

CORESTA
Task Force Genetically
Modified Tobacco –
Collaborative Study on
Detection Methods

**PROFICIENCY TESTING TRIALS
FOR DETECTION OF GM
TOBACCO**

Final Report to Participants

May 2002

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GM TOBACCO**

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CONTENTS

Executive Summary	1
Round 1 Information	2
Round 1 Results	5
Round 1 Summary of Participants' Methodologies	8
Round 2 Information	9
Round 2 Results	11
Technical Discussion and Conclusions	15

Executive Summary

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A project was commissioned by the CORESTA Task Force Genetically Modified Tobacco – Collaborative Study on Detection Methods, to study the state-of-the-art in the detection of GM species in tobacco. 18 Laboratories applied to take part in this study, but 2 of these withdrew at an early stage.

Two rounds of a proficiency test were carried out as part of this study, both of them concerned solely with the detection of GM tobacco in mixtures containing GM and non-GM tobacco at levels between 0% and 5% GM tobacco.

The study has highlighted issues surrounding the ability of laboratories to detect GM tobacco at low levels, and the suitability of certain primers for these analyses.

The results obtained for both rounds have generated data of a high calibre with most participants correctly identifying the majority of GM containing samples. All laboratories were able to detect down to 0.5% GM which is below the cut off limit already decided for food ingredients within the EU.

Round 1

This round was conducted in accordance with the project Protocol (May 2001, as amended October 2001). The samples, together with positive and negative controls were despatched on November 7, 2001 with a result deadline of December 14, 2001, which was extended to December 24, 2001 as some participants experienced delays with delivery of samples and/or replacement primers. Out of the 16 possible participants 15 submitted results. 35S primers were included with each sample set, together with instructions for use, and a suggested extraction protocol. Participants were encouraged, however, to use their own procedures as the objective of a proficiency test is to study routine procedures used in participating laboratories.

Samples were labelled 1 through 8, which equated to samples A to H as described in the protocol. The details of each sample are given below.

Sample Information

Sample Identifier	Description
A (1)	2% GM Tobacco
B (2)	Blank
C (3)	0.1% GM Tobacco
D (4)	1% GM Tobacco
E (5)	5% GM Tobacco
F (6)	Blank
G (7)	0.1% GM Tobacco
H (8)	0.5% GM Tobacco

Participants were not given these sample descriptions; all samples were supplied "blind". Participants were not requested to provide proof of DNA amplifiability, i.e. by sending gel pictures or real-time PCR trace files.

Homogeneity Data

Table 1: The number of positive readings for each sample, out of the 3 amplifications carried out using Primer Set P35sA/B.

Sample	Replicate Analyses															
	1		2		3		4		5		6		7		8	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
A (1)	3	3	3	3	2	3	3	3	3	3	3	3	3	3	3	3
B (2)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C (3)	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
D (4)	2	3	3	3	3	3	3	3	3	3	3	2	3	3	3	3
E (5)	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
F (6)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G (7)	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
H (8)	3	2	3	1	2	1	2	1	0	3	0	3	0	0	0	0

Table 2: The number of positive readings for each sample, out of the 3 amplifications carried out using Primer Set NOS1/3.

Sample	Replicate Analyses															
	1		2		3		4		5		6		7		8	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
A (1)	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
B (2)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C (3)	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0
D (4)	3	3	3	3	3	3	3	2	3	3	2	3	3	3	3	3
E (5)	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
F (6)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G (7)	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0
H (8)	1	2	3	0	1	1	3	3	0	2	3	1	0	0	0	0

The samples are homogeneous, with the exception to sample H, according to the system described in the protocol for the assessment of sample homogeneity.

It is also noted that although samples C and G (the same sample) are homogeneous, the laboratory failed to detect GM species at the 0.1% level at which they were prepared.

