**EUROPEAN AND MEDITERRANEAN PLANT PROTECTION ORGANIZATION**
**ORGANISATION EUROPEENNE ET MEDITERRANEENNE POUR LA PROTECTION DES PLANTES**
(11-17239)

**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.

| | | |
|---|---|---|
| **Target Organism** | Pospiviroid | |
| **Short description** | Development and validation of a real-time RT-PCR assay for generic detection of Pospiviroids | |
| **Laboratory contact details** | Netherlands Institute for Vectors, Invasive plants and Plant health<br>P.O. Box 9102, 6700 HC Wageningen, Netherlands | |
| **Date and reference of the validation report** | 2012-04-04 - NRC-ref: 2010.molbio.015 | |
| **Validation process according to EPPO Standard PM 7/98:** | Yes | |
| **Reference of the test description** | N/R<br>M. Botermans, B.T.L.H. van de Vossenberg, J.Th.J. Verhoeven, J.W. Roenhorst, M. Hooftman, R. Dekter, E.T.M. Meekes (2013) Development and validation of a real-time RT-PCR assay for generic detection of Pospiviroids. Journal of Virological Methods 187 43– 50 | |
| **Is the test the same as described in the EPPO DP?** | | |
| **Is the lab accredited for this test?** | No | |
| **Plant species tested (if relevant)** | Solanum lycopersicum | |
| **Matrices tested (if relevant)** | leaves | |
| | | |
| ***List of methods used*** | | |
| **Method for extraction / isolation / baiting of target organism from matrix** | | |
| **Molecular methods, e.g. hybridization, PCR and real time PCR** | X | RNA isolation using the RNeasy Plant Mini Kit (Qiagen), followed by real-time RT-PCR |
| **Serological methods: IF, ELISA, Direct Tissue Blot Immuno Assay** | | |
| **Plating methods: selective isolation** | | |
| **Bioassay methods: selective enrichment in host plants, baiting, plant test and grafting.** | | |
| **Pathogenicity test** | | |

| | | |
|---|---|---|
| **Fingerprint methods: protein profiling, fatty acid profiling & DNA profiling** | | |
| **Morphological and morphometrical methods intended for identification** | | |
| **Biochemical methods: e.g. enzyme electrophoresis, protein profiling** | | |
| **Other** | | |
| **Analytical sensitivity (= limit of detection)** | | |
| **What is smallest amount of target that can be detected reliably?** | Undiluted infected leaf sap was considered 100% infected. Starting with undiluted plant sap from infected hosts up to 10^7 times dilutes in sap of healthy tomato leaves, all pospiviroids species were detected up to a relative infection rate of 0.13% (=770 times diluted) | |
| **Diagnostic sensitivity** | | |
| **Proportion of infected/infested samples tested positive compared to results from the standard test , see appendix 2 of PM 7/98** | 100 % | |
| **Specify the standard test** | 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. | |
| **Analytical specificity** | | |
| **Specificity value** | 100 % | |
| **Number of strains/populations of target organisms tested** | 28 pospiviroid strains, see table 1 Botermans et al. (2013) | |
| **Number of non-target organisms tested** | 8 non -ospiviroid strains and 8 tomato viruses, see table 1 Botermans et al. (2013) | |
| **Cross reacts with (specify the species)** | | |
| **Diagnostic Specificity** | | |
| **Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test** | 100 % | |
| **Specify the standard test** | 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. | |
| **Reproducibility** | | |
| **Provide the calculated % of agreement for a given level of the pest (see PM 7/98)** | 100% at undiluted, 500 x, and 1000x diluted | |

| | |
|---|---|
| **Repeatability** | |
| **Provide the calculated % of agreement for a given level of the pest (see PM 7/98)** | 100% at undiluted, 500 x, and 1000x diluted |
| **Test performance study** | |
| **Test performance study?** | Yes |
| **Include brief details of the test performance study and its output.It available, provide a link to published article/report** | Three laboratories participated in the inter-laboratory comparison: Dutch General Inspection Service for Agricultural Seed and Seed Potatoes (NAK, Emmeloord), Naktuinbouw (Roelofarendsveen) and the National Reference Centre of the National Plant Protection Organization (Wageningen). Sixteen samples of tomato leaves infected with PSTVd, TASVd or Tomato chlorotic dwarf viroid (TCDVd) at different relative infection rates were tested at the three laboratories (Table 6, Botermans et al. 2013) |
| **Other information** | |
| **Any other information considered useful**<br>**e.g. robustness, ease of performing the test, etc.** | For tomato leaves the assay is not influenced by the matrix (selectivity). To determine the robustness of the test an alternative RNA extraction kit was used: the Sbeadex maxi plant kit on a Kingfisher KF96 system, and Real-time RT-PCRs were carried out on different real-time PCR machines. The test results were similar for all samples tested. |
| | |
| The following complementary files are available online: | • Development and validation of a real-time RT-PCR assay for generic detection of pospiviroids |