codex alimentarius commission

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

WORLD HEALTH ORGANIZATION

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Agenda Item 7a) **CX/MAS 02/8**

JOINT FAO/WHO FOOD STANDARDS PROGRAMME

CODEX COMMITTEE ON METHODS OF ANALYSIS AND SAMPLING

Twenty-fourth Session
Budapest, Hungary, 18-22 November 2002

CONSIDERATION OF METHODS FOR THE DETECTION AND IDENTIFICATION OF FOODS DERIVED FROM BIOTECHNOLOGY

METHODS SUBMITTED BY THE AD HOC INTERGOVERNMENTAL TASK FORCE ON FOODS DERIVED FROM BIOTECHNOLOGY

The First Session of the *Ad hoc* Intergovernmental Task Force on Foods Derived from Biotechnology agreed to initiate work on the methods for the detection and identification of foods derived from biotechnology and asked for information on appropriate analytical methods in CL 2000/29-FBT/MAS. This question was further considered by the Second and Third Sessions of the Task Force.

The Third Session of the Task Force considered document CX/FBT 02/9 including the information on methods and the comments provided by member countries in response to circular letter CL 2001/18-FBT (ALINORM 03/34, paras. 91-95). It agreed on a list of methods based on Annex 1 of CX/FBT 02/9 and methods later reported by Japan and the United States. The Task Force made the following recommendations:

- to forward to the CCMAS for its consideration the agreed list of methods (CRD 12, Appendices 1-3)
- to propose to the CCMAS to consider further methods of analysis with respect to foods derived from biotechnology on the basis of the proposals from member countries
- to propose through the Codex Alimentarius Commission that FAO, WHO and the FAO/IAEA Joint Division for Nuclear Techniques in Food and Agriculture encourage the development and maintenance of information on methods under development or not yet validated in co-operation with national/regional institutes

The 23rd Session of the Committee on Methods of Analysis and Sampling noted the request from the Committee on Food Labelling to consider the methods of analysis for foods derived from biotechnology and the work initiated in the Task Force on Foods Derived from Biotechnology. The Committee agreed that it should exercise a general co-ordinating role as regards methods for the detection or identification of foods derived from biotechnology and that it was ready to consider the proposals made by the Task Force at its next session. The Committee also agreed that the work of relevant international organizations would be taken into account in the process and invited them to provide relevant information in this area (ALINORM 01/23, paras 10-12).

The methods submitted by the Task Force on Foods Derived from Biotechnology are presented in the attached Annex for consideration by the Committee.

ANNEX

CODEX *AD HOC* INTERGOVERNMENTAL TASK FORCE ON FOODS DERIVED FROM BIOTECHNOLOGY

METHODS VALIDATED BY INTERLABORATORY STUDIES*

From the methods reported by the member countries, those which have been selected for this list have been validated in interlaboratory studies with at least 5 participating laboratories and which meet Codex criteria for the selection of methods of analysis[1].

Most of the methods are based on the polymerase chain reaction (PCR). They are suitable to either screen for or to specifically detect recombinant DNA (rDNA). Several PCR methods can also be used to quantify the amount of rDNA.

Two of the reported methods are based on the detection of a heterologous protein.

The list is organised as follows:

- Each method is referred to a food source and/or the target for which it has been designed (first column).
- For PCR based methods the primer sequences and the size of the amplicons are given (columns 2 and 3).
- The reporting countries and notifiers are indicated in column 4.
- Information on the status and the type of method (screening for common heterologous genetic elements, qualitative detection or quantification of rDNA) is given in columns 5 - 8.
- A data sheet is added for each method providing information about performance criteria.

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* Appendices 1, 2 and 3 of CRD 12 of March 1, 2002 have been compiled. The methods submitted by Japan (Appendix 2 of March 1, 2002) have been amended considering additional data provided by Japan in April 2002.

1 Codex Alimentarius Commission Procedural Manual, 12 th Edition, p.65 and Codex Alimentarius Checklist of Information, Volume 13-1994, Chapter 1.2 Design, Conduct and Reporting of Results of Collaborative Study Supporting the Endorsement of the Method

I. Validated Methods - Summary

Food Source / Target	*Primers*	*Size of Amplicon*	*Reported by*	*National Standard*	*Screening*	*Qual.*	*Quant.*
CaMV 35S Promotor							
35S-1/35S-2	f: 5'- GCTCCTACAAATGCCATCA r: 5'- GATAGTGGGATTGTGCGTCA	195 bp		☐	☑	☑	☐
			European Commission Joint Research Centre (JRC)				
35S-cf3/35S-cr4	f: 5'- CCACGTCTTCAAAGCAAGTGG r: 5'- TCCTCTCCAAATGAAATGAACTTCC	123 bp		☐	☑	☑	☐
			European Commission Joint Research Centre (JRC)				
			Ireland State Laboratory				
Maize Bt 11 (Novartis)							
Bt11 3-5'/Bt11 3-3'	f: 5'- Wako Pure Chemical Code No. 316-04871 r: 5'- Wako Pure Chemical Code No. 316-04871	128 bp		☑	☐	☑	☐
			Japan National Food Research Institute (NFRI)				
IVS2-2/PAT-B	f: 5'- CTGGGAGGCCAAGGTATCTAAT r: 5'- GCTGCTGTAGCTGGCCTAATCT	189 bp		☐	☐	☑	☐
			Germany Federal Institue for Health Protection of Consumers (BgVV)				
Q_Bt11 3-5'/Bt11 3-3'/Bt11Taq	f: 5'- Wako Pure Chemical Code No. 318-05051 r: 5'- Wako Pure Chemical Code No. 318-05051	128 bp		☑	☐	☐	☑
			Japan National Food Research Institute (NFRI)				

Food Source / Target	Primers	Size of Amplicon	Reported by	National Standard	Screening	Qual.	Quant.
Maize Event 176 (Maximizer, Novartis)							
Cry03/Cry04	f: 5'- CTCTCGCCGTTCATGTCCGT r: 5'- GGTCAGGCTCAGGCTGATGT	211 bp	**Germany** Federal Institue for Health Protection of Consumers (BgVV)	☐	☐	☑	☐
Cry05/Cry06	f: 5'- CCGCAGCCGATCCAACAATG r: 5'- GCTGATGTCGATGGGGGTGTAG	134 bp	**Germany** Federal Institue for Health Protection of Consumers (BgVV)	☐	☐	☑	☐
E176 2-5'/E176 2-3'	f: 5'- Wako Pure Chemical Code No. 313-04881 r: 5'- Wako Pure Chemical Code No. 313-04881	100 bp	**Japan** National Food Research Institute (NFRI)	☑	☐	☑	☐
Q_E176 2-5'/E176 2-3'/E176Taq	f: 5'- Wako Pure Chemical Code No. 315-05061 r: 5'- Wako Pure Chemical Code No. 315-05061	100 bp	**Japan** National Food Research Institute (NFRI)	☑	☐	☐	☑
Maize MON810 (Yield Gard Corn, Monsanto)							
M810 2-5'/M810 2-3'	f: 5'- Wako Pure Chemical Code No. 313-04901 r: 5'- Wako Pure Chemical Code No. 313-04901	113 bp	**Japan** National Food Research Institute (NFRI)	☑	☐	☑	☐
Protein_CrylAb	f: 5'- r: 5'-	- bp	**USA** United States Department of Agriculture	☐	☐	☐	☑
Q_M810 2-5'/M810 2-3'/M810Taq	f: 5'- Wako Pure Chemical Code No. 319-05081 r: 5'- Wako Pure Chemical Code No. 319-05081	113 bp	**Japan** National Food Research Institute (NFRI)	☑	☐	☐	☑
VW01/VW03	f: 5'- TCGAAGGACGAAGGACTCTAACG r: 5'- TCCATCTTTGGGACCACTGTCG	170 bp	**Germany** Federal Institue for Health Protection of Consumers (BgVV)	☐	☐	☑	☐

Food Source / Target	*Primers*	*Size of Amplicon*	*Reported by*	*National Standard*	*Screening*	*Qual.*	*Quant.*
Maize Roundup Ready (GA21, Monsanto)							
GA21 3-5'/GA21 3-3'	**f:** 5'- Wako Pure Chemical Code No. 315-04841 **r:** 5'- Wako Pure Chemical Code No. 315-04841	133 bp	**Japan** National Food Research Institute (NFRI)	☑	☐	☑	☐
Q_GA21 3-5'/GA21 3-3'/GA21Taq	**f:** 5'- Wako Pure Chemical Code No. 317-05021 **r:** 5'- Wako Pure Chemical Code No. 317-05021	133 bp	**Japan** National Food Research Institute (NFRI)	☑	☐	☐	☑
Maize StarLink (CBH 351, Aventis)							
CaM03-5'/CBH02-3'	**f:** 5'- CCTTCGCAAGACCCTTCCTCTATA **r:** 5'- GTAGCTGTCGGTGTAGTCCTCGT	170 bp	**Japan** National Institute of Health Sciences (NIHS)	☑	☐	☑	☐
Cry9C-5'/35Ster-3'	**f:** 5'- CCTATAGCTTCCCTTCTTCC **r:** 5'- TGCTGTAATAGGGCTGATGA	171 bp	**Japan** National Institute of Health Sciences (NIHS)	☑	☐	☑	☐
Protein_Cry9c 11-20	**f:** 5'- **r:** 5'-	- bp	**USA** United States Department of Agriculture	☐	☐	☐	☑
Protein_Cry9c 11-21	**f:** 5'- **r:** 5'-	- bp	**USA** United States Department of Agriculture	☐	☐	☐	☑

Food Source / Target	Primers	Size of Amplicon	Reported by	National Standard	Screening	Qual.	Quant.
Maize T25 (Liberty link, Aventis Crop Science formerly AgrEv							
Q_T25 1-5'/T25 1-3'/T25Taq	f: 5'- Wako Pure Chemical Code No. 312-05071 r: 5'- Wako Pure Chemical Code No. 312-05071	149 bp	**Japan** National Food Research Institute (NFRI)	☑	☐	☐	☑
T25 1-5'/T25 1-3'	f: 5'- Wako Pure Chemical Code No. 310-04891 r: 5'- Wako Pure Chemical Code No. 310-04891	149 bp	**Japan** National Food Research Institute (NFRI)	☑	☐	☑	☐
NOS-Terminator							
HA-NOS118-f/HA-NOS118r	f: 5'- GCATGACGTTATTTATGAGATGGG r: 5'- GACACCGCGCGCGATAATTTATCC	118 bp	**European Commission** Joint Research Centre (JRC) **Ireland** State Laboratory	☐	☑	☑	☐
NOS-1/NOS-3	f: 5'- GAATCCTGTTGCCGGTCTTG r: 5'- TTATCCTAGTTTGCGCGCTA	180 bp	**European Commission** Joint Research Centre (JRC)	☐	☑	☑	☐
Papaya 55-1, 66-1 (Cornell University)							
CaM3-5'/GUSn-3'	f: 5'- CCTTCGCAAGACCCTTCCTCTATA r: 5'- TCGTTAAAACTGCCTGGCAC	250 bp	**Japan** National Institute of Health Sciences (NIHS)	☑	☐	☑	☐
NosC-5'/CaMVN-3'	f: 5'- TTACGGCGAGTTCTGTTAGG r: 5'- CATGTGCCTGAGAAATAGGC	207 bp	**Japan** National Institute of Health Sciences (NIHS)	☑	☐	☑	☐

Food Source / Target	*Primers*	*Size of Amplicon*	*Reported by*	*National Standard*	*Screening*	*Qual.*	*Quant.*
Potato NewLeaf Plus (RBMT21-129, RBMT21-350, RBMT22-0							
p-FMV02-5'/PLRV01-3'	**f:** 5'- AAATAACGTGGAAAAGAGCTGTCCTGA **r:** 5'- AAAAGAGCGGCATATGCGGTAAATCTG	234 bp	**Japan** National Institute of Health Sciences (NIHS)	☑	☐	☑	☐
PLRV-rep1-5'/PLRV-rep1-3'	**f:** 5'- CTTCTTTCACGGAGTTCCAG **r:** 5'- TCGTCATTAAACTTGACGAC	172 bp	**Japan** National Institute of Health Sciences (NIHS)	☑	☐	☑	☐
Potato NewLeaf Y (RBMT15-101, SEMT15-02, SEMT15-15, M							
p-FMV05-5'/PVY02-3'	**f:** 5'- AAAAGAGCTGTCCTGACAGC **r:** 5'- TCCTCCTGCATCAATTGTGT	225 bp	**Japan** National Institute of Health Sciences (NIHS)	☑	☐	☑	☐
PVY01-5'/PVY01-3'	**f:** 5'- GAATCAAGGCTATCACGTCC **r:** 5'- CATCCGCACTGCCTCATACC	161 bp	**Japan** National Institute of Health Sciences (NIHS)	☑	☐	☑	☐

Food Source / Target	*Primers*	*Size of Amplicon*	*Reported by*	*National Standard*	*Screening*	*Qual.*	*Quant.*
Soybean Roundup Ready (Monsanto)							
35S-af2/Petu-r1	f: 5'- TGATGTGATATCTCCACTGACG r: 5'- TGTATCCCTTGAGCCATGTTGT	171 bp	**Germany** Federal Institue for Health Protection of Consumers (BgVV)	☑	☐	☑	☐
Protein_EPSPS	f: 5'- r: 5'-	- bp	**European Commission** Joint Research Centre (JRC) **USA** United States Department of Agriculture	☐	☐	☐	☑
Q_RR1-F/RR1-R/Probe	f: 5'- CATTTGGAGAGGACACGCTGA r: 5'- GAGCCATGTTGTTAATTTGTGCC	74 bp	**Germany** Federal Institue for Health Protection of Consumers (BgVV) **Germany** GeneScan Europe AG, c/o BioInside GmbH	☐	☐	☐	☑
Q_RRS01-5'/RRS01-3'/RRSTaq	f: 5'- Wako Pure Chemical Code No. 314-05151 r: 5'- Wako Pure Chemical Code No. 314-05151	121 bp	**Japan** National Food Research Institute (NFRI)	☑	☐	☐	☑
RRS01-5'/RRS01-3'	f: 5'- Wako Pure Chemical Code No. 315-04961 r: 5'- Wako Pure Chemical Code No. 315-04961	121 bp	**Japan** National Food Research Institute (NFRI)	☑	☐	☑	☐

II. Validated Methods - Data Sheets

Validated method	**35S-1/35S-2**
Food Source / Target	**CaMV 35S Promotor**
Sender	Guy Van den Eede Joint Research Centre (JRC) European Commission

Number of laboratories: 27

Further information:

- ***Specificity*** specific primers for CamV 35S promotor plus confirmation by means of Xmn I restriction endonuclease digestion.
- ***Limit of detection*** 0.1 % (0 % negative control)

	relative	***Number of copies***
	0.1 %	

- ***False-positive*** 5.4 %
- ***False-negative*** 6.5 %

Further information for quantitative methods:

- ***Limit of quantification***

	relative	***Number of copies***

- ***Precision***
- ***Repeatability***
- ***Reproducability***

Further documentation:

- ***References*** Lipp M., Anklam E, Brodmann, P., Pietsch, K and Pauwels J. (1999) Results of a screening method of genetically modified organisms in soy beans and maize. Food Control 10: 379-383.
- ***Description of the study*** The study was performed both for the CaMV 35S promotor sequence and the nos-terminator sequence detection.
- ***Sample description*** Each laboratory received 32 unknown specimen and 4 known samples for analysis. 25 % of the specimen were free of GMO (0 %), 75 % of the specimen contained GMO. The percentages of CaMV specific contents ranged between 0,1 % and 2 %.
- ***Matrices*** Raw material

Others:

- Excluded labs	3	no conclusive results	
- Labs with uncomplete results	0		
- Total number of samples	736		
- Positive samples	552		
- Outlying results	1	***Outlying tests***	wrong sensitivity adjustment

- ***Comments:***

Validated method	**35S-cf3/35S-cr4**
Food Source / Target	**CaMV 35S Promotor**
Sender	Guy Van den Eede Joint Research Centre (JRC) European Commission

Number of laboratories: 23

Further information:

- ***Specificity*** specific primers for CamV 35S promotor

- ***Limit of detection***

	relative	*Number of copies*
- False-positive	3.9 %	
- False-negative	1.9 %	

Further information for quantitative methods:

- ***Limit of quantification***

	relative	*Number of copies*
- Precision		
- Repeatability		
- Reproducability		

Further documentation:

- ***References*** Lipp M., Bluth, A., Eyquem F., Kruse L., Schimmel H., Van den Eede G. And Anklam E. (2001): Validation of a method based on polymerase chain reaction for the detection of genetically modified organisms in various processed foodstuffs. Eur. Food. Technol. 212: 497-504.
- ***Description of the study*** The study was performed both for the CaMV 35S promotor sequence and the nos-terminator sequence detection.
- ***Sample description*** GMO amount in samples: 2 %, 100 %, in biscuits: 10 % / CaMV-specific content: 0,4 % - 100 %
- ***Matrices*** Procesed food (cooked maize grit - polenta, infant formula, biscuits, meal of acidified soybeans).

Others:

- Excluded labs	1	use of different set of primers	
- Labs with uncomplete results	5		
- Total number of samples	690		
- Positive samples	460		
- Outlying results	5	***Outlying tests***	high amount of non-correct results

- ***Comments:*** Also reported by IRELAND / State Laboratory

Validated method	**IVS2-2/PAT-B**
Food Source / Target	**Maize Bt 11 (Novartis)**
Sender	Jutta Zagon Federal Institue for Health Protection of Consumers (BgVV) Germany

Number of laboratories: 18

Further information:

- Specificity No amplification was observed using DNA from non-transgenic maize and from the transgenic Roundupready soybean as well as from the transgenic maize-lines Bt-176, T25 and MON810.