RESULTS of ROUND 1

Sample	A		B		C	
	Supplied	Own	Supplied	Own	Supplied	Own
	Primer	Primer	Primer	Primer	Primer	Primer
Laboratory ID						
2	Yes	Yes	No	No	Yes	Yes
3	No	Yes	No	No	No	No
4	Yes		No		Yes	
5	Yes	Yes	No	No	No	Yes
6	Yes	Yes	No	No	No	Yes
7	Yes	Yes	No	No	No	No
8						
9	Yes	Yes	No	Yes	No	No
10	Yes	Yes	No	No	Yes	Yes
12	Yes	Yes	No	No	Yes	Yes
13	Yes	Yes	No	No	Yes	Yes
14	Yes	Yes	No	No	No	No
15	Yes	Yes	No	No	Yes	Yes
16	Yes		No		No	
17	Yes		No		Yes	
18	Yes	Yes	Yes	Yes		Yes
Number of Labs finding GM	14/15	12/12	1/15	1/12	7/14	7/12
Percentage of Labs finding GM	93.3	100.0	6.7	8.3	50.0	58.3
Number of Labs not finding GM	1/15	0/12	14/15	11/12	7/14	5/12
Percentage of Labs not finding GM	6.7	0.0	93.3	91.7	50.0	41.7
Sample Descriptor (Nominal)	2% GM		Blank		0.1% GM	
Laboratories 1 and 11 withdrew from the trial at an early stage						

Sample	D		E		F	
	Supplied	Own	Supplied	Own	Supplied	Own
	Primer	Primer	Primer	Primer	Primer	Primer
Laboratory ID						
2	Yes	Yes	Yes	Yes	No	No
3	No	No	No	Yes	No	No
4	Yes		Yes		No	
5	Yes	Yes	Yes	Yes	No	No
6	Yes	Yes	Yes	Yes	No	No
7	Yes	Yes	Yes	Yes	No	No
8						
9	Yes	Yes	Yes	Yes	No	No
10	Yes	Yes	Yes	Yes	No	No
12	Yes	Yes	Yes	Yes	No	No
13	Yes	Yes	Yes	Yes	No	No
14	Yes	Yes	Yes	Yes	No	No
15	Yes	Yes	Yes	Yes	No	No
16	Yes		Yes		No	
17	Yes		Yes		No	
18	Yes	Yes	No	No	No	No
Number of Labs finding GM	14/15	11/12	13/15	11/12	0/15	0/12
Percentage of Labs finding GM	93.3	91.7	86.7	91.7	0.0	0.0
Number of Labs not finding GM	1/15	1/12	2/15	1/12	15/15	12/12
Percentage of Labs not finding GM	6.7	8.3	13.3	8.3	100.0	100.0
Sample Descriptor (Nominal)	1% GM		5% GM		Blank	
Laboratories 1 and 11 withdrew from the trial at an early stage						

Sample	G		H	
	Supplied	Own	Supplied	Own
	Primer	Primer	Primer	Primer
Laboratory ID				
2	Yes	Yes	Yes	Yes
3	No	No	No	No
4	Yes		Yes	
5	No	Yes	Yes	Yes
6	Yes	Yes	Yes	Yes
7	No	No	No	Yes
8				
9	No	Yes	Yes	Yes
10	Yes	Yes	Yes	Yes
12	Yes	Yes	Yes	Yes
13	Yes	Yes	Yes	Yes
14	No	Yes	Yes	Yes
15	Yes	Yes	Yes	Yes
16	Yes		Yes	
17	No		Yes	
18		Yes	Yes	Yes
Number of Labs finding GM	8/15	10/12	13/15	11/12
Percentage of Labs finding GM	53.3	83.3	86.7	91.7
Number of Labs not finding GM	7/15	2/12	2/15	1/12
Percentage of Labs not finding GM	46.7	16.7	13.3	8.3
Sample Descriptor (Nominal)	0.1% GM		0.5% GM	
Laboratories 1 and 11 withdrew from the trial at an early stage				

