## 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.

| Laboratory contact details                                      | Netherlands Institute for Vectors, Invasive plants<br>and Plant health<br>P.O. Box 9102, 6700 HC Wageningen, Netherlands   |
|---|--|
| Short description of the test                                   | Detection of Tomato brown rugose fruit virus by LAMP (Agdia AmplifyRP) in seeds of tomato and pepper   |
| Date, reference of the validation report                        | 2021-12-01 - Euphresco 2019-A-327 project report   |
| Link to other validation data                                   | - Euphresco 2019-A-327 project report Detection of Tomato brown rugose fruit virus by real time RT PCR (Menzel and Winter, 2021) in seeds of tomato and pepper - Euphresco 2019-A-327 project report Detection of Tomato brown rugose fruit virus by real time RT PCR (Abiopep) in seeds of tomato and pepper Euphresco 2019-A-327 project report Detection of Tomato brown rugose fruit virus by conventional RT PCR (Loewe kit) in seeds of tomato and pepper - Euphresco 2019-A-327 project report Detection of Tomato brown rugose fruit virus by conventional RT PCR (Alkowni et al., 2019) in seeds of tomato and pepper - Euphresco 2019-A-327 project report Detection of Tomato brown rugose fruit virus by LAMP (Sarkes et al., 2020) in seeds of tomato and pepper - Euphresco 2019-A-327 project report Detection of Tomato brown rugose fruit virus by real time RT PCR (ISHI-Veg test) in seeds of tomato and pepper |
| 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? | Euphresco  |
| If yes, please specify  | Euphresco 2019-A-327   |
|   |  |
| Description of the test   |  |
|   |  |
| Organism(s)   | Tomato brown rugose fruit virus / Tobamovirus fructirugosum (TOBRFV)   |
| Detection / identification                                      | detection  |
| Method(s)   | Extraction Molecular Extraction DNA RNA Molecular LAMP   |

| Potorones of the test description                   |  |  |  |
|---|--|--|--|
| Reference of the test description                   | Reference of the test description                    |  |  |
| As or adapted from an EPPO diagnostic protocol      | yes  |  |  |
| EPPO Diagnostic Protocol name                       | PM 7/146 Tomato brown rugose fruit virus (version 1) |  |  |
| As or adapted from an IPPC diagnostic protocol      | no   |  |  |
| Is the test modified compared to the reference test | no   |  |  |
| Other information                                   |  |  |  |
| Other details on the test                           | GH+ buffer   |  |  |
| Method: Molecular Extraction DNA RNA                |  |  |  |
| Reference of the test description                   |  |  |  |
| As or adapted from an EPPO diagnostic protocol      | yes  |  |  |
| EPPO Diagnostic Protocol name                       | PM 7/146 Tomato brown rugose fruit virus (version 1) |  |  |
| As or adapted from an IPPC diagnostic protocol      | no   |  |  |
| Is the test modified compared to the reference test | yes Centrifugation at 4°C                            |  |  |
| Kit   |  |  |  |
| Is a kit used                                       | yes  |  |  |
| Manufacturer name                                   | QIAGEN   |  |  |
| Specify the kit used                                | RNeasy Plant Mini Kit                                |  |  |
| Kit used following the manufacturer's instructions? | no Centrifugation at 4°C                             |  |  |
| Other information                                   |  |  |  |
| Method: Molecular LAMP                              |  |  |  |
| Reference of the test description                   |  |  |  |
| As or adapted from an EPPO diagnostic protocol      | no   |  |  |
| As or adapted from an IPPC diagnostic protocol      | no   |  |  |
| Reference of the test                               | Agdia kit  |  |  |
| Kit   |  |  |  |
| Is a kit used                                       | yes  |  |  |
| Manufacturer name                                   | AGDIA  |  |  |
| Specify the kit used                                | AmplifyRP® XRT for ToBRFV (XCS 66800)                |  |  |
| Kit used following the manufacturer's instructions? | no minor modifications (see report)                  |  |  |

| Other information   |   |  |
|---|---|--|
| Performance Criteria :  |   |  |
| Organism 1.:  | Tobamovirus fructirugosum(TOBRFV)   |  |
| Analytical sensitivity  |   |  |
| What is smallest amount of target that can be detected reliably?  | Preliminary study on tomato seeds spiked with ToBRFV: 10^-4 with GH+ buffer for extraction and 1uL RNA 10^-4 with phosphate buffer for extraction and 1 uL RNA 10^-1 with phosphate buffer for extraction and 10uL of raw extract. Did not work with GH+ buffer for extraction and 10uL of raw extract. |  |
| Diagnostic sensitivity  |   |  |
| Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98 | Based on the results of 3 laboratories Tomato: 81.4% Pepper: 42.9%  |  |
| Standard test(s)  | Comparison with samples of known status   |  |
| Diagnostic Specificity  |   |  |
| Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test        | Based on the results of 3 laboratories Tomato: 100% Pepper: 100%  |  |
| Specify the test(s)   | Comparison with samples of known status   |  |
| Test performance study  |   |  |
| Test performance study?   | yes   |  |
| Brief details of the test performance study and its output.It available, link to published article/report                     | Test performance study organized in the framework of the Euphresco project 2019-A-327 involving 26 laboratories from 16 countries. The performance of this test is based on data from 3 laboratories.   |  |
|   |   |  |
| The following complementary files are available online:   | Report_2019-A-327_Euphresco   |  |

Creation date: 2022-01-06 10:48:54 - Last update: 2022-01-14 16:52:34