

**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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| <b>Target Organism</b>  | Diabrotica virgifera virgifera   |  |
| <b>Short description</b>  | Morphological identification of D.virgifera in pheromone traps   |  |
| <b>Laboratory contact details</b>   | ILVO Institute for Agricultural and Fisheries Research<br>Burg. Van Gansberghelaan 96, 9820 Merelbeke, Belgium |  |
| <b>Date and reference of the validation report</b>  | 2011-05-19 - F16_I09   |  |
| <b>Validation process according to EPPO Standard PM 7/98:</b>                                   | No   |  |
| <b>Reference of the test description</b>  | PM 7/036(1)  |  |
| <b>Is the test the same as described in the EPPO DP?</b>  | Yes  |  |
| <b>Is the lab accredited for this test?</b>   | Yes  |  |
| <b>Plant species tested (if relevant)</b>   | Not relevant   |  |
| <b>Matrices tested (if relevant)</b>  | Pheromone traps, artificially infected with adults of Diabrotica virgifera                                     |  |
| <b>List of methods used</b>   |  |  |
| <b>Method for extraction / isolation / baiting of target organism from matrix</b>               | X  | Visual inspection of the pheromone traps with stereomicroscope, using a lattice work (A→P, 1→25) to localize the beetles on the trap |
| <b>Molecular methods, e.g. hybridization, PCR and real time PCR</b>                             |  |  |
| <b>Serological methods: IF, ELISA, Direct Tissue Blot Immuno Assay</b>                          |  |  |
| <b>Plating methods: selective isolation</b>   |  |  |
| <b>Bioassay methods: selective enrichment in host plants, baiting, plant test and grafting.</b> |  |  |
| <b>Pathogenicity test</b>   |  |  |
| <b>Fingerprint methods: protein profiling, fatty acid profiling &amp; DNA profiling</b>         |  |  |
| <b>Morphological and morphometrical methods intended for identification</b>                     | X  | Morphological identification using stereomicroscope and checklist (F03_I07) with most  |

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|   |  | important morphological characters of the beetle, same morphological characters as described in PM 7/36 (1). Before the analysis starts there is a control of a beetle (standard reference material from Hungary) with checklist F03_I11   |
| <b>Biochemical methods: e.g. enzyme electrophoresis, protein profiling</b>  |  |  |
| <b>Other</b>  |  |  |
| <b>Analytical sensitivity (= limit of detection)</b>  |  |  |
| <b>What is smallest amount of target that can be detected reliably?</b>   |  | 1 individual beetle (In the validation test 10 pheromone traps were used, artificially infected with one <i>D. virgifera</i> each, on different places on the traps. Four analysts checked the traps and noticed the place on the trap (f.e. B23, G8))   |
| <b>Diagnostic sensitivity</b>   |  |  |
| <b>Proportion of infected/infested samples tested positive compared to results from the standard test , see appendix 2 of PM 7/98</b> |  | Not done   |
| <b>Specify the standard test</b>  |  | Not relevant   |
| <b>Analytical specificity</b>   |  |  |
| <b>Specificity value</b>  |  |  |
| <b>Number of strains/populations of target organisms tested</b>   |  | Not done   |
| <b>Number of non-target organisms tested</b>  |  | Not done   |
| <b>Cross reacts with (specify the species)</b>  |  | Not done   |
| <b>Diagnostic Specificity</b>   |  |  |
| <b>Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test</b>         |  | Not done   |
| <b>Specify the standard test</b>  |  |  |
| <b>Reproducibility</b>  |  |  |
| <b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>  |  | 100% (10 pheromone traps (negative traps already used in maize fields), each of them artificially infected with 1 adult of <i>D. virgifera</i> . On each pheromone trap there was a large diversity of other insects, belonging to different orders. Four analysts checked the pheromone traps on four different days) |
| <b>Repeatability</b>  |  |  |
| <b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>  |  | 100% (10 pheromone traps (negative traps already used in maize fields), each of them artificially infected with 1 adult of <i>D. virgifera</i> . On each pheromone trap there was a large diversity of other insects, belonging to different orders. Five replicates.)   |
| <b>Test performance study</b>   |  |  |

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| <b>Test performance study?</b>  | Yes   |
| <b>Include brief details of the test performance study and its output. If available, provide a link to published article/report</b> | Interlaboratory test with 5 participating labs, including DCP. Each lab received 5 artificially infected pheromone traps. Same situation for each lab: the number of the beetles on the traps, as well as the location on the traps was identical. Result: one lab had a success rate of 71% (36,4% false positives and 18,2% false negatives). The other labs and DCP: success rate 100% (false positives and false negatives 0%). |
| <b><u>Other information</u></b>   |   |
| <b>Any other information considered useful<br/>e.g. robustness, ease of performing the test, etc.</b>                               |   |