilutes in sap of healthy tomato leaves, all PSTVd and TCDVd strains were detected up to a relative infection rate of 10% (=10 times diluted) .                                                                         |
| <b>Diagnostic sensitivity</b>                                                                                                        |                                                                                                                                                                                                                                                                                                                                                                            |
| <b>Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98</b> | 1                                                                                                                                                                                                                                                                                                                                                                          |
| <b>Standard test(s)</b>                                                                                                              | PCR sequencing of the complete viroid genome is considered the standard test. This can be achieved with primers Vid-FW/Vid-RE (CLVd, PSTVd and TCDVd) and 3H1/2H1 (PSTVd, TCDVd, MPVd and TPMVd). For other pospiviroids primers Posp1-FW/Posp1-RE are used to sequence the partial viroid genome.                                                                         |
| <b>Analytical specificity - inclusivity</b>                                                                                          |                                                                                                                                                                                                                                                                                                                                                                            |
| <b>Number of strains/populations of target organisms tested</b>                                                                      | 7 pospiviroid strains, see table 4 validation report                                                                                                                                                                                                                                                                                                                       |
| <b>Specificity value</b>                                                                                                             | 1                                                                                                                                                                                                                                                                                                                                                                          |
| <b>Analytical specificity - exclusivity</b>                                                                                          |                                                                                                                                                                                                                                                                                                                                                                            |
| <b>Number of non-target organisms tested</b>                                                                                         | 10 (4 Avsunviroidae and 6 Pospiviroidae strains), see table 6 validation report                                                                                                                                                                                                                                                                                            |

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| <b>Specificity value</b>                                                                                                             |                                                                                                                                                                                                                                                                                                      |
| <b><u>Diagnostic Specificity</u></b>                                                                                                 |                                                                                                                                                                                                                                                                                                      |
| <b>Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test</b>        | 1                                                                                                                                                                                                                                                                                                    |
| <b>Specify the test(s)</b>                                                                                                           | PCR sequencing of the complete viroid genome is considered the standard test. This can be achieved with primers Vid-FW/Vid-RE (CLVd, PSTVd and TCDVd) and 3H1/2H1 (PSTVd, TCDVd, MPVd and TPMVd). For other pospiviroids primers Pospi1-FW/Pospi1-RE are used to sequence the partial viroid genome. |
| <b><u>Reproducibility</u></b>                                                                                                        |                                                                                                                                                                                                                                                                                                      |
| <b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>                                             | 100% at undiluted, 2x and 4x diluted                                                                                                                                                                                                                                                                 |
| <b><u>Repeatability</u></b>                                                                                                          |                                                                                                                                                                                                                                                                                                      |
| <b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>                                             | 100% at undiluted, 2x and 4x diluted                                                                                                                                                                                                                                                                 |
| <b>Organism 2.:</b>                                                                                                                  | <b>Pospiviroid fusituberis(PSTVD0)</b>                                                                                                                                                                                                                                                               |
| <b><u>Analytical sensitivity</u></b>                                                                                                 |                                                                                                                                                                                                                                                                                                      |
| <b>What is smallest amount of target that can be detected reliably?</b>                                                              | Undiluted leaf sap was considered 100% infected. Starting with undiluted plant sap from PSTVd and TCDVd infected hosts up to 10 <sup>7</sup> times dilutes in sap of healthy tomato leaves, all PSTVd and TCDVd strains were detected up to a relative infection rate of 10% (=10 times diluted) .   |
| <b><u>Diagnostic sensitivity</u></b>                                                                                                 |                                                                                                                                                                                                                                                                                                      |
| <b>Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98</b> | 1                                                                                                                                                                                                                                                                                                    |
| <b>Standard test(s)</b>                                                                                                              | PCR sequencing of the complete viroid genome is considered the standard test. This can be achieved with primers Vid-FW/Vid-RE (CLVd, PSTVd and TCDVd) and 3H1/2H1 (PSTVd, TCDVd, MPVd and TPMVd). For other pospiviroids primers Pospi1-FW/Pospi1-RE are used to sequence the partial viroid genome. |
| <b><u>Analytical specificity - inclusivity</u></b>                                                                                   |                                                                                                                                                                                                                                                                                                      |
| <b>Number of strains/populations of target organisms tested</b>                                                                      | 7 pospiviroid strains, see table 4 validation report                                                                                                                                                                                                                                                 |
| <b>Specificity value</b>                                                                                                             | 1                                                                                                                                                                                                                                                                                                    |
| <b><u>Analytical specificity - exclusivity</u></b>                                                                                   |                                                                                                                                                                                                                                                                                                      |
| <b>Number of non-target organisms tested</b>                                                                                         | 10 (4 Avsunviroidae and 6 Pospiviroidae strains), see table 6 validation report                                                                                                                                                                                                                      |
| <b>Specificity value</b>                                                                                                             |                                                                                                                                                                                                                                                                                                      |
| <b><u>Diagnostic Specificity</u></b>                                                                                                 |                                                                                                                                                                                                                                                                                                      |

|                                                                                                                               |                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
|-------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test</b> | 1                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
| <b>Specify the test(s)</b>                                                                                                    | PCR sequencing of the complete viroid genome is considered the standard test. This can be achieved with primers Vid-FW/Vid-RE (CLVd, PSTVd and TCDVd) and 3H1/2H1 (PSTVd, TCDVd, MPVd and TPMVd). For other pospiviroids primers Pospi1-FW/Pospi1-RE are used to sequence the partial viroid genome.                                                                                                                                                     |
| <b><u>Reproducibility</u></b>                                                                                                 |                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
| <b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>                                      | 100% at undiluted, 2x and 4x diluted                                                                                                                                                                                                                                                                                                                                                                                                                     |
| <b><u>Repeatability</u></b>                                                                                                   |                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
| <b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>                                      | 100% at undiluted, 2x and 4x diluted                                                                                                                                                                                                                                                                                                                                                                                                                     |
| <b>Test performance study</b>                                                                                                 |                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
| <b>Test performance study?</b>                                                                                                | no                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
| <b>Other information</b>                                                                                                      |                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
| <b>Any other information considered useful</b>                                                                                | To determine the robustness of the test two different reaction mixes were used to generate PCR products: OneStep RT-PCR kit (Qiagen) and SuperScript One-Step RT-PCR System with Platinum Taq (Invitrogen). The latter yielded the best results. Different extraction buffers were used. Using a GH plus buffer with a heating step yielded the best results. Freezing the samples prior to analysis did not influence the qualitative results obtained. |
|                                                                                                                               |                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
| The following complementary files are available online:                                                                       | <ul style="list-style-type: none"> <li>• <a href="#">Validation of a conventional RT-PCR test for detection and identification of PSTVd, TCDVd, MPVd and TPMVd using primers described by Shamoul et al. (1997). (In Dutch)</a></li> </ul>                                                                                                                                                                                                               |

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