**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** | Globodera pallida<br>Globodera rostochiensis | |
| **Short description** | Diagnosis of Globodera pallida and Globodera rostochiensis (potato cyst nematodes) using Taqman® real-time PCR | |
| **Laboratory contact details** | Fera<br>Sand Hutton, YO41 1LZ York, United Kingdom | |
| **Date and reference of the validation report** | 2013-09 - Potato Coucil Project Report 2009/15: Validation of quantitative DNA detection systems for PCN. Ref: R287 | |
| **Validation process according to EPPO Standard PM 7/98:** | Yes | |
| **Reference of the test description** | PM 7/040<br>Based on: Bulman S. R. & Marshall J. W. (1997) Differentiation of Australasian potato cyst nematode (PCN) populations using the polymerase chain reaction (PCR). New Zealand Journal of Crop and Horticultural Science 25: 123-129. | |
| **Is the test the same as described in the EPPO DP?** | Yes | |
| **Is the lab accredited for this test?** | Yes | |
| **Plant species tested (if relevant)** | | |
| **Matrices tested (if relevant)** | Dissected cyst and eggs. Half is retained if morphological examination is required | |
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| ***List of methods used*** | | |
| **Method for extraction / isolation / baiting of target organism from matrix** | X | Conventional flotation method to isolate cysts (Wye washer, following EPPO diagnostic protocol PM 7/40 (3)) |
| **Molecular methods, e.g. hybridization, PCR and real time PCR** | X | Spin column based DNA extraction and Real-time PCR<br>PCR developed from Bulman S. R. & Marshall J. W. (1997)<br>Applied Biosystems TaqMan Universal master mix II, no UNG (4440043)<br>50˚C for 2 min; 95˚C for 10 min; 95˚C 15 sec, 60˚C 1 min (40 repeats) |
| **Serological methods: IF, ELISA, Direct Tissue Blot Immuno Assay** | | |
| **Plating methods: selective isolation** | | |
| **Bioassay methods: selective** | | |

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| **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** | | |

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| **<u>Analytical sensitivity (= limit of detection)</u>** | |
| **What is smallest amount of target that can be detected reliably?** | DNA from single cyst detectable at 1000 fold dilution |
| **<u>Diagnostic sensitivity</u>** | |
| **Proportion of infected/infested samples tested positive compared to results from the standard test , see appendix 2 of PM 7/98** | G. pallida 100%<br>G. rostochiensis 100% |
| **Specify the standard test** | The TaqMan assay was compared to the standard conventional PCR assay of Bulman & Marshall, 1997. |
| **<u>Analytical specificity</u>** | |
| **Specificity value** | |
| **Number of strains/populations of target organisms tested** | 20+ strains of G. pallida (see validation report)<br>20+ strains of G. rostochiensis (see validation report) |
| **Number of non-target organisms tested** | Strains of G. tabacum (see validation report)<br>Strains of G. achillae/millefolii (see validation report) |
| **Cross reacts with (specify the species)** | G. tabacum, however this gives a distinct profile and be differentiated for G.pallida and G.rostochiensis |
| **<u>Diagnostic Specificity</u>** | |
| **Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test** | G. pallida 87.1%<br>G. rostochiensis 93.75%<br>The testing gave no false negatives for either species. |
| **Specify the standard test** | The TaqMan assay was compared to the standard conventional PCR assay of Bulman & Marshall, 1997 and morphological identification. |
| **<u>Reproducibility</u>** | |
| **Provide the calculated % of agreement for a given level of the pest (see PM 7/98)** | The testing has been successful carried out by multiple users on all equipment over several days |
| **<u>Repeatability</u>** | |
| **Provide the calculated % of agreement for a given level of the pest (see PM 7/98)** | Positive controls used on every run produce repeatable results. |

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| **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** | 100% correct identification results over recent proficieny tests. |
| **Other information** | |
| **Any other information considered useful**<br>**e.g. robustness, ease of performing the test, etc.** | The Globodera pallida probe is known to cross-react slightly with Globodera rostochiensis DNA. The cross reaction will show as a slight increase in delta Rn in the FAM channel (G. pallida) as the delta Rn increases exponentially in the TET channel (G. rostochiensis). This cross reaction is only observed when a sample is positive for G. rostochiensis. |
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| The following complementary files are available online: | • Validation of quantitative DNA detection systems for PCN Ref: R287<br>• Validation report; speciation of Globodera pallida and G. rostochiensis (potato cyst nematodes) using TaqMan® real-time PCR |