- Limit of detection Based on the assumption of 1 copy of the genetic construct per genome (AGBIOS database: http://www.agbios.com) and the genome size of 2.65x10^9 base pairs (http://www.rbgkew.org.uk/cval/homepage.html) the absolute detection limit with 50 ng DNA from the specific plant species (with a relative GMO content of 0.1 % into ground seeds) introduced in PCR is 20 genome equivalents.

relative 0.1 % ***Number of copies*** 20

- False-positive 3

- False-negative 3

Further information for quantitative methods:

- Limit of quantification

relative ***Number of copies***

- Precision

- Repeatability

- Reproducibility

Further documentation:

- References * Collection of official methods under article 35 of German Federal Foods Act; Methods of sampling and analysis of foods, tobacco products, cosmetics and commodity goods/Federal Health Office, Loose leaf edition as of November 1999, Berlin, Köln, Beuth Verlag GmbH, No L xx.xx.xx
* Broll et al. (paper in preparation)

- Description of the study National collaborative study in the year 1999.

- Sample description

- Matrices

Others:

- Excluded labs	2	no data submitted
- Labs with uncomplete results	0	
- Total number of samples	108	
- Positive samples	32	
- Outlying results		***Outlying tests***

- Comments:

Validated method	**Q_Bt11 3-5'/Bt11 3-3'/Bt11Taq**
Food Source / Target	**Maize Bt 11 (Novartis)**
Sender	Akihiro HINO National Food Research Institute (NFRI) Japan

Number of laboratories: 14

Further information:

- Specificity

No amplification was observed using DNA from non-transgenic maize, soy, rice, wheat and barley as well as from the transgenic maize-lines GA21, T25, Event176 and MON810 and the transgenic soy-line Roundup Ready soy.

- Limit of detection

relative 0.1 % ***Number of copies***

- False-positive

- False-negative

Further information for quantitative methods:

- Limit of quantification

The plasmid DNA containing sequences of PCR products amplified by specific primer pairs was used as reference molecule. Standard curves were calibrated using the five concentrations of reference molecules such as 20, 125, 1,500, 20,000 and 250,000 copies per reaction. Almost (21/22) of the measurement copies of 0.10 % Bt11 were below 20 copies, whereas all (28/28) of the measurment copies of 0.50 % Bt11 were above 20 copies. Therefore we conclude that limit of quantification for Bt11 was 0.50 %.

relative 0.50 % ***Number of copies*** 20

- Precision

Sample (% GM)	0.10	0.50	1.0	5.0	10.0
Mean value (% GM)	0.091	0.510	1.15	6.08	12.1
Biaz (%)	-9.0	+2.0	+14.7	+21.6	+21.1

- Repeatability

Sample (% GM)	0.10	0.50	1.0	5.0	10.0
Sr	0.020	0.121	0.216	0.830	1.258
r (2.8 x Sr)	0.057	0.338	0.606	2.325	3.524
RSDr (%)	22.3	23.7	18.9	13.7	10.4

- Reproducability

Sample (% GM)	0.10	0.50	1.0	5.0	10.0
SR	0.016	0.105	0.216	0.786	1.389
R (2.8 x SR)	0.046	0.293	0.605	2.200	3.889
RSDR (%)	18.0	20.5	18.8	12.9	11.5

Further documentation:

- References

Kuribara H., Shindo Y., Matsuoka, T., Takubo K., Futo S., Aoki N., Hirao T., Akiyama H., Goda Y., Toyoda M., Hino A.: Quantification Methods using Novel Reference Molecules for Detection of Genetically Modified Maize and Soybean. Journal of AOAC International (Submitted)

Shindo Y., Kuribara H., Matsuoka T., Futo S., Sawada C., Shono J., Akiyama H., Goda Y., Toyoda M., Hino A.: Validation Studies of Real-time PCR Analyses for Line Specific Quantification of Genetically Modified Maize and Soybean Using New Reference Molecules. Journal of AOAC International (Submitted)

- Description of the study

Bt11 maize DNA quantification method using plasmid DNA as reference molecules. Interlab validation with 14 participating laboratories conducted by NFRI in 2001.

- Sample description

Totally twelve maize blind samples were sent to each participant. The maize blind samples were designed as six pairs of blind duplicates including 0, 0.10, 0.50, 1.0, 5.0 and 10 % of Bt11, GA 21, T25, Event176 and MON810 maize (i.e., the maize 1.0 % blind sample consisted 1.0 % Bt11, 1.0 % GA21, 1.0 % T25, 1.0 % Event176, 1.0 % MON810, and 95 % non-GMO for a total of 5.0 % GMO). The blank sample, 0 % GM maize, was only used to remove the invalid laboratories before statistical analysis.

- Matrices

Raw materials

Others:

- Excluded labs 0

- Labs with uncomplete results 0

- Total number of samples 168

- Positive samples 140

- Outlying results 6 ***Outlying tests*** Cochran's and Grubb's test

- Comments:

Validated method	**Cry03/Cry04**
Food Source / Target	**Maize Event 176 (Maximizer, Novartis)**
Sender	Jutta Zagon Federal Institue for Health Protection of Consumers (BgVV) Germany

Number of laboratories: 15

Further information:

- Specificity

No amplification was observed using DNA from non-transgenic maize and from the transgenic Roundup Ready soybean as well as from the transgenic maize-lines Bt-11, T25 and MON810.

- Limit of detection

Based on the assumption of 1 copy of the genetic construct per genome (AGBIOS database: http://www.agbios.com) and the genome size of 2.65x10^9 base pairs (http://www.rbgkew.org.uk/cval/homepage.html) the absolute detection limit with 25 ng DNA from the specific plant species (with a relative GMO content of 0.1 % into ground seeds) introduced in PCR is 10 genome equivalents.

relative 0.1 % ***Number of copies*** 10

- False-positive 1

- False-negative 6

Further information for quantitative methods:

- Limit of quantification

relative ***Number of copies***

- Precision

- Repeatability

- Reproducability

Further documentation:

- References

Hupfer, C, Hotzel, H, Sachse, K, Engel, K-H (1998): Detection of the genetic modification in heat treated products of Bt maize by polymerase chain reaction. Lm. Unters. Forsch. 206 (Band A), pp.203-207. Collection of official methods under article 35 of German Federal Foods Act; Methods of sampling and analysis of foods, tobacco products, cosmetics and commodity goods/Federal Health Office, Loose leaf edition as of November 1999, Berlin, Köln, Beuth Verlag GmbH, No L xx.xx.xx

- Description of the study

- Sample description 11 labs received 3 samples, 3 labs 4 and 1 lab 8 samples.

- Matrices

Others:

- Excluded labs 0

- Labs with uncomplete results 0

- Total number of samples 53

- Positive samples 43

- Outlying results 0 ***Outlying tests***

- Comments:

The high number of false-positive results is due to a probable contamination of the samples, which have been sent to the participants.

Validated method	**Cry05/Cry06**
Food Source / Target	**Maize Event 176 (Maximizer, Novartis)**
Sender	Jutta Zagon Federal Institue for Health Protection of Consumers (BgVV) Germany

Number of laboratories: 18

Further information:

- Specificity

No amplification was observed using DNA from non-transgenic maize and from the transgenic Roundupready soybean as well as from the transgenic maize-lines Bt-11, T25 and MON810.

- Limit of detection

Based on the assumption of 1 copy of the genetic construct per genome (AGBIOS database: http://www.agbios.com) and the genome size of 2.65x10^9 base pairs (http://www.rbgkew.org.uk/cval/homepage.html) the absolute detection limit with 25 ng DNA from the specific plant species (with a relative GMO content of 0.1 % into ground seeds) introduced in PCR is 10 genome equivalents.

relative 0.1 % ***Number of copies*** 10

- False-positive 0

- False-negative 0

Further information for quantitative methods:

- Limit of quantification

relative ***Number of copies***

- Precision

- Repeatability

- Reproducability

Further documentation:

- References

Hupfer, C, Hotzel, H, Sachse, K, Engel, K-H (1998): Detection of the genetic modification in heat treated products of Bt maize by polymerase chain reaction. Lm. Unters. Forsch. 206 (Band A), pp.203-207. Collection of official methods under article 35 of German Federal Foods Act; Methods of sampling and analysis of foods, tobacco products, cosmetics and commodity goods/Federal Health Office, Loose leaf edition as of November 1999, Berlin, Köln, Beuth Verlag GmbH, No L xx.xx.xx

- Description of the study

- Sample description

- Matrices

Others:

- Excluded labs 2

- Labs with uncomplete results 0

- Total number of samples 108

- Positive samples 32

- Outlying results 0 ***Outlying tests***

- Comments:

Validated method	**Q_E176 2-5'/E176 2-3'/E176Taq**
Food Source / Target	**Maize Event 176 (Maximizer, Novartis)**
Sender	Akihiro HINO National Food Research Institute (NFRI) Japan

Number of laboratories: 14

Further information:

- Specificity

No amplification was observed using DNA from non-transgenic maize, soy, rice, wheat and barley as well as from the transgenic maize-lines Bt11, GA21, T25, and MON810 and the transgenic soy-line Roundup Ready soy.

