

Final Report

Proficiency Study: DNA-Based Testing Laboratories

**Grain Inspection, Packers and Stockyards Administration
Technical Services Division
Biotechnology Branch**

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DNA-Based Testing Proficiency Study

Summary

The United States Department of Agriculture (USDA) Grain Inspection, Packers and Stockyards Administration (GIPSA) conducted a proficiency study to assess the capability and reliability of DNA-based testing for U.S. approved biotechnology events in corn. Private and government laboratories in the U.S. and Europe participated in this study. Batches of corn were prepared to contain various combinations of U.S. approved biotechnology corn events, and samples shipped to the participating laboratories for analysis. No methods for analyses were specified, and the laboratories processed the samples in a routine manner.

GIPSA prepared nine batches of corn to contain each of the following biotechnology-derived corn events at a concentration of approximately 0.1 % (weight/weight basis): T25 (Aventis), CBH351 (Aventis), MON810 (Monsanto), GA21 (Monsanto), E176 (Syngenta) and Bt11 (Syngenta). One batch of corn was prepared that contained no biotechnology events. Two samples from each batch (20 grams each), a total of twenty samples, were randomized and shipped to the participating laboratories. Each sample set included a description of the study, a Chain of Custody document, and a data reporting form.

Twelve laboratories agreed to participate in the study, but only eleven laboratories provided results in a timely manner. Participants included five private testing laboratories in the U.S., two U.S. government laboratories, and four testing laboratories in Europe (government and private). The laboratories were asked to provide qualitative results for each of the events, and for the 35S promoter and NOS terminator. Three laboratories provided both qualitative and quantitative results, but the quantitative data is not included in this report.

Seven of the eleven laboratories reported results for all events (Laboratories 2, 3, 5, 6, 7, 8 and 9).

- Laboratories 2, 7 and 9 received a perfect score, i.e.; they correctly analyzed all samples.
- Laboratories 3, 5 and 6 analyzed the majority of samples correctly, but had a small percentage of false negatives and/or false positives.
- Laboratory 8 had a very high percentage of false negatives and a small percentage of false positives.

Four laboratories could not analyze for all events (Laboratory 1, 4, 10 and 11).

- Laboratories 1, 4, 10 and 11 did not report results for GA21
- Laboratories 1 and 11 did not report results for CBH351
- Laboratory 4 did not report results for T25 and MON810
- Laboratories 1, 10 and 11 had a high percentage of false negatives and/or false positives for various events.

Conclusions

This study showed that the capability of laboratories to analyze for biotechnology events varied significantly, i.e., some laboratories could analyze for all events, while other laboratories could analyze for select events. Overall, the performance of the laboratories was generally good, but some laboratories had a significant number of false positives and false negatives.

Purpose of Proficiency Study

The purpose of the study was to assess the capability and reliability of DNA-based testing to detect the presence of corn derived through the use of modern biotechnology and commercially produced in the U.S.

Study Materials

Control corn. Control corn used to prepare samples was obtained from a private grain handling organization, and from GIPSA stored inventories (1995-crop year). All corn was washed and dried prior to grinding. All corn was then ground using a Hammermill, analyzed for biotechnology events, and stored in separate 4 kg batches at approximately 4° C prior to use.

The control corn obtained from the private source was analyzed by Polymerase Chain Reaction (PCR) and found to contain low levels of E176 and MON810. This corn was used to prepare batches that contained E176 and MON810. Subsequent analyses of this corn, conducted during and after completion of the study, showed that it also contained low levels of T25 and Bt11. The corn from GIPSA inventories was analyzed by PCR and found to be free of all biotechnology events. This corn was used to prepare the batches that did not contain MON810 and E176.

Biotechnology-derived corn. All biotechnology-derived corn was obtained from the Life Science Companies that commercialized the particular corn event. The study included six biotechnology events:

- T25 (Aventis)
- CBH351 (Aventis)
- MON810 (Monsanto)
- GA21 (Monsanto)
- E176 (Syngenta)
- Bt11 (Syngenta)

Each of the biotechnology-derived corn materials was analyzed by PCR and/or protein-based testing technologies to verify the purity of the corn. After verification, approximately 50 grams of each corn event was ground using a cryogenic grinder, and stored in a freezer at -20° C.

Preparation of Challenge Samples

Grinding studies were conducted to establish operational parameters to produce a homogenous mixture of the control corn and the biotechnology-derived corn at the 0.1 % level. A total of ten (10) batches of corn were prepared for the Proficiency Study: Nine batches containing various biotechnology events, and one batch that did not contain any biotechnology events. Table 1 below shows the preparation of each batch and the source of control corn used. Since the control corn from the private source contained low levels of E176 and MON810, this corn was used to prepare those batches that contained E176 and MON810. The corn that was free of all biotechnology events was used to prepare those batches that did not contain E176 and MON810.

Prior to preparation of the batches, GIPSA developed and validated a procedure to produce homogeneous mixtures containing biotechnology events at the 0.1 % level. The target level of 0.1 % was deemed to be a reasonable level based on the capabilities of the methodology.

After the batches were prepared and samples distributed to the participating laboratories, additional analyses were conducted, and the corn obtained from a private grain company was found to contain low levels of T25 and Bt11. The batches prepared from this corn were then reanalyzed. Batch 3 was found to contain low levels of T25, and batch 9 was found to contain low levels of Bt11. The level of T25 and Bt11 in these samples appeared to be well below the 0.1 % target level, and therefore, laboratories were not penalized for classifying samples from these batches as either negative or positive for these particular events.

Table 1. Preparation of Challenge Samples

Batch No.	Biotechnology Event						Corn
	T25	CBH351	MON810	GA21	E176	Bt11	Used
Batch 1	Negative	Negative	Negative	Negative	Negative	Negative	TSD
Batch 2	Negative	Positive	Positive	Positive	Positive	Positive	Private
Batch 3	Positive ¹	Negative	Positive	Positive	Positive	Positive	Private
Batch 4	Negative	Negative	Negative	Positive	Positive	Positive	Private
Batch 5	Positive	Negative	Negative	Negative	Positive	Positive	Private
Batch 6	Positive	Positive	Negative	Negative	Negative	Positive	TSD
Batch 7	Positive	Positive	Positive	Negative	Negative	Negative	TSD
Batch 8	Positive	Positive	Positive	Positive	Negative	Negative	TSD
Batch 9	Positive	Positive	Positive	Positive	Positive	Positive ²	Private
Batch 10	Positive	Positive	Positive	Positive	Positive	Positive	Private

¹Batch 3 inadvertently contained low levels of T25, confirmed by subsequent analyses, and supported by observations from the Proficiency Study.

²Batch 9 inadvertently contained low levels of Bt11, confirmed by subsequent analyses, and supported by observations from the Proficiency Study.

Proficiency Study Samples and Documents

Each laboratory received the following:

- Twenty (20) ground samples, two from each batch, 20 grams per sample
- Letter on the intent on the study, the materials to be shipped, and a projected completion date
- Brief description of the Proficiency Study
- Chain of Custody document
- Data report form

Each laboratory was asked to provide the following information:

- A description of the DNA-based testing technology used in their particular laboratory
- The estimated minimum detectable limit (MDL) for each biotechnology event
- Qualitative analyses for each biotechnology event, including the 35S promoter and NOS terminator
- Quantitative analyses for each biotechnology event, including the 35S promoter and NOS terminator (Note: Quantitative analytical results were optional for all events)

Proficiency Study Participants

Twelve laboratories originally agreed to participate in the study, but one laboratory was unable to complete the sample analyses within the study timeframes. Therefore, eleven (11) laboratories reported results that are summarized in this report:

- Five (5) U.S. private laboratories
- Two (2) U.S. government laboratories
- Four (4) foreign laboratories (government and private laboratories)

Result and Discussion

Each laboratory was asked to submit their data electronically or by hard copy. Table 2 shows each laboratory's capability with respect to the biotechnology events.

Table 2. Laboratory Data Submissions

<u>Laboratory ID</u>	<u>Events Reported</u>	<u>Comments</u>
Laboratory 1	35S, NOS, T25, MON810, E176 and Bt11	No data submitted for CBH351 and GA21 Qualitative results only
Laboratory 2	35S, NOS, T25, CBH351, MON810, GA21 E176 and Bt11	Data for all events submitted Qualitative results only
Laboratory 3	35S, NOS, T25, CBH351, MON810, GA21 E176 and Bt11	Data for all events submitted Qualitative results only
Laboratory 4	35S, NOS, CBH351, E176 and Bt11	No data submitted for T25, MON810 and GA21 Qualitative results only
Laboratory 5	35S, NOS, T25, CBH351, MON810, GA21 E176 and Bt11	Data for all events submitted Qualitative and quantitative results
Laboratory 6	35S, NOS, T25, CBH351, MON810, GA21 E176 and Bt11	Data for all events submitted Qualitative and quantitative results
Laboratory 7	35S, NOS, T25, CBH351, MON810, GA21 E176 and Bt11	Data for all events submitted Qualitative and quantitative results
Laboratory 8	35S, NOS, T25, CBH351, MON810, GA21 E176 and Bt11	Data for all events submitted Qualitative results only
Laboratory 9	35S, NOS, T25, CBH351, MON810, GA21 E176 and Bt11	Data for all events submitted Qualitative results only
Laboratory 10	35S, NOS, T25, CBH351, MON810, E176 and Bt11	No data submitted for GA21 Qualitative results only
Laboratory 11	35S, NOS, T25, MON810, E176 and Bt11	No data submitted for CBH351 and GA21 Qualitative results only

This study showed that the capability to analyze for all events and the performance on the individual events varied among laboratories. Seven of the eleven laboratories reported results for all events, and four laboratories reported results for select events. Three laboratories correctly analyzed all samples, and the performance of the remaining laboratories varied significantly. A summary of laboratory performance follows.

- Three laboratories analyzed all samples correctly for all events: Laboratories 2, 7 and 9.
- Three laboratories analyzed the samples for all events, but had some false negatives and/or false positives: Laboratories 3, 5 and 6.
- One laboratory analyzed for all events, but had a high percentage of false negatives and a low percentage of false positives: Laboratory 8.
- Four laboratories did not report results for all events, with variable levels of false negatives and/or false positives found: Laboratories 1, 4, 10 and 11.

Conclusions

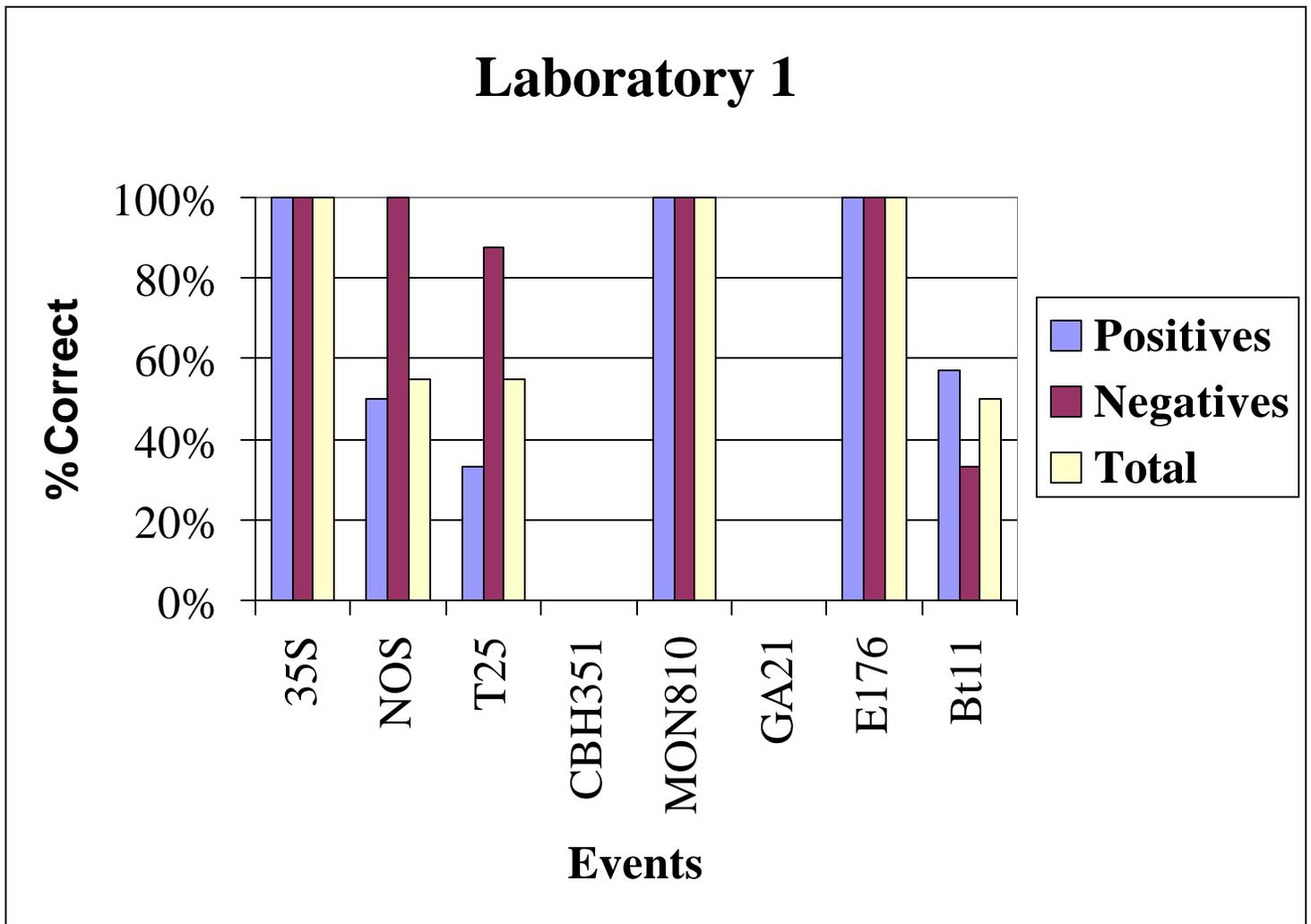
This study showed that the capability of laboratories to analyze for biotechnology events varied significantly, i.e., some laboratories could analyze for all events, while other laboratories could analyze for select events. Overall, the performance of the laboratories was generally good, but some laboratories had a significant number of false positives and false negatives.

Individual Laboratory Data: Laboratory 1

This laboratory submitted data for 35S, NOS, T25, MON810, E176 and Bt11. The laboratory was not able to analyze for CBH351 and GA21. This laboratory demonstrated good performance on 35S, MON810 and E176, but false negatives and/or false positives were observed for NOS, T25 and Bt11.

	Event (% Correct)							
	<u>35S</u>	<u>NOS</u>	<u>T25</u>	<u>CBH351</u>	<u>MON810</u>	<u>GA21</u>	<u>E176</u>	<u>Bt11</u>
Positives	100%	50%	33%	N/A	100%	N/A	100%	57%
Negatives	100%	100%	88%	N/A	100%	N/A	100%	33%
Total	100%	55%	55%	N/A	100%	N/A	100%	50%

Note: Based on results obtained, T25 and Bt11 may have been present in select samples, and the laboratory was not penalized for identifying those samples as containing T25 and Bt11.

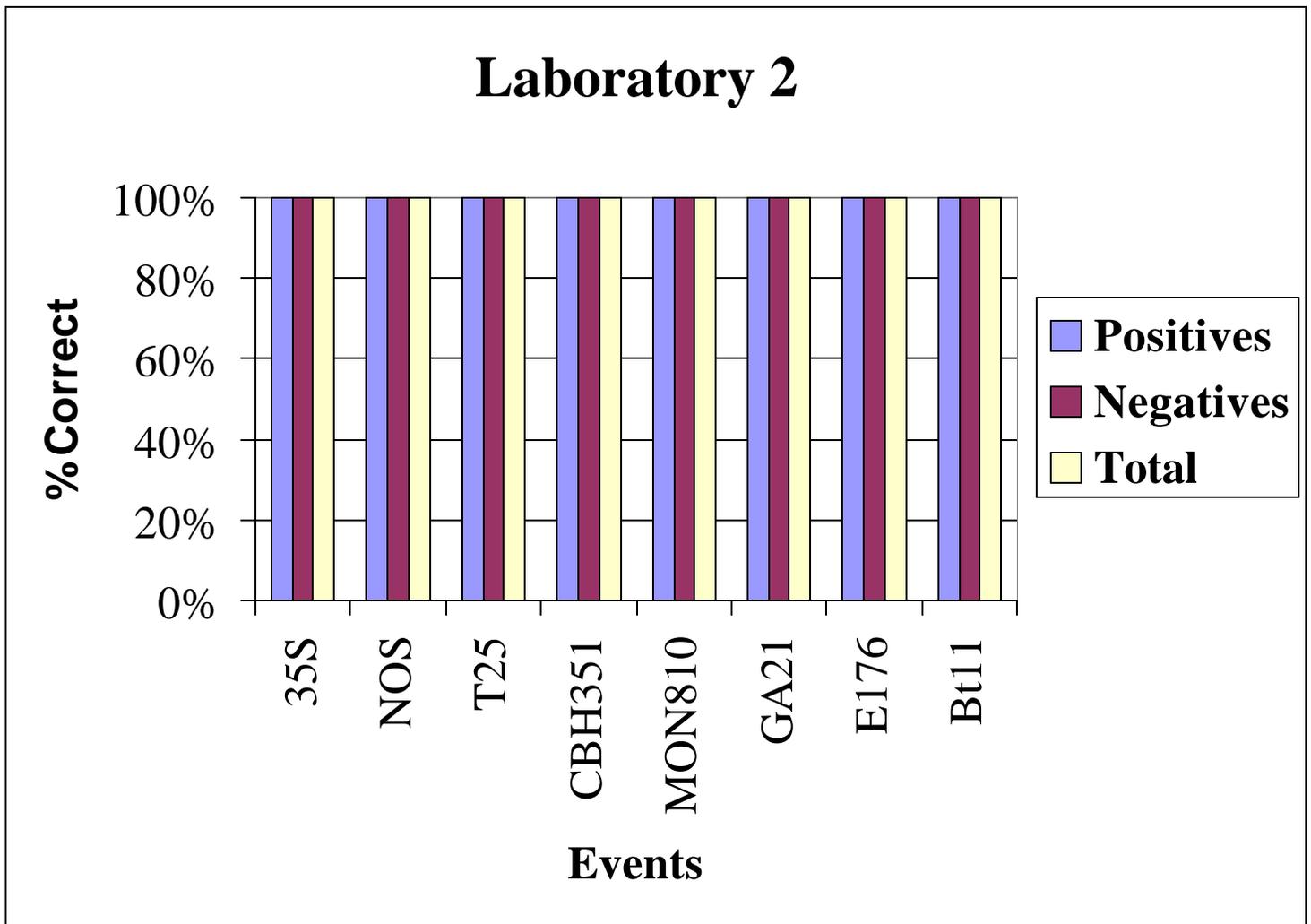


Individual Laboratory Data: Laboratory 2

This laboratory submitted data for all events, and demonstrated good performance on 35S, NOS, T25, CBH351, MON810, GA21, E176 and Bt11.

	Event (% Correct)							
	<u>35S</u>	<u>NOS</u>	<u>T25</u>	<u>CBH351</u>	<u>MON810</u>	<u>GA21</u>	<u>E176</u>	<u>Bt11</u>
Positives	100%	100%	100%	100%	100%	100%	100%	100%
Negatives	100%	100%	100%	100%	100%	100%	100%	100%
Total	100%	100%	100%	100%	100%	100%	100%	100%

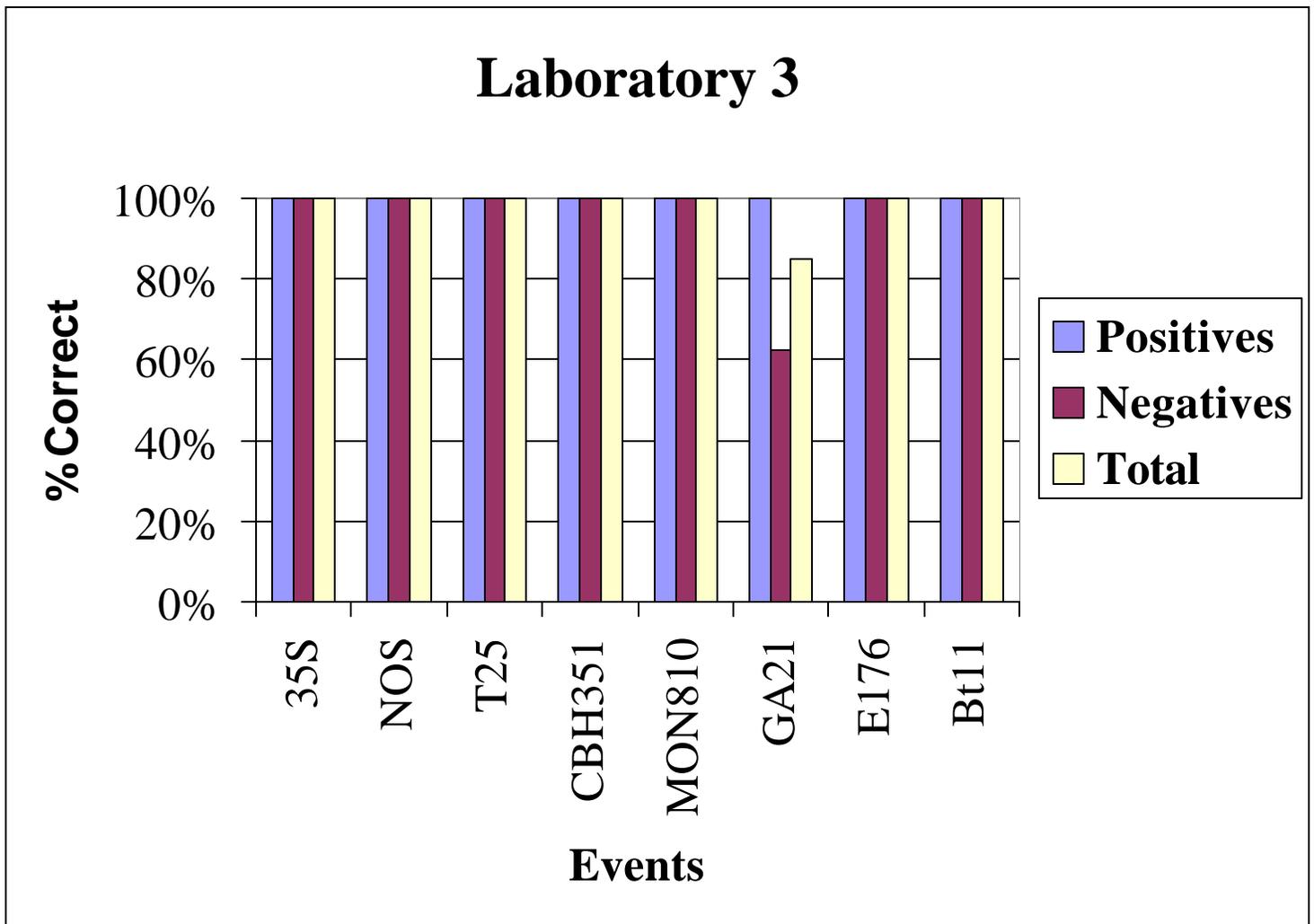
Note: Based on results obtained, T25 and Bt11 may have been present in select samples, and the laboratory was not penalized for identifying those samples as containing T25 and Bt11.



Individual Laboratory Data: Laboratory 3

This laboratory submitted data for all events, and demonstrated good performance on 35S, NOS, T25, CBH351, MON810, E176 and Bt11, but false positives were observed for GA21.

	Event (% Correct)							
	<u>35S</u>	<u>NOS</u>	<u>T25</u>	<u>CBH351</u>	<u>MON810</u>	<u>GA21</u>	<u>E176</u>	<u>Bt11</u>
Positives	100%	100%	100%	100%	100%	100%	100%	100%
Negatives	100%	100%	100%	100%	100%	63%	100%	100%
Total	100%	100%	100%	100%	100%	85%	100%	100%

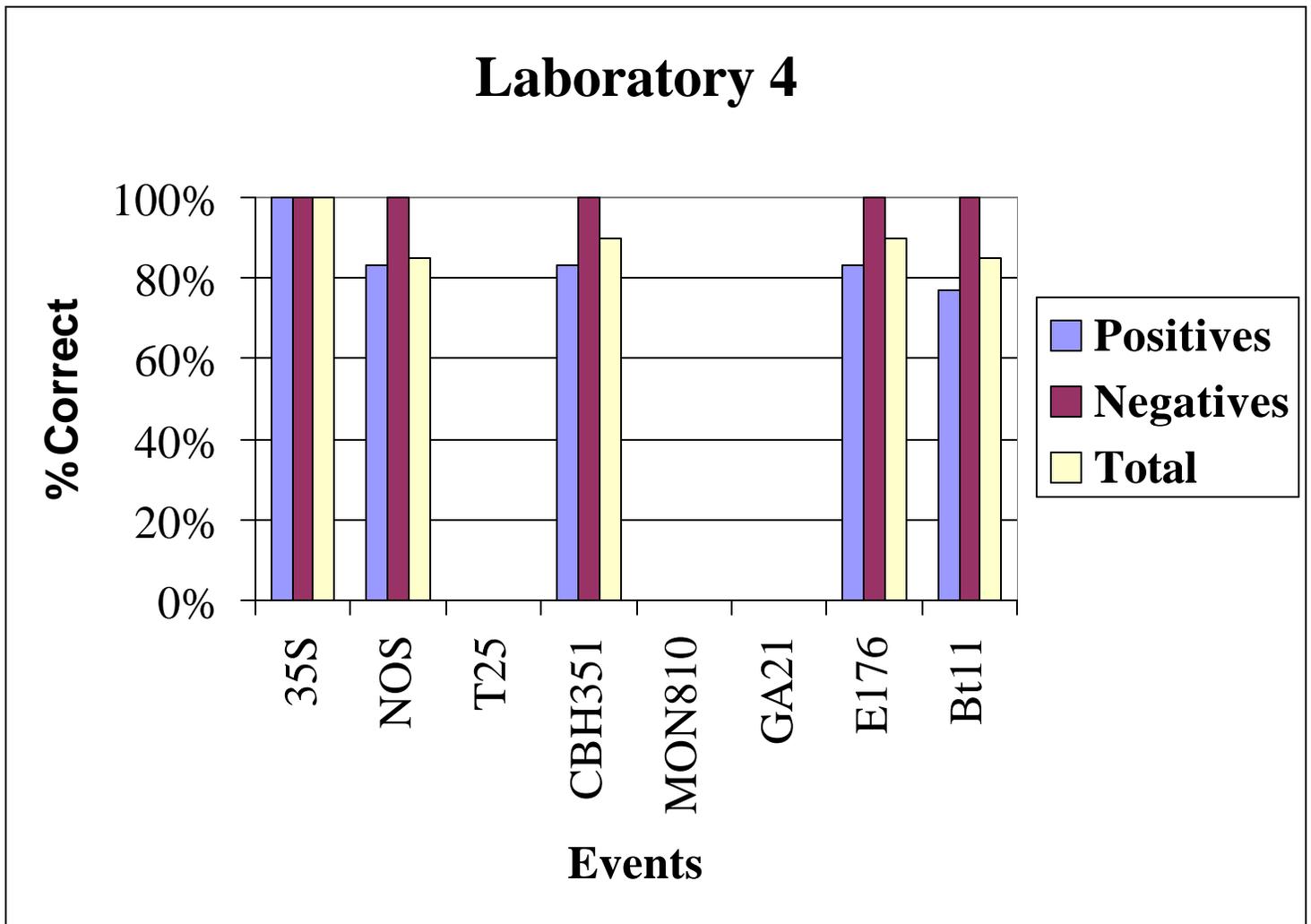


Individual Laboratory Data: Laboratory 4

This laboratory submitted data for 35S, NOS, CBH351, E176 and Bt11. The laboratory was not able to analyze for T25, MON810 and GA21. This laboratory demonstrated good performance on 35S, but false negatives were observed for NOS, CBH351, E176 and Bt11.

	Event (% Correct)							
	<u>35S</u>	<u>NOS</u>	<u>T25</u>	<u>CBH351</u>	<u>MON810</u>	<u>GA21</u>	<u>E176</u>	<u>Bt11</u>
Positives	100%	83%	N/A	83%	N/A	N/A	83%	77%
Negatives	100%	100%	N/A	100%	N/A	N/A	100%	100%
Total	100%	85%	N/A	90%	N/A	N/A	90%	85%

Note: Based on results obtained, T25 and Bt11 may have been present in select samples, and the laboratory was not penalized for identifying those samples as containing T25 and Bt11.

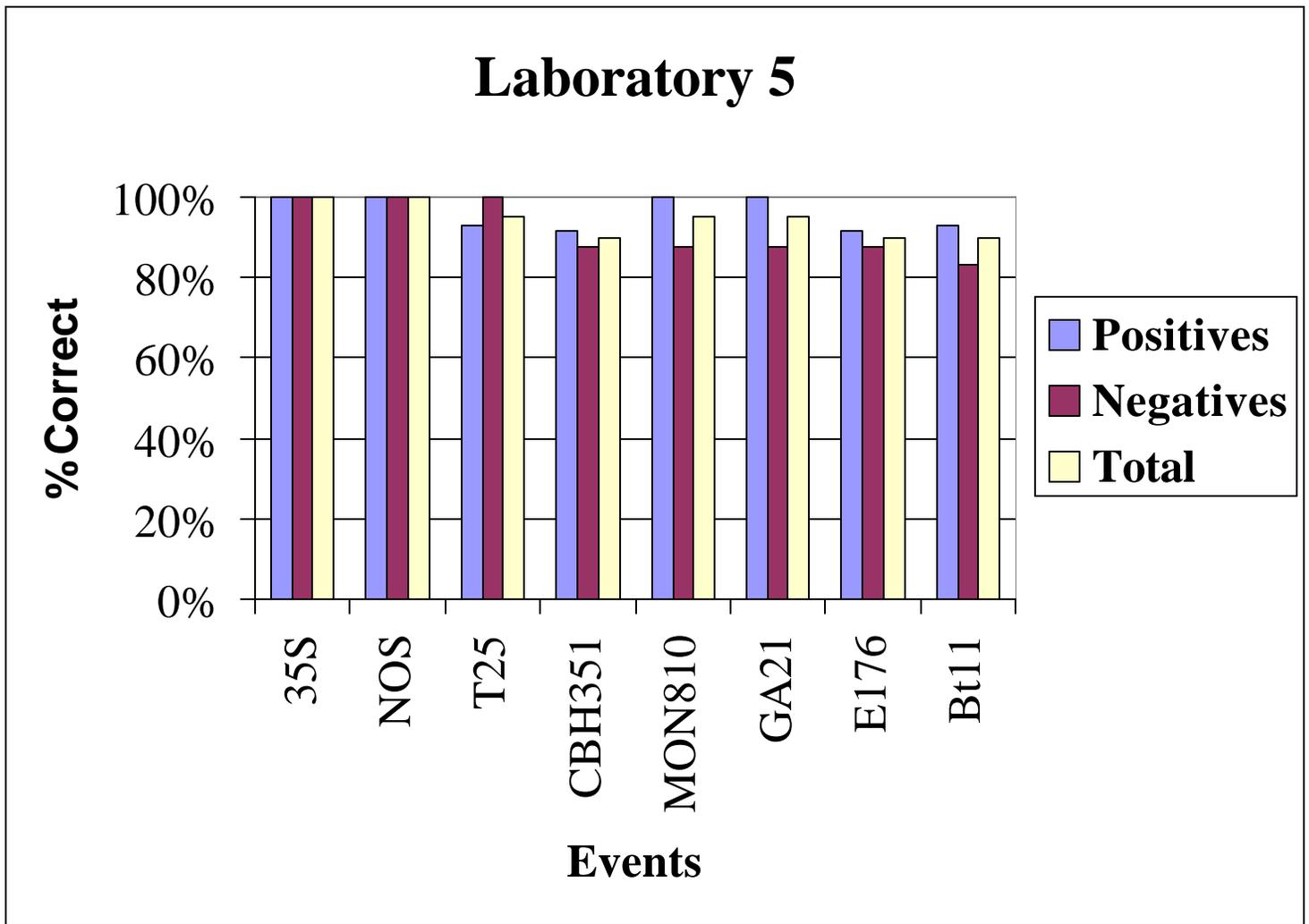


Individual Laboratory Data: Laboratory 5

This laboratory submitted data for all events and demonstrated good performance on 35S and NOS, but false negatives and/or false positives were observed for T25, CBH351, MON810, GA21, E176 and Bt11.

	Event (% Correct)							
	<u>35S</u>	<u>NOS</u>	<u>T25</u>	<u>CBH351</u>	<u>MON810</u>	<u>GA21</u>	<u>E176</u>	<u>Bt11</u>
Positives	100%	100%	93%	92%	100%	100%	92%	93%
Negatives	100%	100%	100%	88%	88%	88%	88%	83%
Total	100%	100%	95%	90%	95%	95%	90%	90%

Note: Based on results obtained, T25 and Bt11 may have been present in select samples, and the laboratory was not penalized for identifying those samples as containing T25 and Bt11.

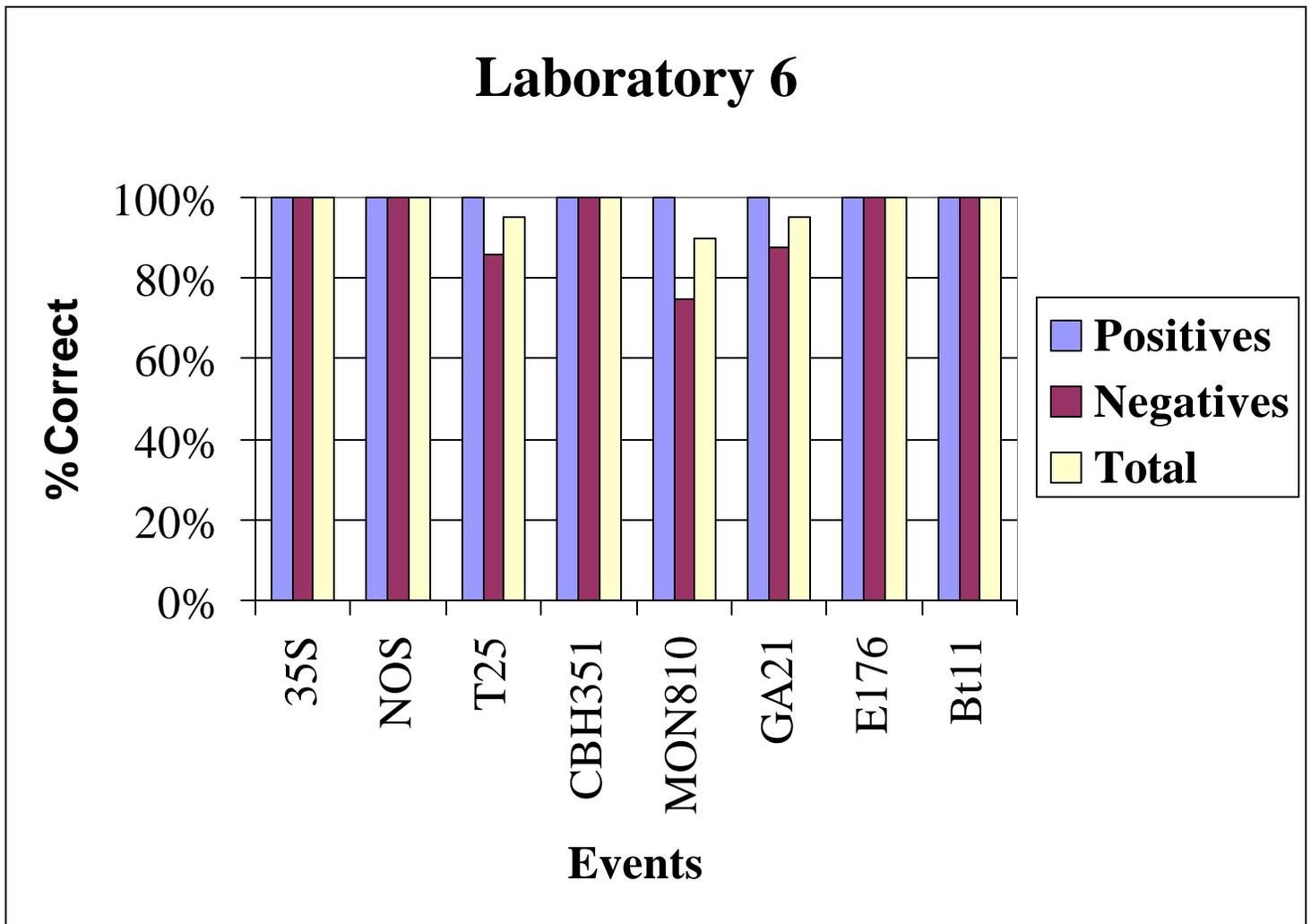


Individual Laboratory Data: Laboratory 6

This laboratory submitted data for all events, and demonstrated good performance on 35S, NOS, CBH351, E176 and Bt11, but false positives were observed for T25, MON810 and GA21.

	Event (% Correct)							
	<u>35S</u>	<u>NOS</u>	<u>T25</u>	<u>CBH351</u>	<u>MON810</u>	<u>GA21</u>	<u>E176</u>	<u>Bt11</u>
Positives	100%	100%	100%	100%	100%	100%	100%	100%
Negatives	100%	100%	86%	100%	75%	88%	100%	100%
Total	100%	100%	95%	100%	90%	95%	100%	100%

Note: Based on results obtained, T25 and Bt11 may have been present in select samples, and the laboratory was not penalized for identifying those samples as containing T25 and Bt11.

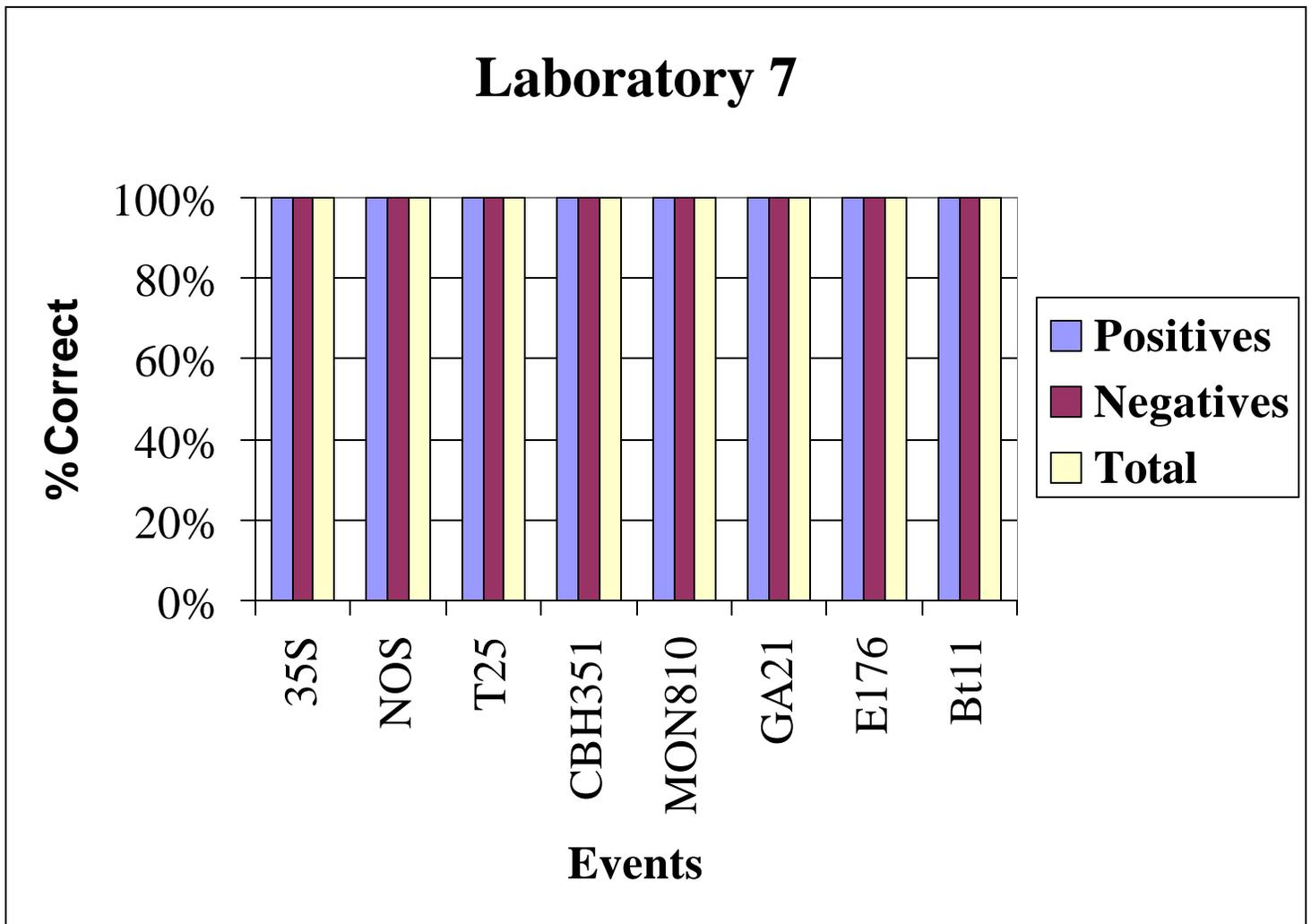


Individual Laboratory Data: Laboratory 7

This laboratory submitted data for all events, and demonstrated good performance on 35S, NOS, T25, CBH351, MON810, GA21, E176 and Bt11.

	Event (% Correct)							
	<u>35S</u>	<u>NOS</u>	<u>T25</u>	<u>CBH351</u>	<u>MON810</u>	<u>GA21</u>	<u>E176</u>	<u>Bt11</u>
Positives	100%	100%	100%	100%	100%	100%	100%	100%
Negatives	100%	100%	100%	100%	100%	100%	100%	100%
Total	100%	100%	100%	100%	100%	100%	100%	100%

Note: Based on results obtained, T25 and Bt11 may have been present in select samples, and the laboratory was not penalized for identifying those samples as containing T25 and Bt11.

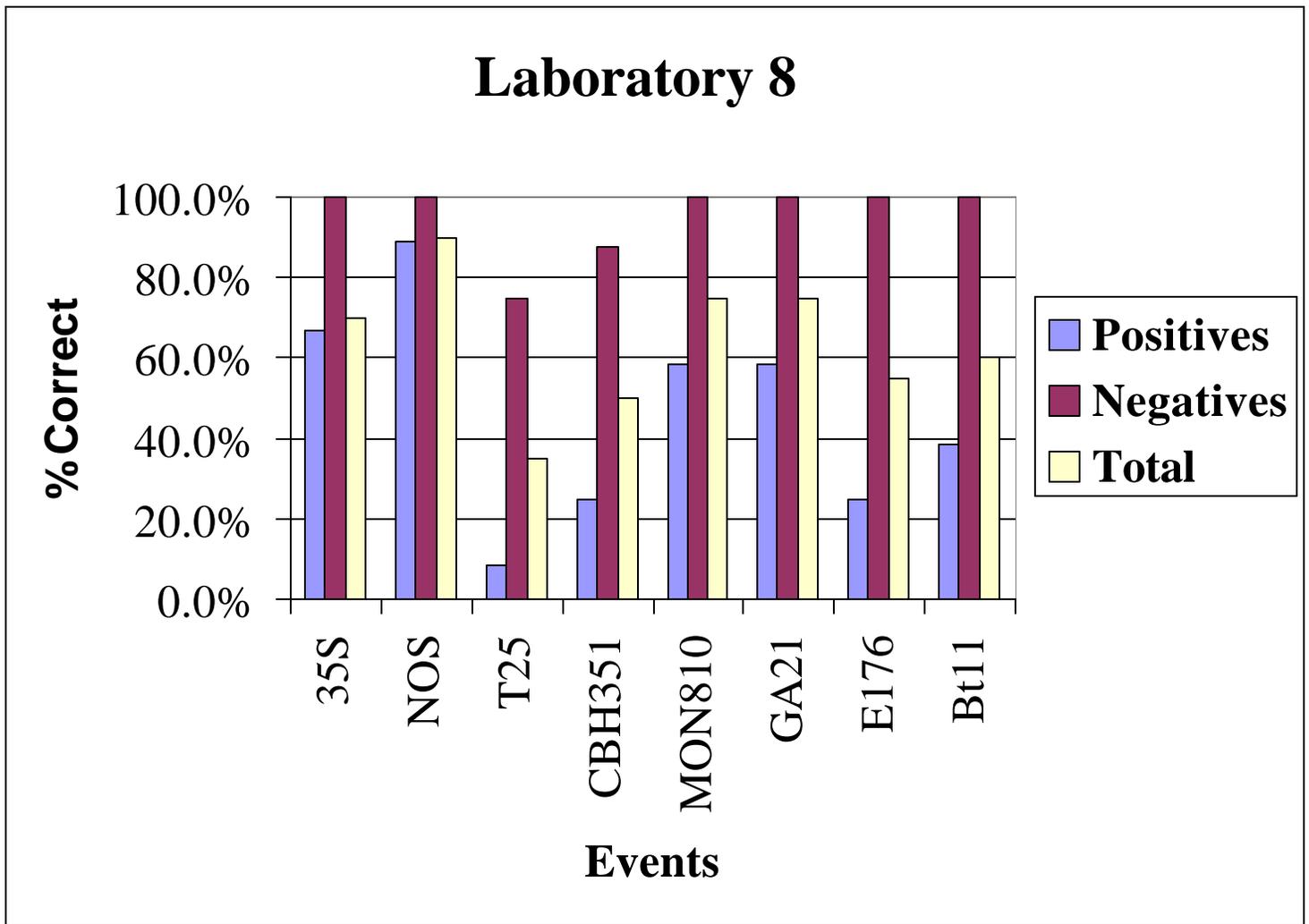


Individual Laboratory Data: Laboratory 8

This laboratory submitted data for all events, but false negatives and/or false positives were observed for 35S, NOS, T25, CBH351, MON810, GA21, E176 and Bt11.

	Event (% Correct)							
	<u>35S</u>	<u>NOS</u>	<u>T25</u>	<u>CBH351</u>	<u>MON810</u>	<u>GA21</u>	<u>E176</u>	<u>Bt11</u>
Positives	67%	89%	8%	25%	58%	58%	25%	39%
Negatives	100%	100%	75%	88%	100%	100%	100%	100%
Total	70%	90%	35%	50%	75%	75%	55%	60%

Note: Based on results obtained, T25 and Bt11 may have been present in select samples, and the laboratory was not penalized for identifying those samples as containing T25 and Bt11.

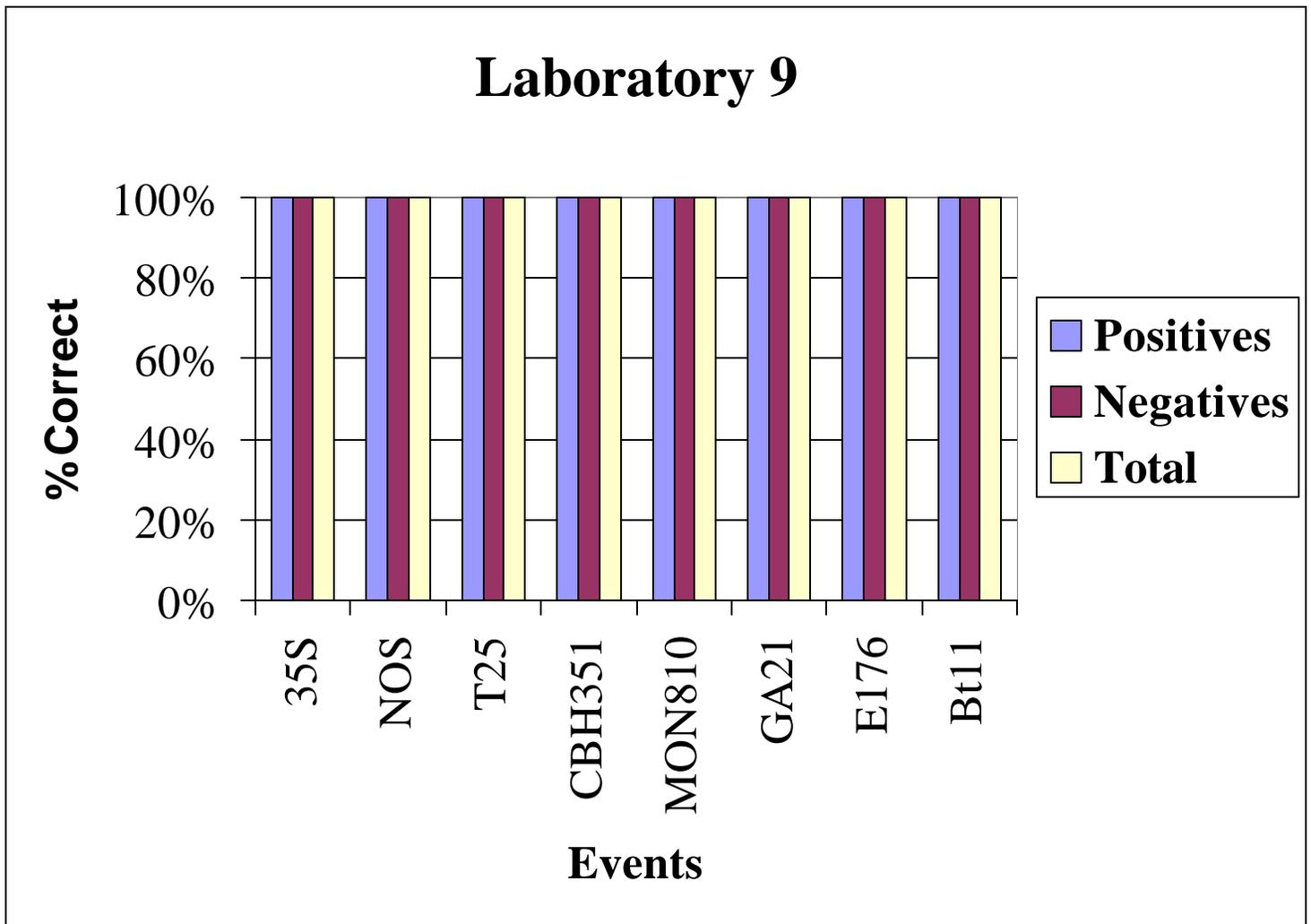


Individual Laboratory Data: Laboratory 9

This laboratory submitted data for all events, and demonstrated good performance on 35S, NOS, T25, CBH351, MON810, GA21, E176 and Bt11.

	Event (% Correct)							
	<u>35S</u>	<u>NOS</u>	<u>T25</u>	<u>CBH351</u>	<u>MON810</u>	<u>GA21</u>	<u>E176</u>	<u>Bt11</u>
Positives	100%	100%	100%	100%	100%	100%	100%	100%
Negatives	100%	100%	100%	100%	100%	100%	100%	100%
Total	100%	100%	100%	100%	100%	100%	100%	100%

Note: Based on results obtained, T25 and Bt11 may have been present in select samples, and the laboratory was not penalized for identifying those samples as containing T25 and Bt11.

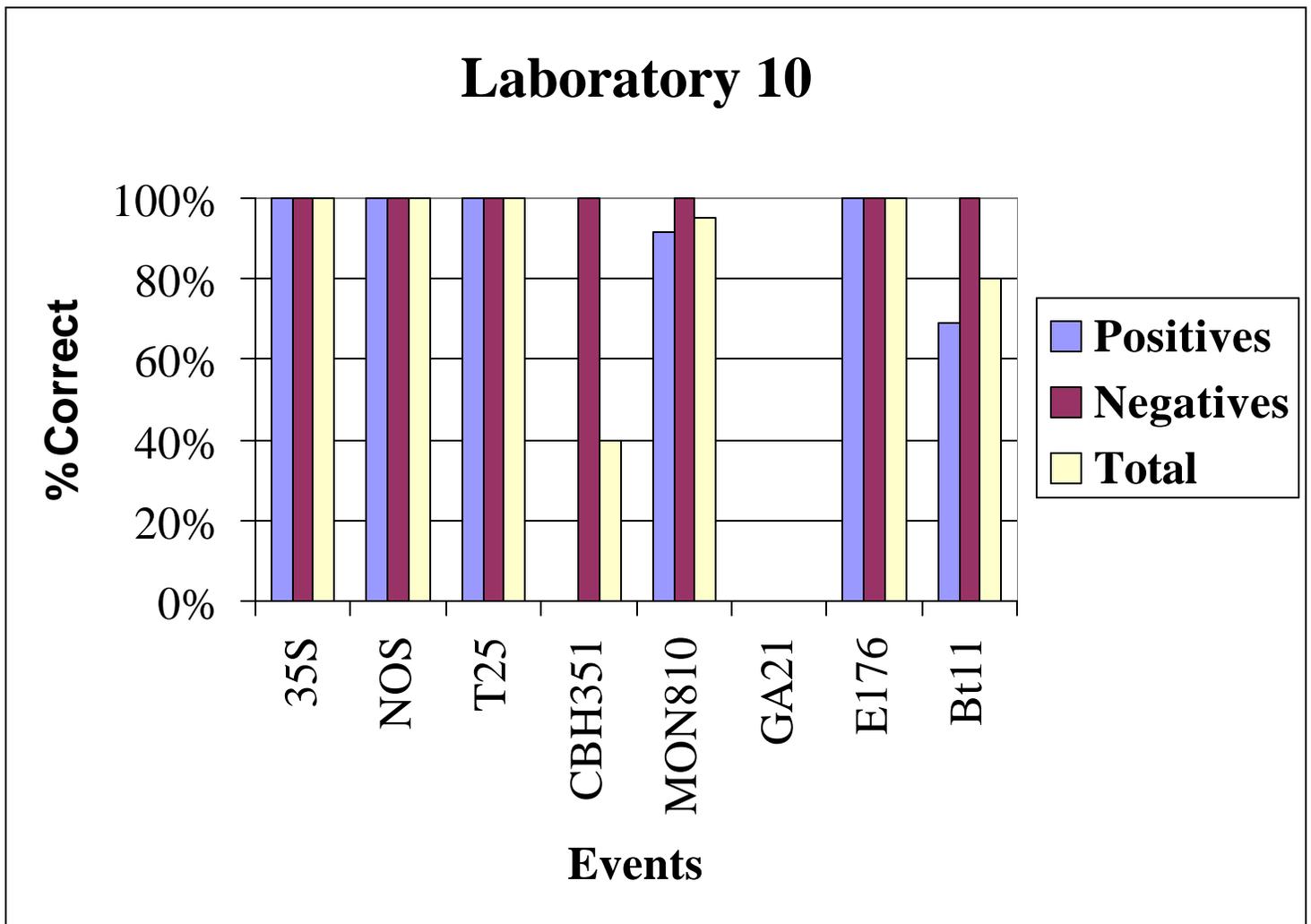


Individual Laboratory Data: Laboratory 10

This laboratory submitted data for 35S, NOS, T25, CBH351, MON810, E176 and Bt11. The laboratory was not able to analyze for GA21. The laboratory demonstrated good performance for 35S, NOS, T25, MON810 and E176, but false negatives and/or false positives were observed for CBH351 and Bt11.

	Event (% Correct)							
	<u>35S</u>	<u>NOS</u>	<u>T25</u>	<u>CBH351</u>	<u>MON810</u>	<u>GA21</u>	<u>E176</u>	<u>Bt11</u>
Positives	100%	100%	100%	0%	92%	N/A	100%	69%
Negatives	100%	100%	100%	100%	100%	N/A	100%	100%
Total	100%	100%	100%	40%	95%	N/A	100%	80%

Note: Based on results obtained, T25 and Bt11 may have been present in select samples, and the Laboratory was not penalized for identifying those samples as containing T25 and Bt11.



Individual Laboratory Data: Laboratory 11

This laboratory submitted data for 35S, NOS, T25, MON810, E176 and Bt11. The laboratory was not able to analyze for CBH351 and GA21. The laboratory demonstrated good performance NOS and T25, but false negatives and /or false negatives were observed for 35S, MON810, E176 and Bt11.

	Event (% Correct)							
	<u>35S</u>	<u>NOS</u>	<u>T25</u>	<u>CBH351</u>	<u>MON810</u>	<u>GA21</u>	<u>E176</u>	<u>Bt11</u>
Positives	83%	94%	92%	N/A	67%	N/A	67%	50%
Negatives	100%	100%	100%	N/A	100%	N/A	100%	100%
Total	85%	95%	95%	N/A	80%	N/A	80%	70%

