

**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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| Laboratory contact details | Council for Agricultural Research and Economics- Research Centre for Plant Protection and Certification Via Carlo Giuseppe Bertero, 22, 00156 Rome, Italy |
| Short description of the test | Real Time PCR for the identification of <i>Phyllosticta citricarpa</i> (van Gent-Pelzer et al., 2007) |
| Date, reference of the validation report | 2014-01-01 - |
| 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? | |
| If yes, please specify | |
| Description of the test | |
| Organism(s) | <i>Phyllosticta citricarpa</i> (GUIGCI) |
| Detection / identification | |
| Matrix(ces) tested | Fruits, Pure culture Fungal pure culture and lemon fruit |
| Plant species tested | Citrus limon |
| Method(s) | Molecular Extraction DNA RNA Molecular real time PCR |
| Method: Molecular Extraction DNA RNA | |
| Reference of the test description | |
| As or adapted from an EPPO diagnostic protocol | |
| New test being considered for inclusion in the next version of the EPPO diagnostic protocol? | |
| As or adapted from an IPPC diagnostic protocol | |
| Is the test modified compared to the reference test | |
| Kit | |
| Is a kit used | yes |

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| Manufacturer name | MACHEREY-NAGEL |
| Specify the kit used | Nucleospin plant II kit |
| Kit used following the manufacturer's instructions? | yes |
| Other information | |
| Other details on the test | |
| Method: Molecular real time PCR | |
| Reference of the test description | |
| As or adapted from an EPPO diagnostic protocol | yes |
| New test being considered for inclusion in the next version of the EPPO diagnostic protocol? | |
| EPPO Diagnostic Protocol name | PM 7/017 Guignardia citricarpa (version 2) |
| Name of the test | Real-time PCR (van Gent-Pelzer et al., 2007) |
| As or adapted from an IPPC diagnostic protocol | |
| Is the test modified compared to the reference test | yes |
| Kit | |
| Is a kit used | |
| Other information | |
| Reaction type | Simplex - Probe |
| Other details on the test | See details in report |
| Are the performance characteristics included in the EPPO diagnostic protocol? | no |
| Performance Criteria : | |
| Organism 1.: | Phyllosticta citricarpa(GUIGCI) |
| Analytical sensitivity | |
| 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 | |
| Standard test(s) | |
| Analytical specificity - inclusivity | |
| Number of strains/populations of target organisms tested | 3 target strains |
| Specificity value | 100% |
| Analytical specificity - exclusivity | |
| Number of non-target organisms tested | 3 non-target strains (see validation report) |

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|---|--|
| Specificity value | 100% no cross reaction |
| Cross reacts with | |
| Diagnostic Specificity | |
| Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test | |
| Specify the test(s) | |
| 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 |
| Brief details of the test performance study and its output. It available, 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 samples (3 containing no DNA and 3 containing DNA of non-target strains) were tested. The results showed: -100% relative sensitivity -100% relative specificity -100% repeatability -100% reproducibility |
| Other information | |
| Any other information considered useful | When verifying the performance criteria cross reactions with the non-target organism <i>Phyllosticta citriasiana</i> 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 <i>P. citriasiana</i> . |
| The following complementary files are available online: | <ul style="list-style-type: none"> • Validation process of the Real Time PCR for the identification of <i>Phyllosticta citricarpa</i> (van Gent-Pelzer et al., 2007) |

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