

USDA/GIPSA Proficiency Program
Testing for the Presence of Biotechnology Events in Corn and Soybeans
May 2009 Sample Distribution Results

Purpose of USDA/GIPSA Proficiency Program

Through the USDA/GIPSA Proficiency Program, USDA seeks to improve the overall performance of testing for biotechnology-derived grains and oil seeds. The USDA/GIPSA Proficiency Program helps organizations identify areas of concern and take corrective actions to improve testing accuracy, capability and reliability.

Program Description

In this round of the USDA/GIPSA Proficiency Program sample distribution, one set of samples was used for both qualitative and quantitative analyses. The samples were fortified with various combinations and concentrations of transgenic traits, and participants had the choice of providing qualitative and/or quantitative results. Scoring of the participant's qualitative results was done by computing the percentage of correctly reported transgenic traits in the samples (Tables 1 to 34, and Figure 1). The percentage false positive and percentage false negative were calculated by dividing the number of incorrectly reported results by the number of provided negatives or provided positives that were distributed to the participants. To assess accuracy of individual participants' submitted quantitative results for a specified transgenic event, z-scores (reported value – fortification value / standard deviation) were computed for each reported quantification result (Tables 37 to 49). Tests for outliers and z-scores assume a normal distribution. At the 0% and 0.1% fortification levels, the distributions are not likely normal and are probably skewed. A result greater than 0 for the 0.0% spike level would probably be considered an outlier. At the 0.1% level, outlier tests will likely declare more outliers than should be declared. Some judgment will be necessary when interpreting data at these low levels. For levels higher than 0.1%, outliers were not included in the standard deviation used to compute the z-scores. Z-scores that are > 2 should be scrutinized by the participating lab. Those that are > 3 are clearly suspect and corrective action should be taken by the participating laboratory. Prior to computing the z-scores, outliers in the distribution of values were eliminated by use of the "Grubb's Test for Outliers." To evaluate the performance as a group (i.e., inter-laboratory variation), a summary table (Table 51) was prepared to show the accuracy and precision of the composite quantification results at each fortification level for the various transgenic events.

Sample Composition

The corn samples contained various combinations and concentrations of the following transgenic traits: T25, CBH351, MON810, GA21, Bt-176, Bt-11, NK603, Herculex, MON863, Herculex RW, MIR 604, Event 3272; or no events (i.e., negative corn sample). The various transgenic concentration levels were produced on a percentage weight-weight basis (%w/w). A calculated amount of ground transgenic corn was blended to homogeneity with a calculated amount of non-transgenic corn to produce concentrations ranging from 0.1 to 2.0% of a specified event. The soybean samples were either non-transgenic soybeans, or fortified soybean samples containing 0.1 to 0.5% of the transgenic glyphosate-tolerant soybeans (RoundUp Ready®). Each participant received six corn and three soybean samples. Each sample contained approximately 15 grams of ground material.

Program Participants

Participants included organizations from Africa, Asia, Europe, North America, and South America. Each participant received a study description and a data report form by electronic mail, and included with the samples. Participants submitted results by electronic mail, FAX, or regular mail. No analytical methodologies were specified, and organizations used both DNA- and protein-based testing technologies. Forty-eight organizations received samples in the May 2009 round of proficiency testing, and 42 organizations submitted results.

- Sixteen participants submitted **qualitative** results only (12 DNA- and 4 protein-based),
- Eleven submitted **quantitative** results only, and
- Fifteen participants submitted a combination of **qualitative** and **quantitative** results.

In this report, participating organizations are identified by a confidential “Participant Identification Number.” Appendix I identifies those organizations who gave GIPSA permission to list them as participants in the USDA/GIPSA Proficiency Program; some listed organizations requested anonymity.

Data Summary Results

Data submitted by the participants are summarized in this report primarily in tables and figures. Participants reported their results on a qualitative basis, quantitative basis, or a combination of both qualitative and quantitative bases. Qualitative results were reported as the presence or absence of a particular event in each sample. Quantitative results were reported as the concentration of a particular event in the sample. Due to the complexity of the data, this report summarizes the data as follows:

Qualitative Data Summaries.

This section summarizes qualitative sample analysis data:

- Table 1: Qualitative results for corn fortified with 35S for all participants (DNA-based assays).
- Table 2: Percentages of correct results, false negatives, and false positives in qualitative reports for 35S for all participants.
- Table 3: Qualitative results for corn fortified with NOS for all participants (DNA-based assays).
- Table 4: Percentages of correct results, false negatives, and false positives in qualitative reports for NOS for all participants.
- Table 5: Qualitative results for corn fortified with T25 for all participants (DNA-based assays).

- Table 6: Percentages of correct results, false negatives, and false positives in qualitative reports for T25 for all participants.
- Table 7: Qualitative results for corn fortified CBH351 with for all participants (DNA-based assays).
- Table 8: Percentages of correct results, false negatives, and false positives in qualitative reports for CBH351 for all participants.
- Table 9: Qualitative results for corn fortified with MON810 for all participants (DNA-based assays).
- Table 10: Percentages of correct results, false negatives, and false positives in qualitative reports for MON810 for all participants.
- Table 11: Qualitative results for corn fortified with GA21 for all participants (DNA-based assays).
- Table 12: Percentages of correct results, false negatives, and false positives in qualitative reports for GA21 for all participants.
- Table 13: Qualitative results for corn fortified with Bt176 for all participants (DNA-based assays).
- Table 14: Percentages of correct results, false negatives, and false positives in qualitative reports for Bt176 for all participants.
- Table 15: Qualitative results for corn fortified with Bt-11 for all participants (DNA-based assays).
- Table 16: Percentages of correct results, false negatives, and false positives in qualitative reports for Bt-11 for all participants.
- Table 17: Qualitative results for corn fortified with NK603 for all participants. (DNA-based assays).
- Table 18: Percentages of correct results, false negatives, and false positives in qualitative reports for NK603 for all participants.
- Table 19: Qualitative results for corn fortified with Herculex for all participants (DNA-based assays).
- Table 20: Percentages of correct results, false negatives, and false positives in qualitative reports for Herculex for all participants.

- Table 21: Qualitative results for corn fortified with MON863 for all participants (DNA-based assays).
- Table 22: Percentages of correct results, false negatives, and false positives in qualitative reports for MON863 for all participants.
- Table 23: Qualitative results for corn fortified with Herculex RW for all participants (DNA-based assays).
- Table 24: Percentages of correct results, false negatives, and false positives in qualitative reports for Herculex RW for all participants.
- Table 25: Qualitative results for corn fortified with MIR604 for all participants (DNA-based assays).
- Table 26: Percentages of correct results, false negatives, and false positives in qualitative reports for MIR604 for all participants.
- Table 27: Qualitative results for corn fortified with Event 3272 for all participants (DNA-based assays).
- Table 28: Percentages of correct results, false negatives, and false positives in qualitative reports for Event 3272 for all participants.
- Table 29: Qualitative results for soybeans fortified with CP4 EPSPS (Roundup Ready) for all participants (DNA-based assays).
- Table 30: Percentages of correct results, false negatives, and false positives in qualitative reports for CP4 EPSPS for all participants.
- Table 31: Composite percentages of correct results, false negatives, and false positives in qualitative reports for each transgenic event for all participants (DNA-based assays).
- Figure 1: Group average of percentage correct for Qualitative reports on each event (DNA-based assays).
- Table 32: Qualitative results for the detection of transgenic events in corn using Lateral Flow Strip (LFS) Testing (Protein-based testing) for Participants #1843 and #2133.
- Table 33: Qualitative results for soybeans fortified with CP4EPSPS (RUR) for all participants using Lateral Flow Strip (LFS) Testing.
- Table 34: Percentage of correct results in qualitative reports for CP4EPSPS for all participants using Lateral Flow Strip (LFS) Testing.

- Table 35: Qualitative results for soybeans fortified with CP4EPSPS for all participants using Enzyme-Linked Immunosorbent Assay (ELISA) (Protein-based testing).
- Table 36: Percentage of correct results in qualitative reports for CP4EPSPS for all participants using Enzyme-Linked Immunosorbent Assay (ELISA) (Protein-based testing).

Quantitative Data Summaries. This section summarizes quantitative sample analysis data: (z-scores were purposefully left blank in Tables 37- 49 on non-fortified (0.0%) samples since a z-score assumes a normal distribution and the interpretation may be distorted).

- Table 37: Quantitative results and z-scores for corn fortified with T25 for all participants (DNA-based assays).
- Table 38: Quantitative results and z-scores for corn fortified with CBH351 for all participants (DNA-based assays).
- Table 39: Quantitative results and z-scores for corn fortified with MON810 for all participants (DNA-based assays).
- Table 40: Quantitative results and z-scores for corn fortified with GA21 for all participants (DNA-based assays).
- Table 41: Quantitative results and z-scores for corn fortified with Bt176 for all participants (DNA-based assays).
- Table 42: Quantitative results and z-scores for corn fortified with Bt11 for all participants (DNA-based assays).
- Table 43: Quantitative results and z-scores for corn fortified with NK603 for all participants (DNA-based assays).
- Table 44: Quantitative results and z-scores for corn fortified with Herculex for all participants (DNA-based assays).
- Table 45: Quantitative results and z-scores for corn fortified with MON863 for all participants (DNA-based assays).
- Table 46: Quantitative results and z-scores for corn fortified with Herculex RW for all participants (DNA-based assays).
- Table 47: Quantitative results and z-scores for corn fortified with MIR604 for all participants (DNA-based assays).
- Table 48: Quantitative results and z-scores for corn fortified with Event 3272 for all participants (DNA-based assays).

- Table 49: Quantitative results for soybeans fortified with CP4EPSPS (RUR) using Enzyme-Linked Immunosorbent Assay (ELISA) (Protein-based testing) for Participant # 1754 (only this participant submitted results).
- Table 50: Quantitative results and z-scores for soybeans fortified with CP4 EPSPS for all participants (DNA-based assays).
- Table 51: Descriptive statistics for participants reported quantifications relative to GIPSA fortification levels using DNA-based assays.
- Appendix I: List of organizations who wished to be identified as a participant in the GIPSA May 2009 Proficiency Program.

Table 1: Qualitative results for corn fortified with 35S for all participants (DNA-based assays) (N = negative; P = positive; Incorrect results are shown in boldface).

35S	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Participant Number	N	P	P	P	P	P
1751	N	P	P	P	P	P
1754	N	P	P	P	P	P
1761	N	P	P	P	P	P
1764	N	P	P	P	P	P
1770	N	P	P	P	P	P
1774	N	P	P	P	P	P
1844	N	P	P	P	P	P
1847	N	P	P	P	P	P
1854	N	P	P	P	P	P
1858	N	P	P	P	P	P
1859	N	P	P	P	P	P
1862	N	P	P	P	P	P
1870	N	P	P	P	P	P
1891	N	P	P	P	P	P
1892	N	P	P	P	P	P
2057	N	P	P	P	P	P
2100	N	P	P	P	P	P
2108	N	P	P	P	P	P
2132	N	P	P	P	P	P
2675	N	P	P	P	P	P
2678	N	P	P	P	P	P
2691	N	P	P	P	P	P
2692	N	P	P	P	P	P
2694	N	P	P	P	P	P
2705	N	P	P	P	P	P
2716	N	P	P	P	P	P
2720	N	P	P	P	P	P
2724	N	P	P	P	P	P
2725	N	P	P	P	P	P
2822	N	P	P	P	P	P
2824	N	P	P	P	P	P
N, Results	31	31	31	31	31	31
# Negative	31	0	0	0	0	0
# Positive	0	31	31	31	31	31
% Correct	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
% Incorrect	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%

Table 2: Percentages of correct results, false negatives, and false positives in qualitative reports for 35S for all participants.

Total # Reported results	186
# Incorrect	0
% Correct	100.0%
# Provided Positives (P)	155
# False Negative	0
% False Negative	0.0%
# Provided Negatives (N)	31
# False Positive	0
% False Positive	0.0%

Table 3: Qualitative results for corn fortified with NOS for all participants (DNA-based assays) (N = negative; P = positive; Incorrect results are shown in boldface).

NOS	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Participant Number	N	P	P	P	P	P
1751	N	P	P	P	P	P
1754	N	P	P	P	P	P
1761	N	P	P	P	P	P
1764	N	P	P	P	P	P
1770	N	P	P	P	P	P
1774	N	P	P	P	P	P
1844	N	P	P	P	P	P
1847	N	P	P	P	P	P
1854	N	P	P	NR	P	P
1858	N	P	P	P	P	P
1859	N	P	P	P	P	P
1870	N	P	P	P	P	P
1891	N	P	P	P	P	P
1892	N	P	P	P	P	P
2057	N	P	P	P	P	P
2108	N	P	P	P	P	P
2132	N	P	P	P	P	P
2675	N	P	P	P	P	P
2678	N	P	P	P	P	P
2691	N	P	P	P	P	P
2692	N	P	P	P	P	P
2694	N	P	P	P	P	P
2705	N	P	P	P	P	P
2716	N	P	P	P	P	P
2724	N	P	P	P	P	P
2822	N	P	P	P	P	P
N, Results	26	26	26	25	26	26
# Negative	26	0	0	0	0	0
# Positive	0	26	26	25	26	26
% Correct	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
% Incorrect	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%

Table 4: Percentages of correct results, false negatives, and false positives in qualitative reports for NOS for all participants.

Total # Reported results	155
# Incorrect	0
% Correct	100.0%
# Provided Positives (P)	129
# False Negative	0
% False Negative	0.0%
# Provided Negatives (N)	26
# False Positive	0
% False Positive	0.0%

Table 5: Qualitative results for corn fortified with T25 for all participants (DNA-based assays) (N = negative; P = positive; Incorrect results are shown in boldface).

T25	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Participant Number	0.0%	2.0%	0.1%	0.0%	0.8%	1.0%
1751	N	P	P	P	P	P
1761	N	P	P	P	P	P
1773	N	P	P	N	P	P
1774	N	P	P	P	P	P
1788	N	P	P	P	P	P
1844	N	P	P	N	P	P
1854	N	P	P	P	P	P
1859	N	P	P	N	P	P
1862	N	P	P	N	P	P
1892	N	P	P	P	P	P
2132	N	P	P	P	P	P
2692	N	P	P	N	P	P
2694	N	P	P	N	P	P
2705	N	P	P	P	P	P
2822	N	P	P	N	P	P
N, Results						
	15	15	15	15	15	15
# Negative	15	0	0	7	0	0
# Positive	0	15	15	8	15	15
% Correct	100.0%	100.0%	100.0%	53.3%	100.0%	100.0%
% Incorrect	0.0%	0.0%	0.0%	46.7%	0.0%	0.0%

Table 6: Percentages of correct results, false negatives, and false positives in qualitative reports for T25 for all participants.

Total # Reported results	90
# Incorrect	8
% Correct	91.1%
# Provided Positives	60
# False Negative	0
% False Negative	0.0%
# Provided Negatives	30
# False Positive	8
% False Positive	26.7%

Table 7: Qualitative results for corn fortified CBH351 with for all participants (DNA-based assays) (N = negative; P = positive; Incorrect results are shown in boldface).

CBH351	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Participant Number	0.0%	0.1%	0.0%	0.0%	0.0%	1.0%
1751	N	P	N	P	N	N
1770	N	P	N	N	N	P
1773	N	P	N	P	P	P
1774	N	P	N	N	N	P
1844	N	P	N	N	N	P
1854	N	P	N	N	P	P
1859	N	P	N	N	N	P
1891	N	P	N	P	N	P
1892	N	P	N	N	N	P
2692	N	P	N	N	P	P
2694	N	P	N	N	N	P
N, Results						
	11	11	11	11	11	11
# Negative	11	0	11	8	8	1
# Positive	0	11	0	3	3	10
% Correct	100.0%	100.0%	100.0%	72.7%	72.7%	90.9%
% Incorrect	0.0%	0.0%	0.0%	27.3%	27.3%	9.1%

Table 8: Percentages of correct results, false negatives, and false positives in qualitative reports for CBH351 for all participants.

Total # Reported results	66
# Incorrect	7
% Correct	89.4%
# Provided Positives	22
# False Negative	1
% False Negative	4.6%
# Provided Negatives	44
# False Positive	6
% False Positive	13.6%

Table 9: Qualitative results for corn fortified with MON810 for all participants (DNA-based assays) (N = negative; P = positive; Incorrect results are shown in boldface).

MON810	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Participant Number	0.0%	2.0%	0.8%	0.0%	0.1%	1.0%
1751	N	P	P	N	P	N
1761	N	P	P	N	P	P
1773	N	P	P	N	P	P
1774	N	P	P	N	P	P
1788	N	P	P	N	P	P
1844	N	P	P	N	P	P
1854	N	P	P	N	P	P
1858	N	P	P	N	P	P
1859	N	P	P	N	P	P
1862	N	P	P	N	P	P
1892	N	P	P	N	P	P
2132	N	P	P	N	P	P
2569	N	P	P	N	P	P
2691	N	P	P	N	P	P
2692	N	P	P	N	P	P
2705	N	P	P	N	P	P
2724	N	P	P	N	P	P
2822	N	P	P	N	P	P
2824	N	P	P	N	N	P
N, Results						
	19	19	19	19	19	19
# Negative						
	19	0	0	19	1	1
# Positive						
	0	19	19	0	18	18
% Correct						
	100.0%	100.0%	100.0%	100.0%	94.7%	94.7%
% Incorrect						
	0.0%	0.0%	0.0%	0.0%	5.3%	5.3%

Table 10: Percentages of correct results, false negatives, and false positives in qualitative reports for MON810 for all participants.

Total # Reported results	114
# Incorrect	2
% Correct	98.3%
# Provided Positives	76
# False Negative	2
% False Negative	2.6%
# Provided Negatives	38
# False Positive	0
% False Positive	0.0%

Table 11: Qualitative results for corn fortified with GA21 for all participants (DNA-based assays) (N = negative; P = positive; Incorrect results are shown in boldface).

GA21	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Participant Number	0.0%	0.0%	0.4%	0.8%	0.4%	0.1%
1751	N	P	P	N	P	P
1761	N	N	P	P	P	P
1774	N	P	P	P	P	P
1788	N	N	P	P	P	P
1844	N	N	P	P	P	P
1854	N	N	P	P	P	P
1858	N	N	P	P	P	P
1859	N	N	P	P	P	P
1862	N	N	P	P	P	P
1892	N	N	P	P	P	P
2569	N	N	P	P	P	P
2691	N	N	P	P	P	P
2692	N	N	P	P	P	P
2705	N	N	P	P	P	P
2822	N	N	P	P	P	P
2824	N	N	P	P	P	P
N, Results	16	16	16	16	16	16
# Negative	16	14	0	1	0	0
# Positive	0	2	16	15	16	16
% Correct	100.0%	87.5%	100.0%	93.8%	100.0%	100.0%
% Incorrect	0.0%	12.5%	0.0%	6.3%	0.0%	0.0%

Table 12: Percentages of correct results, false negatives, and false positives in qualitative reports for GA21 for all participants.

Total # Reported results	96
# Incorrect	3
% Correct	96.9%
# Provided Positives	64
# False Negative	1
% False Negative	1.6%
# Provided Negatives	32
# False Positive	2
% False Positive	6.3%

Table 13: Qualitative results for corn fortified with Bt176 for all participants (DNA-based assays) (N = negative; P = positive; Incorrect results are shown in boldface).

Bt176	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Participant Number	0.0%	1.5%	0.0%	0.8%	0.1%	0.1%
1761	N	P	N	P	P	P
1773	N	P	N	P	P	P
1774	N	P	N	P	P	P
1788	N	P	N	P	P	N
1844	N	P	N	P	P	P
1854	N	P	N	P	P	P
1858	N	P	N	P	P	P
1859	N	P	N	P	P	P
1862	N	P	N	P	P	P
1892	N	P	N	P	P	P
2132	N	P	N	P	P	P
2569	N	P	N	P	P	P
2691	N	P	N	P	P	P
2692	N	P	N	P	P	P
2705	N	P	N	P	P	P
2724	N	P	N	P	P	P
2822	N	P	N	P	N	N
N, Results	17	17	17	17	17	17
# Negative	17	0	17	0	1	2
# Positive	0	17	0	17	16	15
% Correct	100.0%	100.0%	100.0%	100.0%	94.1%	88.2%
% Incorrect	0.0%	0.0%	0.0%	0.0%	5.9%	11.8%

Table 14: Percentages of correct results, false negatives, and false positives in qualitative reports for Bt176 for all participants.

Total # Reported results	102
# Incorrect	3
% Correct	97.1%
# Provided Positives	68
# False Negative	3
% False Negative	4.4%
# Provided Negatives	34
# False Positive	0
% False Positive	0.0%

Table 15: Qualitative results for corn fortified with Bt11 for all participants (DNA-based assays) (N = negative; P = positive; Incorrect results are shown in boldface).

Bt11	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Participant Number	0.0%	0.1%	0.5%	0.4%	0.0%	0.0%
1761	N	P	P	P	N	N
1773	N	P	P	P	N	N
1774	N	P	P	P	N	N
1788	N	P	P	P	N	N
1844	N	P	P	P	N	N
1854	N	P	P	N	P	P
1858	N	P	P	P	N	N
1859	N	P	P	P	N	N
1862	N	P	P	P	N	N
1892	N	P	P	P	N	N
2132	N	P	P	P	N	N
2569	N	P	P	P	N	N
2691	N	P	P	P	N	N
2692	N	P	P	P	N	N
2705	N	P	P	P	N	N
2724	N	P	P	P	N	N
2822	N	P	P	P	N	N
2824	N	P	P	P	N	N
N, Results	18	18	18	18	18	18
# Negative	18	0	0	1	17	17
# Positive	0	18	18	17	1	1
% Correct	100.0%	100.0%	100.0%	94.4%	94.4%	94.4%
% Incorrect	0.0%	0.0%	0.0%	5.6%	5.6%	5.6%

Table 16: Percentages of correct results, false negatives, and false positives in qualitative reports for Bt11 for all participants.

Total # Reported results	108
# Incorrect	3
% Correct	97.2%
# Provided Positives	72
# False Negative	1
% False Negative	1.4%
# Provided Negatives	36
# False Positive	2
% False Positive	5.6%

Table 17: Qualitative results for corn fortified with NK603 for all participants. (DNA-based assays) (N = negative; P = positive).

NK603	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Participant Number	0.0%	1.0%	0.1%	0.4%	0.0%	2.0%
1751	N	P	N	P	N	P
1761	N	P	P	P	N	P
1774	N	P	P	P	N	P
1788	N	P	P	P	N	P
1844	N	P	P	P	N	P
1854	N	P	P	P	P	P
1858	N	P	P	P	N	P
1859	N	P	P	P	N	P
1862	N	P	P	P	N	P
2569	N	P	P	P	N	P
2691	N	P	P	P	P	P
2692	N	P	P	P	N	P
2705	N	P	P	P	N	P
2822	N	P	P	P	N	P
2824	N	P	P	P	N	P
N, Results	15	15	15	15	15	15
# Negative	15	0	1	0	13	15
# Positive	0	15	14	15	2	0
% Correct	100.0%	100.0%	93.3%	100.0%	86.7%	100.0%
% Incorrect	0.0%	0.0%	6.7%	0.0%	13.3%	0.0%

Table 18: Percentages of correct results, false negatives, and false positives in qualitative reports for NK603 for all participants.

Total # Reported results	90
# Incorrect	3
% Correct	96.7%
# Provided Positives	60
# False Negative	1
% False Negative	1.7%
# Provided Negatives	30
# False Positive	2
% False Positive	6.7%

Table 19: Qualitative results for corn fortified with Herculex for all participants (DNA-based assays) (N = negative; P = positive; Incorrect results are shown in boldface).

Herculex	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Participant Number	0.0%	0.0%	0.5%	0.0%	0.8%	0.1%
1751	N	P	P	P	P	P
1761	N	N	P	N	P	P
1773	N	N	P	N	P	P
1774	N	N	P	N	P	P
1844	N	N	P	N	P	P
1854	N	P	P	N	P	P
1859	N	N	P	N	P	P
1862	N	N	P	N	P	P
2569	N	N	P	N	P	P
2691	N	N	P	N	P	P
2692	N	N	P	N	P	P
2705	N	N	N	N	N	N
2822	N	N	P	P	P	P
2824	N	N	N	N	N	N
N, Results	14	14	14	14	14	14
# Negative	14	12	2	12	2	2
# Positive	0	2	12	2	12	12
% Correct	100.0%	85.7%	85.7%	85.7%	85.7%	85.7%
% Incorrect	0.0%	14.3%	14.3%	14.3%	14.3%	14.3%

Table 20: Percentages of correct results, false negatives, and false positives in qualitative reports for Herculex for all participants.

Total # Reported results	84
# Incorrect	10
% Correct	88.1%
# Provided Positives	42
# False Negative	6
% False Negative	14.3%
# Provided Negatives	42
# False Positive	4
% False Positive	9.5%

Table 21: Qualitative results for corn fortified with MON863 for all participants (DNA-based assays) (N = negative; P = positive; Incorrect results are shown in boldface).

MON863	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Participant Number	0.0%	0.0%	0.5%	1.5%	0.4%	0.0%
1751	N	N	P	P	N	P
1761	N	N	P	P	P	N
1773	N	N	P	P	P	N
1774	N	N	P	P	P	N
1788	N	N	P	P	P	N
1844	N	N	P	P	P	N
1854	N	N	P	P	P	P
1859	N	N	P	P	P	N
2569	N	N	P	P	P	N
2691	N	N	P	P	P	N
2692	N	N	P	P	P	N
2705	N	N	P	P	P	N
2822	N	N	P	P	P	N
2824	N	N	P	P	P	N
N, Results	14	14	14	14	14	14
# Negative	14	14	0	0	1	12
# Positive	0	0	14	14	13	2
% Correct	100.0%	100.0%	100.0%	100.0%	92.9%	85.7%
% Incorrect	0.0%	0.0%	0.0%	0.0%	7.1%	14.3%

Table 22: Percentages of correct results, false negatives, and false positives in qualitative reports for MON863 for all participants.

Total # Reported results	84
# Incorrect	3
% Correct	96.4%
# Provided Positives	42
# False Negative	1
% False Negative	2.4%
# Provided Negatives	42
# False Positive	2
% False Positive	4.8%

Table 23: Qualitative results for corn fortified with Herculex RW for all participants (DNA-based assays) (N = negative; P = positive).

Herculex RW	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Participant Number	0.0%	0.0%	0.5%	0.8%	0.1%	2.0%
1761	N	N	P	P	P	P
1773	N	N	P	P	P	P
1774	N	N	P	P	P	P
1844	N	N	P	P	N	P
1859	N	N	P	P	P	P
2569	N	N	P	P	P	P
2705	N	N	P	P	P	P
2822	N	N	P	N	P	P
N, Results	8	8	8	8	8	8
# Negative	8	8	0	1	1	0
# Positive	0	0	8	7	7	8
% Correct	100.0%	100.0%	100.0%	87.5%	87.5%	100.0%
% Incorrect	0.0%	0.0%	0.0%	12.5%	12.5%	0.0%

Table 24: Percentages of correct results, false negatives, and false positives in qualitative reports for Herculex RW for all participants.

Total # Reported results	48
# Incorrect	2
% Correct	95.8%
# Provided Positives	32
# False Negative	2
% False Negative	6.3%
# Provided Negatives	16
# False Positive	0
% False Positive	0.0%

Table 25: Qualitative results for corn fortified with MIR604 for all participants (DNA-based assays).

MIR604	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Participant Number	0.0%	0.0%	0.1%	0.5%	1.5%	0.5%
1761	N	N	P	P	P	P
1773	N	N	P	P	P	P
1844	N	N	P	P	P	P
1859	N	N	P	P	P	P
2822	N	N	P	P	P	P
2824	P	N	P	P	P	P
N, Results						
	6	6	6	6	6	6
# Negative	5	6	0	0	0	0
# Positive	1	0	6	6	6	6
% Correct	83.3%	100.0%	100.0%	100.0%	100.0%	100.0%
% Incorrect	16.7%	0.0%	0.0%	0.0%	0.0%	0.0%

Table 26: Percentages of correct results, false negatives, and false positives in qualitative reports for MIR604 for all participants.

# Reported results	36
# Incorrect	1
% Correct	97.2%
# Provided Positives	24
# False Negative	0
% False Negative	0.0%
# Provided Negatives	12
# False Positive	1
% False Positive	8.3%

Table 27: Qualitative results for corn fortified with Event 3272 for all participants (DNA-based assays) (N = negative; P = positive; Incorrect results are shown in boldface).

Event 3272	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Participant Number	0.0%	0.5%	0.0%	0.0%	0.0%	0.1%
1773	N	P	N	N	N	P
1774	N	P	N	N	N	P
1844	N	P	N	N	N	P
1859	N	P	N	N	N	P
1870	N	P	N	N	N	P
1891	N	P	N	N	N	P
2822	N	P	N	N	N	P
N, Results						
	7	7	7	7	7	7
# Negative	7	0	7	7	7	0
# Positive	0	7	0	0	0	7
% Correct	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
% Incorrect	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%

Table 28: Percentages of correct results, false negatives, and false positives in qualitative reports for Event 3272 for all participants.

# Reported results	42
# Incorrect	0
% Correct	100.0%
# Provided Positives	14
# False Negative	0
% False Negative	0.0%
# Provided Negatives	28
# False Positive	0
% False Positive	0.0%

Table 29: Qualitative results for soybeans fortified with CP4 EPSPS (Roundup Ready) for all participants (DNA-based assays) (N = negative; P = positive; Incorrect results are shown in boldface).

CP4 EPSPS	Sample 1	Sample 2	Sample 3
Participant Number	0.5%	0.1%	0.0%
1751	P/P		N
1761	P	P	N
1788	P	P	N
1844	P	P	N
1854	N	P	P
1858	P		N/N
1859	P	P	N
1892	P	P	N
2100	P	P	P
2108	P	N	N
2691	P	P	N
2692	P	P	N
2724	P	P	N
2822	P	P	N
N, Results			
	15	12	15
# Negative			
	1	1	13
# Positive			
	14	11	2
% Correct			
	93.3%	91.7%	86.7%
% Incorrect			
	6.7%	8.3%	13.3%

Table 30: Percentages of correct results, false negatives, and false positives in qualitative reports for CP4 EPSPS (Roundup Ready) for all participants.

Total # Reported results	42
# Incorrect	4
% Correct	90.5%
# Provided Positives	27
# False Negative	2
% False Negative	7.4%
# Provided Negatives	15
# False Positive	2
% False Positive	13.3%

Table 31: Composite percentages of correct results, false negatives, and false positives in qualitative reports for each transgenic event for all participants (DNA-based assays).

N = total number of results submitted for an event; %False Negative = [# False Negatives / # Provided Positives] x 100; %False Positives = [#False Positives / # Provided Negatives] x100.

Event	35S	NOS	T25	CBH351	MON810	GA21	Bt176	
N, Results	186	155	90	66	114	96	102	
Reported Incorrect	0	0	8	7	2	3	3	
% Correct	100.0%	100.0%	91.1%	89.4%	98.3%	96.9%	97.1%	
N, Provided Positives	155	130	60	22	76	64	68	
N, False Negatives	0	0	0	1	2	1	3	
% False Negative	0.0%	0.0%	0.0%	4.6%	2.6%	1.6%	4.4%	
N, Provided Negatives	31	26	30	44	38	32	34	
N, False Positives	0	0	8	6	0	2	0	
% False Positives	0.0%	0.0%	26.7%	13.6%	0.0%	6.3%	0.0%	
Event	Bt11	NK603	Herculex	MON863	HerculexRW	MIR604	EV3272	RUR
N, Results	108	90	84	84	48	36	42	42
Reported Incorrect	3	3	10	3	2	1	0	4
% Correct	97.2%	96.7%	88.1%	96.4%	95.8%	97.2%	100.0%	90.5%
N, Provided Positives	72	60	42	42	32	24	14	27
N, False Negatives	1	1	6	1	2	0	0	2
% False Negative	1.4%	1.7%	14.3%	2.4%	6.3%	0.0%	0.0%	7.4%
N, Provided Negatives	36	30	42	42	16	12	28	15
N, False Positives	2	2	4	2	0	1	0	2
% False Positives	5.6%	6.7%	9.5%	4.8%	0.0%	8.3%	0.0%	13.3%

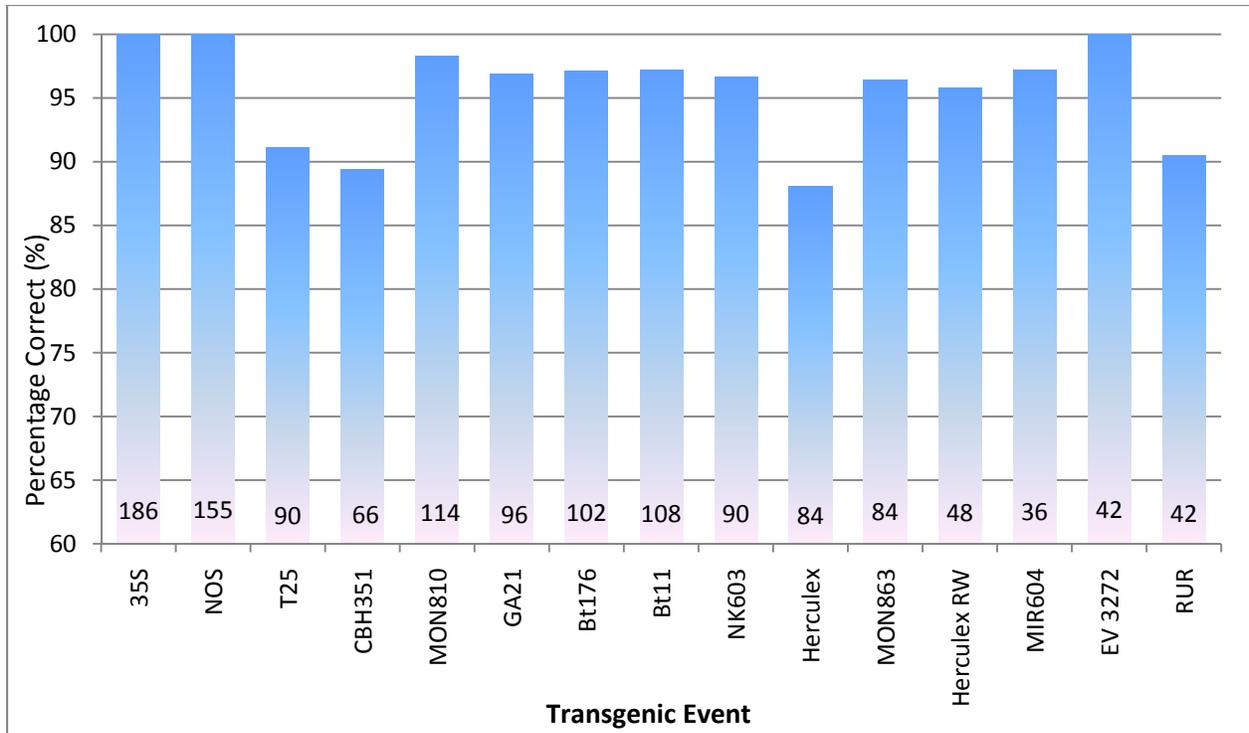


Figure 1: Group average of percentage correct for qualitative reports on each event (DNA-based assays). Embedded numbers represent the total number of reported results for that event. Data are shown on a composite basis (i.e., all participants' results combined) extracted from the percentage correct scores in Table 31.

Table 32: Qualitative results for the detection of transgenic events in corn using Lateral Flow Strip (LFS) Testing (Protein-based testing) for Participants #1843 and #2133.

Participant Number 1843		Transgenic Event					Participant Number 2133		Transgenic Event
Sample Number	T25	NK603	Cry 1 Ab	Herculex	Mon863	Sample Number	NK 603		
1	< 1.0	< 0.5	< 1.0	< 0.5	< 0.5	1	N		
2	≥ 1.0	≥ 0.5	< 1.0	< 0.5	< 0.5	2	P		
3	< 1.0	< 0.5	< 1.0	≥ 0.5	≥ 0.5	3	N		
4	< 1.0	≥ 0.5*	< 1.0	< 0.5	≥ 0.5	4	P		
5	≥ 1.0*	< 0.5	< 1.0	≥ 0.5	≥ 0.5*	5	N		
6	≥ 1.0	≥ 0.5	< 1.0	< 0.5	< 0.5	6	P		
Total # Reported results	6	6	6	6	6	Total # Reported results	6		
# Incorrect	0	0	2	0	0	# Incorrect	1		
% Correct	100.0%	100.0%	66.7%	100.0%	100.0%	% Correct	83.3%		
# Provided Positives^a	3	3	2	2	3	# Provided Positives	4		
# False Negative	0	0	2	0	0	# False Negative	1		
% False Negative	0.0%	0.0%	100.0%	0.0%	0.0%	% False Negative	25.0%		
# Provided Negatives^b	3	3	4	4	3	# Provided Negatives	2		
# False Positive	0	0	0	0	0	# False Positive	0		
% False Positive	0.0%	0.0%	0.0%	0.0%	0.0%	% False Positive	0.0%		
LODs	1.0%	0.5%	1.0%	0.5%	0.5%	LODs	Not Provided		

^aSamples fortified **at or above** the participant's LOD are considered in this table as provided positives. In some instances, the actual fortified amount is below the participants reported LOD (i.e. T25, NK603 and Mon863). A * denotes a detection with the method even though the sample was fortified below the participants' LOD and constituted a provided positive sample in the results.

^bOnly samples fortified **below** the participants LOD where a negative result was reported, are considered in this table as provided negatives.

Participant 2133

Participant did not provide a LOD. Therefore, all samples fortified with NK603 at all levels were included in the report.

Table 33: Qualitative results for soybeans fortified with CP4EPSPS (RUR) for all participants using Lateral Flow Strip (LFS) Testing (N = negative; P = positive).

CP4 EPSPS (RUR)	Sample 1	Sample 2	Sample 3
Participant Number	0.5%	0.1%	0.0%
1764	P	P	N
1843	P	P	N
1851	P	P/P	Not Provided
2133	Not Provided	P/P	N
N, Results	3	6	3
# Negative	0	0	3
# Positive	3	6	0
% Correct	100.0%	100.0%	100.0%
% Incorrect	0.0%	0.0%	0.0%

Table 34: Percentage of correct results in qualitative reports for CP4EPSPS for all participants using Lateral Flow Strip (LFS) Testing.

Total # Reported results	12
# Incorrect	0
% Correct	100.0%
# Provided Positives	9
# False Negative	0
% False Negative	0.0%
# Provided Negatives	3
# False Positive	0
% False Positive	0.0%

Table 35: Qualitative results for soybeans fortified with CP4EPSPS for all participants using Enzyme-Linked Immunosorbent Assay (ELISA) (Protein-based testing).

CP4 EPSPS (RUR)	Sample 1	Sample 2	Sample 3
Participant Number	0.5%	0.10%	0.00%
1751	P/P	Not Provided	N
2817	P	N	P

Table 36: Percentage of correct results in qualitative reports for CP4EPSPS for all participants using Enzyme-Linked Immunosorbent Assay (ELISA) (Protein-based testing).

Total # Reported results	6
# Incorrect	2
% Correct	66.7%
# Provided Positives	4
# False Negative	1
% False Negative	25.0%
# Provided Negatives	2
# False Positive	1
% False Positive	50.0%

Table 37: Quantitative results and z-scores for corn fortified with T25 for all participants (DNA-based assays). Values highlighted in yellow indicate z-scores outside of the expected range, i.e., $z > 2$. Quantifications marked in red indicate values determined to be either: (1) outliers by the “Grubb’s Test for Outliers”; (2) a quantitative value for a non-fortified sample (i.e. a false positive result); or (3) a negative value for a fortified sample (i.e. a false negative result).

Event: T25												
%w/w Fortification Level	0.0%		2.0%		0.1%		0.0%		0.8%		1.0%	
Participant Number	Result	z-score										
1754	0.0		1.30	-0.67	0.10	0.00	0.0		0.70	-0.19	0.50	-0.70
1764	0.0		0.70	-1.25	*0.6	9.56	0.4		0.50	-0.57	1.30	0.42
1769	0.0		0.81	-1.14	0.04	-1.24	0.0		0.27	-1.01	0.34	-0.92
1770	0.0		2.40	0.38	0.10	0.00	0.0		0.90	0.19	1.10	0.14
1780	0.0		1.58	-0.40	0.08	-0.38	0.0		0.75	-0.09	0.81	-0.26
1783	0.0		3.81	1.74	0.12	0.38	0.0		1.93	2.14	2.56	2.18
1870	0.0		2.20	0.19	0.20	1.91	0.0		1.30	0.95	1.10	0.14
1891	0.0		3.00	0.96	*0.4	5.74	0.0		1.50	1.33	2.10	1.53
2057	0.0		2.00	0.00	0.10	0.00	0.0		0.80	0.00	1.00	0.00
2128	0.0		1.24	-0.73	0.10	0.00	0.0		0.50	-0.57	0.40	-0.84
2675	0.0		0.19	-1.74	0.00	-1.91	0.0		0.06	-1.40	0.09	-1.27
2716	0.0		2.50	0.48	0.10	0.00	0.0		1.02	0.42	1.00	0.00

Table 38: Quantitative results and z-scores for corn fortified with CBH351 for all participants (DNA-based assays). Z-scores outside of the expected range of $z > 2$ were not observed in this data set except that one false negative result, highlighted in red, was reported.

Event: CBH351									
%w/w Fortification Level	0.0%		0.0%		0.5%		3.0%		
Participant Number	Result	z-score	Result	z-score	Result	z-score	Result	z-score	
1754	0.0		0.0		0.20	-1.4	1.80	-0.8	
1755	0.0		0.0		0.20	-1.5	1.10	-1.3	
1778	0.0		0.0		0.40	-0.5	3.10	0.1	
1870	0.0		0.0		0.40	-0.5	1.90	-0.7	
2044	0.0		0.0		0.00	-2.4	0.30	-1.8	
2057	0.0		0.0		0.52	0.1	4.70	1.1	
2723	0.0		0.0		0.57	0.3	3.00	0.0	

Table 39: Quantitative results and z-scores for corn fortified with MON810 for all participants (DNA-based assays). Values highlighted in yellow indicate z-scores outside of the expected range, i.e., $z > 2$. Quantifications marked in red indicate values determined to be either: (1) outliers by the “Grubb’s Test for Outliers”; (2) a quantitative value for a non-fortified sample (i.e. a false positive result); or (3) a negative value for a fortified sample (i.e. a false negative result).

Event: MON810												
%w/w Fortification Level	0.0%		2.0%		0.8%		0.0%		0.1%		1.0%	
Participant Number	Result	z-score										
1754	0.0		1.10	-1.37	0.40	-1.80	0.0		0.10	0.00	0.60	-1.65
1764	0.0		1.60	-0.61	0.50	-1.35	0.0		0.10	0.00	0.70	-1.24
1769	0.0		1.16	-1.28	0.45	-1.57	0.0		0.07	-1.02	0.54	-1.90
1770	0.0		1.40	-0.91	0.60	-0.90	0.0		0.10	0.00	0.80	-0.83
1780	0.0		1.41	-0.90	0.70	-0.45	0.0		0.09	-0.29	0.75	-1.03
1783	0.0		1.46	-0.82	0.58	-0.99	0.0		0.10	0.00	0.65	-1.44
1788	0.0		0.48	-2.31	0.19	-2.74	0.0		P	N/A	0.29	-2.93
1847	0.0		0.83	-1.78	0.23	-2.56	0.0		0.02	-2.33	0.37	-2.60
1870	0.0		1.70	-0.46	0.80	0.00	0.0		0.10	0.00	0.80	-0.83
1891	0.0		0.80	-1.82	0.40	-1.80	0.0		0.10	0.00	0.60	-1.65
2057	0.0		2.00	0.00	0.80	0.00	0.0		0.10	0.00	0.90	-0.41
2128	0.0		1.42	-0.88	0.36	-1.97	0.0		0.10	0.00	0.60	-1.65
2675	0.0		2.38	0.58	0.30	-2.24	0.2		0.03	-2.04	*2.34	5.53
2692	0.0		1.01	-1.50	0.37	-1.93	0.0		0.10	0.00	0.45	-2.27
2694	0.0		0.92	-1.64	0.35	-2.02	0.0		0.04	-1.75	0.43	-2.35
2716	0.0		2.98	1.49	0.80	0.00	0.0		0.10	0.00	1.00	0.00
2720	0.0		0.26	-2.64	0.00	-3.59	0.0		0.00	-2.92	0.00	-4.13
2822	0.0		1.00	-1.52	0.40	-1.80	0.0		0.10	0.00	0.70	-1.24

Table 40: Quantitative results and z-scores for corn fortified with GA21 for all participants (DNA-based assays). Values highlighted in yellow indicate z-scores outside of the expected range, i.e., $z > 2$. Quantifications marked in red indicate values determined to be either: (1) outliers by the “Grubb’s Test for Outliers”; (2) a quantitative value for a non-fortified sample (i.e. a false positive result); or (3) a negative value for a fortified sample (i.e. a false negative result).

Event: GA21												
%w/w Fortification Level	0.0%		0.0%		0.4%		0.8%		0.4%		0.1%	
Participant Number	Result	z-score										
1754	0.0		0.0		0.10	-2.43	0.20	-1.89	0.10	-1.32	0.10	0.00
1764	0.0		0.0		0.50	0.81	1.10	0.95	0.50	0.44	0.40	1.32
1769	0.0		0.0		0.30	-0.81	0.71	-0.30	0.24	-0.70	0.19	0.37
1770	0.0		0.0		0.30	-0.81	0.70	-0.32	0.20	-0.88	0.20	0.44
1773	0.0		0.0		0.10	-2.43	0.30	-1.58	0.10	-1.32	0.10	0.00
1780	0.0		0.0		0.43	0.24	0.75	-0.16	0.51	0.48	0.26	0.70
1783	0.0		0.0		0.27	-1.06	0.42	-1.20	0.19	-0.92	0.17	0.31
1870	0.0		0.0		0.30	-0.81	0.70	-0.32	0.40	0.00	0.20	0.44
1891	0.0		0.0		0.15	-2.03	0.50	-0.95	0.20	-0.88	0.10	0.00
2057	0.0		0.0		0.40	0.00	0.80	0.00	0.30	-0.44	0.40	1.32
2128	0.0		0.0		0.12	-2.27	0.28	-1.64	0.13	-1.19	0.04	-0.26
2675	0.0		0.0		*1.38	7.95	1.40	1.89	0.65	1.10	0.34	1.06
2692	0.0		0.0		0.31	-0.73	0.43	-1.17	0.64	1.05	0.29	0.84
2694	0.0		0.0		0.29	-0.89	0.65	-0.47	0.23	-0.75	0.20	0.44
2716	0.0		0.0		0.33	-0.57	0.79	-0.03	0.45	0.22	0.52	1.85
2720	0.0		0.0		*1.30	7.30	*3.0	6.93	0.87	2.06	0.98	3.87

Table 41: Quantitative results and z-scores for corn fortified with Bt176 for all participants (DNA-based assays). Values highlighted in yellow indicate z-scores outside of the expected range, i.e., $z > 2$. Quantifications marked in red indicate values determined to be either: (1) outliers by the “Grubb’s Test for Outliers”; (2) a quantitative value for a non-fortified sample (i.e. a false positive result); or (3) a negative value for a fortified sample (i.e. a false negative result).

Event: Bt176												
%w/w Fortification Level	0.0%		1.5%		0.0%		0.8%		0.1%		0.1%	
Participant Number	Result	Z-score										
1754	0.0		1.10	-0.83	0.0		0.50	-1.32	0.10	0.00	0.10	0.00
1764	0.0		0.90	-1.24	0.0		0.50	-1.32	0.10	0.00	0.00	-1.84
1769	0.0		1.04	-0.96	0.0		0.52	-1.23	0.09	-0.25	0.10	-0.09
1770	0.0		0.90	-1.24	0.0		0.50	-1.32	0.10	0.00	0.10	0.00
1780	0.0		1.44	-0.12	0.0		0.73	-0.31	0.13	0.76	0.09	-0.18
1783	0.0		0.93	-1.18	0.0		0.52	-1.23	0.10	0.00	0.10	0.00
1788	0.0		0.41	-2.26	0.0		0.18	-2.73	0.10	0.00	0.00	-1.84
1870	0.0		1.20	-0.62	0.0		0.50	-1.32	0.09	-0.25	0.04	-1.11
1891	0.0		2.10	1.24	0.0		0.80	0.00	0.20	2.53	0.20	1.84
2057	0.0		2.00	1.04	0.0		0.70	-0.44	0.10	0.00	0.10	0.00
2128	0.0		1.74	0.50	0.0		0.85	0.22	0.13	0.76	0.10	0.00
2675	0.0		1.90	0.83	0.0		1.10	1.32	0.14	1.01	0.13	0.55
2692	0.0		1.51	0.02	0.0		0.80	0.00	0.11	0.25	0.13	0.55
2694	0.0		1.09	-0.85	0.0		0.56	-1.06	0.09	-0.25	0.09	-0.18
2716	0.0		1.89	0.81	0.0		1.00	0.88	0.10	0.00	0.13	0.55
2822	0.0		1.40	-0.21	0.0		0.70	-0.44	0.00	-2.53	0.00	-1.84

Table 42: Quantitative results and z-scores for corn fortified with Bt11 for all participants (DNA-based assays). Values highlighted in yellow indicate z-scores outside of the expected range, i.e., $z > 2$. Quantifications marked in red indicate values determined to be either: (1) outliers by the “Grubb’s Test for Outliers”; (2) a quantitative value for a non-fortified sample (i.e. a false positive result); or (3) a negative value for a fortified sample (i.e. a false negative result).

Event: Bt11												
%w/w Fortification Level	0.0%		0.1%		0.5%		0.4%		0.0%		0.0%	
Participant Number	Result	z-score										
1754	0.0		0.10	0.00	0.40	-0.75	0.30	-0.81	0.0		0.0	
1764	0.0		0.00	-1.27	0.50	0.00	0.40	0.00	0.0		0.0	
1769	0.0		0.10	-0.06	0.48	-0.15	0.26	-1.17	0.0		0.0	
1770	0.0		0.10	0.00	0.50	0.00	0.60	1.61	0.0		0.0	
1780	0.0		0.08	-0.25	0.52	0.15	0.36	-0.32	0.0		0.0	
1783	0.0		0.10	0.00	0.76	1.94	0.40	0.00	0.0		0.0	
1788	0.0		P	N/A	0.30	-1.49	0.13	-2.18	0.0		0.0	
1870	0.0		0.10	0.00	0.50	0.00	0.30	-0.81	0.0		0.0	
1891	0.0		0.10	0.00	0.40	-0.75	0.20	-1.61	0.0		0.0	
2057	0.0		0.10	0.00	0.35	-1.12	0.25	-1.21	0.0		0.0	
2128	0.0		0.09	-0.13	0.47	-0.22	0.35	-0.40	0.0		0.0	
2675	0.0		0.04	-0.76	0.30	-1.49	0.19	-1.69	0.0		0.0	
2692	0.0		0.27	2.15	*1.51	7.55	*1.54	9.19	0.0		0.0	
2694	0.0		0.03	-0.89	0.31	-1.42	0.21	-1.53	0.0		0.0	
2716	0.0		0.10	0.00	0.50	0.00	0.31	-0.73	0.0		0.0	
2822	0.0		0.30	2.53	0.70	1.49	0.50	0.81	0.0		0.0	

Table 43: Quantitative results and z-scores for corn fortified with NK603 for all participants (DNA-based assays). Values highlighted in yellow indicate z-scores outside of the expected range, i.e., $z > 2$. Quantifications marked in red indicate values determined to be either: (1) outliers by the “Grubb’s Test for Outliers”; (2) a quantitative value for a non-fortified sample (i.e. a false positive result); or (3) a negative value for a fortified sample (i.e. a false negative result).

Event: NK603												
%w/w Fortification Level	0.0%		1.0%		0.1%		0.4%		0.0%		2.0%	
Participant Number	Result	z-score										
1754	0.0		0.60	-1.78	0.10	0.00	0.40	0.00	0.0		0.80	-2.55
1764	0.0		0.50	-2.23	0.00	-2.21	0.30	-0.85	0.0		1.30	-1.49
1769	0.0		0.79	-0.96	0.09	-0.22	0.29	-0.98	0.0		1.62	-0.82
1770	0.0		0.30	-3.12	0.10	0.00	0.20	-1.70	0.0		0.60	-2.98
1773	0.0		0.30	-3.12	0.10	0.00	0.10	-2.56	0.0		0.50	-3.19
1780	0.0		0.45	-2.45	0.08	-0.44	0.34	-0.51	0.0		0.96	-2.21
1783	0.0		0.75	-1.12	0.15	1.10	0.48	0.68	0.0		1.59	-0.87
1847	0.0		0.21	-3.53	0.02	-1.77	0.19	-1.79	0.0		0.48	-3.23
1870	0.0		0.90	-0.45	0.10	0.00	0.30	-0.85	0.0		1.40	-1.28
1891	0.0		0.60	-1.78	0.10	0.00	0.20	-1.70	0.0		0.90	-2.34
2057	0.0		0.75	-1.12	0.10	0.00	0.30	-0.85	0.0		1.40	-1.28
2128	0.0		0.32	-3.03	0.05	-1.10	0.15	-2.13	0.0		0.54	-3.10
2675	0.0		0.94	-0.27	0.06	-0.88	0.36	-0.34	0.0		1.82	-0.38
2692	0.0		0.62	-1.70	0.11	0.22	0.46	0.51	0.0		1.22	-1.66
2694	0.0		0.51	-2.19	0.07	-0.66	0.22	-1.53	0.0		0.88	-2.38
2716	0.0		0.80	-0.89	0.10	0.00	0.38	-0.17	0.0		1.51	-1.04
2822	0.0		0.40	-2.68	0.20	2.21	0.50	0.85	0.0		1.90	-0.21

Table 44: Quantitative results and z-scores for corn fortified with Herculex for all participants (DNA-based assays). Values highlighted in yellow indicate z-scores outside of the expected range, i.e., $z > 2$. Quantifications marked in red indicate values determined to be either: (1) outliers by the “Grubb’s Test for Outliers”; (2) a quantitative value for a non-fortified sample (i.e. a false positive result); or (3) a negative value for a fortified sample (i.e. a false negative result).

Event: Herculex												
%w/w Fortification Level	0.0%		0.0%		0.5%		0.0%		0.8%		0.1%	
Participant Number	Result	z-score										
1754	0.0		0.0		0.20	-1.74	0.0		0.40	-1.82	0.10	0.00
1769	0.0		0.0		0.26	-1.42	0.0		0.41	-1.78	0.05	-1.21
1770	0.0		0.0		0.20	-1.74	0.0		0.40	-1.82	0.10	0.00
1780	0.0		0.0		0.36	-0.81	0.0		0.49	-1.41	0.07	-0.73
1783	0.0		0.0		0.13	-2.14	0.0		0.22	-2.64	0.00	-2.42
1847	0.0		0.0		0.11	-2.26	0.0		0.33	-2.14	0.03	-1.70
1870	0.0		0.0		0.30	-1.16	0.0		0.30	-2.28	0.05	-1.21
1891	0.0		0.0		0.40	-0.58	0.0		0.40	-1.82	0.10	0.00
2057	0.0		0.0		0.55	0.29	0.0		0.80	0.00	0.15	1.21
2128	0.0		0.0		0.36	-0.81	0.0		0.64	-0.73	0.02	-1.94
2675	0.0		0.0		0.11	-2.26	0.0		0.19	-2.78	0.04	-1.45
2692	0.0		0.0		0.32	-1.04	0.0		0.41	-1.78	0.05	-1.21
2694	0.0		0.0		0.21	-1.68	0.0		0.32	-2.19	0.03	-1.70
2716	0.0		0.0		0.72	1.27	0.0		0.98	0.82	0.10	0.00

Table 45: Quantitative results and z-scores for corn fortified with MON863 for all participants (DNA-based assays). Values highlighted in yellow indicate z-scores outside of the expected range, i.e., $z > 2$. Quantifications marked in red indicate values determined to be either: (1) outliers by the “Grubb’s Test for Outliers”; (2) a quantitative value for a non-fortified sample (i.e. a false positive result); or (3) a negative value for a fortified sample (i.e. a false negative result).

Event: MON863												
%w/w Fortification Level	0.0%		0.0%		0.5%		1.5%		0.4%		0.0%	
Participant Number	Result	z-score										
1754	0.0		0.0		0.70	2.08	1.20	-0.66	0.50	1.41	0.0	
1764	0.0		0.0		0.60	1.04	1.80	0.66	0.60	2.83	0.0	
1769	0.0		0.0		0.65	1.56	1.93	0.95	0.61	2.90	0.0	
1770	0.0		0.0		0.60	1.04	1.80	0.66	0.50	1.41	0.0	
1780	0.0		0.0		0.60	1.04	1.60	0.22	0.42	0.28	0.0	
1783	0.0		0.0		0.89	4.06	2.23	1.61	0.54	1.98	0.0	
1870	0.0		0.0		0.50	0.00	1.10	-0.88	0.40	0.00	0.0	
1891	0.0		0.0		0.60	1.04	1.80	0.66	0.50	1.41	0.0	
2057	0.0		0.0		0.60	1.04	1.60	0.22	0.40	0.00	0.0	
2128	0.0		0.0		*0.24	-2.70	0.78	-1.59	*0.15	-3.53	0.0	
2675	0.0		0.0		0.61	1.14	2.22	1.59	0.57	2.40	0.0	
2692	0.0		0.0		*1.18	7.07	2.43	2.05	*0.81	5.80	0.0	
2694	0.0		0.0		0.60	1.04	1.71	0.46	0.54	1.98	0.0	
2716	0.0		0.0		0.55	0.52	1.51	0.02	0.50	1.41	0.0	

Table 46: Quantitative results and z-scores for corn fortified with Herculex RW for all participants (DNA-based assays). Values highlighted in yellow indicate z-scores outside of the expected range, i.e., $z > 2$. Quantifications marked in red indicate values determined to be either: (1) outliers by the “Grubb’s Test for Outliers”; (2) a quantitative value for a non-fortified sample (i.e. a false positive result); or (3) a negative value for a fortified sample (i.e. a false negative result).

Event: Herculex RW												
%w/w Fortification Level	0.0%		0.0%		0.5%		0.8%		0.1%		2.0%	
Participant Number	Result	z-score										
1754	0.0		0.0		1.00	1.25	1.40	1.14	0.20	2.34	3.30	1.80
1780	0.0		0.0		0.88	0.95	0.97	0.32	0.13	0.70	2.95	1.32
1783	0.0		0.0		0.56	0.15	0.88	0.15	0.10	0.00	2.35	0.49
1870	0.0		0.0		1.00	1.25	1.10	0.57	0.10	0.00	3.20	1.66
1891	0.0		0.0		1.70	3.01	2.30	2.86	*0.7	14.01	3.90	2.63
2057	0.0		0.0		0.60	0.25	1.00	0.38	0.10	0.00	2.50	0.69
2694	0.0		0.0		0.87	0.93	1.07	0.51	0.18	1.87	2.95	1.32
2716	0.0		0.0		0.40	-0.25	0.51	-0.55	0.10	0.00	1.50	-0.69

Table 47: Quantitative results and z-scores for corn fortified with MIR604 for all participants (DNA-based assays). Values in yellow indicate z-scores outside of expected range i.e., $z > 2$. No values were determined to be outliers by the Grubb's Test for Outliers in this data set.

Event: MIR604												
%w/w Fortification Level	0.0%		0.0%		0.1%		0.5%		1.5%		0.5%	
Participant Number	Result	z-score										
1754	0.0		0.0		0.10	0.00	0.30	-1.49	1.20	-0.62	0.40	-0.53
1769	0.0		0.0		0.06	-1.35	0.39	-0.86	0.96	-1.13	0.26	-1.28
1774	0.0		0.0		0.02	-2.55	0.05	-3.40	0.10	-2.89	0.06	-2.37
1780	0.0		0.0		0.14	1.20	0.38	-0.90	1.40	-0.21	0.40	-0.53
1870	0.0		0.0		0.10	0.00	0.30	-1.49	0.90	-1.24	0.40	-0.53
1891	0.0		0.0		0.10	0.00	0.30	-1.49	0.90	-1.24	0.30	-1.06
2057	0.0		0.0		0.10	0.00	0.30	-1.49	1.50	0.00	0.50	0.00
2128	0.0		0.0		0.09	-0.30	0.18	-2.39	0.71	-1.63	0.25	-1.33
2675	0.0		0.0		0.06	-1.20	0.22	-2.09	0.36	-2.36	0.27	-1.22
2694	0.0		0.0		0.11	0.30	0.48	-0.15	1.56	0.12	0.37	-0.69
2716	0.0		0.0		0.10	0.00	0.52	0.15	1.50	0.00	0.81	1.65

Table 48: Quantitative results and z-scores for corn fortified with Event 3272 for all participants (DNA-based assays). All values provide acceptable z-scores and no values were determined to be outliers by the Grubb's Test for Outliers in this data set.

Event: Event 3272												
%w/w Fortification Level	0.0%		0.5%		0.0%		0.0%		0.0%		0.1%	
Participant Number	Result	z-score										
1769	0.0		0.43	-0.29	0.0		0.0		0.0		0.08	-0.37
1780	0.0		0.43	-0.27	0.0		0.0		0.0		0.07	-0.44
2057	0.0		0.80	1.16	0.0		0.0		0.0		0.20	1.46
2128	0.0		0.17	-1.27	0.0		0.0		0.0		0.05	-0.73

Table 49: Quantitative results for soybeans fortified with CP4EPSPS (RUR) using Enzyme-Linked Immunosorbent Assay (ELISA) (Protein-based testing) for Participant # 1754 (only this participant submitted results). Quantifications marked in red indicate a false negative result using this method.

Event: RUR			
%w/w Fortification Level	0.5%	0.1%	0.0%
Participant Number	Result	Result	Result
1754	0.30	0.00	0.00

Table 50: Quantitative results and z-scores for soybeans fortified with CP4 EPSPS (RUR) for all participants (DNA-based assays). Values highlighted in yellow indicate z-scores outside of expected range, i.e., $z > 2$ and quantifications marked in red indicate values determined to be outliers by the Grubb's Test for Outliers or a positive value for a non-fortified sample (i.e. a false positive result).

Event: RUR						
%w/w Fortification Level	0.5%		0.1%		0.0%	
Participant Number	Result	z-score	Result	z-score	Result	z-score
1754	0.50	0.00	0.10	0.00	0.0	
1764	0.60	0.51	0.10	0.00	0.0	
1769	0.95	2.28	0.14	1.32	0.0	
1770	0.40	-0.51	0.10	0.00	0.0	
1773	0.70	1.02	0.10	0.00	0.10	
1774	0.25	-1.27	*0.25	4.95	0.00	
1780	0.49	-0.05	0.1/0.09	0.00/-0.33	Not Provided	
1783	0.53	0.15	0.11	0.33	0.0	
1788	0.47	-0.15	0.15	1.65	0.0	
1847	0.36	-0.71	0.10	0.00	0.0	
1858	0.60	0.51	Not Provided	N/A	0.0/0.0	
1862	0.31	-0.96	0.09	-0.33	0.0	
1870	0.50	0.00	0.10	0.00	0.0	
1891	Not Provided	N/A	0.1/0.15	0.00/1.65	0.0	
1892	0.30	-1.02	0.10	0.00	0.0	
2057	0.50	0.00	0.10	0.00	0.0	
2128	0.42	-0.41	0.08	-0.66	0.0	
2132	0.92	2.13	*1.36	41.56	0.0	
2692	*7.45	35.27	*3.09	98.62	0.0	
2694	0.89	1.98	0.16	1.98	0.0	
2705	0.50	0.00	0.06	-1.32	0.0	
2716	*1.5	5.08	*0.51	13.52	0.0	
2720	0.71	1.07	0.17	2.31	0.0	
2725	0.62	0.61	0.15	1.65	0.0	

Table 51: Descriptive statistics for participants reported quantifications relative to GIPSA fortification levels using DNA-based assays. % Relative standard deviation ($\%RSD_R$) = [standard deviation/mean value x 100]; % Relative error = [reported value – fortified value/fortified value x 100]. Outliers were determined by the Grubb’s Test for Outliers and **excluded** from calculations involving reported mean, standard deviation, % relative deviation, and % relative error but were **included** in the range of results.

Transgenic Event	Reported Results (N)	Fortification (%w/w)	Reported Mean	Standard Deviation	% Relative Standard Deviation	% Relative Error	Range of Results
T25	12	0.1	0.09	0.05	55.6%	-10.0%	0.0-0.60
T25	12	0.8	0.85	0.53	62.4%	6.3%	0.06-1.93
T25	12	1.0	1.03	0.72	69.9%	3.0%	0.09-2.56
T25	12	2.0	1.81	1.04	57.5%	-9.5%	0.19-3.81
CBH351	3	0.1	0.20	0.1	50.0%	100.0%	0.10-0.30
CBH351	3	1.0	1.17	0.29	24.8%	17.0%	1.00-1.50
MON810	18	0.1	0.08	0.03	37.5%	-20.0%	0.0-0.1
MON810	18	0.8	0.46	0.22	47.8%	-42.5%	0.0-0.8
MON810	18	1.0	0.60	0.24	40.0%	-40.0%	0.0-2.34
MON810	18	2.0	1.33	0.66	49.6%	-33.5%	0.26-2.98
GA21	16	0.1	0.28	0.23	82.1%	180.0%	0.04-0.98
GA21	16	0.4	0.28	0.12	42.9%	-30.0%	0.10-1.38
GA21	16	0.4	0.36	0.23	63.9%	-10.0%	0.10-0.87
GA21	16	0.8	0.65	0.32	49.2%	-18.8%	0.20-3.00
Bt176	16	0.1	0.11	0.04	36.4%	10.0%	0.0-0.2
Bt176	16	0.1	0.09	0.05	55.6%	-10.0%	0.0-0.2
Bt176	16	0.8	0.65	0.23	35.4%	-18.8%	0.18-1.10
Bt176	16	1.5	1.35	0.48	35.6%	-10.0%	0.41-2.10
Bt11	16	0.1	0.11	0.08	73.0%	10.0%	0.0-0.3
Bt11	16	0.4	0.32	0.12	37.5%	-20.0%	0.13-1.54
Bt11	16	0.5	0.47	0.13	27.7%	-6.0%	0.30-1.51
NK603	17	0.1	0.09	0.05	55.6%	-10.0%	0.0-0.2
NK603	17	0.4	0.30	0.12	40.0%	-25.0%	0.10-0.50
NK603	17	1.0	0.57	0.22	38.6%	-43.0%	0.21-0.94
NK603	17	2.0	1.14	0.47	41.2%	-43.0%	0.48-1.90
Herculex	14	0.1	0.06	0.04	66.7%	-40.0%	0.0-0.15
Herculex	14	0.5	0.30	0.17	56.7%	-40.0%	0.11-0.72
Herculex	14	0.8	0.45	0.22	48.9%	-43.8%	0.19-0.98
MON863	14	0.4	0.51	0.07	13.7%	27.5%	0.40-0.81
MON863	14	0.5	0.63	0.1	15.9%	26.0%	0.24-1.18
MON863	14	1.5	1.69	0.45	26.6%	12.7%	0.78-2.43
HerculexRW	8	0.1	0.13	0.04	30.8%	30.0%	0.10-0.70
HerculexRW	8	0.5	0.88	0.4	45.5%	76.0%	0.40-1.70
HerculexRW	8	0.8	1.15	0.53	46.1%	43.8%	0.51-2.30
HerculexRW	8	2.0	2.83	0.72	25.4%	41.5%	1.50-3.90
MIR604	11	0.1	0.09	0.03	33.3%	-10.0%	0.02-0.14
MIR604	11	0.5	0.31	0.13	41.9%	-38.0%	0.05-0.52
MIR604	11	0.5	0.37	0.19	51.4%	-26.0%	0.06-0.81
MIR604	11	1.5	1.01	0.48	47.5%	-32.7%	0.10-1.56
EV3272	4	0.1	0.10	0.07	70.0%	0.0%	0.05-0.20
EV3272	4	0.5	0.46	0.26	56.5%	-8.0%	0.17-0.80
RUR	23	0.1	0.11	0.03	27.3%	10.0%	0.06-3.09
RUR	25	0.5	0.50	0.2	40.0%	0.0%	0.25-7.45

Summary of Findings

- **Qualitative Sample Analysis**

DNA-based Testing. The method of DNA-based testing for the qualitative determination of events was by a conventional polymerase chain reaction assay (PCR) which generally has a sensitivity of 0.01% w/w transgenic event. The lowest fortification level in this round of proficiency testing was 0.1% w/w; therefore, if the event was present it should be detectable by a given laboratory that employs conventional PCR. As evidenced by the summary of performance scores (**Table 31** and **Figure 1**), eleven of the fifteen transgenic events were correctly detected with greater than or equal to 95% reliability. This was a moderate decline over the performance in the November 2008 round wherein thirteen of the fourteen events were detected with greater than or equal to 95% reliability. The only events that tested with less than 95% reliability included: T25 (91.1%), CBH351 (89.4%), Herculex (88.1%), and Roundup Ready Soy (90.5%). The occurrence of T25 in this category was similar to the trends observed in the May 2008, November 2008 and other previous reports while CBH351, Herculex and Roundup Ready Soy provided greater than 95% reliability in the November 2008 distribution. The failure of these events to test with greater than 95% reliability was due to a higher incidence of false positives (T25= 26.7%, CBH351 = 13.6%, RUR Soy = 13.3%) and not false negatives. The only exception to this trend was Herculex which gave a higher false negative rate (14.3%) compared with false negatives (2.2%).

Protein-based Testing. The principle methods of protein-based testing were lateral flow strips (LFS) and enzyme-linked immunosorbent assay (ELISA). The LFS test has a sensitivity ranging between 0.125 – 1.0% w/w for corn events and 0.1% w/w for soybean event RUR (Strategic Diagnostics Inc., 2001 & 2003). ELISA has a sensitivity of 0.5 - 1% w/w for corn and soy events (Ahmed, 2004). Laboratories demonstrated proficiency when using protein-based methods to detect the presence of biotechnology-derived traits in maize that were fortified above their reported LOD (**Table 32**). Laboratories demonstrated reasonable proficiency, with 2 of 6 false positive and 1 of 12 false negative results, when using protein-based methods to detect the presence of the CP4EPSPS protein in samples fortified with the RoundUp Ready trait (**Tables 33 to 35**).

- **Quantitative Sample Analysis**

DNA-based Testing. The method of DNA-based testing for the quantitative determination of transgenic event was by real-time quantitative PCR. This analytical method has a limit of detection (LOD) of 0.01% w/w and a limit of quantification (LOQ) of approximately 0.1% w/w for a specified event (Ahmed, 2004; Lipp et. al., 2005).

Composite Performance Assessment. These data combined the participants' reported quantifications and evaluated the group's performance by considering the mean value of reported results of all participants (**Table 51**). Because test samples were fortified ranging from 0.1 – 2.0% w/w of a particular event, it was expected that qPCR technologies would detect the traits in all of the fortified samples but not in non-fortified samples. With regard to the detection specificity of qPCR, a disperse number of detects in non-fortified samples were

observed (i.e. false positive results, see **Tables 37, 39, and 50**). A greater number of false negative results were observed for the different traits, (i.e. see **Tables 38, 41, 42, 43, 44, and 49**). Another expectation was that the inter-laboratory variation observed in reported quantifications, as measured by the % Relative Standard Deviation, should be higher in samples fortified at lower amounts (e.g. 0.1% w/w) as compared to the variation observed in samples fortified at higher amounts (e.g. 2.0% w/w). With regard to this inverse relationship between variability (%RSD_R) in reported quantifications and fortification level, the trend held true for CBH351, GA21, Bt-11, NK603, Herculex, and Event 3272 (**Table 51**). This inverse relationship has been observed in the quantitative data from previous rounds of USDA/GIPSA proficiency sample distributions. Though similar trends in these characteristics of inter-laboratory variation were observed, the amount of this variation was for the most part greater than the acceptance criteria of $\leq 35\%$ as established by the Joint Research Council/ENGL (<http://gmo-crl.jrc.ec.europa.eu>). As established by the Joint Research Council/ENGL, the acceptance criterion for trueness is that the percentage relative error in the result should be $\leq 25\%$ in comparison to an accepted reference value—in this case the reference value was the %w/w fortification of the samples. In this round of proficiency testing, there were forty-three trials of inter-laboratory quantifications (i.e., total number of events at the total number of fortification levels) and in twenty-three of those trials the **inter-laboratory relative error** was observed to be $\leq 25\%$ (**Table 51**). This is similar to what was observed in the November 2008 distribution whereby twenty-two of thirty eight trials were observed to be $\leq 25\%$. Thus, these results were approximately 53% concordant with the acceptance criteria for trueness as established by the Joint Research Council/ENGL. Furthermore, there was a tendency for the reported quantifications to be moderately under-estimated (low bias) as evidenced by the observation that approximately 60% of the quantification trials had percentage relative error values that were negative (**Table 51**). This same trend of a low bias in the quantifications in comparison to accepted values was observed in the quantitative data from previous rounds of our proficiency sample distributions which can be found at: <http://www.gipsa.usda.gov/GIPSA/webapp?area=home&subject=grpi&topic=iws-prof-rep>.

Individual Performance Assessment. The performance of each participating laboratory for quantifying transgenic events in the proficiency samples can be observed by inspecting Tables 35 through 47. To assess the accuracy of their reported quantifications, z-scores were computed. Laboratories with z-scores above +2 or below -2 were noted and highlighted in yellow because their result was greater than two standard deviations from the expected value. Interpretation of z-scores assumes that the data have a normal distribution. Data from samples with lower fortification levels (e.g., 0.1% w/w) may not be normally distributed and caution should be used when interpreting these z-scores.

In this round of inter-laboratory proficiency testing, the %RSD_R for several of the transgenic events was greater than 35% for samples that were fortified above 0.1% (**Table 51**). This observation could be due to numerous confounders including zygoty, lack of standardization, the presence of inhibitors in the reaction mix, etc. Monitoring and improving the performance of laboratories that use PCR technologies for the detection and/or quantification of transgenic events in corn and soybeans will improve the reliability of testing methods and the marketing of these commodities. The USDA/GIPSA proficiency testing

program should be complementary to other quality assurance measures that laboratories use to improve their analytical capabilities.

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Trait check Bt1 corn grain lateral flow test kit user guide. November 2001. Strategic Diagnostics, Inc., Newark, DE, part no. 3099998.

Trait check RUR bulk grain lateral flow test kit user guide. November 2003. Strategic Diagnostics, Inc., Newark, DE, part no. 3099956.

***Note: The transgenic seed or grain used to prepare these samples was made available to GIPSA by the Life Science Organizations. Care was taken to ensure the transgenic material was either essentially 100% positive for the event, or adjusted accordingly. The fortified samples were prepared using a process that has been verified to produce homogenous mixes, and representative samples were analyzed to ensure proper fortification and homogeneity. Reference standards are now commercially available for all transgenic traits used in this proficiency program and GIPSA encourages the use of these reference materials when developing internal validated methods.**

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