

7th Workshop of the Italian Network of the GM food and feed testing
laboratories for the official control

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Pilot application of pre-spotted plates for GM detection and future perspective

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DISCLAIMER: The contents of this presentation are the views of the authors and do not necessarily represent an official position of the European Commission.



Mandatory labelling if
GM event $> 0.9\%$



- Screen
- Identify
- Quantify

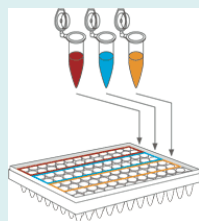


Solution:

Multi-target & ready-to-use
tool: the pre-spotted plate
(PSP)

Pre-Spotted Plates (PSP) for GMO detection

PSP



RTi-PCR plastic support whose wells are spotted with dried primers & probes targeting chosen DNA sequences (GMO detection assays)

Advantage



Perform up to 96 assays in a single experiment, with limited steps

Pre-spotted plates pilot project

Objective:

To compare the use of the PSPs to the use of current control procedure while generating data to assess:

1. Reliability and performance of the PSPs
2. Costs and benefits
3. Practical advantages and disadvantages
4. Need for future systematic production

Multi-target ready-to-use PCR plates



NEW Screening-PSP

	1	2	3	4	5	6	7	8	9	10	11	12
A	HMG	p35s	HMG	p35s	HMG	p35s	HMG	p35s	HMG	p35s	HMG	p35s
B	Lec	tNOS	Lec	tNOS	Lec	tNOS	Lec	tNOS	Lec	tNOS	Lec	tNOS
C	CruA	CTP2-EPSPS	CruA	CTP2-EPSPS	CruA	CTP2-EPSPS	CruA	CTP2-EPSPS	CruA	CTP2-EPSPS	CruA	CTP2-EPSPS
D	Sah7	PAT	Sah7	PAT	Sah7	PAT	Sah7	PAT	Sah7	PAT	Sah7	PAT
E	UGP	BAR	UGP	BAR	UGP	BAR	UGP	BAR	UGP	BAR	UGP	BAR
F	PLD	Cry1Ab	PLD	Cry1Ab	PLD	Cry1Ab	PLD	Cry1Ab	PLD	Cry1Ab	PLD	Cry1Ab
G	GS	CV127	GS	CV127	GS	CV127	GS	CV127	GS	CV127	GS	CV127
H	DAS-40278	DP-305423	DAS-40278	DP-305423	DAS-40278	DP-305423	DAS-40278	DP-305423	DAS-40278	DP-305423	DAS-40278	DP-305423
	Replicate 1		Replicate 2		Replicate 1		Replicate 2		Positive		Negative	
	Sample 1				Sample 2				Controls			



Event-sp. PSP

	1	2	3	4	5	6	7	8	9	10	11	12
A	HMG	E3272	E98140	BT11	BT176	DAS 40278	DAS 59122	GA21	MIR162	MIR604	MON810	MON863
B	MON 87460	MON 88017	MON 89034	NK603	T25	TC1507	LEC	A2704	A5547	CV127	DP 305423	DP 356043
C	FG72	GTS 40-3-2	MON 87701	MON 89788	CruA	GT73	MS1	MS8	RF1	RF2	RF3	T45
D	Topas 19/2	Sah7	E281	E3006	GHB119	GHB614	LL Cotton25	MON 1445	MON 15985	MON531	MON 88913	T304
E	HMG	E3272	E98140	BT11	BT176	DAS 40278	DAS 59122	GA21	MIR162	MIR604	MON810	MON863
F	MON 87460	MON 88017	MON 89034	NK603	T25	TC1507	LEC	A2704	A5547	CV127	DP 305423	DP 356043
G	FG72	GTS 40-3-2	MON 87701	MON 89788	CruA	GT73	MS1	MS8	RF1	RF2	RF3	T45
H	Topas 19/2	Sah7	E281	E3006	GHB119	GHB614	LL Cotton25	MON 1445	MON 15985	MON531	MON 88913	T304
	Sample 1				Sample 2							

All GM events from 4 species listed in EU register
Maize, Soybean, Oilseed rape and Cotton

- 6 Element-sp. Methods
- 7 Taxon-sp. Methods
- 3 Event-sp. Methods

Development of a GMO Screening System

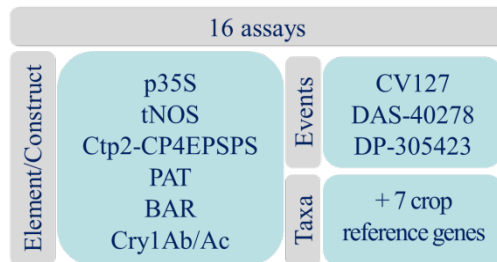
I. Which assays?

III. Building a screening strategy: combine PSP with a Decision Support System

JRC GMO-Matrix³



GMOMETHODS
database⁴



II. Assay standardization for use on PSP (and re-assessment of method performance)

Sample 1 rep 1	Sample 1 rep 2	Sample 2 rep 1	Sample 2 rep 2	Positive Control	Negative Control
hmg	p35s	hmg	p35s	hmg	p35s
lec	nOS	lec	nOS	lec	nOS
CruA	CTP2-CP4EPSPS	CruA	CTP2-CP4EPSPS	CruA	CTP2-CP4EPSPS
sah7	pat	sah7	pat	sah7	pat
ugp	bar	ugp	bar	ugp	bar
pld	Cry1Ab/Ac	pld	Cry1Ab/Ac	pld	Cry1Ab/Ac
gs	CV 127	gs	CV 127	gs	CV 127
DAS 40278	DP 305423	DAS 40278	DP 305423	DAS 40278	DP 305423

+

JRC GMO-Matrix³

Web!

The following events match the selected pattern:

	CaMV P-35S	T-nos	CTP2-CP4 EPSPS	pat	bar	cry1Ab/Ac	DAS-40278-9	CV127	DP-305423-1
GMO Event NK603 Maize (MON-00603-6)	2	2	2	0	0	0	0	0	0
GMO Event MON88017 Maize (MON-88017-3)	2	2	2	0	0	0	0	0	0

The following events can also be present:

	CaMV P-35S	T-nos	CTP2-CP4 EPSPS	pat	bar	cry1Ab/Ac	DAS-40278-9	CV127	DP-305423-1
GMO Event 98140 Maize (DP-098140-6)	2	0	0	0	0	0	0	0	0
GMO Event GA21 Maize (MON-00021-9)	0	2	0	0	0	0	0	0	0

Best
identification
strategy!

Pilot project layout

Confront lab internal procedure against PSP in terms of reliability, time, costs and practicability

