



Twinning BA/12/IB/AG 01 “Further strengthening of capacities of phytosanitary sector in the fields of plant protection products, plant health and seeds and seedlings, including phytosanitary laboratories and phytosanitary inspections”

Training course on agricultural nematology

Mostar, March 7-11, 2016

Validation of diagnostic methods is very important activity in introduction of quality systems in the laboratory.

The validation of an analytical test method is performed to show that it is suitable for the purpose it is used.

**Validation of methods of extraction and
identification of nematodes with
morphometrics**

Validation of identification of nematodes with morphometrics

- In Central Laboratory of MISPHSI in Toruń, validation of extraction of nematodes and their identification with morphometrics was performed in Accordance with inner procedure and EPPO Standard. PM7/98.
- The following documents were prepared:
 - plan of validation (signed by performer);
 - form containing data of origin of material used in validation;
 - forms for filling with validation data (signed by performers);
 - report of validation (signed by performer and accepted by the Director of Laboratory by her sign which means approval of method for application);

Plan of validation (general scheme of content)

| | | |
|---|--|------|
| Name of method (including tested object and matrix) | | Date |
| No of procedure /norm/standard | | |
| Scope of procedure use | | |
| Aim | | |

| No. | Performance criteria | Planned means of realisation | Date of realisation |
|-----|----------------------|------------------------------|---------------------|
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| | Confirmed by: |
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Report of validation (general scheme of content)

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|---|-------------------|
| Name of method (including tested object and matrix) | |
| No of procedure /norm/standard | |
| Scope of procedure use | |
| Date of test performance | from to..... |
| Personnel participating in validation process | |
| Personnel responsible for validation | |

| No. | Performance criteria | Comparison | | Evaluation of obtained results (satisfactory/not satisfactory) |
|-----|----------------------|---------------|----------------|--|
| | | Assumed value | Obtained value | |
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Evaluation of validation results (including delimitation of methods).....

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| | Confirmed: by |
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Validation of extraction of cyst nematodes from soil was performed as follows

Extraction with automatic soil sample extractor

Analytical sensitivity, repeatability: Establishment the number of positive results of examination of samples contaminated with different *Globodera* cyst number (2, 10, 20 pieces per 200 ml of soil) using different extraction parameters of soil sample extractor (time of the working cycle and breaks in work).

Analytical specificity: Not performed as not applicable.

Selectivity: Establishment the number of positive results of examination of samples contaminated with the same *Globodera* cyst number (20 pieces per 200 ml of soil) using different extraction parameters of soil sample extractor (time of the working cycle and breaks in work) and with flask method; evaluation of the extent of washing of the samples (i.e. full washing or any remains on the sieve).

Repeatability: Analyses of results of extraction of cysts from soil samples taken from at least 3 localities where *Globodera* cysts are known to occur and 3 localities where they are not known to occur.

Reproducibility: Ring test on extraction of *Globodera* cysts from soil samples taken from a locality where *Globodera* cysts are known to occur by morphometrics 18 laboratories.

Evaluation of budget of uncertainty: Calculations basing on calculation of all possible factors which may cause uncertainty of results.

Validation of identification of cyst nematodes to genus level was performed as follows

Morphological and morphometrical methods intended for identification

Analytical sensitivity: Establishment the minimum number of individuals to perform a reliable identification with a minimum of three repetitions whenever possible; cyst from *Globodera* genus in number 1, 2, 5 and 10 cysts were used.

Analytical specificity: Screening against a range of morphologically similar target and non target organisms. Screening using mixtures of cysts from *Globodera* and *Heterodera* was performed in at least three replications.

Selectivity: Not performed as not applicable.

Repeatability: 8 identifications of the same slide as a blind test within a range of morphologically similar target and non target organisms were done; slides of *Globodera* and *Heterodera* cysts were used

Reproducibility: Identification of a set containing of slides of *Globodera* and *Heterodera* in at least 8 replications (different combination of slides in each replication) by 2 analyticians.

Evaluation of budget of uncertainty: Calculations basing on calculation of all possible factors which may cause uncertainty of results.

Validation of identification of cyst nematodes from *Globodera* genus to species was performed as follows

Morphological and morphometrical methods intended for identification

Analytical sensitivity: Establishment the minimum number of individuals to perform a reliable identification with a minimum of three repetitions whenever possible. *G. rostochiensis*, *G. pallida* were used.

Analytical specificity: Screening against a range of morphologically similar target and non target organisms. Screening using *G. rostochiensis* and *G. pallida* was performed

Selectivity: Not performed as not applicable.

Repeatability: 8 identifications of the same slide as a blind test within a range of morphologically similar target and non target organisms were done. *G. rostochiensis*, *G. pallida* and *G. artemisiae* were used.

Reproducibility: Ring test on identification of *G. pallida* and *G. rostochiensis* in microscopic slides to species by morphometrics by 17 laboratories.

Evaluation of budget of uncertainty: Calculations basing on calculation of all possible factors which may cause uncertainty of results.

Validation of extraction of nematodes from *Bursaphelenchus* genus from wood was performed as follows

Analytical sensitivity, repeatability: Not performed as there is not possible to contaminate wood samples.

Analytical specificity: Not performed as not applicable.

Selectivity, repeatability: Establishment the number of positive results of examination of wood coming from at least from three localities where *Bursaphelenchus mucronatus* was found and at least three localities where *Bursaphelenchus* spp. are absent but other nematodes occur; incubation time (10 days) and extraction time (4 days) is the same for all samples, but type of filters used on sieves placed in Baermann funnels is different

Reproducibility: Ring test on extraction of nematodes from wood samples coming from localities where *Bursaphelenchus* spp. were present and absent. by 18 laboratories.

Evaluation of budget of uncertainty: Calculations basing on calculation of all possible factors which may cause uncertainty of results.

Validation of identification of nematodes from *Bursaphelenchus* genus, „*xylophilus*” group was performed as follows

Morphological and morphometrical methods intended for identification

Analytical sensitivity Establishment the minimum number of individuals to perform a reliable identification with a minimum of three repetitions whenever possible. *Bursaphelenchus xylophilus*, *B. mucronatus* were used.

Analytical specificity Screening against a range of morphologically similar target and non target organisms. Screening using *Bursaphelenchus xylophilus*, *B. mucronatus* and *Bursaphelenchus* out of „*xylophilus*” group was performed

Selectivity Not performed as not applicable.

Repeatability 8 identifications of the same slide as a blind test within a range of morphologically similar target and non target organisms were done *Bursaphelenchus xylophilus*, *B. mucronatus*, *Bursaphelenchus* out of „*xylophilus*” group and *Aphelenchodes* spp. were used

Reproducibility Ring test on identification of in 10 microscopic slides to *Bursaphelenchus* genus, „*xylophilus*” group by morphometrics by 17 laboratories; nematodes from and out of this group and nematodes from Aphelenchida, but out of *Bursaphelenchus* genus were used; ring test on identification of nematodes extracted from wood (see previous slide), by 18 laboratories.

Evaluation of budget of uncertainty Calculations basing on calculation of all possible factors which may cause uncertainty of results.