Details of Primers Used by Participants in Round 1

- Participant 2: Directed to Nitrate reductase, P35S and Thos
- Participant 3: 35SPROF: 5' gctcctacaaatgcatca 3' (19 mer)
35SPROR1: 5' gatagtgggattgtgcgtca 3' (20 mer)
- Participant 4: Supplied primers
- Participant 5: Nested PCR approach targeting the 35S promoter: first round amplification (40 cycles) was followed by second round PCR (40 cycles).
- Participant 6: CaMV 35S Promoter PB20/PB21
- Participant 7: real-time TaqMan[®] PCR
- Participant 8: No results reported
- Participant 9: p35SA and p35SB:
35S F200: 5' ctacaaatgcatcattgcg 3' (20 mer)
35S R-2: 5' gggctcttgcaaggatagtg 3' (20 mer)
- Participant 10: 35S-1: 5' gctcctacaaatgcatca 3' (19 mer)
35S-2: 5' gatagtgggattgtgcgtca 3' (19 mer)
- Participant 12: No details provided
- Participant 13: NPT II fragment, amplicon size 327bp.
- Participant 14: Own 35S primer sequence
- Participant 15: 35S Promoter and NOS Terminator as published in document CEN/TC 275/WG 11N 149
- Participant 16: Supplied primers
- Participant 17: Supplied primers
- Participant 18: DNA 260/280:1.4-1.6:
35sf: 5' gctcctacaaatgcatca 3' (19 mer)
35sr: 5' gatagtgggattgtgcgtca 3' (20 mer)
nosf: 5' gaatcctgtgccggtcttg 3' (20 mer)
nosr: 5' gacaccgcgcgataatttatcc 3' (24 mer)

Participants 7, 12, 13 and 14 did not provide any details of the in-house primers they used.

Round 2

This round was conducted in accordance with the project Protocol (May 2001, as amended October 2001). The samples, together with positive and negative controls were despatched on March 21, 2002 with a result deadline of April 19, 2002. Out of the 16 possible participants 14 submitted results. Two sets of 35S primers and 1 set of NOS primers were included with each sample set, together with instructions for use. Participants were encouraged however, to use their own procedures as the objective of a proficiency test is to study routine procedures used in participating laboratories. A questionnaire was provided for participants to complete the details of their procedures and in-house primers.

Samples were labelled A through H. The details of each sample are given below.

Sample Information

Sample Identifier	Description
A	Blank
B	1% GM Tobacco
C	0.1% GM Tobacco
D	5% GM Tobacco
E	0.1% GM Tobacco
F	0.5% GM Tobacco
G	Blank
H	2% GM Tobacco

Participants were not given these sample descriptions; all samples were supplied "blind". Participants were again not requested to provide proof of DNA amplifiability, i.e. by sending gel pictures or real-time PCR trace files.

The samples used were from the same batch as was prepared for the first round of the study. No further homogeneity testing was deemed necessary by the project team.

RESULTS of ROUND 2

Sample	A					B				
	Supplied 35S	Supplied 35S	In-house 35S	Supplied	In-house	Supplied 35S	Supplied 35S	In-house 35S	Supplied	In-house
	Primer (1)	Primer (2)	Primer	NOS Primer	NOS Primer	Primer (1)	Primer (2)	Primer	NOS Primer	NOS Primer
Laboratory ID										
1										
2	No	No	No	No	No	Yes	Yes	Yes	Yes	Yes
3	Yes	No	Yes (faint)	Yes (faint)	-	Yes	Yes	Yes	Yes (faint)	-
4	No	No	-	No	-	Yes	Yes	-	No	-
5	No	No	No	No	-	Yes	Yes	Yes	Yes	-
6	No	No	No	No	No	Yes	Yes	Yes	Yes	Yes
7	-	-	No	-	No	-	-	Yes	-	Yes
8	No	No	-	No	-	No	No	-	No	-
9										
10	No	No	No	No	No	Yes	Yes	Yes	Yes	Yes
12	No	No	No/No	No	No/No	Yes	Yes	Yes/Yes	Yes	Yes/Yes
13	No	No	No	No	No	Yes	Yes	Yes	Yes	Yes
14	No	No	-	No	-	Yes	Yes	-	Yes	-
15	-	No	-	-	No	-	Yes	-	-	Yes
16	No	No	-	No	-	Yes	Yes	-	Yes	-
17	No	No	No	No	No	Yes	Yes	Yes	Yes	Yes
18										
Number of Labs finding GM	1	0	1	1	0	11	12	9	10	8
Percentage of Labs finding GM	8.3%	0.0%	12.5%	8.3%	0.0%	91.7%	92.3%	100.0%	83.3%	100.0%
Number of Labs not finding GM	11	13	7	11	8	1	1	0	2	0
Percentage of Labs not finding GM	91.7%	100.0%	87.5%	91.7%	100.0%	8.3%	7.7%	0.0%	16.7%	0.0%
Sample Descriptor (Nominal)	Blank					1%				
Laboratories 1 and 11 withdrew from the trial before the first round										

Sample	C					D				
	Supplied 35S	Supplied 35S	In-house 35S	Supplied	In-house	Supplied 35S	Supplied 35S	In-house 35S	Supplied	In-house
	Primer (1)	Primer (2)	Primer	NOS Primer	NOS Primer	Primer (1)	Primer (2)	Primer	NOS Primer	NOS Primer
Laboratory ID										
1										
2	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
3	Yes	No	Yes	No	-	Yes	Yes	Yes	Yes	-
4	No	Yes	-	No	-	Yes	Yes	-	-	No
5	No	No	Yes	No	-	Yes	Yes	Yes	Yes	-
6	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
7	-	-	Yes	-	Yes	-	-	Yes	-	Yes
8	No	No	-	No	-	No	No	-	No	-
9										
10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
12	Yes	Yes	Yes/Yes	Yes	Yes/Yes	Yes	Yes	Yes/Yes	Yes	Yes/Yes
13	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes
14	No	No	-	No	-	Yes	Yes	-	Yes	-
15	-	Yes	-	-	Yes	-	Yes	-	-	Yes
16	Yes	Yes	-	No	-	Yes	Yes	-	Yes	-
17	No	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes
18										
Number of Labs finding GM	7	9	9	6	6	11	12	9	10	9
Percentage of Labs finding GM	58.3%	69.2%	100.0%	54.5%	75.0%	91.7%	92.3%	100.0%	83.3%	100.0%
Number of Labs not finding GM	5	4	0	5	2	1	1	0	2	0
Percentage of Labs not finding GM	41.7%	30.8%	0.0%	45.5%	25.0%	8.3%	7.7%	0.0%	16.7%	0.0%
Sample Descriptor (Nominal)	0.1%					5%				
Laboratories 1 and 11 withdrew from the trial before the first round										

Sample	E					F				
	Supplied 35S	Supplied 35S	In-house 35S	Supplied	In-house	Supplied 35S	Supplied 35S	In-house 35S	Supplied	In-house
	Primer (1)	Primer (2)	Primer	NOS Primer	NOS Primer	Primer (1)	Primer (2)	Primer	NOS Primer	NOS Primer
Laboratory ID										
1										
2	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
3	No	No	No	No	-	Yes	Yes	Yes	Yes	-
4	Yes	Yes	-	No	-	Yes	Yes	-	No	-
5	No	No	Yes	No	-	No	Yes	Yes	Yes	-
6	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes
7	-	-	Yes	-	Yes	-	-	Yes	-	Yes
8	No	No	-	No	-	No	No	-	No	-
9										
10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
12	Yes	Yes	Yes/Yes	Yes	Yes/Yes	Yes	Yes	Yes/Yes	Yes	Yes/Yes
13	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
14	Yes?	Yes	-	Yes?	-	No	Yes	-	Yes	-
15	-	Yes	-	-	Yes	-	Yes	-	-	Yes
16	Yes	Yes	-	No	-	Yes	Yes	-	Yes	-
17	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	No	Yes
18										
Number of Labs finding GM	9	10	8	5	8	9	12	9	9	8
Percentage of Labs finding GM	75.0%	76.9%	88.9%	41.7%	100.0%	75.0%	92.3%	100.0%	75.0%	100.0%
Number of Labs not finding GM	3	3	1	7	0	3	1	0	3	0
Percentage of Labs not finding GM	25.0%	23.1%	11.1%	58.3%	0.0%	25.0%	7.7%	0.0%	25.0%	0.0%
Sample Descriptor (Nominal)	0.1%					0.5%				
Laboratories 1 and 11 withdrew from the trial before the first round										

Sample	G					H				
	Supplied 35S	Supplied 35S	In-house 35S	Supplied	In-house	Supplied 35S	Supplied 35S	In-house 35S	Supplied	In-house
	Primer (1)	Primer (2)	Primer	NOS Primer	NOS Primer	Primer (1)	Primer (2)	Primer	NOS Primer	NOS Primer
Laboratory ID										
1										
2	No	No	No	No	No	Yes	Yes	Yes	Yes	Yes
3	No	No	No	No	-	Yes	Yes	Yes	Yes	-
4	No	No	-	No	-	Yes	Yes	-	No	-
5	No	No	No	No	-	Yes	Yes	Yes	Yes	-
6	No	No	No	No	No	Yes	Yes	Yes	Yes	Yes
7	-	-	No	-	No	-	-	Yes	-	Yes
8	No	No	-	No	-	No	No	-	No	-
9										
10	No	No	No	No	No	Yes	Yes	Yes	Yes	Yes
12	No	No	No/No	No	No/No	Yes	Yes	Yes/Yes	Yes	Yes/Yes
13	No	No	No	No	No	Yes	Yes	Yes	Yes	Yes
14	No	No	-	No	-	Yes	Yes	-	Yes	-
15	-	No	-	-	No	-	Yes	-	-	Yes
16	No	No	-	No	-	Yes	Yes	-	Yes	-
17	No	No	No	No	No	Yes	Yes	Yes	Yes	Yes
18										
Number of Labs finding GM	0	0	0	0	0	11	12	9	10	8
Percentage of Labs finding GM	0.0%	0.0%	0.0%	0.0%	0.0%	91.7%	92.3%	100.0%	83.3%	100.0%
Number of Labs not finding GM	12	13	9	12	8	1	1	0	2	0
Percentage of Labs not finding GM	100.0%	100.0%	100.0%	100.0%	100.0%	8.3%	7.7%	0.0%	16.7%	0.0%
Sample Descriptor (Nominal)	Blank					2%				
Laboratories 1 and 11 withdrew from the trial before the first round										

Technical Discussion of Results

Round 1

A difference is apparent between the number of labs detecting GM material in the first and second batches of 0.1% material. This may be a reflection of the relatively low amount of target transgenic DNA present which would lead to batch to batch variability such as that observed.

Only one lab detected GM tobacco in the blank material and this was only in one of the two samples supplied. This suggests that all of the labs are operating a high standard of GLP, minimising cross contamination at the DNA extraction and PCR level.

The application of in-house primers gave slightly better results. This may be a reflection of operator experience and familiarity with each of the systems involved.

Round 2

Overall laboratory performance was slightly better than that achieved in round 1 with a higher percentage of labs detecting GM material in each sample using the variety of primer sets employed. As might be expected, the greatest level of variation in detection occurred with the 0.1% samples but most labs were able to detect GM material with at least one of the primer pairs used. However, the most sensitive method gave different results for the two batches of 0.1% samples. This probably reflects the low amount of target DNA present leading to variation between different amplification reactions.

Primer sets specific for the 35S promoter gave a better performance than those targetted against the NOS sequence. In both cases, those tests using in-house assays and extraction techniques performed better overall as observed in Round 1, probably reflecting operator experience and familiarity.

Only one laboratory detected GM material in the blank sample, suggesting that participants are continuing to operate high standards of GLP.

Overall

The results obtained for both rounds have generated data of a high calibre with most participants correctly identifying the majority of GM containing samples. The vast majority of laboratories were able to detect down to 0.5% GM which is below the cut off limit already decided for food ingredients within the EU.