

# 2<sup>nd</sup> ISTA Proficiency Test on GMO Testing on *Zea mays* (MON810)

## Summary of the Results

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### 1. AIM

The aim of this 2<sup>nd</sup> ISTA Proficiency Test on GMO Testing is to check the ability of individual laboratories to detect and, on a voluntary basis, to quantify the presence of GM seeds in samples of conventional seed of *Zea mays* L.

The object of data analysis will not be to identify deviating laboratories but to compile the performances in the laboratories and to provide data for the laboratories' internal performance data base.

### 2. EXPERIMENTAL DESIGN

For this proficiency test two different maize seed lots were provided: a non GM and a MON810 GM seed lot. The MON810 material was kindly provided by KWS Saat AG, Germany. For checking genetic purity, 30,000 seeds of the negative seed lot were tested and proved to be negative. This test was made in the laboratory of Norbert Leist, Staatliche Landwirtschaftliche Untersuchungs- und Forschungsanstalt Augustenberg, Germany. From the positive seed lot 400 seeds were individually tested and proved to be positive. These results were kindly provided by KWS Saat AG.

The samples to be sent out were prepared by Michael Kruse and his personnel of the Institute of Plant Breeding, Seed Science and Population Genetics, University of Hohenheim. Each participating laboratory received a set of 10 maize samples. Samples were numbered randomly from 1 - 10, for example, positive samples due to MON810 sent to laboratory 1 could have the same or a different number as compared to those sent to laboratory 2. Each sample contained about 3000 seeds (determined by weight) and was labelled only with a random sample and

laboratory number. Based on the testing of the negative seed lot, the potential accidental content of positive seed is below 0.01% with 95% confidence.

Three samples were negative (no GM seeds added) and 7 samples were positive. The positive samples were made positive by adding seeds from the MON810 seed lot to negative seeds. For three samples, 7.3g seeds from the MON810 seed lot were added to 1036.7g negative seeds so that the expected value for the GMO content in these positive samples is 0.7% by weight (approximately 21 seeds in 3000). For four samples, 14.6g seeds from the MON810 seed lot were added to 1029.4 g negative seeds so that the expected value for the GMO content in these positive samples is 1.4% by weight (approximately 42 seeds in 3000). To avoid cross contamination, the negative samples were prepared first, whereas the positive samples were prepared after sealing all negative bags. The samples were packed in aluminium foil bags to avoid any cross contamination during sample handling and shipment.

The shipment of the samples to the participants started on 28 February 2003 after the laboratories signed the Material Transfer Agreement. No laboratory reported open bags per shipment.

#### *Obligatory qualitative test*

Only a qualitative result was requested. Laboratories could use the method they thought appropriate for this test. The results for the qualitative test, i.e. a sample is positive or negative, had been submitted for each sample along with the sample identification number provided by ISTA. Participants were not expected to identify the events in the positive samples.

#### *Optional quantification of GMO in positive samples*

On a voluntary basis laboratories could do a quantification of the GMO content in the positive samples by either a semi-quantitative test (sub-sampling strategy) or by a quantitative test.

#### **Semi-quantitative test (sub-sampling strategy)**

A semi-quantitative test using the sub-sampling strategy was optional to the participants. The participants should report as a result of this test whether the GMO content in the test sample was above or below the level of 1% and the testing plan, i.e. number of sub-samples, size of sub-samples (number of seeds), the number of positive sub-samples per sample, the false positive and false negative rate which was used for calculation of results, and the decision rule (maximum number of positive samples to accept = 1%). The laboratories could use the method they thought appropriate for this test. The SEED-CALC programme was recommended to use for designing the testing plan (available on the ISTA Website).

#### **Quantitative test**

The quantitative test was also optional. This quantitative test is for checking the ability of the laboratories to quantify the GMO content in a sample. The participants should report the quantitative estimated value of the GMO content of the test sample, the 95% confidence interval lower and upper limit, and should give information about the method used. The laboratories could use the method they thought appropriate. It was optional to report if the result is above or below 1%.

*The Summary of Results is continued on page 16*

3. RESULTS

The acceptance of results for the evaluation was closed on 27 June 2003. 51 laboratories out of 52 received the samples and 47 submitted their results. All 47 laboratories reported qualitative results that could be evaluated. 13 laboratories reported semi-quantitative test results that could be evaluated. 19 laboratories reported quantitative test results, with evaluable data, and performed in total 20 test series since one laboratory applied two different methods.

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. There was no identification or quantification requested. So, for a given sample, the result reported by the laboratory can be either correct or false.

Out of the 47 laboratories:

- 40 laboratories reported results without any false results, all 10 tested samples were classified correctly. This is 85% of the laboratories.
- 96.4% of the 470 samples were reported correctly by the 40 laboratories.
- In total, 7 laboratories reported false results, 3 laboratories reported both, false positive results and false negative results, 2 laboratories only false negatives and 2 laboratories only false positives.
- 5 laboratories reported false positive results (between 1 and 3 out of the 3 negative samples (1/3) and 3/3) with a total number of 7 out of 141 negative samples tested. This is 10.6% of the laboratories and 5.0% of the negative samples.
- 5 laboratories reported false negative results (between 1/7 and 3/7) with a total number of 10 out of 329 positive samples tested. This is 10.6% of the laboratories and 3% of the posi-

Table 1: Number and percentage of all, negative and positive samples reported incorrectly.

	# of samples tested	# of samples reported incorrectly	# of samples reported incorrectly
All samples	470	17	3.6%
Negative Samples	141	7	5.0%
Positive samples all	329	10	3.0%
0.7% GMO content	141	8	5.7%
1.4% GMO content	188	2	1.1%

Table 2: The table shows the negative samples (column A to C, light yellow), the samples with a GMO content of <1% (0.7%, column D to G, dark yellow) and of >1% (1.4%, column H to K, orange), respectively. Further, information about the testing plan, i.e. number of sub-samples, size of sub-samples, maximum number of positive samples to accept =1%, and the false positive and false negative rate is given. The rows are sorted first by number of sub-sample and second by size of sub-samples. If a laboratory reported that a sample with a GMO content of 0.7% has a GMO content >1% or that a sample with a GMO content of 1.4% has a content <1%, the cells are marked red. If a laboratory reported false positive or false negative results the cell is also marked red and pos and neg, respectively.

Lab	Classification of 10M seeds reported by the laboratory										Information about Testing Plan:				
	A	B	C	D	E	F	G	H	I	K	# of sub-samples	size of sub-samples	Max.# of pos. samples to accept > 1%	fpr	fnr
1											3	100	0	0.00	0.00
2											3	400	2	1.00	1.00
3											3	1000	2	0.00	0.00
4											4	60	2	0.00	0.00
5											5	70	0	0.00	0.00
6											5	150	2	0.00	0.00
7											5	200g***	1		
8											6	75	0		
9											0	200	3 resp. 4***	0.01	0.01
10											10	300	5	0.01	0.01
11											3-20	150	0 out of 3 or 7 out of 13		
12											30	100	70		
13											20	100	70		

\* Test plan reported by laboratory:

	A	B	C	D	E	F	G	H	I	K
#	20	19	19	13	13	3	13	3	3	3

\*\* 200g is approximately 600 seeds.  
 \*\*\* 3 at 99% CL; 4 at 95% CL.

tive samples.

- With respect to the spiking level, 5 laboratories reported false negative results with positive samples of a 0.7% GMO content. Between 1/3 and 2/3 samples were classified falsely as negative with a total number of 8 samples out of the 141 positive samples of 0.7% GMO content. These are 10.6% of the laboratories and 5.7% of the 0.7% content samples.

- With respect to the spiking level, 2 laboratories reported false negative results with positive samples of a 1.4% GMO content. Each classified 1 out of 4 samples falsely as negative with a total number of 2 samples out of the 188 positive samples of 1.4% GMO content. These are 4.3% of the laboratories and 1.1% of the 1.4% content samples.

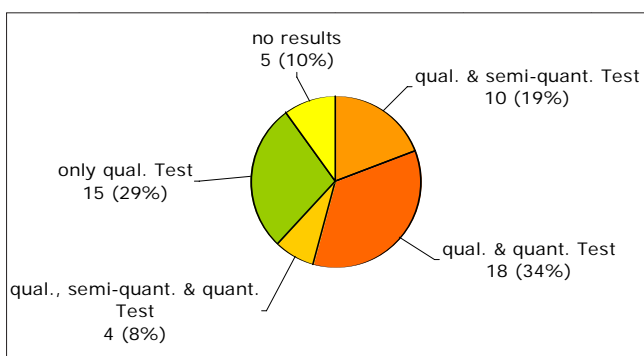


Figure 1: Performed tests by the participating laboratories.

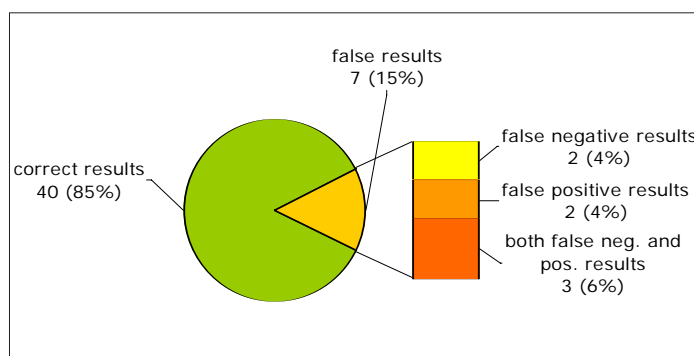


Figure 2: Percentage of laboratories reporting correct and false results.

**3.2 The Semi-quantitative Results**

Each laboratory reported for the individual sample as a result of this test whether the GMO content is below or above 1% as well as the testing plan. One laboratory out of 13, which used this approach, classified all samples correctly above or below 1% GMO content. Eleven laboratories had difficulties in categorizing the samples accordingly. Between one and five samples were classified falsely. More misclassifications are reported in case of a low number of sub-samples and if only a fraction of the whole samples was used for testing.

**3.3. The Quantitative Results**

Nineteen laboratories, performing the quantitative test in 20 test series, reported for the individual test sample the estimated value of the GMO content as the percentage of e.g. haploid genomes, DNA or seed by mass (Fig. 3 and 4).

Twenty one laboratories reported (in 22 test series) for each sample if the value was above or below 1% (Fig. 5). Three laboratories did not report values for the GMO content of the samples but categorised the samples. Four laboratories classified correctly. Eight laboratories reported the samples with the 0.7% GMO content as above 1%. Between 1 and 3 out of 3 samples were classified falsely. Nine laboratories reported (in 10 test series) the samples with the 1.4% GMO content as below 1%. Between 1 and 4 out of 4 samples were reported falsely. ■

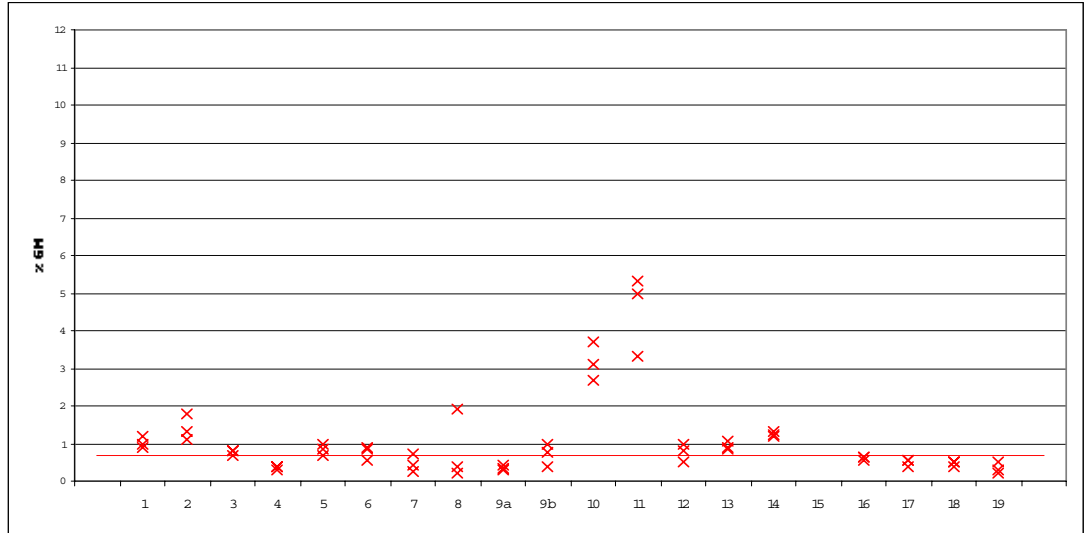


Figure 3: Percentage of GMO (based on e.g. haploid genomes or seed by mass) reported by the laboratories for the seed samples with a spiking level of 0.7% (3 samples per laboratory). (The results of laboratory 15 exceed 12%.)

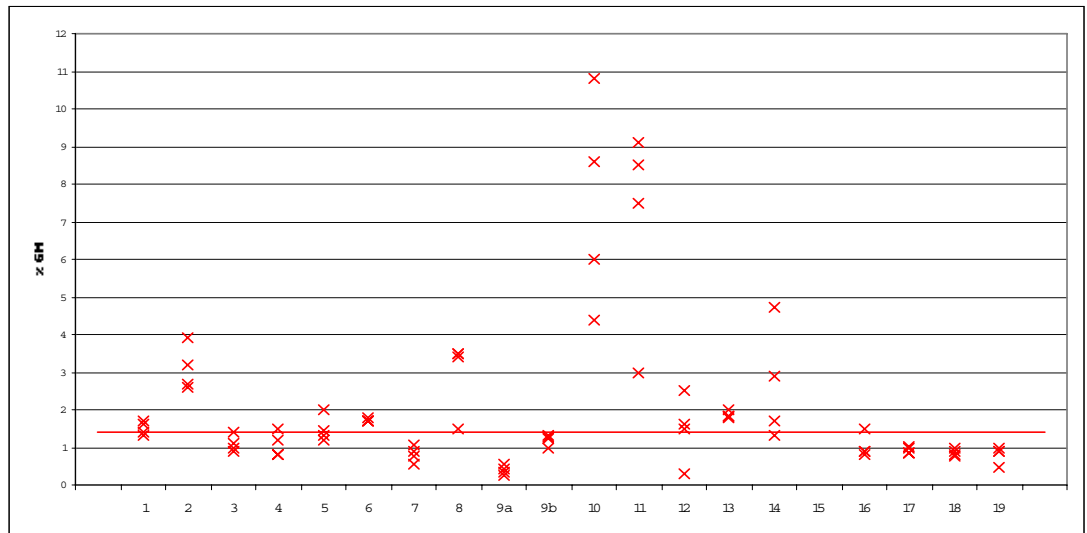


Figure 4: Percentage of GMO (based on e.g. haploid genomes or seed by mass) reported by the laboratories for the seed samples with a spiking level of 1.4% (4 samples per laboratory). (The results of laboratory 15 exceed 12%.)

lab	Classification for negative and positive samples (<1% and >1%)										
	A	B	C	D	E	F	G	H	I	J	K
1	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow
2	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow
3	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow
4	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow
5	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow
6	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow
7	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow
8	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow
9a	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow
9b	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow
10	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow
11	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow
12	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow
13	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow
14	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow
15	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow
16	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow
17	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow
18	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow
19	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow

Figure 5: Classification for negative (light yellow) and positive samples: <1% (dark yellow) and >1% (orange), reported by the laboratories performing the quantitative test. If a laboratory reported for samples with a spiking level of 0.7% that the GM content was >1% and for samples with a spiking level of 1.4% that the GM content was <1%, respectively, the relevant table cell is marked red. Laboratory in the first row did not report a classification for the samples.

**Announcement of the 3<sup>rd</sup> ISTA Proficiency Test on GMO Testing**

The 3<sup>rd</sup> proficiency test will start in October 2003. Laboratories interested in participating should please contact the ISTA Secretariat for more information.

Application forms are also available on request:  
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 fax: +41 1 838 6001