**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.

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| **Target Organism** | Phyllosticta citricarpa | |
| **Short description** | Real Time PCR for the identification of Phyllosticta citricarpa (van Gent-Pelzer et al., 2007) | |
| **Laboratory contact details** | Council for Agricultural Research and Economics– Research Centre for Plant Protection and Certification<br>Via Carlo Giuseppe Bertero, 22, 00156 Rome, Italy | |
| **Date and reference of the validation report** | 2014 - | |
| **Validation process according to EPPO Standard PM 7/98:** | Yes | |
| **Reference of the test description** | N/R<br>Modification from EPPO PM 7/17(2), 2009. Guignardia citricarpa. EPPO Bulletin, 39, 318–327. | |
| **Is the test the same as described in the EPPO DP?** | | |
| **Is the lab accredited for this test?** | Yes | |
| **Plant species tested (if relevant)** | Citrus lemon | |
| **Matrices tested (if relevant)** | Fungal pure culture and lemon fruit | |
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| ***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 | van Gent-Pelzer M.P.E,. van Brouwershaven I.R., Kox L.F.F., Bonants P.J.M., 2007. A Taqman PCR method for routine diagnosis of the quarantine fungus Guignardia citricarpa. Journal of Phytopathology, 155, 357–363. |
| **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** | | |

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| **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?** | 10 fg of DNA | |
| **Diagnostic sensitivity** | | |
| **Proportion of infected/infested samples tested positive compared to results from the standard test , see appendix 2 of PM 7/98** | | |
| **Specify the standard test** | | |
| **Analytical specificity** | | |
| **Specificity value** | 100% | |
| **Number of strains/populations of target organisms tested** | 3 target strains | |
| **Number of non-target organisms tested** | 3 non-target strains (see validation report) | |
| **Cross reacts with (specify the species)** | no cross reaction | |
| **Diagnostic Specificity** | | |
| **Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test** | | |
| **Specify the standard test** | | |
| **Reproducibility** | | |
| **Provide the calculated % of agreement for a given level of the pest (see PM 7/98)** | 100% | |
| **Repeatability** | | |
| **Provide the calculated % of agreement for a given level of the pest (see PM 7/98)** | 100% | |
| **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** | The robustness of the method was verified through a Test Performance Study among 6 laboratories. For each lab 6 positive samples (3 containing the target DNA slightly above the relative limit of detection and 3 containing the target DNA ten times the relative limit of detection) and 6 negative | |

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| | samples (3 containing no DNA and 3 containing DNA of non-target strains) were tested. The results showed:<br>-100% relative sensitivity<br>-100% relative specificity<br>-100% repeatability<br>-100% reproducibility |
| **Other information** | |
| **Any other information considered useful**<br>**e.g. robustness, ease of performing the test, etc.** | When verifying the performance criteria cross reactions wih the non-target organism Phyllosticta citriasiana was noted so the protocol was slightly changed and a new validation was performed. It is suggested to use the amplification commercial kit in RealTime-PCR to avoid no specific amplification with P. citriasiana. |
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| The following complementary files are available online: | • Validation process of the Real Time PCR for the identification of Phyllosticta citricarpa (van Gent-Pelzer et al., 2007) |