

6th ISTA Proficiency Test on GMO Testing on *Brassica napus* L.



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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 oil rape seed, *Brassica napus*.

2. Experimental Design

Samples were either negative, i.e. did not contain any transgenic events, or positive, i.e. contained the transgenic event GT73 (synonym RT73). When preparing the positive samples different quantities of GT73 seeds were mixed with non-GM seeds. The genetic purity was tested prior to the sample preparation.

The GT73 seeds in the samples have the figwort mosaic virus (FMV) 35S promotor, the CP4 EPSPS and goxv247 genes. The CP4 EPSPS gene encoding the CP4 EPSPS protein (5-enolpyruvylshikimate-3-phosphate synthase) confers tolerance to the glyphosate herbicide (the active ingredient in Roundup Ready®). The gox247 gene encoding a modified version of glyphosate oxidase, a bacterial enzyme from *Ochrobactrum anthropi* improves the affinity of the enzyme for glyphosate.

Each participating laboratory received a set of 10 oil rape seed samples each containing about 3300 seeds. The positive samples were made positive by adding a defined number of seeds from the GT73 seed lot to the negative seeds. For each sample, the non-GM seeds were weighted and the GM seeds were counted and the weight determined. Three samples were negative and seven samples were positive. Two samples contained 0.3% GT73 seeds, three samples contained 0.6% and two samples contained 1.2% GT73 seeds.

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

3. Results

Fifty laboratories received samples. Forty-nine submitted their results. Fourteen laboratories submitted only qualitative results. Twelve laboratories performed the quantification using the sub-sampling strategy. Twenty-three laboratories reported quantitative results performing a quantitative test (e.g. RT-PCR). One laboratory did not report data.

The identity of the individual laboratories is kept confidential.

3.1 Descriptive Statistics of the Qualitative Results

Each laboratory reported for the individual sample whether this is a negative sample or a positive sample. This could be either derived from the quantitative test result, or from a separate test on the sample. For a given sample, the result reported by the laboratory can be either correct or false (Table 1).

Out of the 49 laboratories:

- Forty-five laboratories classified all ten tested samples correctly. These are 91.8% of the laboratories.
- 97.6% of the 489¹ samples were reported correctly by the 49 laboratories.
- In total, four laboratories reported results falsely reporting both, false positives and false negatives. These are 8.2% of the laboratories.
- The four laboratories reported false positives (between one (1/3) and three (3/3) out of the three negative samples) with a total number of eight out of 147 negative samples tested. These are 5.4% of the negative samples.

Table 1: Number and percentage of samples for which false results were reported.

	# of samples tested	# of false results	% of false results
All samples	489	12	2.4
Negative samples	147	8	5.4
Positive samples			
all	342	4	1.2
0.3% GT73 content	97	2	2.0
0.6% GT73 content	147	1	0.7
1.2% GT73 content	98	1	1.0

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• The four laboratories reported false negatives (1/7) with a total number of four out of 342¹ positive samples tested. These are 1.2% of the positive samples. Two laboratories reported false negatives for the samples with a spiking level of 0.3%. Both classified 1/3 samples falsely as negative with a total number of two samples out of 97¹. These are 2.0% of the samples. One laboratory reported false negatives for the samples with a spiking level of 0.6%. It classified 1/3 samples falsely as negative. This is one sample out of 147 or 0.7% of the 0.6% GM seed samples. One laboratory reported false negatives for the samples with a spiking level of 1.2%. It classified 1/3 samples falsely as negative. This is one sample out of 98 or 1.0% of the 1.2% GM seed samples.

3.2 The Quantitative Results

Twelve laboratory reported the number of sub-samples tested, the size of the sub-samples (number of seeds) and the number of positive sub-samples per sample (see Table 2). These elements were used by the laboratory to compute the estimate of the percentage of GM seeds (Figure 1).

¹Laboratory 13 reported data for nine samples, i.e. only for one of the two samples with a spiking level of 0.3%.

The seedcalc6 programme was recommended to use for designing the testing plan and to perform the computation (freely available on the ISTA Website).

This quantitative test was for checking the ability of the laboratories to quantify the GM seeds in a sample. The laboratories could use the method they thought appropriate. The results were given in percentage of GM seeds in the sample.

Twenty-three laboratories performed the quantitative test and reported for the individual test sample the estimated value of the GM content as the percentage GM seeds in number of seeds, percentage mass of GM seeds or percentage GMO DNA copies (Figure 2).

Table 3 shows the overall performance of the laboratory regarding the different spiking levels: 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 results show the

lowest variation for the spiking level 0.6% and similar variation for 0.3% and 1.2%. These variation coefficients are similar to the ones of previous test rounds. The relative error (% relative error = [reported value – true value]/true value*100) shows the closeness of agreement between the reported value (test result) and the true value. There is no significant difference between the relative errors.

3.3 Summary

The percentage of laboratories reporting correct qualitative results for all samples was higher as in the previous tests, i.e. >90% of labs made no misclassification, and >97% of samples were reported correctly.

The test plan selected by the laboratory for the sub-sampling quantification had a big influence on the results, i.e. test plans with a lower number of sub-samples led to results with a higher variation and distance from the true value and to missing values for the higher spiking level of 1.2%.

The quantitative results showed similar variation for the spiking levels (VC~50%) and there was a high number of laboratories overestimating the true value. ■

CALL FOR REGISTRATION

8th and 9th ISTA Proficiency Test on GMO Testing

Since GMO testing has been included in the ISTA Accreditation Programme, the participation in the ISTA Proficiency Tests on GMO Testing is compulsory for those laboratories which have GMO testing methods in their scope of accreditation. The ISTA GMO Proficiency Test Programme on GMO Testing is also open to all laboratories involved in GM seed testing. Your laboratory can select the method appropriate to detect the presence or absence of GM seeds, to quantify and to identify their presence in samples of conventional seeds.

8th ISTA Proficiency Test on GMO Testing on *Glycine max* (L.) Merr.

Your laboratory will receive 14 soybean test samples each of 3000 seeds, either containing GM seeds or not.

Registration deadline: December 31, 2006

9th ISTA Proficiency Test on GMO Testing on *Zea mays* L.

Your laboratory will receive maize samples, either containing GM seeds or not.

Registration deadline: April 30, 2007

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

Laboratories interested in participating should contact the:

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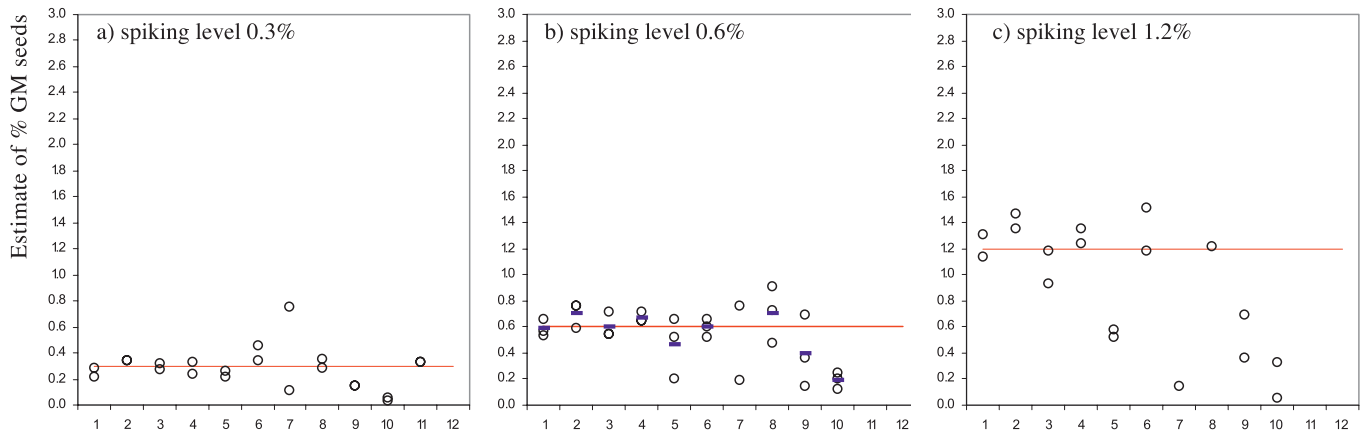


Figure 1: The results of the sub-sampling quantification: Estimates of the percentage of GM seeds reported by the laboratory (circles) for each sample of the different spiking levels: 0.3% (a), 0.6% (b) and 1.2% (c) GT73 seeds and the mean of these estimates for each laboratory (black short line). The mean is only shown in case that at least three estimates were reported as values. The red line shows the spiking level.

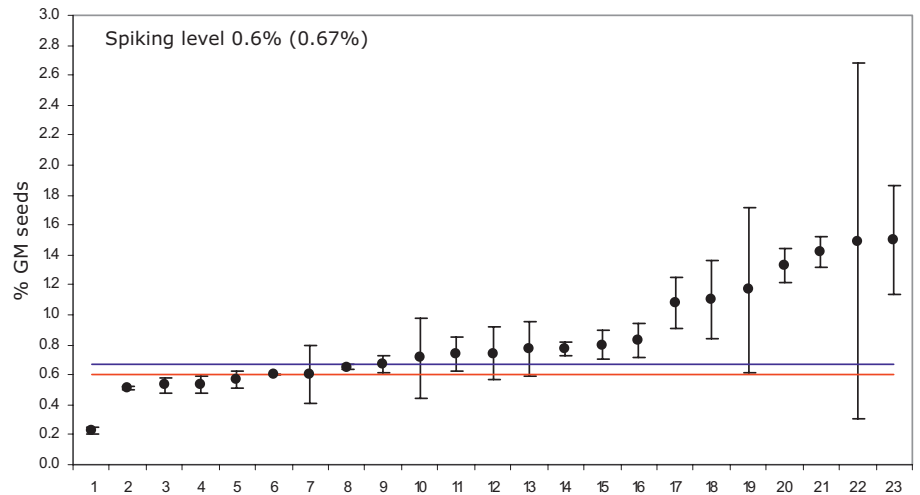


Figure 2: The results of the quantification: Estimates of the percentage of GM seeds reported by the laboratory (circles) for each sample of the spiking levels 0.6% (0.67% GT73 seeds, and the mean of these estimates for each laboratory and the standard deviation.

Table 2: The table shows testing plans used by the laboratories (lab #), i.e. number of sub-samples and the size of sub-samples (in number of seeds).

Lab #	# of sub-samples	Size of sub-samples
1	~300	10
2	~90	30
3, 4	~30	~110
5, 6	20-25	~150
7, 8	15	210-220
9, 10, 11	5-10	330-550
12	2-3	

Table 3: 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 (%)	Replicates	Mean ± SD (%)	Variation coefficient.	Relative error (%)
0.3/0.33	65	0.38 ± 0.22	59.3	17.9
0.6/0.67	94	0.80 ± 0.40	49.6	19.0
1.2/1.35	61	1.57 ± 0.90	57.9	20.0