- Limit of detection

relative 0.1 % ***Number of copies***

- False-positive

- False-negative

Further information for quantitative methods:

- Limit of quantification

The plasmid DNA containing sequences of PCR products amplified by specific primer pairs was used as reference molecule. Standard curves were calibrated using the five concentrations of reference molecules such as 20, 125, 1,500, 20,000 and 250,000 copies per reaction. Almost (23/24) of the measurement copies of 0.10 % Event 176 were above 20 copies. Therefore we conclude that limit of quantification for Event 176 was 0.10 %.

relative 0.10 % ***Number of copies*** 20

- Precision

Sample (% GM)	0.10	0.50	1.0	5.0	10.0
Mean value (% GM)	0.111	0.492	0.923	5.00	9.62
Biaz (%)	+11.3	-1.6	-7.7	0.00	-3.8

- Repeatability

Sample (% GM)	0.10	0.50	1.0	5.0	10.0
Sr	0.018	0.029	0.066	0.406	0.554
r (2.8 x Sr)	0.051	0.080	0.184	1.137	1.552
RSDr (%)	16.3	5.8	7.1	8.1	5.8

- Reproducability

Sample (% GM)	0.10	0.50	1.0	5.0	10.0
SR	0.024	0.051	0.106	0.559	0.917
R (2.8 x SR)	0.066	0.142	0.296	1.565	2.566
RSDR (%)	21.3	10.3	11.4	11.2	9.5

Further documentation:

- References

Kuribara H., Shindo Y., Matsuoka, T., Takubo K., Futo S., Aoki N., Hirao T., Akiyama H., Goda Y., Toyoda M., Hino A.: Quantification Methods using Novel Reference Molecules for Detection of Genetically Modified Maize and Soybean. Journal of AOAC International (Submitted)

Shindo Y., Kuribara H., Matsuoka T., Futo S., Sawada C., Shono J., Akiyama H., Goda Y., Toyoda M., Hino A.: Validation Studies of Real-time PCR Analyses for Line Specific Quantification of Genetically Modified Maize and Soybean Using New Reference Molecules. Journal of AOAC International (Submitted)

- Description of the study

Event 176 maize DNA quantification method using plasmid DNA as reference molecules. Interlab validation with 14 participating laboratories conducted by NFRI in 2001.

- Sample description

Totally twelve maize blind samples were sent to each participant. The maize blind samples were designed as six pairs of blind duplicates including 0, 0.10, 0.50, 1.0, 5.0 and 10 % of Bt11, GA 21, T25, Event176 and MON810 maize (i.e., the maize 1.0 % blind sample consisted 1.0 % Bt11, 1.0 % GA21, 1.0 % T25, 1.0 % Event176, 1.0 % MON810, and 95 % non-GMO for a total of 5.0 % GMO). The blank sample, 0 % GM maize, was only used to remove the invalid laboratories before statistical analysis.

- Matrices

Raw materials

Others:

- Excluded labs 1

- Labs with uncomplete results 0

- Total number of samples 156

- Positive samples 130

- Outlying results 8 ***Outlying tests*** Cochran's and Grubb's test

- Comments:

Validated method	**Protein_CrylAb**
Food Source / Target	**Maize MON810 (Yield Gard Corn, Monsanto)**
Sender	F. Edward Scarbrough United States Department of Agriculture USA

Number of laboratories: 40

Further information:

- Specificity Antibodies are specific for Bacillus thuringiensis Cry1Ab protein. The test does not react with other Bt proteins including Cry1Ac, Cry1F, Cry9c, Cry2A, Cry3Bb in corn.

- Limit of detection

relative 0.15 % ***Number of copies***

- False-positive

- False-negative

Further information for quantitative methods:

- Limit of quantification

relative 0.5 % ***Number of copies***

- Precision

- Repeatability RSDr = 6-18 %

- Reproducability RSDR = 13-24 %

Further documentation:

- References Bt Cry1Ab-Modified Corn in Corn Flour - ELISA Method
American Association of Cereal Chemists Approved Method 11-10 (First Approval November 8, 2000)

Stave, J.W., Magin, K., Schimmel, H., Lawruk, T., Wheling, P., and Bridges, A. 2000, AACC collaborative study of a protein method for detection of genetically modified corn. Cereal Foods World 45: 497-501

- Description of the study An ELISA method for quantitative determination of corn Cry1Ab protein has been validated by an international collaborative study to AOAC/AACC/IUPAC/ISO guidelines.

- Sample description Samples used in the study were different percentages of MON810 corn blended with control corn. (0, 0.3, 0.5, 0.75, 1.0, 1.25, and 2.0 % MON810 corn)

- Matrices Corn flour

Others:

- Excluded labs 0

- Labs with uncomplete results 0

- Total number of samples 640

- Positive samples 560

- Outlying results 10 ***Outlying tests*** Cochran's and Grubb's test's

- Comments: Both antibody reagents were raised against recombinant BtCry1Ab expressed in E. Coli. The microtiter plate is coated with purified monoctonal antibody and detection is achieved using a biotinylated rabbit polyclonal antibody to Cry1Ab.

Validated method	**Q_M810 2-5'/M810 2-3'/M810Taq**
Food Source / Target	**Maize MON810 (Yield Gard Corn, Monsanto)**
Sender	Akihiro HINO National Food Research Institute (NFRI) Japan

Number of laboratories: 14

Further information:

- Specificity

No amplification was observed using DNA from non-transgenic maize, soy, rice, wheat and barley as well as from the transgenic maize-lines Bt11, GA21, T25 and Event176 and the transgenic soy-line Roundup Ready soy.

- Limit of detection

relative 0.1 % ***Number of copies***

- False-positive

- False-negative

Further information for quantitative methods:

- Limit of quantification

The plasmid DNA containing sequences of PCR products amplified by specific primer pairs was used as reference molecule. Standard curves were calibrated using the five concentrations of reference molecules such as 20, 125, 1,500, 20,000 and 250,000 copies per reaction. Almost (19/22) of the measurement copies of 0.10 % MON810 were below 20 copies, whereas almost (26/26) of the measurement copies of 0.50 % MON810 were above 20 copies. Therefore we conclude that limit of quantification for MON810 was 0.50 %.

relative 0.50 % ***Number of copies*** 20

- Precision

Sample (% GM)	0.10	0.50	1.0	5.0	10.0
Mean value (% GM)	0.125	0.547	1.05	4.78	9.82
Biaz (%)	+25.0	+9.4	+4.6	-4.3	-1.8

- Repeatability

Sample (% GM)	0.10	0.50	1.0	5.0	10.0
Sr	0.040	0.082	0.124	0.647	1.028
r (2.8 x Sr)	0.113	0.231	0.347	1.813	2.879
RSDr (%)	32.3	15.1	11.8	13.5	10.5

- Reproducability

Sample (% GM)	0.10	0.50	1.0	5.0	10.0
SR	0.033	0.107	0.158	0.569	1.140
R (2.8 x SR)	0.091	0.301	0.443	1.593	3.191
RSDR (%)	26.1	19.6	15.1	11.9	11.6

Further documentation:

- References

Kuribara H., Shindo Y., Matsuoka, T., Takubo K., Futo S., Aoki N., Hirao T., Akiyama H., Goda Y., Toyoda M., Hino A.: Quantification Methods using Novel Reference Molecules for Detection of Genetically Modified Maize and Soybean. Journal of AOAC International (Submitted)

Shindo Y., Kuribara H., Matsuoka T., Futo S., Sawada C., Shono J., Akiyama H., Goda Y., Toyoda M., Hino A.: Validation Studies of Real-time PCR Analyses for Line Specific Quantification of Genetically Modified Maize and Soybean Using New Reference Molecules. Journal of AOAC International (Submitted)

- Description of the study

MON810 maize DNA quantification method using plasmid DNA as reference molecules. Interlab validation with 14 participating laboratories conducted by NFRI in 2001.

- Sample description

Totally twelve maize blind samples were sent to each participant. The maize blind samples were designed as six pairs of blind duplicates including 0, 0.10, 0.50, 1.0, 5.0 and 10 % of Bt11, GA 21, T25, Event176 and MON810 maize (i.e., the maize 1.0 % blind sample consisted 1.0 % Bt11, 1.0 % GA21, 1.0 % T25, 1.0 % Event176, 1.0 % MON810, and 95 % non-GMO for a total of 5.0 % GMO). The blank sample, 0 % GM maize, was only used to remove the invalid laboratories before statistical analysis.

- Matrices

Raw materials

Others:

- Excluded labs 0

- Labs with uncomplete results 0

- Total number of samples 168

- Positive samples 140

- Outlying results 12 ***Outlying tests*** Cochran's and Grubb's test

- Comments:

Validated method	**VW01/VW03**
Food Source / Target	**Maize MON810 (Yield Gard Corn, Monsanto)**
Sender	Jutta Zagon Federal Institue for Health Protection of Consumers (BgVV) Germany

Number of laboratories: 16

Further information:

- *Specificity*: No amplification was observed using DNA from non-transgenic maize and from the transgenic Roundupready soybean as well as from the transgenic maize-lines Bt-176, Bt-11 and T25.
- *Limit of detection*: Based on the assumption of 1 copy of the genetic construct per genome (AGBIOS database: http://www.agbios.com) and the genome size of 2.65x10^9 base pairs (http://www.rbgkew.org.uk/cval/homepage.html) the absolute detection limit with 50 ng DNA from the specific plant species (with a relative GMO content of 0.1 % into ground seeds) introduced in PCR is 20 genome equivalents.

  relative 0.1 % *Number of copies* 20
- *False-positive*: 0
- *False-negative*: 0

Further information for quantitative methods:

- *Limit of quantification*

  relative *Number of copies*
- *Precision*
- *Repeatability*
- *Reproducability*

Further documentation:

- *References*: Collection of official methods under article 35 of German Federal Foods Act; Methods of sampling and analysis of foods, tobacco products, cosmetics and commodity goods/Federal Health Office, Loose leaf edition as of November 1999, Berlin, Köln, Beuth Verlag GmbH, No L xx.xx.xx
- *Description of the study*: International collaborative study in the year 2000.
- *Sample description*
- *Matrices*

Others:

- *Excluded labs*: 1 — no data submitted
- *Labs with uncomplete results*: 0
- *Total number of samples*: 80
- *Positive samples*: 30
- *Outlying results* *Outlying tests*
- *Comments:*

Validated method	**Q_GA21 3-5'/GA21 3-3'/GA21Taq**
Food Source / Target	**Maize Roundup Ready (GA21, Monsanto)**
Sender	Akihiro HINO National Food Research Institute (NFRI) Japan

Number of laboratories: 14

Further information:

- Specificity

No amplification was observed using DNA from non-transgenic maize, soy, rice, wheat and barley as well as from the transgenic maize-lines Bt11, Event176, T25, and MON810 and the transgenic soy-line Roundup Ready soy.

- Limit of detection

relative 0.1 % ***Number of copies***

- False-positive

- False-negative

Further information for quantitative methods:

- Limit of quantification

The plasmid DNA containing sequences of PCR products amplified by specific primer pairs was used as reference molecule. Standard curves were calibrated using the five concentrations of reference molecules such as 20, 125, 1,500, 20,000 and 250,000 copies per reaction. Almost (20/24) of the measurement copies of 0.10 % GA21 were above 20 copies. Therefore we conclude that limit of quantification for GA21 was 0.10 %.

relative 0.10 % ***Number of copies*** 20

- Precision

Sample (% GM)	0.10	0.50	1.0	5.0	10.0
Mean value (% GM)	0.095	0.538	1.12	5.83	11.5
Biaz (%)	-5.4	+7.7	+20.2	+16.6	+15.0

- Repeatability

Sample (% GM)	0.10	0.50	1.0	5.0	10.0
Sr	0.019	0.068	0.148	0.476	0.907
r (2.8 x Sr)	0.054	0.189	0.414	1.332	2.539
RSDr (%)	20.5	12.6	12.3	8.2	7.9

- Reproducability

Sample (% GM)	0.10	0.50	1.0	5.0	10.0
SR	0.019	0.117	0.224	0.927	1.565
R (2.8 x SR)	0.055	0.329	0.627	2.597	4.382
RSDR (%)	20.6	21.8	18.6	15.9	13.6

Further documentation:

- References

Kuribara H., Shindo Y., Matsuoka, T., Takubo K., Futo S., Aoki N., Hirao T., Akiyama H., Goda Y., Toyoda M., Hino A.: Quantification Methods using Novel Reference Molecules for Detection of Genetically Modified Maize and Soybean. Journal of AOAC International (Submitted)

Shindo Y., Kuribara H., Matsuoka T., Futo S., Sawada C., Shono J., Akiyama H., Goda Y., Toyoda M., Hino A.: Validation Studies of Real-time PCR Analyses for Line Specific Quantification of Genetically Modified Maize and Soybean Using New Reference Molecules. Journal of AOAC International (Submitted)

- Description of the study

GA21 maize DNA quantification method using plasmid DNA as reference molecules. Interlab validation with 14 participating laboratories conducted by NFRI in 2001.

- Sample description

Totally twelve maize blind samples were sent to each participant. The maize blind samples were designed as six pairs of blind duplicates including 0, 0.10, 0.50, 1.0, 5.0 and 10 % of Bt11, GA 21, T25, Event176 and MON810 maize (i.e., the maize 1.0 % blind sample consisted 1.0 % Bt11, 1.0 % GA21, 1.0 % T25, 1.0 % Event176, 1.0 % MON810, and 95 % non-GMO for a total of 5.0 % GMO). The blank sample, 0 % GM maize, was only used to remove the invalid laboratories before statistical analysis.

- Matrices

Raw materials

Others:

- Excluded labs 1

- Labs with uncomplete results 0

- Total number of samples 156

- Positive samples 130

- Outlying results 4 ***Outlying tests*** Cochran's and Grubb's test

- Comments:

Validated method	**CaM03-5'/CBH02-3'**
Food Source / Target	**Maize StarLink (CBH 351, Aventis)**
Sender	Hiroshi Akiyama National Institute of Health Sciences (NIHS) Japan

Number of laboratories: 14

Further information:

- Specificity No amplification was observed using DNA from non-transgenic maize, soybeaans, rice, wheat and barley as well as from the transgenic maize-lines GA21, T25, Event176, and MON810 and the transgenic soy-line Roundup Ready soy.

- Limit of detection

relative	0.10 %	***Number of copies***

- False-positive 2

- False-negative 0

Further information for quantitative methods:

- Limit of quantification

relative	***Number of copies***

- Precision

- Repeatability

- Reproducibility

Further documentation:

- References Takeshi Matsuoka, Hideo Kuribara, Seiko Suefuji, Hirohito Miura, Yuko Kusakabe, Hiroshi Akiyama, Yukihiro Goda, Kenji Isshiki, Masatake Toyoda and Akihiro Hino (2001): A Detection Method for Recombinant DNA from Genetically Modified Maize CBH351. J. Food Hyg. Doc. Japan 42: 197-201.

- Description of the study National Collaborative study in the year 2002

- Sample description Five maize blind samples were sent to the participants as duplicates.
Positive samples: 0.1 % and 1 %.

- Matrices Raw materials

Others:

- Excluded labs 0

- Labs with uncomplete results 0

- Total number of samples 84

- Positive samples 56

- Outlying results 0 ***Outlying tests***

- Comments:

Validated method	**Cry9C-5'/35Ster-3'**
Food Source / Target	**Maize StarLink (CBH 351, Aventis)**
Sender	Hiroshi Akiyama National Institute of Health Sciences (NIHS) Japan

Number of laboratories: 14

Further information:

- Specificity No amplification was observed using DNA from non-transgenic maize, soybeaans, rice, wheat and barley as well as from the transgenic maize-lines GA21, T25, Event176, and MON810 and the transgenic soy-line Roundup Ready soy.

- Limit of detection

relative 0.10 % ***Number of copies***

- False-positive 2

- False-negative 0

Further information for quantitative methods:

- Limit of quantification

relative ***Number of copies***

- Precision

- Repeatability

- Reproducibility

Further documentation:

- References Takeshi Matsuoka, Hideo Kuribara, Seiko Suefuji, Hirohito Miura, Yuko Kusakabe, Hiroshi Akiyama, Yukihiro Goda, Kenji Isshiki, Masatake Toyoda and Akihiro Hino (2001): A Detection Method for Recombinant DNA from Genetically Modified Maize CBH351. J. Food Hyg. Doc. Japan 42: 197-201.

- Description of the study National Collaborative study in the year 2002

- Sample description Five maize blind samples were sent to the participants as duplicates.
Positive samples: 0.1 % and 1 %.

- Matrices Raw materials

Others:

- Excluded labs 0

- Labs with uncomplete results 0

- Total number of samples 84

- Positive samples 56

- Outlying results 0 ***Outlying tests***

- Comments:

Validated method	**Protein_Cry9c 11-20**
Food Source / Target	**Maize StarLink (CBH 351, Aventis)**
Sender	F. Edward Scarbrough United States Department of Agriculture USA

Number of laboratories: 40

Further information:

- Specificity Antibodies are specific for Bacillus thuringiensis Cry 9c protein. The test does not react with other Bt proteins including Cry1Ab, Cry1Ac, Cry1F, Cry2A, Cry3Bb in corn.

- Limit of detection

relative 0.006 % ***Number of copies***

- False-positive

- False-negative

Further information for quantitative methods:

- Limit of quantification

relative 0.02 % ***Number of copies***

- Precision

- Repeatability RSDr = 10-31 % Flour
RSDr = 11-33 % Meal

- Reproducability RSDR = 12-38 % Flour
RSDR = 17-50 % Meal

Further documentation:

- References Starlink Corn in Corn Flour and Corn Meal - ELISA Method,
American Association of Cereal Approved Method 11-20 (First Approval October 17, 2001)

- Description of the study An ELISA method for quantitative determination of corn Cry9c protein has been validated by an international collaborative study to AOAC/AACC/IUPAC/ISO guidelines.

- Sample description Samples used in the study were different percentages of Starlink corn blended with control corn. (0, 0.01, 0.025, 0.05, 0.075 % Starlink corn))

- Matrices Corn flour and corn meal

Others:

- Excluded labs 2 Two laboratories unable to complete did not pass method quality assurance checks.

- Labs with uncomplete results 1

- Total number of samples 480

- Positive samples 384

- Outlying results 30 ***Outlying tests*** Cochran's and Grubb's test's

- Comments: Both antibody reagents were raised against recombinant Bt Cry9c expressed in E.coli. The microtiter plate is coated with purified rabbit polyclonal antibody, and detection is achieved using a horseradish peroxidase-labeled monoclonal antibody to Cry9c.

Validated method	**Protein_Cry9c 11-21**
Food Source / Target	**Maize StarLink (CBH 351, Aventis)**
Sender	F. Edward Scarbrough United States Department of Agriculture USA

Number of laboratories: 26

Further information:

- Specificity Polyclonal antibody based test does not distinguish between Cry9C endotoxin and certain other compounds, but detects their presence to differing degrees.

- Limit of detection

relative 0.006 % ***Number of copies***

- False-positive

- False-negative

Further information for quantitative methods:

- Limit of quantification

relative 0.02 % ***Number of copies***

- Precision

- Repeatability RSDr = 10-31 % Flour
RSDr = 11-33 % Meal

- Reproducability RSDR = 15-20 % Flour
RSDR = 25-70 % Meal

Further documentation:

- References ELISA Method for Starlink Corn in Corn Flour and Corn Meal
American Association of Cereal Chemists Approved Method 11-21 (First Approval October 17, 2001)

- Description of the study An ELISA method for quantitative determination of corn Cry9c protein has been validated by an international collaborative study to AOAC/AACC/IUPAC/ISO guidelines.

- Sample description Samples used in the study were different percentages of Starlink corn blended with control corn. (0, 0.01, 0.025, 0.05, 0.075 % Starlink corn)

- Matrices Corn flour and corn meal

Others:

- Excluded labs 0

- Labs with uncomplete results 0

- Total number of samples 520

- Positive samples 416

- Outlying results 26 ***Outlying tests*** Cochran's and Grubb's test's

- Comments:

Validated method	**Q_T25 1-5'/T25 1-3'/T25Taq**
Food Source / Target	**Maize T25 (Liberty link, Aventis Crop Science formerly AgrEvo)**
Sender	Akihiro HINO National Food Research Institute (NFRI) Japan

Number of laboratories: 14

Further information:

- Specificity

No amplification was observed using DNA from non-transgenic maize, soy, rice, wheat and barley as well as from the transgenic maize-lines Bt11, GA 21, Event176 and MON810 and the transgenic soy-line Roundup Ready soy.

- Limit of detection

relative 0.1 % ***Number of copies***

- False-positive

- False-negative

Further information for quantitative methods:

- Limit of quantification

The plasmid DNA containing sequences of PCR products amplified by specific primer pairs was used as reference molecule. Standard curves were calibrated using the five concentrations of reference molecules such as 20, 125, 1,500, 20,000 and 250,000 copies per reaction. Almost (22/22) of the measurement copies of 0.10 % T25 were below 20 copies, whereas almost (27/28) of the measurement copies of 0.50 % T25 were above 20 copies. Therefore we conclude that limit of quantification for T25 was 0.50 %.

relative 0.50 % ***Number of copies*** 20

- Precision

Sample (% GM)	0.10	0.50	1.0	5.0	10.0
Mean value (% GM)	0.139	0.577	1.20	5.58	10.8
Biaz (%)	+38.6	+15.3	+20.0	+11.6	+8.1

- Repeatability

Sample (% GM)	0.10	0.50	1.0	5.0	10.0
Sr	0.033	0.162	0.082	0.690	1.439
r (2.8 x Sr)	0.092	0.455	0.228	1.932	4.030
RSDr (%)	23.7	28.2	6.8	12.4	13.3

- Reproducability

Sample (% GM)	0.10	0.50	1.0	5.0	10.0
SR	0.037	0.159	0.138	0.827	1.591
R (2.8 x SR)	0.103	0.446	0.386	2.317	4.456
RSDR (%)	26.5	27.6	11.5	14.8	14.7

Further documentation:

- References

Kuribara H., Shindo Y., Matsuoka, T., Takubo K., Futo S., Aoki N., Hirao T., Akiyama H., Goda Y., Toyoda M., Hino A.: Quantification Methods using Novel Reference Molecules for Detection of Genetically Modified Maize and Soybean. Journal of AOAC International (Submitted)

Shindo Y., Kuribara H., Matsuoka T., Futo S., Sawada C., Shono J., Akiyama H., Goda Y., Toyoda M., Hino A.: Validation Studies of Real-time PCR Analyses for Line Specific Quantification of Genetically Modified Maize and Soybean Using New Reference Molecules. Journal of AOAC International (Submitted)

- Description of the study

T25 maize DNA quantification method using plasmid DNA as reference molecules. Interlab validation with 14 participating laboratories conducted by NFRI in 2001.

- Sample description

Totally twelve maize blind samples were sent to each participant. The maize blind samples were designed as six pairs of blind duplicates including 0, 0.10, 0.50, 1.0, 5.0 and 10 % of Bt11, GA 21, T25, Event176 and MON810 maize (i.e., the maize 1.0 % blind sample consisted 1.0 % Bt11, 1.0 % GA21, 1.0 % T25, 1.0 % Event176, 1.0 % MON810, and 95 % non-GMO for a total of 5.0 % GMO). The blank sample, 0 % GM maize, was only used to remove the invalid laboratories before statistical analysis.

- Matrices

Raw materials

Others:

- Excluded labs 0

- Labs with uncomplete results 0

- Total number of samples 168

- Positive samples 140

- Outlying results 8 ***Outlying tests*** Cochran's and Grubb's test

- Comments:

Validated method	**HA-NOS118-f/HA-NOS118r**
Food Source / Target	**NOS-Terminator**
Sender	Guy Van den Eede Joint Research Centre (JRC) European Commission

Number of laboratories: 23

Further information:

- Specificity specific primers for the nos-terminator.

- Limit of detection 0.4 % (0 % negative control)

relative 0.4 % ***Number of copies***

- False-positive 1.8 %

- False-negative 2.1 %

Further information for quantitative methods:

- Limit of quantification

relative ***Number of copies***

- Precision

- Repeatability

- Reproducability

Further documentation:

- References Lipp M., Bluth, A., Eyquem F., Kruse L., Schimmel H., Van den Eede G. And Anklam E. (2001): Validation of a method based on polymerase chain reaction for the detection of genetically modified organisms in various processed foodstuffs. Eur. Food. Technol. 212: 497-504

- Description of the study The study was performed both for the CaMV 35S promotor sequence and the nos-terminator sequence detection.

- Sample description Each laboratory received 30 unknown specimen and 4 known samples for analysis. 10 specimen were free of GMO (0 %), 20 specimen contained total GMO at different extents (2 %, 100 % - 10 % in the case of bisciuts). The percentages of CaMV specific contents ranged between 0.4 % and 100 %.

- Matrices Processed food (cooked maize grit - polenta-, infant formula, biscuits, meal of acified soybeans).

Others:

- Excluded labs 1 use of different set of primers

- Labs with uncomplete results 5

- Total number of samples

- Positive samples

- Outlying results 5 ***Outlying tests*** high amount of non-correct results

- Comments: Also reported by IRELAND / State Laboratory

Validated method	**NOS-1/NOS-3**
Food Source / Target	**NOS-Terminator**
Sender	Guy Van den Eede Joint Research Centre (JRC) European Commission

Number of laboratories: 27

Further information:

- ***Specificity*** specific primers for the nos-terminator plus confirmation by means of Nsi I restriction endonuclease digestion.
- ***Limit of detection*** 0.1 % (0 % negative control)

	relative	***Number of copies***
	0.1 %	

- ***False-positive*** 1.2 %
- ***False-negative*** 4.9 %

Further information for quantitative methods:

- ***Limit of quantification***

	relative	***Number of copies***

- ***Precision***
- ***Repeatability***
- ***Reproducability***

Further documentation:

- ***References*** Lipp M., Anklam E, Brodmann, P., Pietsch, K and Pauwels J. (1999) Results of a screening method of genetically modified organisms in soy beans and maize. Food Control 10: 379-383.
- ***Description of the study*** The study was performed both for the CaMV 35S promotor sequence and the nos-terminator sequence detection.
- ***Sample description*** Each laboratory received 32 unknown specimen and 4 known samples for analysis. 25 % of the specimen were free of GMO (0 %), 75 % of the specimen contained GMO. The percentages of CaMV specific contents ranged between 0,1 % and 2 %.
- ***Matrices*** Raw material

Others:

- ***Excluded labs*** 3 no conclusive results
- ***Labs with uncomplete results*** 0
- ***Total number of samples*** 736
- ***Positive samples*** 552
- ***Outlying results*** 1 ***Outlying tests*** wrong sensitivity adjustment
- ***Comments:***

Validated method	**CaM3-5'/GUSn-3'**
Food Source / Target	**Papaya 55-1, 66-1 (Cornell University)**
Sender	Hiroshi Akiyama National Institute of Health Sciences (NIHS) Japan

Number of laboratories: 5

Further information:

- Specificity No amplification was observed using DNA from non-transgenic papaya, maize, soybeaans, rice, wheat and barley as well as from the transgenic maize-lines GA21, T25, Event176, and MON810 and the transgenic soy-line Roundup Ready soy.

- Limit of detection

relative 0.05 % ***Number of copies***

- False-positive 0

- False-negative 0

Further information for quantitative methods:

- Limit of quantification

relative ***Number of copies***

- Precision

- Repeatability

- Reproducibility

Further documentation:

- References Yukihiro Goda, Takuya Asano, Masaaki Shibuya, Akihiro Hino, Masatake Toyoda (2001): Detection of Recombinant DNA from Genetically Modified Papaya. J. Food Hyg. Japan 42: 231-236.

- Description of the study National Collaborative study in the year 2001

- Sample description Five maize blind samples were sent to the participants as duplicates.

- Matrices Raw materials

Others:

- Excluded labs 0

- Labs with uncomplete results 0

- Total number of samples 50

- Positive samples 30

- Outlying results 0 ***Outlying tests***

- Comments:

Validated method	**NosC-5'/CaMVN-3'**
Food Source / Target	**Papaya 55-1, 66-1 (Cornell University)**
Sender	Hiroshi Akiyama National Institute of Health Sciences (NIHS) Japan

Number of laboratories: 5

Further information:

- Specificity No amplification was observed using DNA from non-transgenic papaya, maize, soybeaans, rice, wheat and barley as well as from the transgenic maize-lines GA21, T25, Event176, and MON810 and the transgenic soy-line Roundup Ready soy.

- Limit of detection

relative 0.05 % ***Number of copies***

- False-positive 0

- False-negative 0

Further information for quantitative methods:

- Limit of quantification

relative ***Number of copies***

- Precision

- Repeatability

- Reproducibility

Further documentation:

- References Yukihiro Goda, Takuya Asano, Masaaki Shibuya, Akihiro Hino, Masatake Toyoda (2001): Detection of Recombinant DNA from Genetically Modified Papaya. J. Food Hyg. Japan 42: 231-236.

- Description of the study National Collaborative study in the year 2001

- Sample description Five maize blind samples were sent to the participants as duplicates.

- Matrices Raw materials

Others:

- Excluded labs 0

- Labs with uncomplete results 0

- Total number of samples 50

- Positive samples 30

- Outlying results 0 ***Outlying tests***

- Comments:

Validated method	**p-FMV02-5'/PLRV01-3'**
Food Source / Target	**Potato NewLeaf Plus (RBMT21-129, RBMT21-350, RBMT22-082, Monsanto)**
Sender	Hiroshi Akiyama National Institute of Health Sciences (NIHS) Japan

Number of laboratories: 14

Further information:

- Specificity No amplification was observed using DNA from non-transgenic potato, soybeaans, rice, wheat and barley as well as from the transgenic maize-lines GA21, T25, Event176, and MON810 and the transgenic soy-line Roundup Ready soy, the transgenic maize-NewLeaf and NewLeaf Y.

- Limit of detection

relative 0.10 % ***Number of copies***

- False-positive 0

- False-negative 0

Further information for quantitative methods:

- Limit of quantification

relative ***Number of copies***

- Precision

- Repeatability

- Reproducibility

Further documentation:

- References Hiroshi Akiyama, Kazue Sugimoto, Misao Matsumoto, Kazuto Isuzugawa, Masaaki Shibuya, Yukihiro Goda and Masatake Toyoda (2002): A Detection Method of Recombinant DNA from Genetically Modified Potato NewLeaf Plus potato and Detection of NewLeaf Plus in Snack. J. Food Hyg. Doc. Japan 43: 24-29.

- Description of the study National Collaborative study in the year 2002

- Sample description Five maize blind samples were sent to the participants as duplicates.
Positive samples: 0.1 % and 1 %

- Matrices Raw materials

Others:

- Excluded labs 0

- Labs with uncomplete results 0

- Total number of samples 140

- Positive samples 56

- Outlying results 0 ***Outlying tests***

- Comments:

Validated method	**PLRV-rep1-5'/PLRV-rep1-3'**
Food Source / Target	**Potato NewLeaf Plus (RBMT21-129, RBMT21-350, RBMT22-082, Monsanto)**
Sender	Hiroshi Akiyama National Institute of Health Sciences (NIHS) Japan

Number of laboratories: 14

Further information:

- Specificity No amplification was observed using DNA from non-transgenic potato, soybeaans, rice, wheat and barley as well as from the transgenic maize-lines GA21, T25, Event176, MON810 and the transgenic soy-line Roundup Ready soy, the transgenic maize-NewLeaf and NewLeaf Y.

- Limit of detection

relative 0.10 % ***Number of copies***

- False-positive 7

- False-negative 0

Further information for quantitative methods:

- Limit of quantification

relative ***Number of copies***

- Precision

- Repeatability

- Reproducibility

Further documentation:

- References Hiroshi Akiyama, Kazue Sugimoto, Misao Matsumoto, Kazuto Isuzugawa, Masaaki Shibuya, Yukihiro Goda and Masatake Toyoda (2002): A Detection Method of Recombinant DNA from Genetically Modified Potato NewLeaf Plus potato and Detection of NewLeaf Plus in Snack. J. Food Hyg. Doc. Japan 43: 24-29.

- Description of the study National Collaborative study in the year 2002

- Sample description Five maize blind samples were sent to the participants as duplicates.
Positive samples: 0.1 % and 1 %.

- Matrices Raw materials

Others:

- Excluded labs 0

- Labs with uncomplete results 0

- Total number of samples 140

- Positive samples 56

- Outlying results 0 ***Outlying tests***

- Comments:

Validated method	**p-FMV05-5'/PVY02-3'**
Food Source / Target	**Potato NewLeaf Y (RBMT15-101, SEMT15-02, SEMT15-15, Monsanto)**
Sender	Hiroshi Akiyama National Institute of Health Sciences (NIHS) Japan

Number of laboratories: 14

Further information:

- Specificity No amplification was observed using DNA from non-transgenic potato, soybeaans, rice, wheat and barley as well as from the transgenic maize-lines GA21, T25, Event176, MON810, the transgenic soy-line Roundup Ready soy, the transgenic potato-NewLeaf and NewLeaf Plus.

- Limit of detection

relative 0.10 % ***Number of copies***

- False-positive 3

- False-negative 0

Further information for quantitative methods:

- Limit of quantification

relative ***Number of copies***

- Precision

- Repeatability

- Reproducability

Further documentation:

- References Hiroshi Akiyama, Takahiro Watanabe, Tiseko Wakui, Masaaki Shibuya, Yukihiro Goda and Masatake Toyoda: A Detection Method of Recombinant DNA from Genetically Modified Potato NewLeaf Y potato. J. Food Hyg. Doc. Japan (submitted).

- Description of the study National Collaborative study in the year 2002

- Sample description Five maize blind samples were sent to the participants as duplicates.
Positive samples: 0.1 % and 1 %.

- Matrices Raw materials

Others:

- Excluded labs 0

- Labs with uncomplete results 0

- Total number of samples 140

- Positive samples 56

- Outlying results 0 ***Outlying tests***

- Comments:

Validated method	**PVY01-5'/PVY01-3'**
Food Source / Target	**Potato NewLeaf Y (RBMT15-101, SEMT15-02, SEMT15-15, Monsanto)**
Sender	Hiroshi Akiyama National Institute of Health Sciences (NIHS) Japan

Number of laboratories: 14

Further information:

- Specificity No amplification was observed using DNA from non-transgenic potato, soybeaans, rice, wheat and barley as well as from the transgenic maize-lines GA21, T25, Event176, MON810 and the transgenic soy-line Roundup Ready soy, the transgenic potato-NewLeaf and NewLeaf Plus.

- Limit of detection

	relative 0.10 %	***Number of copies***

- False-positive 0

- False-negative 0

Further information for quantitative methods:

- Limit of quantification

	relative	***Number of copies***

- Precision

- Repeatability

- Reproducibility

Further documentation:

- References Hiroshi Akiyama, Takahiro Watanabe, Tiseko Wakui, Masaaki Shibuya, Yukihiro Goda and Masatake Toyoda: A Detection Method of Recombinant DNA from Genetically Modified Potato NewLeaf Y potato. J. Food Hyg. Doc. Japan (submitted).

- Description of the study National Collaborative study in the year 2002

- Sample description Five maize blind samples were sent to the participants as duplicates.
Positive samples: 0.1 % and 1 %.

- Matrices Raw materials

Others:

- Excluded labs 0

- Labs with uncomplete results 0

- Total number of samples 140

- Positive samples 56

- Outlying results 0 ***Outlying tests***

- Comments:

Validated method	**35S-af2/Petu-r1**
Food Source / Target	**Soybean Roundup Ready (Monsanto)**
Sender	Jutta Zagon Federal Institue for Health Protection of Consumers (BgVV) Germany

Number of laboratories: 15

Further information:

- Specificity No amplification was observed using DNA from non-transgenic soybeans, potatoes, tomatoes, maize and sugar beets as well as from the transgenic maize-lines Bt-176, Bt-11 and T25 and MON810.

- Limit of detection Based on the assumption of 1 copy of the genetic construct per genome (AGBIOS database: http://www.agbios.com) and the genome size of 1.13x10^9 base pairs (http://www.rbgkew.org.uk/cval/homepage.html) the absolute detection limit with 50 ng DNA from the specific plant species (with a relative GMO content of 0.1 % into ground seeds) introduced in PCR is 20 genome equivalents.

relative 0.1 % ***Number of copies*** 40

- False-positive 5

- False-negative 0

Further information for quantitative methods:

- Limit of quantification

relative ***Number of copies***

- Precision

- Repeatability

- Reproducability

Further documentation:

- References Repost of the EU-tender No. XXIV/98/A3/001. Development of qualitative as well as quantitative detection methods to identify a genetic modification in soybean and maize (2000). Internet: http://www.europa.eu.int/comm/food/fs/biotech/biotech02_en.html.

- Description of the study European collaborative study in the year 2000.

- Sample description 12 laboratories received 3 samples, 2 received 5 samples and 1 received 9 samples.

- Matrices

Others:

- Excluded labs 0

- Labs with uncomplete results 0

- Total number of samples 55

- Positive samples 45

- Outlying results ***Outlying tests***

- Comments: The high number of false positive results is due to a contamination of the non-GMO samples which have been sent to the participants.

Validated method	**Protein_EPSPS**
Food Source / Target	**Soybean Roundup Ready (Monsanto)**
Sender	Guy Van den Eede Joint Research Centre (JRC) European Commission

Number of laboratories: 38

Further information:

- ***Specificity*** mAb and polyclonal Ab specificity testing
- ***Limit of detection*** 0.35 % (0 % negative control)

	relative	***Number of copies***
	0.35 %	

- ***False-positive***
- ***False-negative***

Further information for quantitative methods:

- ***Limit of quantification***

	relative	***Number of copies***

- ***Precision***
- ***Repeatability*** RSDr = 7 %
- ***Reproducability*** RSDR = 10 %

Further documentation:

- ***References*** Lipp M., Anklam E. (2000): Validation of an immunoassay for detection and quantification of a genetically modified soybean in food fractions using materials: interlaboratory study. Journal of AOAC International 83: 919-927
- ***Description of the study*** Immunoscreening
- ***Sample description*** The percentages of Roundup Ready Soybean specific contents ranged between 0.5 %, 1 % and 2 % plus a 0 % negative control.
- ***Matrices*** Raw material (soybean standard materials)

Others:

- ***Excluded labs*** 6 various mistakes
- ***Labs with uncomplete results*** 1
- ***Total number of samples***
- ***Positive samples***
- ***Outlying results*** ***Outlying tests***
- ***Comments:*** Also reported by USA / United States Department of Agriculture
mAb: 39B10.1 murine monoclonal antibody against CP4 EPSPS protein expressed in E. Coli.
Polyclonal Ab: Rabbit anti- CP4 EPSPS protein expressed in E. Coli.

Validated method	**Q_RR1-F/RR1-R/Probe**
Food Source / Target	**Soybean Roundup Ready (Monsanto)**
Sender	Jutta Zagon Federal Institue for Health Protection of Consumers (BgVV) Germany

Number of laboratories: 14

Further information:

- Specificity

No amplification was observed using DNA from non-transgenic soybeans, potatoes, tomatoes, maize and sugar beets as well as from the transgenic maize-lines Bt-176, Bt-11 and T25 and MON810.

- Limit of detection

Based on the assumption of 1 copy of the genetic construct per genome (AGBIOS database: http://www.agbios.com) and the genome size of 1.13x10^9 base pairs (http://www.rbgkew.org.uk/cval/homepage.html) the absolute detection limit with 1 ng DNA from the specific plant species (with a relative GMO content of 0.1 % into ground seeds) introduced in PCR is 1 genome equivalents.

relative 0.1 % ***Number of copies*** 1

- False-positive 0

- False-negative 0

Further information for quantitative methods:

- Limit of quantification

Based on the assumption of 1 copy of the genetic construct per genome (AGBIOS database: http://www.agbios.com) and the genome size of 1.13x10^9 base pairs (http://www.rbgkew.org.uk/cval/homepage.html) the absolute detection limit with 10 ng DNA from the specific plant species (with a relative GMO content of 0.1 % into ground seeds) introduced in PCR is 10 genome equivalents.

relative 0.1 % ***Number of copies*** 10

- Precision

Sample (% GM)	0.1	0.5	1	2	5	2 (TVP)
Mean value (% GM)	0.11	0.49	1.00	2.26	4.91	1.71
Recovery (%)	108.75	98.71	99.86	113.23	98.27	85.38

- Repeatability

Sample (% GM)	0.1	0.5	1	2	5	2 (TVP)
Sr (%)	0.02	0.12	0.17	0.20	0.56	0.39
RSDr (%)	14.17	25.04	16.80	8.63	11.33	22.64

- Reproducability

Sample (% GM)	0.1	0.5	1	2	5	2 (TVP)
SR (%)	0.02	0.12	0.27	0.60	0.95	0.48
RSDR (%)	17.78	25.04	26.82	26.54	19.34	27.98

Further documentation:

- References Broll et al. (paper in preparation)

- Description of the study European collaborative study in the year 2000 with ABI7700.

- Sample description

- Matrices

Others:

- Excluded labs 0

- Labs with uncomplete results 0

- Total number of samples 98

- Positive samples 84

- Outlying results 3 ***Outlying tests*** Cochran-test

- Comments: Also reported by GERMANY / GeneScan Europe AG

Validated method	**Q_RRS01-5'/RRS01-3'/RRSTaq**
Food Source / Target	**Soybean Roundup Ready (Monsanto)**
Sender	Akihiro HINO National Food Research Institute (NFRI) Japan

Number of laboratories: 13

Further information:

- Specificity

No amplification was observed using DNA from non-transgenic maize, soy, rice, wheat and barley as well as from the transgenic maize-lines Bt11, GA21, T25, Event176 and MON810.

- Limit of detection

relative 0.1 % ***Number of copies***

- False-positive

- False-negative

Further information for quantitative methods:

- Limit of quantification

The plasmid DNA containing sequences of PCR products amplified by specific primer pairs was used as reference molecule. Standard curves were calibrated using the five concentrations of reference molecules such as 20, 125, 1,500, 20,000 and 250,000 copies per reaction. Almost (18/22) of the measurement copies of 0.10 % Roundup Ready soy were above 20 copies. Therefore we conclude that limit of quantification for Roundup Ready soy was 0.10 %.

relative 0.10 % ***Number of copies*** 20

- Precision

Sample (% GM)	0.10	0.50	1.0	5.0	10.0
Mean value (% GM)	0.108	0.571	1.16	5.76	11.7
Biaz (%)	+8.1	+14.3	+16.1	+15.1	+17.2

- Repeatability

Sample (% GM)	0.10	0.50	1.0	5.0	10.0
Sr	0.015	0.068	0.129	0.435	0.993
r (2.8 x Sr)	0.041	0.191	0.362	1.219	2.779
RSDr (%)	13.4	12.0	11.2	7.6	8.5

- Reproducability

Sample (% GM)	0.10	0.50	1.0	5.0	10.0
SR	0.014	0.091	0.161	0.660	1.246
R (2.8 x SR)	0.040	0.255	0.451	1.849	3.489
RSDR (%)	13.4	15.9	13.9	11.5	10.6

Further documentation:

- References

Kuribara H., Shindo Y., Matsuoka, T., Takubo K., Futo S., Aoki N., Hirao T., Akiyama H., Goda Y., Toyoda M., Hino A.: Quantification Methods using Novel Reference Molecules for Detection of Genetically Modified Maize and Soybean. Journal of AOAC International (Submitted)

Shindo Y., Kuribara H., Matsuoka T., Futo S., Sawada C., Shono J., Akiyama H., Goda Y., Toyoda M., Hino A.: Validation Studies of Real-time PCR Analyses for Line Specific Quantification of Genetically Modified Maize and Soybean Using New Reference Molecules. Journal of AOAC International (Submitted)

- Description of the study

Roundup Ready soy DNA quantification method using plasmid DNA as reference molecules. Interlab validation with 13 participating laboratories conducted by NFRI in 2001.

- Sample description

Totally twelve soy blind samples were sent to each participant. The soy blind samples were designed as six pairs of blind duplicates including 0, 0.10, 0.50, 1.0, 5.0 and 10 % of Roundup Ready soy. The blank sample, 0 % GM soy, was only used to remove the invalid laboratories before statistical analysis.

- Matrices

Raw materials

Others:

- Excluded labs 0

- Labs with uncomplete results 0

- Total number of samples 144

- Positive samples 120

- Outlying results 2 ***Outlying tests*** Cochran's and Grubb's test

- Comments: