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 | Globodera pallida Globodera rostochiensis | | |
|--|---|--|--|
| Short description | Qualitative detection of viability and identification of Globodera spp. | | |
| Laboratory contact details | Dutch General Inspection Service (NAK) Randweg 14, 8304AS Emmeloord, Netherlands | | |
| Date and reference of the validation report | July 2014 - Qualitative detection of Globodera spp. viability version 2 | | |
| Validation process according to EPPO Standard PM 7/98: | Yes | | |
| Reference of the test description | PM 7/040 FES resea Globodera v.d. Lee. 0 nematode specific R on the art been mad extraction | (2) arch: Validation of the determination of viability of a cysts/eggs based on RNA detection version 3. Theo Quantification of viable eggs of the potato cyst es (Globodera spp.) using either trehalose or RNA- eal-Time PCR Beniers et al. 2014 The test is based ticle of Beniers et al. 2014 but some changes have de (another internal control is used and the m method is automated). | |
| Is the test the same as described in the EPPO DP? | Yes | | |
| Is the lab accredited for this test? | No | | |
| Plant species tested (if relevant) | | | |
| Matrices tested (if relevant) | Potato cysts | | |
| | | | |
| List of methods used | | | |
| Method for extraction / isolation / baiting of target organism from matrix | | | |
| Molecular methods, e.g. hybridization, PCR and real time PCR | Х | Qualitative 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. | | | |

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|---|--|--|--|--|
| 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? | 1 -10 viable juveniles or eggs | | | |
| Diagnostic sensitivity | | | | |
| Proportion of infected/infested samples tested positive compared to results from the standard test , see appendix 2 of PM 7/98 | G. pallida: 100 % G. rostochiensis: 100 % | | | |
| Specify the standard test | -Dead or alive visual assessment using the table according to Beniers, 2008 -Soortbepaling Globodera pallida en Globodera rostochiensis m.b.v. realtime RT-PCR, PRI (niet gepubliceerd) -Validatierapport Nematoden-onderzoek Soortbepaling Globodera pallida en G. rostochiensis m.b.v. real-time PCR, versie 2, NAK, T. Dekker, mei 2012 -EPPO protocol PM7/40 (3), appendix 4 Visual determination | | | |
| Analytical specificity | | | | |
| Specificity value | | | | |
| Number of strains/populations of target organisms tested | 8 strains o Ecnavorna Rookmake 6 strains o C262, Ro2 2013, Ro4 | of G. pallida (Pa2 WUR 248, Pa2 Averis 2013, Pa3 ay, Pa3 er 2009, Pa3 Rookmaker 2013, Pa3 Averis 2013) of G. rostochiensis (Ro1 SCRI, Ro1 Mierenbos, Ro2,3 2,3 PRI 5 F515, Ro5 G1518) | | |
| Number of non-target organisms tested | 6 non-targ Heteroder glycines, l tabacum, Cactodera | get organisms were tested (Heterodera betae, ra Heterodera schachtii, Heterodera trifolii, Globodera n cacti) | | |
| Cross reacts with (specify the species) | No cross r | eactions with other organisms | | |
| Diagnostic Specificity | | | | |
| Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test | G. pallida: G. rostoch | 72.2% iiensis: 86.7% | | |

| Specify the standard test | Dead or alive assessment according to the table in Beniers, 2008. Adapted and inserted in EPPO protocol PM7/40 (3), appendix 4 Visual determination. Species identification of Globodera pallida and Globodera rostochiensis by real-time RT-PCR, PRI (intern report FES) | | |
|--|--|--|--|
| Reproducibility | | | |
| Provide the calculated % of agreement for a given level of the pest (see PM 7/98) | Reproducibility: 100% | | |
| Repeatability | | | |
| Provide the calculated % of agreement for a given level of the pest (see PM 7/98) | Repeatability: 100% | | |
| Test performance study | | | |
| Test performance study? | Νο | | |
| Include brief details of the test performance study and its output.It available, provide a link to published article/report | | | |
| Other information | | | |
| Any other information considered useful e.g. robustness, ease of performing the test, etc. | Various KingFisher apparatus and ABI 7500 apparatus did not influence the outcome of the tests. The above described method will be applied in a qualitative way at the NAK. The new test has been applied to 184 field samples that were analysed by the old and the new method. No false-negative results were found. The new test found 6 positive results that were not detected with the old method. | | |