

5th ISTA Proficiency Test on GMO Testing on *Glycine max* (L.) Merr.

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1. AIM

The aim of the proficiency test is to check the ability of individual laboratories to detect the presence or absence of GM seeds and to quantify their presence in samples of conventional seed of soybean *Glycine max*.

2. EXPERIMENTAL DESIGN

Samples were either negative, i.e. did not contain any transgenic events, or positive, i.e. contained the transgenic event GTS 40-3-2 (GTS40) or A2704-12 (A2704). When preparing the positive samples different quantities of GTS40 or A2704 seeds were mixed with non-GM seeds. The genetic purity was tested prior to the sample preparation.

The GTS40 seeds in the samples have the 35S promotor, the NOS terminator and the CP4 EPSPS gene. The CP4 EPSPS gene encoding the EPSPS protein (5-enolpyruvylshikimate-3-phosphate synthase) confers tolerance to the glyphosate herbicide (the active ingredient in Roundup Ready®). This transgenic event was already used in the 4th ISTA Proficiency Test on GMO Testing. The

A2704 seeds in the samples have two copies of the 35S promotor and a single copy of the PAT gene. The PAT gene encoding the PAT enzyme (phosphinothricin acetyltransferase) confers tolerance to glufosinate ammonium (also known as phosphinothricin herbicide tolerance).

Each participating laboratory received a set of 12 soybean samples each containing about 3000 seeds. The positive samples were made positive by adding a defined number of seeds from the GTS40 or A2704 seed lot to the negative seeds. For each sample, the non-GM seeds were weighed and the GM seeds were counted and the weight determined. For laboratories performing sub-sampling quantification, also the non-GM seeds were counted.

Three samples were negative and nine samples were positive. Five out of the nine positive samples were the same for all laboratories: two samples contained 0.2% GTS40 seeds and three samples contained 1.0% A2704 seeds. Four samples differed in their GMO content between the laboratories. One-

third of the labs received either samples with a GTS40 content of 0.5%, 1.0% or 1.5%. Table 1 and 2 give detailed information about the arrangement of the samples and the spiking levels.

The choice of the method used for testing was at the laboratory's discretion.

3. RESULTS

Sixty-four laboratories received samples. Fifty-eight submitted their results. Three (5%) submitted only qualitative results. Thirteen (20%) performed the quantification using the sub-sampling strategy. Forty-one (64%) reported quantitative results performing a quantitative test. One (2%) submitted results for both tests, sub-sampling quantification and quantitative test and six (9%) did not report data.

3.1 Descriptive Statistics of the Qualitative Results

Each laboratory reported for the individual sample whether this is a negative sample or

Table 1: Detailed information about disposition of the samples.

Label	A-C	D-E	F-I	K-M
# of samples	3	2	4	3
events	-	GTS 40-3-2	GTS 40-3-2	A2704-12
GM spiking level by # of seeds	0%	0.2%	0.5% or 1.0% or 1.5%	1.0%

Table 2: Detailed information about the spiking levels of the test samples: spiking levels by number and by mass of seeds, the number and average weight of GM and non-GM seeds per sample.

GM spiking level by # of seed	0%	0.2%	0.5%	1%	1.5%
Average GM spiking level by mass of seed of GTS 40-3-2	-	0.20% ± 0.01%	0.50% ± 0.02%	0.98% ± 0.03%	1.47% ± 0.04%
Average GM spiking level by mass of seed of A 2704-12	-	-	-	1.01% ± 0.04%	-
# of non GM seeds ¹	3000	2994	2985	2970	2955
# of GM seeds ¹	0	6	15	30	45
Weight of non-GM seeds ²	~412 g	~411 g	~410 g	~407 g	~406 g
Weight of GM seeds (GTS 40-3-2) ²	0 g	~0.81 g	~2.06 g	~4.13 g	~6.06 g

¹samples for laboratories performing sub-sampling quantification.

²samples for laboratories performing a quantitative test, e.g. RT-PCR.

a positive sample. Hence, for a given sample, the result reported by the laboratory can be either correct or false (Figure 1 and Table 3).

Out of the 58 laboratories:

- Thirty-two laboratories classified all 12 tested samples correctly. These are 55.2% of the laboratories.
- 89.1% of the 696 samples were reported correctly by the 58 laboratories.
- In total, 26 laboratories reported results falsely, one laboratory reported both, false positives and false negatives and 25 laboratories only false negatives. No laboratory reported only false positives.
- One laboratory reported false positives (one out of the three negative samples (1/3)) with a total number of one out of 174 negative samples tested. These are 1.7% of the laboratories and 0.6% of the negative samples.
- Twenty-six laboratories reported false negatives (between 1/9 and 3/9) with a total number of 75 out of 522 positive samples tested. These are 44.8% of the laboratories and 14.4% of the positive samples. All 26 laboratories reported false negatives for the A2704 samples with a spiking level of 1.0%. Between 1/3 and 3/3 samples were classified falsely as negative with a total number of 75 samples out of 174. These are 44.8% of the laboratories and 43.1% of the A2704 samples.
- Zero laboratories reported false negatives for the GTS40 samples with a spiking level of 0.2%, 0.5%, 1.0% or 1.5%.

3.2 The Quantitative Results

Fourteen laboratories used the sub-sampling strategy for a quantification. All laboratories reported the number of sub-samples, the size of these sub-samples and the number of positive sub-samples tested. Most laboratories used a testing plan with a high number of sub-samples. All laboratories used all 3000 seeds to create the sub-samples. Figure 2 shows the estimate of the percentage of GM seeds in a sample reported by the laboratories.

Forty-one laboratories performed the quantitative test, e.g. using RT-PCR, and reported for the individual test sample the estimated value of the GM content as the percentage seed in number of seeds or mass of seeds or other units, e.g. percentage of DNA. (Figure 3).

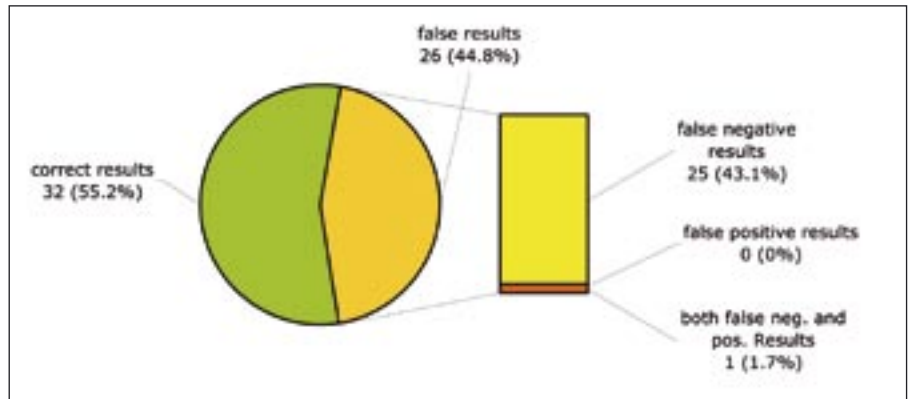


Figure 1: Percentage of laboratories reporting correct and false results.

Table 3: Number and percentage for which false results were reported.

	# of samples tested	# of false results	% of false results
All samples	696	76	10.9%
Negative samples	174	1	0.6%
Positive samples	all	75	14.4%
0.2% GTS40 content	116	0	0.0%
0.5% GTS40 content	76	0	0.0%
1.0% GTS40 content	76	0	0.0%
1.5% GTS40 content	80	0	0.0%
1.0% A2704 content	174	75	43.1%

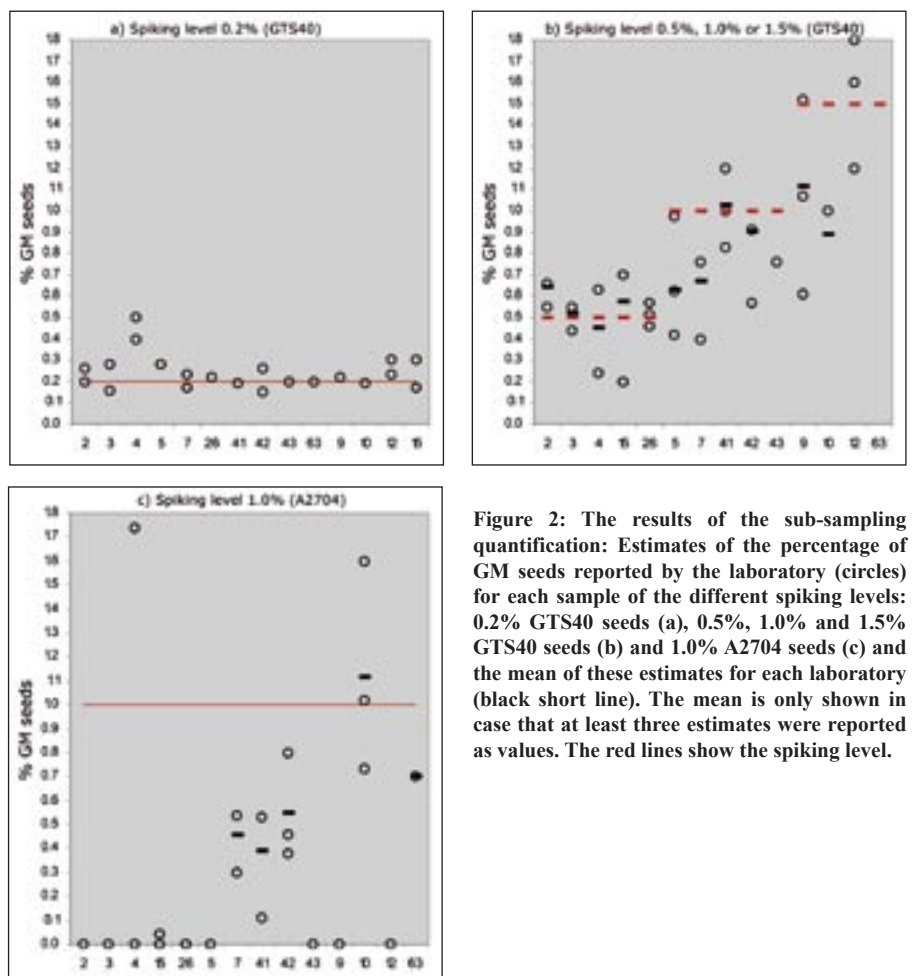


Figure 2: The results of the sub-sampling quantification: Estimates of the percentage of GM seeds reported by the laboratory (circles) for each sample of the different spiking levels: 0.2% GTS40 seeds (a), 0.5%, 1.0% and 1.5% GTS40 seeds (b) and 1.0% A2704 seeds (c) and the mean of these estimates for each laboratory (black short line). The mean is only shown in case that at least three estimates were reported as values. The red lines show the spiking level.

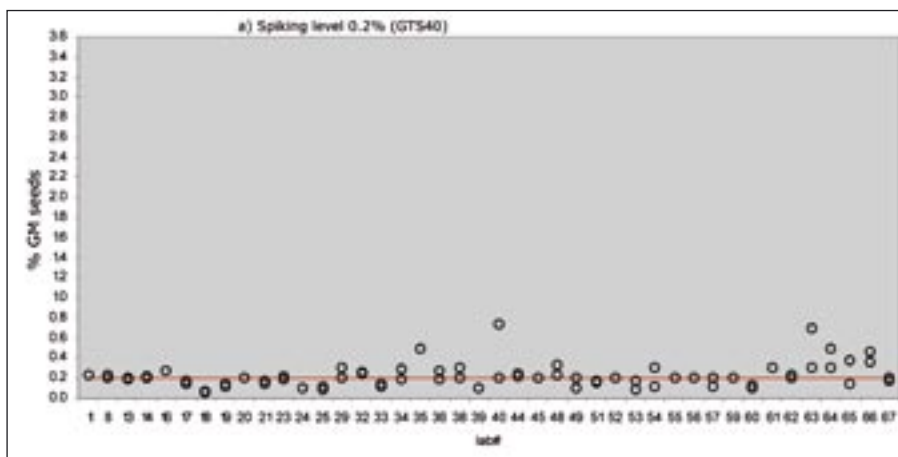


Figure 3: The results of the quantification: Estimates of the percentage of GM seeds reported by the laboratory (circles) for each sample of the different spiking levels: 0.2% GTS seeds (a), 0.5% GTS40 seeds (b), 1.0% GTS seeds (c), 1.5% GTS40 seeds (d), 1.0% A2704 seeds (e) and the mean of these estimates for each laboratory (short line). The mean is only shown in case that at least three estimates were reported as values.

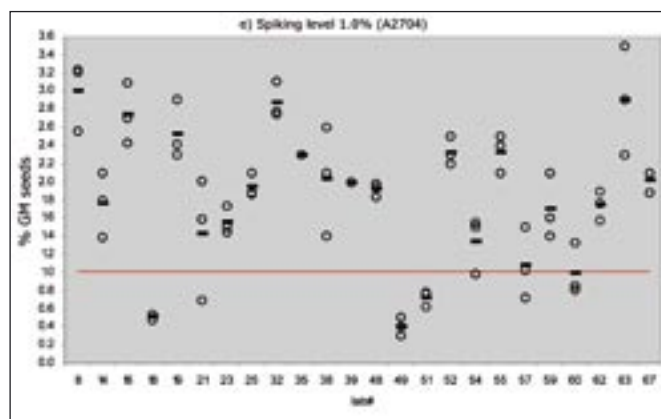
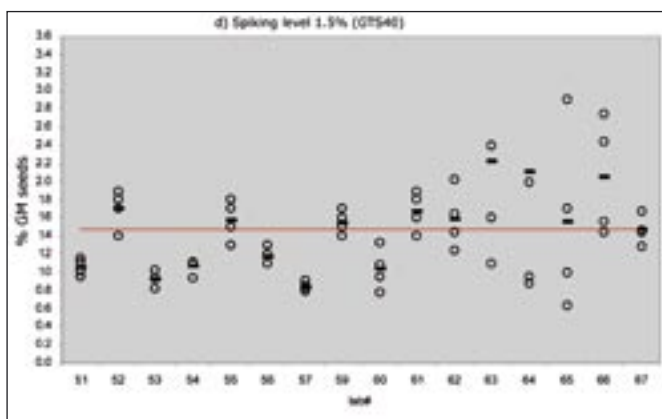
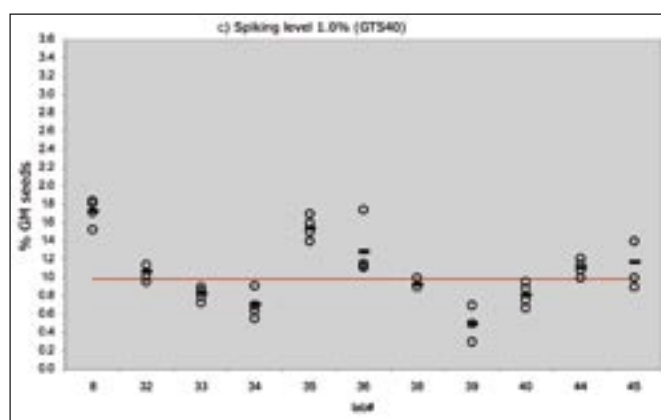
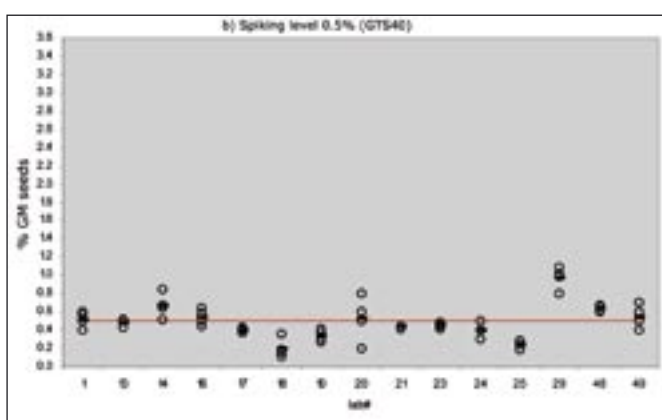


Table 4: Quantitative test - The (overall) mean of the quantitative test results for each spiking level, the standard deviation, the variation coefficient and the relative error among the samples within each spiking level. The standard deviation, the variation coefficient and the relative error in this table are related to the single results per sample and not to the laboratories' means.)

Spiking level and transgenic event	Replicates	Mean ± SD (%)	Variation coefficient (%)	relative error (%)
0.2% GTS40	116	0.23 ± 0.12	51.4	16.4
0.5% GTS40	76	0.48 ± 0.16	33.2	10.9
1.0% GTS40	69	0.99 ± 0.34	34.7	40.5
1.5% GTS40	79	1.47 ± 0.67	45.6	16.4
1.0% A2704	88	1.63 ± 0.85	52.1	82.0

Table 4 shows the overall performance of the laboratory regarding the different sample groups, i.e. spiking level and event: The (overall) mean of the quantitative and sub-sampling test results for each spiking level, the standard deviation, the variation coefficient and the relative error among the samples within each spiking level. The variation coefficient (% variation coefficient =

standard deviation/mean*100) shows the inter-sample variability. The lowest variation coefficient was calculated for the 0.5% and 1.0% spiking level of GTS40 samples with 33.2% and 34.7%. The highest were calculated for the 0.2% GTS40 and the 1.0% A2704 samples with 51.4% and 52.1%, respectively. These variation coefficients are similar to the ones of previous test rounds. The relative er-

ror (% relative error = [reported value – true value]/true value*100) shows the closeness of agreement between the reported value (test result) and the true value. The samples with the lowest relative error are the 0.5% GTS40 samples (10.9%) and with the highest relative error are the 1.0% A2704 samples (82.0%).



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Registration deadline: November 30, 2006

Registration forms and further details on proficiency tests can be found on the ISTA Website at www.seedtest.org

Laboratories interested in participating should please contact the ISTA Secretariat:
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