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Report of the collaborative study for the validation of real time PCR methods for GMO screening

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INTRODUCTION

The Italian Reference Laboratory for GMO analysis (NRL), established in accordance with the Reg(CE) 882/2004 within the Istituto Zooprofilattico Sperimentale delle regioni Lazio e Toscana, organized a collaborative study for the validation of six qualitative real time PCR methods to screen the possible presence of food or feed containing, consisting or derived from genetically modified plants.

Methods tested in the collaborative study are derived from previously published papers and they target sequences from the *Cauliflower Mosaic Virus* derived 35S promoter, from the *Agrobacterium tumefaciens* nos terminator and cp4-epsps gene, from the CTP-cp4epsps construct, from the *Escherichia coli* nptII and the *Streptomyces viridochromogenes* pat genes. The two methods targeting the epsps gene are specific for the wild type version of the gene CP4EPSPS and the junction region between the chloroplast transit peptide (CTP2) and the synthetic version of the gene CP4EPSPS respectively.

The method for the detection of p35S consists in a modified version of that previously described by Pauli et al.: a different forward primer, designed to skip a SNP affecting the amplification efficiency in the detection of maize event DAS1507, is adopted.

Before starting the collaborative study, the NRL carried out an *in house* evaluation of the above mentioned methods in terms of specificity and limit of detection (LOD).

The study took place in April-May 2011 and involved 17 members of the Italian network of GMO official laboratories.

All laboratories received a detailed protocol for the reaction mixtures preparation, the plates set up and the dilution instructions for the assessment of the limit of detection (LOD) together with samples and reagents.

The aim of the collaborative study was to evaluate false positive and false negative rates and LOD of each method through a data pool generated by all laboratories.

METHODS DESCRIPTION

P35S

Target: 35s promoter from Cauliflower Mosaic Virus (GenBank V001401)

Primers and probe sequences:

Name	Type	Sequence 5'-3'	References
35S-F3	Fw	ATg CCT CTg CCg ACA gT	This work
35S-R	Rv	AAg ACg Tgg TTg gAA CgT CTT C	a.
35S-TMP	Probe	FAM-CAA AgA Tgg ACC CCC ACC CAC g-TAMRA	a.

Amplicon size: 84 bp

Reactions were performed in a final volume of 25 µL using 1X TaqMan Universal Master Mix (Applied Biosystems, Foster City, CA), Primer concentration 400 nM, Probe conc. 200 nM

Amplification profile: 50°C, 120''; 95°C, 600''; [95 °C, 15''; 60 °C, 60''] x 50 (data acquisition in the 60°C step)

References:

- a. Pauli U, Liniger M, Schrott M, Schouwey B, Hübner P, Brodmann P and Eugster A (2001) Mitt. Lebensm. Hyg. vol. 92/2: 145-158. [4]
- b. International Organization for Standardization. ISO 21570:2005(E): Annex B. [5]

T-NOS

Target: terminator of the nopaline synthase gene from *Agrobacterium tumefaciens* (GenBank JA201792)

Primers and probe sequences:

Name	Type	Sequence 5'-3'
5F:	Fw	gTA ATg CAT gAC gTT ATT TAT gAg A
4R:	Rv	TAA TTT ATC CTA gTT TgC gCg C
P1:	Probe	FAM-TgC ggg ACT CTA ATC ATA AAA ACC CA-TAMRA

Amplicon size: 104 bp

Reactions were performed in a final volume of 25 µL using 1,25 Units AmpliTaq Gold® DNA Polymerase, 0,30 Units AmpErase® UNG, 800 nM dUTP, 400 nM dATP, 400 nM dCTP, 400 nM dGTP, 1X TaqMan® Buffer A, 4,5 mM MgCl₂ (TaqMan® PCR Core Reagents Kit, Applied Biosystems, Foster City, CA), Primer concentration 300 nM, Probe conc. 200 nM

Amplification profile: 50°C, 120''; 95°C, 600''; [95 °C, 15''; 60 °C, 60''] x 50 (data acquisition in the 60°C step)

Reference: Permingeat HR, Reggiardo MI, Vallejos RH (2002) J Agric Food Chem. 50(16):4431-6. [6]

EPSPS

Target: 5-enolpyruvylshikimate-3-phosphate synthase gene from the strain CP4 of *Agrobacterium tumefaciens* (CP4-EPSPS) (GenBank I43998)

Primers and probe sequences:

Name	Type	Sequence 5'-3'
Sttmf3a	Fw	gCA AAT CCT CTg gCC TTT CC
Sttmr2a	Rv	CTT gCC CgT ATT gAT gAC gTC
Sttmpa	Probe	FAM-TTC ATg TTC ggC ggT CTC gCg-TAMRA

Amplicon size: 146 bp

Reactions were performed in a final volume of 25 µL using 1X TaqMan Universal Master Mix (Applied Biosystems, Foster City, CA), Primer concentration 600 nM, Probe conc. 200 nM

Amplification profile: 50°C, 120''; 95°C, 600''; [95 °C, 15''; 60 °C, 60''] x 50 (data acquisition in the 60°C step)

Reference: Väitilingom M, Pijnenburg H, Gendre F, Brignon P. (1999) *J Agric Food Chem.* 47(12):5261-6 [7]

CTP2-EPSPS

Target: Junction region between the chloroplast transit peptide 2 (CTP2) (GenBank I44002) and the synthetic version of 5-enolpyruvylshikimate-3-phosphate synthase gene from the strain CP4 of *Agrobacterium tumefaciens* (CP4-EPSPS) (GenBank I44001)

Primers and probe sequences:

Name	Type	Sequence 5'-3'
F6	Fw	CCA Cgg CgT gCA TgC
R6	Rv	ACC AgA ggA CTT ACg AgC AgT Tg
P	Probe	FAM-TCA Cgg TgC AAg CAg CCg TCC-TAMRA

Amplicon size: 62 bp

Reactions were performed in a final volume of 25 µL using 1X TaqMan Universal Master Mix (Applied Biosystems, Foster City, CA), Primer concentration 300 nM, Probe conc. 100 nM

Amplification profile: 50°C, 120''; 95°C, 600''; [95 °C, 15''; 60 °C, 60''] x 50 (data acquisition in the 60°C step)

Reference: Alexander TW, Sharma R, Deng MY, Whetsell AJ, Jennings JC, Wang Y, Okine E, Damgaard D, McAllister TA. (2004) *J Biotechnol.* 112(3):255-66. [8]

NPTII

Target: neomycin phosphotransferase II gene (nptII) from *Escherichia coli* (GenBank U00004)

Primers and probe sequences:

Name	Type	Sequence 5'-3'
NPTII-f	Fw	gAT AgC ggT CCg CCA CAC
NPTII-r	Rv	CgA ggA TCT CgT CgT gAC ACA T
NPTII-Pro	Probe	FAM-TTT CCA CCA TgA TAT TCg gCA AgC Agg-TAMRA

Amplicon size: 113 bp

Reactions were performed in a final volume of 25 µL using 1X TaqMan Universal Master Mix (Applied Biosystems, Foster City, CA), Primer concentration 600 nM, Probe conc. 200 nM

Amplification profile: 50°C, 120''; 95°C, 600''; [95 °C, 15''; 60 °C, 60''] x 50 (data acquisition in the 60°C step)

Reference: Yi CX, Zhang J, Chan KM, Liu XK, Hong Y (2008) Anal Biochem 375(1):150-2 [9]

PAT

Target: Phosphinothricin N-acetyltransferase genes (pat) from *Streptomyces viridochromogenes* (GenBank DQ156557)

Primers and probe sequences:

Name	Type	Sequence 5'-3'
KVM-5	Fw	TTg Agg gTg TTg Tgg CTg gTA
KVM-6	Rv	TgT CCA ATC gTA AgC gTT CCT
TaqMan Pat1	Probe	FAM-CTT CCA ggg CCC AgC gTA AgC A-TAMRA

Amplicon size: 68 bp

Reactions were performed in a final volume of 25 µL using 1X TaqMan Universal Master Mix (Applied Biosystems, Foster City, CA), Primer concentration 400 nM, Probe conc. 200 nM

Amplification profile: 50°C, 120''; 95°C, 600''; [95 °C, 15''; 60 °C, 60''] x 50 (data acquisition in the 60°C step)

Reference: Weighardt F, Barbati C, Paoletti C, Querci M, Kay S, De Beuckeleer M, Van den Eede G (2004) J AOAC Int. 87(6):1342-55. [10]

SINGLE LABORATORY EVALUATION OF METHODS

Experimental specificity was assessed by testing in duplicates genomic DNA extracted or derived from certified reference materials representing a total of 34 GM events.

LODs were estimated considering the definition of the limit of detection as the lowest concentration at which a positive sample yields a positive result at least 95% of the time [1]. Serial dilutions of DNA extracted from RM were prepared in order to obtain 70, 35, 18, 9, 4 and 2 copies of target elements per reaction; each dilution level was tested on at least 20 PCR replicates.

Results of the in house evaluation are summarized in the table below.

Table 1: Single laboratory evaluation of screening methods. Columns corresponding to methods display expected results based on sequence information (A = absence; P = presence) and the experimental results (+ = amplification signal detected, - = no amplification signal). The last row reports the LOD.

Species	Events	Methods						
		p35S	tNOS	CP4-EPSPS		NPTII	PAT	
				EPSPS	CTP2-EPSPS			
Maize	3272	A -	P +	A -	-	A -	A -	
	BT10	P +	P +	A -	-	A -	P +	
	BT11	P +	P +	A -	-	A -	P +	
	BT176	P +	A -	A -	-	A -	A -	
	DAS1507	P +	A -	A -	-	A -	P +	
	DAS59122	P +	A -	A -	-	A -	P +	
	GA21	A -	P +	A -	-	A -	A -	
	MIR604	A -	P +	A -	-	A -	A -	
	MON810	P +	A -	A -	-	A -	A -	
	MON863	P +	P +	A -	-	P +	A -	
	MON863x MON810	P +	P +	A -	-	P +	A -	
	MON88017	P +	P +	P +	-	A -	A -	
	MON89034	P +	P +	A -	-	A -	A -	
	NK603	P +	P +	P +	-	A -	A -	
	T25	P +	A -	A -	-	A -	P +	
Soy	A2704-12	P +	A -	A -	-	A -	P +	
	A5547-127	P +	A -	A -	-	A -	P +	
	DP-305423	A -	A -	A -	-	A -	A -	
	DP-356043	P +	A -	A -	-	A -	A -	
	MON40-3-2	P +	P +	P +	-	A -	A -	
	MON89788	A -	A -	P -	+	A -	A -	
Cotton	281-24-236x 3006-210-23	A -	A -	A -	-	A -	P +	
	GHB614	A -	A -	A -	-	A -	A -	
	LLCotton25	P +	P +	A -	-	A -	A -	
	MON1445	P +	P +	P -	+	P +	A -	
	MON15985	P +	P +	A -	-	P +	A -	
	MON531	P +	P +	A -	-	P +	A -	
Rice	LLRice62	P +	A -	A -	-	A -	A -	
Canola	GT73	A -	A -	A -	+	A -	A -	
	MS8	A -	P +	A -	-	A -	A -	
	RF3	A -	P +	A -	-	A -	A -	
	T45	P +	A -	A -	-	A -	P +	
Potato	EH92-527-1	A -	P +	A -	-	P +	A -	
Sugar beet	H7-1	A -	A -	P -	+	A -	A -	
Limit of detection (target copy number)		4	9	4	4	4	9	

PARTICIPATING LABORATORIES

Table 2: List of participants and real time PCR platforms

Laboratories	Device
Azienda Sanitaria Locale 10 Firenze	iCycler (BioRad)
Agenzia Provinciale per la Protezione dell'Ambiente di Bolzano	iCycler (BioRad)
Agenzia Regionale per la Protezione dell'Ambiente del Friuli Venezia Giulia	(Biorad)
Agenzia Regionale per la Protezione dell'Ambiente del Piemonte	iCycler (BioRad)
Agenzia Regionale per la Protezione dell'Ambiente del Veneto	ABI 7000
Agenzia Regionale per la Protezione dell'Ambiente della Valle d'Aosta	IQ5 (BioRad)
Istituto Nazionale di Ricerca per gli Alimenti e la Nutrizione (ex ENSE-Tavazzano)	ABI 7900HT
Istituto Superiore di Sanità	ABI 7700
Istituto Zooprofilattico Sperimentale delle Regioni Abruzzo e Molise	ABI 7900HT
Istituto Zooprofilattico Sperimentale delle Regioni Lazio e Toscana	ABI 7900HT
Istituto Zooprofilattico Sperimentale delle Regioni Lombardia ed Emilia Romagna	ABI StepOnePlus
Istituto Zooprofilattico Sperimentale del Mezzogiorno	ABI 7900HT
Istituto Zooprofilattico Sperimentale delle Regioni Piemonte Liguria Valle d'Aosta	AB step one
Istituto Zooprofilattico Sperimentale della Sardegna	ABI 7900HT Fast
Istituto Zooprofilattico Sperimentale della Sicilia	ABI 7700
Istituto Zooprofilattico Sperimentale delle Regioni Umbria e Marche	ABI 7900HT
Istituto Zooprofilattico Sperimentale delle Venezie	AB 7500 Fast

MATERIALS

Participants received the following materials:

- Positive control samples: 6 positive control samples labelled A+, B+, C+, D+, E+, F+ (one for each PCR target), extracted from RM and diluted at the final concentration of 40 target copies/ μ l. These dilutions were performed taking into account the GM content, the number of inserted target and the zygosity status indicated in the certification report (MON89788 soy was assumed to be homozygous). Haploid genome (HG) copy number estimation was made considering 1HG value of 1.13 pg for soya and 2.725 pg for maize [2].
- Blind samples: 30 unknown samples labelled A1-5, B1-5, C1-5, D1-5, E1-5, F1-5 and diluted at the final concentration of 20 ng/ μ l (sample D3=RM at the concentration of 80 copies/ μ l).
- Reagents: 2X TaqMan® PCR Universal Master Mix, NOS Pre-masterMIX, sterile ultrapure water, Tris-EDTA buffer(1mM Tris-HCl pH 8.0/0.01 mM EDTA), Primers 20 pmol/ μ l, Probe 5 pmol/ μ l.

Control and blind samples were distributed as genomic DNA solutions, the sample composition is displayed in table 3.

Table 3: Composition of blind and control samples used for the collaborative validation study. Blind samples are marked with a letter and a number, positive control samples are labelled with + (FS = field samples; RM = reference materials).

p35S		tNOS	
Sample	Description	Sample	Description
A1	FS: Mixed flour (MON40-3-2>5%; BT11=4,8%)	B1	FS: Maize kernels (BT11=0,75%)
A2	FS: non-GM soy	B2	FS: non-GM maize
A3	FS: Maize kernels (BT11=0,75%)	B3	FS: non-GM soy
A4	FS: Feed (MON40-3-2>5%; MON89788=0,87%)	B4	FS: Feed (MON40-3-2>5%; MON89788=0,87%)
A5	FS: non-GM maize	B5	FS: Mixed flour (MON40-3-2>5%; BT11=4,8%)
A+	RM: MON863 1% (heterozygous, 1 target)	B+	RM: NK603 1% (heterozygous, 1 target)

EPSPS		CTP2-EPSPS	
Sample	Description	Sample	Description
C1	FS: non-GM soy	D1	FS: non-GM soy
C2	FS: Feed (MON40-3-2>5%; NK603=0,13%)	D2	FS: Feed (MON40-3-2>5%; MON89788=0,87%)
C3	FS: non_GM maize	D3	RM: MON89788 100%
C4	FS: Mixed flour (MON40-3-2>5%; BT11=4,8%)	D4	FS: non-GM maize
C5	FS: Maize flour (DAS1507=0,6%; NK603=0,2%)	D5	RM+FS: MON1445 100% + non-GM maize (1:19)
C+	RM: NK603 1% (heterozygous, 2 target)	D+	RM: MON89788 100% (homozygous, 1 target)

NPTII		PAT	
Sample	Description	Sample	Description
E1	RM-FS: MON531 100% + non-GM maize (1:19)	F1	FS: Maize flour (DAS1507=0,6%; NK603=0,2%)
E2	FS: non-GM maize	F2	FS: Mixed flour (MON40-3-2>5%; BT11=4,8%)
E3	RM: MON863 1%	F3	FS: non-GM maize
E4	RM+FS: MON1445 100% + non-GM maize (1:19)	F4	FS: non-GM soy
E5	FS: non-GM soy	F5	FS: Maize kernels (BT11=0,75%)
E+	RM: MON863 1% (heterozygous, 1 target)	F+	RM: BT11 1% (heterozygous, 1 target)

DNA EXTRACTION AND SAMPLE PREPARATION

DNA extraction was performed using a CTAB method [3] for maize and soya flours and field samples. DNeasy plant mini kit (Qiagen, Hilden, Germany) was used to extract DNA from cotton reference materials. All DNA extracted were suspended in 0.2X TE [2 mM Tris-HCl and 0.2 mM EDTA, adjusted to pH 8.0] and the DNA purity was checked by both calculating the ratio of the absorption values at 260/280 nm and carrying out a real-time PCR (monitor run) targeting taxon specific genes on two different amounts of each extracted DNA (200 and 50 ng).

EXPERIMENTAL DESIGN

Blind positive samples were selected for having a relative content of GM material close to the EU legal labelling threshold or to the LOD of the method: when available, field samples have been used, otherwise negative field samples were spiked with GM DNA from Reference materials (table 3). Blind negative samples were prepared from soy and maize kernels tested in ten replicates for all screening targets before distribution.

All laboratories ran two plates for the assessment of false negative/false positive rates and LOD respectively.

In the first plate, five unknown DNA samples were tested in duplicate with each method and results were pooled for the calculation of false negative and false positive rates.

In the second plate, samples were prepared by serial dilutions from positive control samples carried out by each laboratory independently and then tested in three PCR replicates per dilution level.

RESULTS

The calculation of false negative and false positive rates for each method was obtained by pooling the overall results from blind samples: false negative and false positive rates observed were 0% for all methods, except for NPTII method which showed a false positive rate of 1.5% due to one positive replicate occurrence on sample E5 (table 4).

The LOD of each method corresponds to 5 target copies as the result of 51 replicates analysed by the 17 laboratories (table 5).

Table 4: Summary of data results of the collaborative study. The "Exp" column represents expected positive (+) or negative (-) results based on sample composition. In the table the number of positive results and the number of determinations are reported.

p35S				tNOS				EPSPS			
Sample	Exp	positive results	numb. deter.	Sample	Exp	positive results	numb. deter.	Sample	Exp	positive results	numb. deter.
A1	+	34	34	B1	+	34	34	C1	-	0	34
A2	-	0	34	B2	-	0	34	C2	+	34	34
A3	+	34	34	B3	-	0	34	C3	-	0	34
A4	+	34	34	B4	+	34	34	C4	+	34	34
A5	-	0	34	B5	+	34	34	C5	+	34	34
False positive rate (%) = 0% False negative rate (%) = 0%				False positive rate (%) = 0% False negative rate (%) = 0%				False positive rate (%) = 0% False negative rate (%) = 0%			

CTP2-EPSPS				NPTII				PAT			
Sample	Exp	positive results	numb. deter.	Sample	Exp	positive results	numb. deter.	Sample	Exp	positive results	numb. deter.
D1	-	0	34	E1	+	34	34	F1	+	34	34
D2	+	34	34	E2	-	0	34	F2	+	34	34
D3	+	34	34	E3	+	34	34	F3	-	0	34
D4	-	0	34	E4	+	34	34	F4	-	0	34
D5	+	34	34	E5	-	1	34	F5	+	34	34
False positive rate (%) = 0% False negative rate (%) = 0%				False positive rate (%) = 1.5% False negative rate (%) = 0%				False positive rate (%) = 0% False negative rate (%) = 0%			

Table 5: Limit of detection: Number and percentage of positive results per dilution level.

p35S				
target copy n°	Exp	positive results	numb. deter.	% pos. results
30	+	51	51	100
15	+	51	51	100
5	+	51	51	100
1	+	36	51	71

tNOS				
target copy n°	Exp	positive results	numb. deter.	% pos. results
30	+	51	51	100
15	+	51	51	100
5	+	51	51	100
1	+	41	51	80

EPSPS				
target copy n°	Exp	positive results	numb. deter.	% pos. results
30	+	51	51	100
15	+	51	51	100
5	+	50	51	98
1	+	25	51	49

CTP2-EPSPS				
target copy n°	Exp	positive results	numb. deter.	% pos. results
30	+	51	51	100
15	+	51	51	100
5	+	49	51	96
1	+	19	51	37

NPTII				
target copy n°	Exp	positive results	numb. deter.	% pos. results
30	+	51	51	100
15	+	51	51	100
5	+	51	51	100
1	+	42	51	82

PAT				
target copy n°	Exp	positive results	numb. deter.	% pos. results
30	+	51	51	100
15	+	51	51	100
5	+	51	51	100
1	+	26	51	51

The raw data collected by laboratories are reported in the Annex.

DEVIATIONS REPORTED

Lab 4 (see annex) reported no amplification signal on the positive control sample (D+) when tested with the CTP2-EPSPS method. The specificity and LOD plates were assessed after the delivery of a new aliquot of the sample.

Lab 14 (see annex) repeated the LOD assessment for CTP2-EPSPS methods for an error occurred on sample preparation or sample loading.

CONCLUSIONS

The collaborative study results demonstrated that the hereby described multi-screening assay is robust, shows a high specificity and is capable to detect as low as 5 copies of each target.

Reliability of each method has been verified both *in house* through the assessment of specificity on a panel of 34 GM events and through the collaborative study.

In this study, samples were provided to the participants as DNA solutions in accordance with the modular approach for the experimental validation of the different analytical procedures used in GMO testing [11]. A major advantage of this approach is that a validated PCR method can be used in combination with different DNA extraction procedures developed and adopted for specific food and feed matrices.

The validated method is fit for the purpose of multiple screening for the enforcement of EU legislation on GM food and feed and can be adopted by the Italian Network of GMO Laboratories. It is recommended that the multi-screening approach be periodically tested for specificity against newly developed GM events which would eventually become available.

REFERENCES

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ANNEX A: RAW DATA FROM THE COLLABORATIVE STUDY FOR THE VALIDATION OF METHODS FOR GMO SCREENING

Report validation of methods for GMO screening

RESULTS OF COLLABORATIVE STUDY OF P35S METHOD

Sample	Expected	Intralab.	Lab 1	Lab 2	Lab 3	Lab 4	Lab 5	Lab 6	Lab 7	Lab 8	Lab 9	Lab 10	Lab 11	Lab 12	Lab 13	Lab 14	Lab 15	Lab 16	Lab 17	Pos/det.	Accordance %	Ct mean	Ct std
A1	Pos	27.52	28.72	28.20	28.50	29.26	27.19	27.57	26.54	27.01	29.04	26.32	28.28	28.50	27.83	27.78	28.60	28.70	24.75	34	100	27.81	1.15
	Pos	27.59	28.83	28.21	28.40	29.32	27.19	27.58	26.55	26.58	29.27	26.10	28.54	28.20	27.89	27.88	28.70	28.50	24.98	34			
A2	Neg	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	34	100		
	Neg	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	34			
A3	Pos	33.26	35.57	34.54	35.10	34.98	33.02	33.36	32.24	31.98	34.69	32.06	34.01	34.70	33.43	33.21	34.50	35.10	31.39	34	100	33.76	1.29
	Pos	33.31	34.94	33.92	35.50	35.05	33.69	33.39	32.43	32.05	34.43	31.89	34.07	34.70	33.64	33.40	34.90	35.20	30.80	34			
A4	Pos	26.79	28.16	27.71	27.70	27.76	26.45	26.90	26.00	25.82	28.23	25.07	27.78	27.90	27.12	27.11	28.40	27.60	23.53	34	100	27.03	1.21
	Pos	26.93	28.23	27.62	28.00	27.53	26.36	27.09	26.00	25.87	27.99	25.29	27.74	27.90	27.04	27.22	28.10	27.70	24.05	34			
A5	Neg	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	34	100		
	Neg	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	34			
NTC	Neg	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd				
A+	Pos	30.41	31.67	31.34	32.20	31.47	30.24	30.74	29.62	29.93	32.56	29.00	31.58	31.40	30.46	31.16	31.90	31.00	27.61			30.82	1.25
Dilution level (copy n.)		Intralab.	Lab 1	Lab 2	Lab 3	Lab 4	Lab 5	Lab 6	Lab 7	Lab 8	Lab 9	Lab 10	Lab 11	Lab 12	Lab 13	Lab 14	Lab 15	Lab 16	Lab 17	Pos/det.	Positive rate %	Ct mean	Ct std
30		33.89	34.30	34.09	32.60	33.94	32.13	33.46	32.25	32.42	34.31	32.36	33.31	33.40	33.86	33.36	34.80	35.00	31.14	51	100	33.34	1.06
		34.13	34.52	33.57	32.60	34.14	31.90	33.95	32.51	31.73	33.57	32.48	33.77	33.50	33.81	34.10	34.60	35.20	31.52	51			
		33.97	34.68	33.81	33.00	34.52	32.26	33.81	32.16	31.79	33.67	31.56	33.83	33.50	33.66	33.16	34.30	35.00	31.50				
15		34.73	35.13	35.01	34.00	34.61	33.86	34.16	33.01	32.77	34.89	32.87	34.84	35.40	34.94	34.72	35.80	37.60	32.82	51	100	34.54	1.18
		34.88	35.89	35.37	34.00	35.65	32.87	34.44	33.78	33.13	34.40	32.81	35.86	35.20	35.70	34.35	35.60	36.80	33.11	51			
		34.94	35.35	35.00	33.90	34.87	34.01	33.84	33.59	32.63	34.67	32.75	35.01	35.30	35.93	34.30	35.30	36.60	32.93				
5		35.96	36.43	37.23	34.70	37.04	36.42	35.88	35.04	33.82	36.91	34.20	36.93	36.80	36.93	36.37	37.70	38.20	34.23	51	100	36.24	1.13
		38.42	37.83	36.28	35.80	36.11	35.74	36.71	35.02	35.12	35.61	35.60	36.04	37.90	36.28	36.21	38.00	38.70	36.29	51			
		36.18	36.94	37.69	35.40	36.15	34.48	36.36	35.13	34.62	36.12	34.74	36.12	37.30	37.09	35.88	37.60	37.00	35.35				
1		40.20	nd	37.32	39.20	39.31	36.35	nd	nd	nd	nd	37.41	nd	39.30	37.34	39.06	38.60	40.70	37.27	36	71	38.49	1.59
		40.21	nd	37.30	nd	41.71	nd	37.74	37.06	nd	37.09	36.21	39.95	39.70	nd	39.14	37.80	42.40	36.20	51			
		39.24	38.80	38.14	nd	38.77	36.95	nd	37.69	37.03	39.22	nd	nd	39.30	39.20	37.99	40.40	41.60	36.57				
NTC		nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd				

Report validation of methods for GMO screening

RESULTS OF COLLABORATIVE STUDY OF T-NOS METHOD

Sample	Expected	Intralab.	Lab 1	Lab 2	Lab 3	Lab 4	Lab 5	Lab 6	Lab 7	Lab 8	Lab 9	Lab 10	Lab 11	Lab 12	Lab 13	Lab 14	Lab 15	Lab 16	Lab 17	Pos/det.	Accordance %	Ct mean	Ct std
B1	Pos	32.25	33.32	32.97	34.50	35.52	32.26	33.30	31.37	31.26	33.21	30.30	32.60	34.00	32.77	32.23	34.60	33.70	31.23	34	100	32.89	1.47
	Pos	32.17	33.84	32.78	34.90	36.69	32.93	32.51	31.20	30.07	33.00	30.93	32.48	33.30	32.76	32.50	33.80	34.40	31.01	34			
B2	Neg	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	34	100		
	Neg	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	34			
B3	Neg	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	34	100		
	Neg	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	34			
B4	Pos	26.17	27.80	26.87	28.00	28.66	26.70	26.27	25.38	24.48	27.64	24.92	26.96	27.30	26.49	26.66	27.70	27.60	24.14	34	100	26.68	1.33
	Pos	26.10	27.57	26.86	28.10	28.70	26.83	26.23	25.29	24.97	27.30	24.90	27.18	28.20	26.55	25.91	27.80	27.80	23.38	34			
B5	Pos	26.84	28.50	27.71	28.90	29.81	27.59	27.19	26.16	25.51	28.39	25.56	27.90	28.20	27.60	27.51	29.20	28.40	25.14	34	100	27.59	1.32
	Pos	27.04	28.62	27.66	28.70	30.04	27.68	27.32	26.15	25.44	28.07	25.69	27.87	28.10	27.40	27.52	29.00	28.50	24.90	34			
NTC	Neg	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd				
B+	Pos	29.04	30.93	29.90	31.40	32.88	29.83	28.95	28.25	28.06	30.61	27.85	29.94	30.40	29.82	29.74	31.00	30.70	26.88			29.83	1.48
Dilution level (copy n.)		Intralab.	Lab 1	Lab 2	Lab 3	Lab 4	Lab 5	Lab 6	Lab 7	Lab 8	Lab 9	Lab 10	Lab 11	Lab 12	Lab 13	Lab 14	Lab 15	Lab 16	Lab 17	Pos/det.	Positive rate %	Ct mean	Ct std
30		32.01	34.39	32.96	31.60	33.40	32.45	32.65	30.88	31.06	32.10	30.60	33.06	32.60	32.83	32.21	33.90	33.70	30.25	51	100	32.33	1.16
		31.84	33.50	33.08	32.20	33.85	31.30	32.24	30.66	30.50	32.79	30.60	32.70	34.20	32.70	32.47	33.80	33.30	30.84	51			
		32.24	32.94	32.76	31.90	33.84	31.65	31.95	30.54	30.92	32.54	30.74	32.89	32.00	33.09	32.08	33.70	33.60	30.14				
15		33.41	33.96	33.61	33.00	34.20	31.98	33.67	32.68	32.10	33.95	31.38	33.62	34.70	33.80	32.50	34.30	34.50	31.18	51	100	33.32	1.17
		33.19	34.90	34.13	33.50	34.43	32.69	33.43	31.53	32.21	33.13	31.82	33.93	33.30	33.60	32.51	34.60	35.50	31.55	51			
		33.29	33.86	33.67	33.10	34.67	32.84	33.59	32.07	31.83	33.55	31.57	34.44	34.10	33.74	33.03	35.70	34.90	30.61				
5		34.96	35.86	34.86	35.30	35.07	33.80	35.09	33.08	33.25	36.97	33.47	35.57	36.20	36.53	35.93	37.40	36.40	33.13	51	100	34.96	1.18
		34.89	35.03	35.92	34.50	35.17	34.69	34.00	33.14	33.44	34.54	34.17	36.69	35.30	34.78	35.90	36.80	36.10	34.06	51			
		34.49	35.33	35.16	33.30	35.98	33.92	34.70	34.01	33.22	34.67	32.98	35.49	35.70	36.05	35.37	35.90	35.60	33.32				
1		37.18	36.78	38.47	36.70	38.32	36.46	37.23	37.82	34.81	36.64	36.63	37.07	39.30	37.58	nd	38.00	nd	38.45	41	80	37.82	1.11
		36.25	nd	39.40	37.00	nd	37.46	36.51	38.06	38.12	nd	nd	38.06	39.20	39.63	39.17	37.80	38.50	nd	51			
		nd	37.40	37.92	37.70	nd	37.64	37.48	nd	36.99	39.00	35.84	37.47	39.20	38.95	36.90	39.00	40.00	nd				
NTC		nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd				

Report validation of methods for GMO screening

RESULTS OF COLLABORATIVE STUDY OF EPSPS METHOD

Sample	Expected	Intralab.	Lab 1	Lab 2	Lab 3	Lab 4	Lab 5	Lab 6	Lab 7	Lab 8	Lab 9	Lab 10	Lab 11	Lab 12	Lab 13	Lab 14	Lab 15	Lab 16	Lab 17	Pos/det.	Accordance %	Ct mean	Ct std
C1	Neg	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	34	100		
	Neg	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	34			
C2	Pos	24.30	25.30	25.32	25.50	24.96	24.57	24.44	23.39	21.43	25.65	22.79	25.29	25.20	25.01	25.21	25.20	24.80	21.12	34	100	24.46	1.40
	Pos	24.32	25.25	25.30	26.20	25.08	24.70	24.41	23.45	21.38	25.67	22.72	25.37	25.40	24.97	25.21	25.10	25.10	21.32	34			
C3	Neg	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	34	100		
	Neg	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	34			
C4	Pos	27.84	28.91	28.93	28.90	28.23	27.99	28.09	27.09	25.05	29.09	26.52	28.91	28.90	28.45	28.58	28.90	28.80	25.02	34	100	28.06	1.32
	Pos	27.88	28.95	28.90	29.00	28.64	28.19	27.99	26.94	25.13	29.30	26.39	28.95	29.10	28.42	28.60	29.10	28.80	25.18	34			
C5	Pos	31.78	32.76	32.63	33.40	32.61	32.07	31.95	31.02	29.48	33.00	30.24	32.71	33.00	32.48	32.60	33.30	32.90	29.59	34	100	32.08	1.26
	Pos	32.07	32.89	32.83	33.00	32.66	32.20	32.17	31.01	29.01	33.16	30.10	32.95	32.60	32.33	32.73	33.00	32.90	29.59	34			
NTC	Neg	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd				
C+	Pos	30.58	31.81	31.68	31.10	31.46	30.95	30.38	30.00	28.50	31.75	28.99	31.55	31.50	31.32	31.50	31.60	31.40	28.05			30.80	1.20
Dilution level (copy n.)		Intralab.	Lab 1	Lab 2	Lab 3	Lab 4	Lab 5	Lab 6	Lab 7	Lab 8	Lab 9	Lab 10	Lab 11	Lab 12	Lab 13	Lab 14	Lab 15	Lab 16	Lab 17	Pos/det.	Positive rate %	Ct mean	Ct std
30	33.84	34.28	33.93	33.10	34.27	33.05	34.11	32.86	32.75	33.54	32.07	33.93	34.00	34.73	34.78	34.70	35.20	31.02	51	100	33.56	1.22	
	33.97	34.76	33.91	32.70	33.94	32.65	33.54	32.37	31.19	34.06	32.35	32.97	33.70	34.63	34.60	35.40	35.10	31.06	51				
	33.73	34.68	34.08	32.40	33.28	32.68	35.06	32.27	32.13	33.73	32.53	33.96	33.80	33.97	35.15	35.00	35.30	30.30					
15	35.40	35.12	35.19	33.40	34.64	34.11	34.37	33.40	32.55	34.81	33.14	34.68	35.30	35.66	35.96	36.00	36.60	31.69	51	100	34.64	1.22	
	34.90	35.98	35.26	34.40	35.02	34.22	35.10	35.40	33.60	34.81	33.04	34.70	34.20	34.89	35.62	35.40	36.80	32.27	51				
	36.49	35.86	35.45	33.50	35.77	34.14	35.72	32.93	33.33	35.12	32.81	34.96	34.80	35.56	35.65	35.80	36.20	31.96					
5	36.06	nd	38.93	36.80	35.84	36.81	36.28	36.88	35.85	37.00	35.02	36.47	39.50	36.21	37.52	36.30	39.20	33.45	50	98	36.81	1.62	
	36.32	38.89	37.11	34.60	38.61	36.01	37.57	35.13	36.97	36.96	34.32	38.71	35.90	38.07	38.31	40.60	38.80	33.39	51				
	37.19	37.52	37.52	36.10	36.25	34.84	36.55	36.70	34.83	35.91	36.92	37.86	36.80	37.64	37.38	36.90	39.50	33.40					
1	38.46	38.44	nd	37.30	nd	38.27	37.78	nd	35.54	38.72	nd	38.53	40.70	nd	37.77	nd	nd	35.24	25	49	37.81	1.48	
	nd	nd	nd	36.60	nd	nd	nd	36.62	37.73	38.74	nd	nd	nd	38.95	nd	38.90	nd	36.59	51				
	37.51	38.87	nd	nd	nd	38.11	36.76	nd	36.47	nd	nd	39.04	37.70	nd	nd	nd	40.80	35.04					
NTC	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd				

Report validation of methods for GMO screening

RESULTS OF COLLABORATIVE STUDY OF CTP2-EPSPS METHOD

Sample	Expected	Intralab.	Lab 1	Lab 2	Lab 3	Lab 4	Lab 5	Lab 6	Lab 7	Lab 8	Lab 9	Lab 10	Lab 11	Lab 12	Lab 13	Lab 14	Lab 15	Lab 16	Lab 17	Pos/det.	Accordance %	Ct mean	Ct std
D1	Neg	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	34	100		
	Neg	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	34			
D2	Pos	33.40	34.81	34.93	32.70	33.67	32.18	33.40	32.42	30.39	33.57	31.36	34.95	32.10	34.23	33.94	32.70	32.40	30.31	34	100	32.95	1.41
	Pos	33.21	34.97	34.97	32.90	33.84	32.17	33.20	32.47	30.30	33.58	31.50	34.96	32.00	33.97	33.46	33.10	32.70	30.28	34			
D3	Pos	30.79	32.39	32.54	29.60	31.21	29.69	30.84	29.81	28.22	31.60	28.89	32.28	29.40	31.46	31.21	30.70	29.50	27.84	34	100	30.43	1.40
	Pos	30.57	32.23	32.60	29.70	31.09	29.84	30.96	30.05	27.76	31.29	29.13	32.32	29.70	31.44	30.99	30.90	29.70	27.88	34			
D4	Neg	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	34	100		
	Neg	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	34			
D5	Pos	28.01	29.53	29.40	26.80	28.33	27.09	28.16	26.82	24.47	28.50	25.96	29.43	26.30	28.55	27.93	27.00	26.80	25.28	34	100	27.45	1.47
	Pos	27.99	29.52	29.54	26.80	28.49	27.08	28.22	26.90	24.57	28.62	26.00	29.35	26.60	28.61	27.88	27.10	26.70	24.82	34			
NTC	Neg	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd				
D+	Pos	31.94	33.60	33.42	30.70	32.13	30.81	32.09	31.19	28.77	32.95	30.23	33.03	30.90	32.65	32.11	31.60	30.90	29.44			31.56	1.37
Dilution level (copy n.)		Intralab.	Lab 1	Lab 2	Lab 3	Lab 4	Lab 5	Lab 6	Lab 7	Lab 8	Lab 9	Lab 10	Lab 11	Lab 12	Lab 13	Lab 14	Lab 15	Lab 16	Lab 17	Pos/det.	Positive rate %	Ct mean	Ct std
30	35.00	35.96	36.20	33.10	35.70	32.70	35.63	34.30	31.77	33.94	33.41	37.57	33.20	35.69	35.25	34.40	33.70	33.05	51	100	34.33	1.46	
	35.01	35.63	35.96	32.70	35.95	33.61	34.20	34.04	31.85	34.57	33.50	36.67	32.40	35.83	34.31	34.30	34.00	33.01	51				
	35.78	35.56	36.92	33.00	35.28	32.69	35.24	33.72	31.74	33.83	32.87	36.26	33.40	36.51	34.75	34.20	34.70	32.10					
15	35.98	36.93	38.14	34.40	36.99	33.27	36.87	34.19	32.93	35.05	34.93	37.24	34.20	38.29	36.14	35.80	36.00	33.35	51	100	35.52	1.64	
	36.97	36.74	37.13	33.80	35.82	34.60	36.55	35.17	32.52	35.45	34.41	38.54	34.70	39.32	35.51	34.70	35.60	32.24	51				
	36.85	36.73	37.29	33.90	35.75	33.75	35.89	34.37	32.95	35.93	35.45	37.33	35.30	38.62	35.36	36.10	34.80	34.39					
5	38.46	37.88	39.68	35.10	36.70	36.86	nd	35.37	36.24	37.33	35.47	37.84	37.40	38.83	38.76	36.90	36.70	34.99	49	96	37.34	2.19	
	37.50	38.23	38.38	34.60	38.13	35.30	nd	35.88	35.38	36.49	35.52	41.94	37.20	40.50	37.38	36.80	37.50	34.62	51				
	nd	45.99	38.93	35.20	36.87	35.70	43.02	37.12	36.69	38.03	35.35	38.69	36.10	39.78	36.50	37.80	35.90	35.93					
1	39.09	nd	40.45	36.70	39.38	nd	nd	36.53	nd	nd	nd	nd	nd	nd	38.10	38.30	nd	nd	19	37	38.61	2.13	
	nd	nd	nd	37.00	38.75	nd	38.80	nd	nd	38.53	44.96	nd	nd	nd	39.75	37.40	39.50	36.65	51				
	39.90	nd	41.26	nd	nd	nd	nd	nd	35.30	nd	nd	nd	37.50	nd	nd	38.80	nd	nd					
NTC	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd				

Report validation of methods for GMO screening

RESULTS OF COLLABORATIVE STUDY OF NPTII METHOD

Sample	Expected	Intralab.	Lab 1	Lab 2	Lab 3	Lab 4	Lab 5	Lab 6	Lab 7	Lab 8	Lab 9	Lab 10	Lab 11	Lab 12	Lab 13	Lab 14	Lab 15	Lab 16	Lab 17	Pos/det.	Accordance %	Ct mean	Ct std
E1	Pos	27.82	29.53	29.00	29.50	29.17	27.98	28.18	27.10	25.87	28.85	26.34	29.10	28.80	28.28	28.11	29.60	29.50	25.53	34	100	28.27	1.34
	Pos	28.04	29.27	29.02	29.90	28.92	28.07	28.29	27.24	25.54	28.93	26.50	29.22	29.30	28.33	28.17	29.50	29.50	25.15	34			
E2	Neg	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	34	100		
	Neg	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	34			
E3	Pos	30.28	31.58	31.73	32.00	32.09	30.78	30.42	29.57	28.69	31.63	29.07	31.92	31.70	31.24	30.73	32.20	32.20	27.64	34	100	30.94	1.33
	Pos	30.51	31.51	31.67	32.20	31.88	30.53	30.50	29.61	28.95	31.35	29.20	31.79	32.50	31.03	30.78	32.50	32.50	28.19	34			
E4	Pos	27.59	28.64	28.86	29.10	29.20	27.88	27.86	26.95	25.25	28.79	25.91	29.15	29.10	28.29	27.74	29.30	29.70	25.06	34	100	28.06	1.40
	Pos	27.48	28.83	29.03	29.20	28.96	27.78	27.92	27.08	25.20	28.71	26.09	29.17	29.00	28.38	27.88	29.30	29.50	25.11	34			
E5	Neg	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	33.47	nd	nd	nd	33	97		
	Neg	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	34			
NTC	Neg	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd				
E+	Pos	30.37	31.89	31.69	31.80	32.57	30.42	30.36	29.61	29.04	31.39	28.95	31.91	31.50	30.69	30.60	32.50	32.30	27.00			30.84	1.50
Dilution level (copy n.)		Intralab.	Lab 1	Lab 2	Lab 3	Lab 4	Lab 5	Lab 6	Lab 7	Lab 8	Lab 9	Lab 10	Lab 11	Lab 12	Lab 13	Lab 14	Lab 15	Lab 16	Lab 17	Pos/det.	Positive rate %	Ct mean	Ct std
30		33.84	34.03	33.83	32.90	33.35	32.99	33.51	32.01	32.35	33.87	31.93	33.86	33.90	34.21	33.71	35.60	35.40	30.37	51	100	33.40	1.20
		33.90	33.63	33.72	32.60	33.97	32.82	33.42	32.37	31.97	34.43	31.75	33.81	33.70	34.72	34.06	35.40	35.10	31.05	51			
		34.09	33.95	33.75	33.20	33.93	33.00	32.87	32.15	31.99	34.58	32.10	33.95	34.10	33.82	33.23	35.10	34.80	30.32				
15		34.57	35.24	34.89	34.60	34.48	33.98	34.58	33.40	33.14	36.99	33.07	35.06	35.10	34.63	34.39	36.00	36.00	31.86	51	100	34.42	1.23
		34.34	34.52	35.04	34.30	34.83	33.76	34.43	33.42	33.65	35.07	32.20	34.55	35.10	34.89	34.68	35.90	36.20	31.41	51			
		34.70	34.20	34.23	33.60	35.08	34.14	33.82	32.79	32.92	35.95	32.49	35.05	35.60	35.26	34.09	35.80	36.90	32.37				
5		35.61	36.61	35.21	35.70	36.28	36.45	36.25	35.19	34.60	36.91	33.30	37.03	37.30	37.04	35.57	38.80	38.90	33.24	51	100	36.21	1.46
		36.63	36.31	35.95	35.10	36.88	35.82	35.55	36.71	34.69	37.77	34.22	37.96	36.10	35.99	35.82	37.70	38.50	34.92	51			
		35.57	36.29	36.08	35.10	36.17	35.76	36.51	34.44	34.29	37.45	33.94	36.86	39.20	36.88	37.95	37.10	38.70	33.42				
1		37.43	37.81	39.03	41.10	38.69	38.19	36.90	37.33	37.11	33.30	38.54	37.82	nd	39.10	nd	nd	41.80	35.67	42	82	38.18	1.99
		38.24	39.09	40.91	36.40	39.22	nd	39.26	38.34	38.24	32.94	37.00	nd	38.90	40.30	nd	nd	38.20	36.20	51			
		38.97	39.06	nd	38.20	38.87	39.22	37.75	37.21	36.98	33.59	37.18	38.16	41.50	39.44	38.61	38.40	41.90	nd				
NTC		nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd				

Report validation of methods for GMO screening

RESULTS OF COLLABORATIVE STUDY OF PAT METHOD

Sample	Expected	Intralab.	Lab 1	Lab 2	Lab 3	Lab 4	Lab 5	Lab 6	Lab 7	Lab 8	Lab 9	Lab 10	Lab 11	Lab 12	Lab 13	Lab 14	Lab 15	Lab 16	Lab 17	Pos/det.	Accordance %	Ct mean	Ct std
F1	Pos	30.72	32.22	32.12	30.80	31.61	30.49	30.75	29.91	28.83	31.46	29.13	32.02	30.50	31.36	30.99	31.30	30.60	28.40	34	100	30.73	1.14
	Pos	30.72	32.28	32.10	30.80	31.61	30.67	30.65	29.90	28.35	31.60	29.36	32.13	30.40	31.35	30.72	31.50	30.70	28.38	34			
F2	Pos	30.92	32.67	32.50	31.10	32.35	31.48	31.18	30.42	28.73	32.26	29.87	32.82	31.10	31.96	31.71	32.00	31.40	29.37	34	100	31.30	1.17
	Pos	31.14	32.91	32.37	31.10	32.16	31.21	31.49	30.44	28.91	31.88	29.67	32.79	31.10	32.00	31.43	31.90	31.10	28.87	34			
F3	Neg	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	34	100		
	Neg	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	34			
F4	Neg	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	34	100		
	Neg	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	34			
F5	Pos	33.18	34.64	34.61	33.50	34.02	33.67	32.71	32.18	31.40	34.48	32.31	34.90	32.90	34.39	33.41	35.00	33.90	31.54	34	100	33.43	1.16
	Pos	33.49	34.66	34.54	33.40	34.06	33.74	33.14	32.57	30.76	34.02	32.10	34.73	32.80	33.70	33.57	34.80	33.40	31.13	34			
NTC	Neg	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd				
F+	Pos	30.32	31.97	31.90	30.80	31.56	30.16	30.36	29.81	28.82	31.36	28.95	31.70	30.40	30.96	30.55	30.70	30.60	28.16			30.52	1.09
Dilution level (copy n.)		Intralab.	Lab 1	Lab 2	Lab 3	Lab 4	Lab 5	Lab 6	Lab 7	Lab 8	Lab 9	Lab 10	Lab 11	Lab 12	Lab 13	Lab 14	Lab 15	Lab 16	Lab 17	Pos/det.	Positive rate %	Ct mean	Ct std
30	34.08	32.42	34.51	31.50	34.04	32.84	33.41	32.25	32.49	32.85	31.78	34.33	32.90	33.83	33.36	34.30	33.40	31.49	51	100	32.96	1.04	
	33.94	32.25	34.18	32.40	34.11	32.71	33.07	32.57	31.42	33.25	31.97	34.74	32.20	33.87	33.50	34.30	33.50	30.74	51				
	33.54	31.88	34.07	31.80	33.99	33.05	33.10	31.89	31.93	32.77	31.58	34.28	32.40	33.91	33.59	33.50	34.00	30.51					
15	34.34	33.70	35.47	33.00	34.65	33.39	34.28	32.66	33.49	34.52	32.49	35.16	33.80	34.41	34.69	34.90	34.60	32.94	51	100	34.06	0.93	
	34.95	33.29	35.18	32.70	34.77	33.77	34.12	33.75	32.83	33.82	33.24	35.63	34.20	34.89	34.80	35.50	34.40	32.67	51				
	35.60	32.46	34.92	33.20	35.59	34.09	33.95	33.78	32.91	33.97	33.16	35.77	34.10	34.37	34.53	35.60	34.10	32.82					
5	35.61	35.13	37.59	34.70	36.57	34.84	35.97	33.91	35.47	35.20	34.06	38.19	34.20	38.69	36.53	36.70	37.10	33.79	51	100	35.80	1.37	
	37.20	39.80	37.40	33.90	36.27	36.27	35.42	35.21	34.34	34.89	34.35	36.75	35.40	37.14	36.21	37.10	35.70	34.11	51				
	36.53	34.96	36.47	34.20	36.79	34.31	37.71	36.65	34.90	35.51	35.50	36.97	34.70	36.36	35.33	35.80	37.10	33.74					
1	nd	36.83	nd	36.70	38.44	nd	37.01	nd	36.22	nd	nd	nd	38.00	38.08	nd	37.40	nd	35.83	26	51	37.39	1.16	
	40.64	nd	37.94	nd	38.75	nd	nd	nd	nd	nd	36.15	nd	nd	38.99	36.54	39.40	39.40	nd	51				
	36.67	36.29	nd	35.60	nd	nd	37.97	36.65	37.91	36.86	nd	nd	36.70	nd	37.03	nd	39.40	36.10					
NTC	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd				