**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** | Grapevine flavescence dorée phytoplasma |
| **Short description** | Simplex real time PCR for the detection of FD and BN phytoplasmas with an internal control for grapevine - Angelini et al., 2007. |
| **Laboratory contact details** | EUPHRESCO-GRAFDEPI<br>Via Carlo Giuseppe Bertero, 22, 00156 ROMA, Italy |
| **Date and reference of the validation report** | Project EUPHRESCO GRAFDEPI Final Report 2014-07-31 - - 1)http://www.euphresco.net/media/project_reports/grafdepi_final_report.pdf 2)The Euphresco Grafdepi Group, 2015. European interlaboratory comparison of detection methods for "flavescence dorée" phytoplasma: preliminary results. Phytopathogenic Mollicutes doi: 10.5958/2249-4677.2015.00015.8 Vol. 5 (1-Supplement), January 2015, S35-S37 |
| **Validation process according to EPPO Standard PM 7/98:** | Yes |
| **Reference of the test description** | N/R<br>ANGELINI E., BIANCHI G.L., FILIPPIN L., MORASSUTTI C., BORGO M. A new TaqMan method for the identification of phytoplasmas associated with grapevine yellows by real-time PCR assay. Journal of Microbiological Methods, 68 (2007), 613–622 |
| **Is the test the same as described in the EPPO DP?** | |
| **Is the lab accredited for this test?** | No |
| **Plant species tested (if relevant)** | The samples had been provided by different partners of Project GRAFDEPI and belonged to different plant host species. The homogenising and preparation were performed by ANSES-LSV (France).<br>The samples consisted in DNA extracts. The batches' selection was based on methodology proposed in PM7/98 for the evaluation of the performance criteria of analytical methods.<br>Positive samples were from different parts of Europe in order to have a wide<br>diversity of strains for testing the inclusivity of methods.<br>Phytoplasmas in the same group and/or infecting grapevines were also chosen to test different degrees of specificity/exclusivity of methods.<br>13 samples were negative for the Flavescence dorée phytoplasma. 4 of them were healthy Vitis sp.. The other ones were other phytoplasmas of 16SrV group and phytoplasmas from other groups, mixed with DNA extract of healthy grapevine to reach the volume necessary for the ring-test.<br>11 samples were positive for the Flavescence dorée |

| | | |
|---|---|---|
| | phytoplasma. They were DNA extracts of Vitis sp. tested positive by PCR for Flavescence dorée phytoplasmas pure or mixed with different quantities of healthy grapevine or mixed with DNA extracts positive for the 16SrXII group phytoplasmas.<br>When it was possible, supernumerary fractions were produced for each sample to validate their status and for testing the homogeneity of the division during the preparation of tubes for the participants. Then, these supernumerary fractions were randomly chosen in the series of tubes.<br>DNA extracts were amplified in real-time triplex PCR (Pelletier et al., 2009). See Appendix | |
| **Matrices tested (if relevant)** | | |
| | | |
| ***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 | A TaqMan real time PCR methodology performed with specific primers and probes for the detection of FD and BN phytoplasmas and a grapevine internal control (Grapevine chloroplast chaperonin 21 gene).<br>Detection of the single targets must be performed in separate reactions. |
| **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** | | |
| **<u>Analytical sensitivity (= limit of detection)</u>** | | |
| **What is smallest amount of target that can be detected reliably?** | The analytical sensitivity was calulated in five laboratories analyzing three samples at five dilution levels (1/10; 1/100; 1/300; 1/900; 1/2700) in five repetitions. Samples (DNA extracts) came from IPEP (Serbia), ACW (Switzerland) and ANSES (France). The homogenising and preparation were performed by ANSES-LSV (France).<br>Two values are provided:<br>The last dilution level with 100% positive results: less than 1/10 (for all samples) | |

| | |
|---|---|
| | The last dilution level with, at least, one positive result for each sample: 1/2700 (for all samples) |
| **Diagnostic sensitivity** | |
| **Proportion of infected/infested samples tested positive compared to results from the standard test , see appendix 2 of PM 7/98** | Seven laboratories performed this protocol within GRAFDEPI ringtest with a total of 168 results.<br>Determined in 11 samples positive for Flavescence dorée phytoplasma.<br>They were DNA extracts of Vitis sp. tested positive by PCR for FD pure or mixed with different quantities of healthy grapevine or mixed with DNA extracts positive for the 16SrXII group phytoplasmas.<br>Within the ringtest 7 diagnostic methods were compared.<br>Diagnostic sensitivity: 86.67%<br>False negative: (14/168) 8.3% |
| **Specify the standard test** | Other protocols included in the ringtest:<br><br>- Simoultaneous detection of FD and BN phytoplasmas by multiplex nested-PCR (Dairè et al., 1997; Angelini et al., 2001; Clair et al., 2003)<br>- Detection of Flavescence dorée phytoplasma by universal direct PCR and nested 16SrV-group specific PCR<br>- Detection and identification of Flavescence dorée phytoplasma by direct and nested PCR followed by RFLP with Taq I (Martini et al., 1999)<br>- Simplex real time PCR for the detection of FD and BN phytoplasmas with an internal control - (Hren et al., 2007)<br>- Triplex real-time PCR for simultaneous FD and BN phytoplasmas detection with an internal control for grapevine. (Pelletier et al., 2009)<br>- Triplex real time PCR for simultaneous FD and BN phytoplasmas detection with an internal control - (under patent IPADLAB) |
| **Analytical specificity** | |
| **Specificity value** | |
| **Number of strains/populations of target organisms tested** | |
| **Number of non-target organisms tested** | |
| **Cross reacts with (specify the species)** | |
| **Diagnostic Specificity** | |
| **Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test** | Seven laboratories performed this protocol within GRAFDEPI ringtest with a total of 168 results.<br>13 non target samples: 4 healthy grapevines and 9 were other phytoplasmas of 16SrV group and phytoplasmas from other groups.<br>Diagnostic specificity: 66.10% |
| **Specify the standard test** | Other protocols included in the ringtest: |

| | |
|---|---|
| | - Simoultaneous detection of FD and BN phytoplasmas by multiplex nested-PCR (Dairè et al., 1997; Angelini et al., 2001; Clair et al., 2003)<br>- Detection of Flavescence dorée phytoplasma by universal direct PCR and nested 16SrV-group specific PCR<br>- Detection and identification of Flavescence dorée phytoplasma by direct and nested PCR followed by RFLP with Taq I (Martini et al., 1999)<br>- Simplex real time PCR for the detection of FD and BN phytoplasmas with an internal control - (Hren et al., 2007)<br>- Triplex real-time PCR for simultaneous FD and BN phytoplasmas detection with an internal control for grapevine. (Pelletier et al., 2009)<br>- Triplex real time PCR for simultaneous FD and BN phytoplasmas detection with an internal control - (under patent IPADLAB) |
| **Reproducibility** | |
| **Provide the calculated % of agreement for a given level of the pest (see PM 7/98)** | The reproducibility was calulated in five laboratories analyzing three samples at five dilution levels (1/10; 1/100; 1/300; 1/900; 1/2700) in five repetitions. Samples (DNA extracts) came from IPEP (Serbia), ACW (Switzerland) and ANSES (France). The homogenising and preparation were performed by ANSES-LSV (France).<br>Reproducibility: 75.59% |
| **Repeatability** | |
| **Provide the calculated % of agreement for a given level of the pest (see PM 7/98)** | The repeatability was calculated in five laboratories analysing three samples at five dilution levels (1/10; 1/100; 1/300; 1/900; 1/2700) in five repetitions. Samples (DNA extracts) came from IPEP (Serbia), ACW (Switzerland) and ANSES (France). The homogenising and preparation were performed by ANSES-LSV (France)<br>Repeatability: 88.05% |
| **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** | Interlaboratory comparison among 15 laboratories within the EUPHRESCO Project GRAFDEPI<br>(CRA-PAV, Italy; AGES, Austria; CRA-W, Belgium, PPRS, Turkey; INIAV, Portugal; ACW, Switzerland; ILVO, Belgium; DIPSA, Bologna Italy; DISAA, Milan Italy; IPEP, Serbia; NIB, Slovenia; IRTA, Spain; ANSES, France; Cra-VIT, Italy) |
| **Other information** | |
| **Any other information considered useful<br>e.g. robustness, ease of performing the test, etc.** | The ringtest was carried out by 15 laboratories and it is not possible to state if any of them is accredited for this test. |
| | |
| The following complementary files are available online: | • Samples for determination of performance criteria |