INTERNATIONAL SEED TESTING ASSOCIATION (ISTA)

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14th ISTA GMO Proficiency Test

Soybean

(*Glycine max.*)

Technical Protocol

The aim of the proficiency test is to check the ability of individual laboratories in detecting GM seeds and in quantifying and identifying their presence in samples of conventional seeds of soybean. The choice of method is at the participants' discretion. The test leader advises all participants to wash the seeds prior to conducting the analyses.

Additional information:

The result forms allow for additional information next to the analysis results. This optional information is provided at the participants own discretion. However, this data is used to contribute to further improving the programme and we strongly encourage all laboratories to share this information with the test leader, with reassurance that confidentiality arrangements apply accordingly.

Sample description

Each participating laboratory receives 12 numbered soybean seed samples. Individual samples contain approximately 3000 seeds based on the 1000 seed weight. Some of the samples have been spiked with GM seed of any the following events: MON89788 and/or A2704. Reference material suppliers can be found on the ISTA GM information platform for GM seeds (http://www.seedtest.org/en/content---1--1195.html). Sample material was tested for specified events only, NOT all possible contaminants.

1 Qualitative Test (Detection)

Please indicate the positive samples (i.e. containing GM seeds) on the qualitative test result sheet (1.1). The qualitative result can be either derived from the quantitative test result or from a separate test on the sample. Not ticking a box means that for the corresponding sample no GM seeds could be detected.

As voluntary information you may state the method you have used and further details.

2 Quantification of GM seeds in positive samples

Laboratories must decide to **either** perform a sub-sampling quantification (semi-quantitative test, report sheet 2.A.) **or** a quantitative test (report sheet 2.B). The rating for the quantitative results will not take into account the samples with stacked events.

2.A. Sub-sampling Quantification (Semi-quantitative test)

Please report the number of sub-samples tested, the size of sub-samples (in number of seeds), the number of positive sub-samples and the estimate of percentage GM seeds (2.A.1).

Laboratories are advised to use seedcalc8, freely available at the ISTA Website http://www.seedtest.org/en/stats_tool_box_content---1--1143.html, 'Qual Impurity Estimation').

Please state the method you have used (2.A.2.). Additional information regarding the method and its estimated false positive and false negative rate may be included at the laboratory's discretion.

2.B. Quantitative Test

If a laboratory chooses quantification by a quantitative test such as real-time PCR, results shall be reported in result sheet 2.B and 2.C (2 pages). Reporting in 2.B.1 shall be to two decimal places; if an interval is reported (e.g. between 0.10 and 0.90) a single value must be provided alongside.

The laboratory shall report the overall GM concentration for each test sample (2.B.1, Total). Results shall be reported as a percentage based on the number of seeds (column 1) or on the seed sample mass (column 2).

Example:

Calibration curve by mass fraction: Typically, the laboratories use a certified reference material (CRM) by mass fraction (g/kg). There are generally two alternatives to prepare such a calibration curve. The one is to use directly a certain amount of DNA (e.g. 100 ng) extracted from a set of available CRMs (0.1%, 0.5%, 1%, 2%, 5%); the other is to use a high GM % of CRM (e.g. 5% or 10%), to make serial dilutions. Even if the copy number is introduced by multiplying the DNA quantity by the 1C value, the quantitative results obtained using the two types of calibration curve shall be reported as %mass.

The result should be understood as the ratio of the specific trait target to the taxon specific target.

There is the option to report results additionally in other units, such as % DNA copies (column 3). **Participants are advised to report, if ever possible, either in % mass or % number of seeds. The samples' reference values (spiking levels) for these two units are known and are most suitable for the evaluation of participants' performance.** If results are only provided in column three, these will be evaluated against the samples median result values, calculated from all participants using column 3 for reporting.

The raw data related to the quantitative results are of particular interest when evaluating laboratory performance. They should be provided, if ever possible in electronic format, as indicated in the result sheet 2.C.

3 Identification Test

Identification of the events used in each sample is optional. Result sheet 3.1. may be used to indicate the presence or absence of the two events in each sample, or, alternatively, the quantification results for each event.

Please note that no differentiation between stacked/ non-stacked samples is required. However, the 'remarks' section can be used for providing additional findings.

Pease state the method you have used and details, e.g. immunoassay strip test, real-time PCR (3.2). There is an option to provide further information such as grinding method; DNA extraction method; target for PCR, ELISA or bioassay; primer sequences; test kit; method publication/validation references.

The deadline for submitting results to the ISTA Secretariat is before 1 November 2010**.**