Analysis of 7 Known GM positive

- Composite samples
- More than 1 GM event detected



Sc-PSP + Eve-PSP



Analysis of 2 Known GM negative

- Composite samples



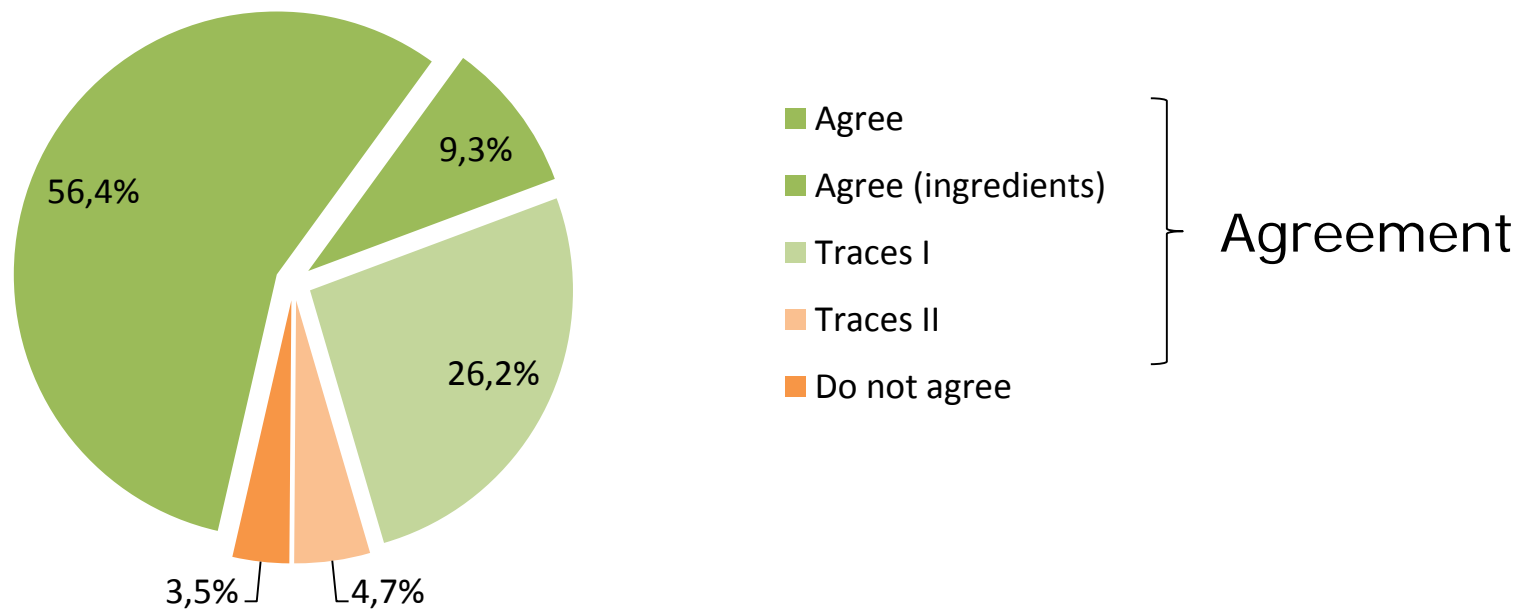
Sc-PSP



Reporting of results

PSPs perform well and are functional (1)

Concordance of results between screening and identification steps



PSPs perform well and are functional (2)

Pilot Study (20 EU NRLs, 135 real-life samples) - workflow



The following events match the selected pattern:

	CaMV P-35S	nos	CP2-CP4 EPSPS	pat	bar	cry1Ab/Ac	DAS-40278-9	CV127	DP-305423-1
Core (Zea mays)	2	2	2	0	0	0	0	0	0
GMO Event N6503 Maize (MON 00603-6)	2	2	2	0	0	0	0	0	0
GMO Event MON810 Maize (MON 810-1)	2	2	2	0	0	0	0	0	0

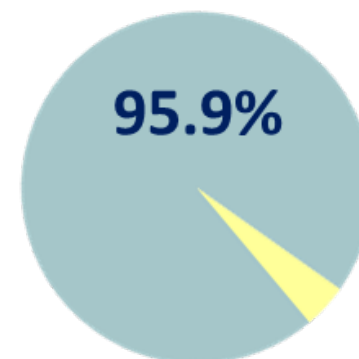
The following events can also be present:

	CaMV P-35S	nos	CP2-CP4 EPSPS	pat	bar	cry1Ab/Ac	DAS-40278-9	CV127	DP-305423-1
Core (Zea mays)	2	0	0	0	0	0	0	0	0
GMO Event N6503 Maize (MON 00603-6)	0	2	0	0	0	0	0	0	0

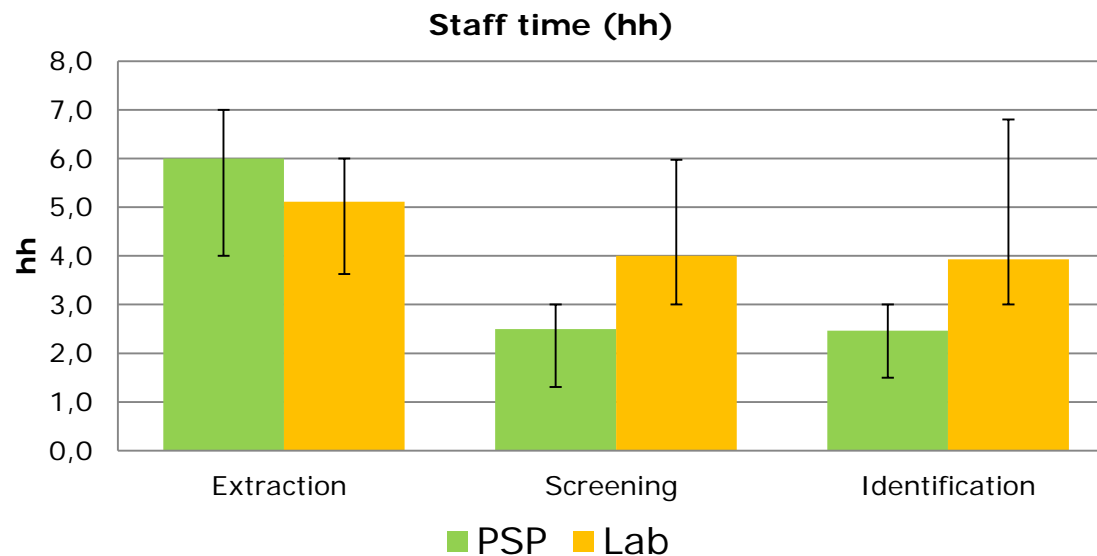


	Hmg	Lec	CruA	Sah7	p35s	tNOS	Ctp2	PAT	BAR	CryI
Sensitivity (%)	97.9	99.6	98.4	82.4	100	98.8	96.6	98.1	87.5	96.9
Specificity (%)	88.2	89.5	92.3	99.6	57.1	88.9	85.7	87.7	96.1	92.7
Concordance (%)	96.7	98.1	95.2	98.5	98.9	97.8	93.0	91.9	95.6	93.7

Performance of the strategy
(PSP + JRC GMO-Matrix)

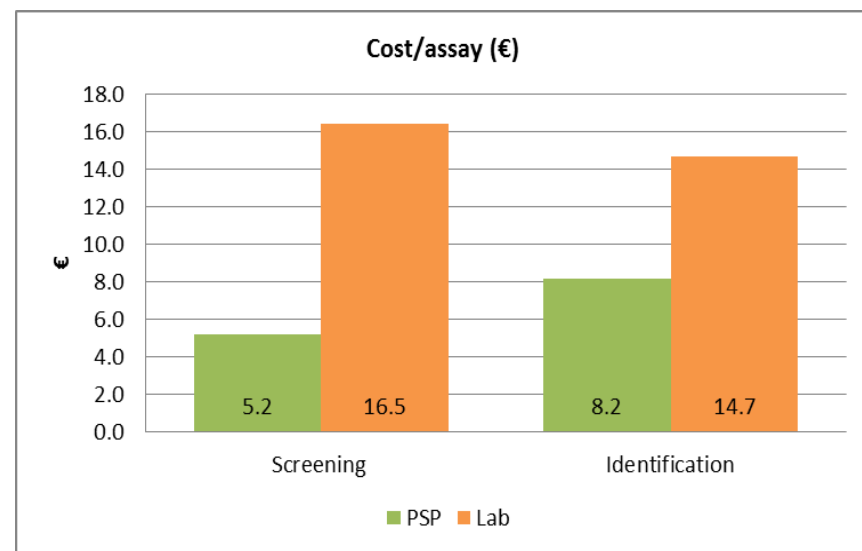
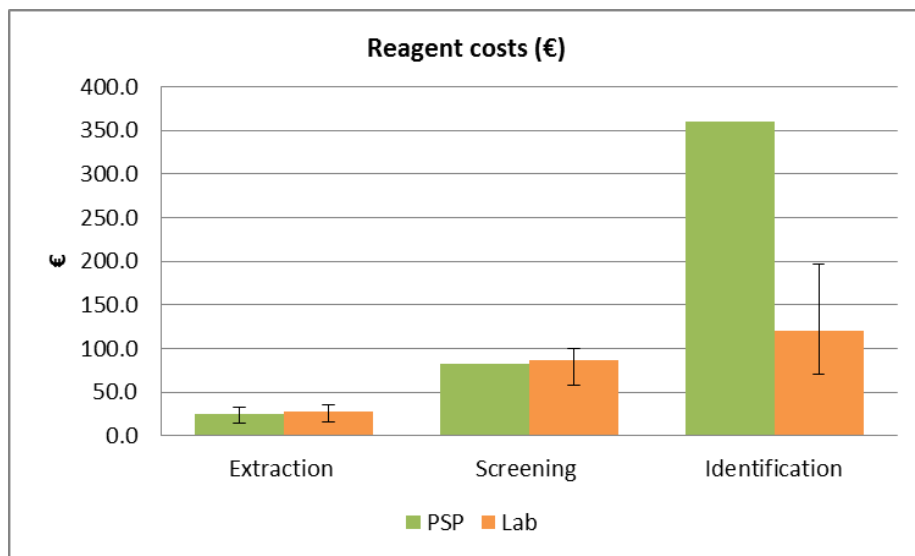


PSP workflow allows a major reduction of staff time



	Extraction		Screening		Identification		Total		Overall time saved
	PSP	Lab	PSP	Lab	PSP	Lab	PSP	Lab	
Median	6.0	5.1	2.5	4.0	2.5	3.9	11.0	13.0	-15.4%
2 nd quartile	4.0	3.6	1.3	3.0	1.5	3.0	6.8	9.6	-29.2%
3 rd quartile	7.0	6.0	3.0	6.0	3.0	6.8	13.0	18.8	-30.9%

Cost benefits when using the PSPs



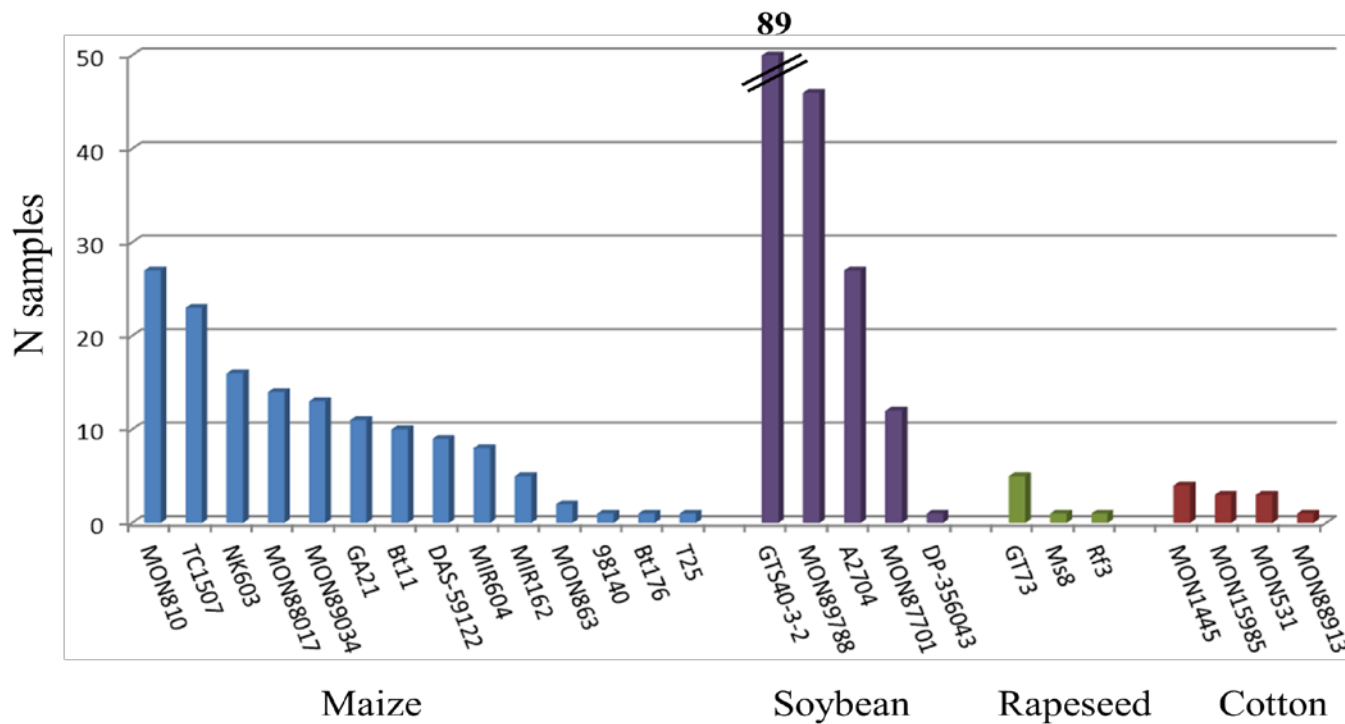
	Extraction Costs (€)		Screening				Identification			
	PSP	Lab	n. tests		Costs (€)		n. tests		Costs (€)	
	PSP	Lab	PSP	Lab	PSP	Lab	PSP	Lab	PSP	Lab
Median	25.0	28.0	16	5.2	83.0	86.0	44	8.1	360.0	119.8
2nd quartile	14.5	15.9	-	4.1	-	57.5	-	4.6	-	70.8
3rd quartile	33.0	34.6	-	7.0	-	100.0	-	12.5	-	197.1

PSPs received a positive evaluation



Better			Comparable			Worse		
9	8	7	6	5	4	3	2	1

2014 GM events profile



Ideas and Perspectives

Technical suggestions already addressed:

- crop-specific ePSP were developed for maize and soy, including the newly approved events (specificity and sensitivity of methods reassessed);
- ePSP reaction volume was scaled down from 50 to 25ul (specificity and sensitivity of methods reassessed);

II. Demonstration project

Overall objective:

The pre-spotted **screening** plate will be introduced into **routine** GMO-control via a demonstration project. To this end about 800 plates will be offered to laboratories that are willing to use them as a routine control approach and to report back to the EURL.

What do you think is missing for the adoption of the PSP?

Aim

1. Survey official lab needs, market analysis (Reg. EC 1829/2003)
2. **plastic supports:** Open the trial to no-ABI instruments
3. 5-10 plates/lab (10-20 samples), no master mix, no control samples.
Are more plates needed to get acquainted before adoption in routine work?
4. Are more data needed to confirm PSP performance?

The survey is coming soon!

Ideas and Perspectives - 2

Missing link: insertion of PSPs in quality system

JRC Scientific and Technical Reports



Verification of analytical methods for GMO testing when implementing interlaboratory validated methods

Guidance document from the European Network of GMO laboratories (ENGL)

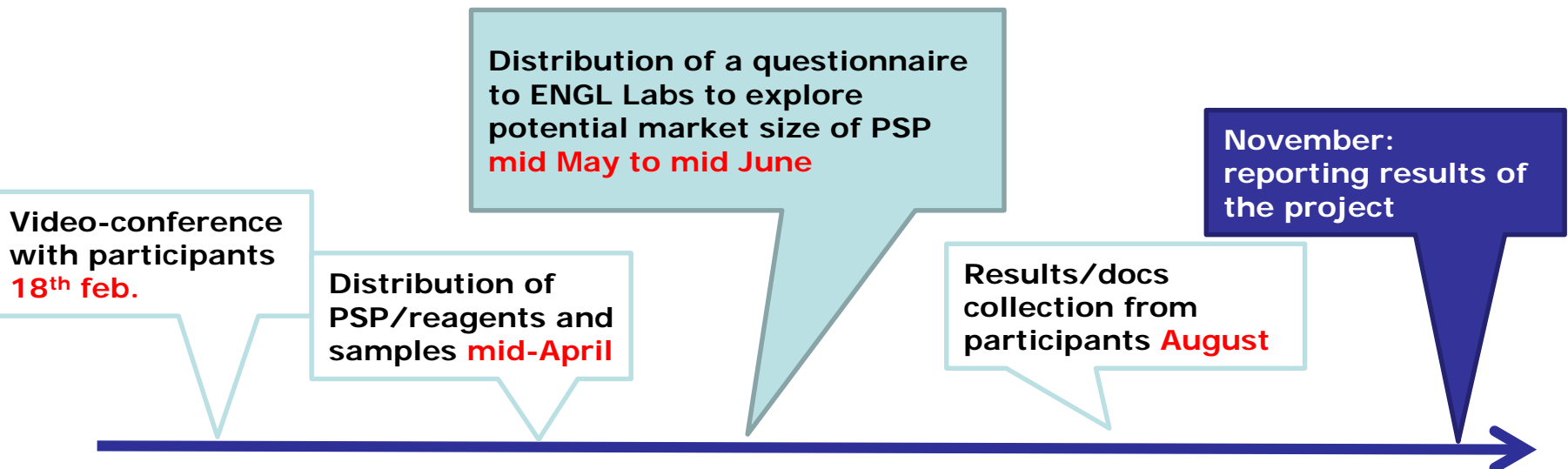
Prepared by the ENGL working group on “Method Verification”



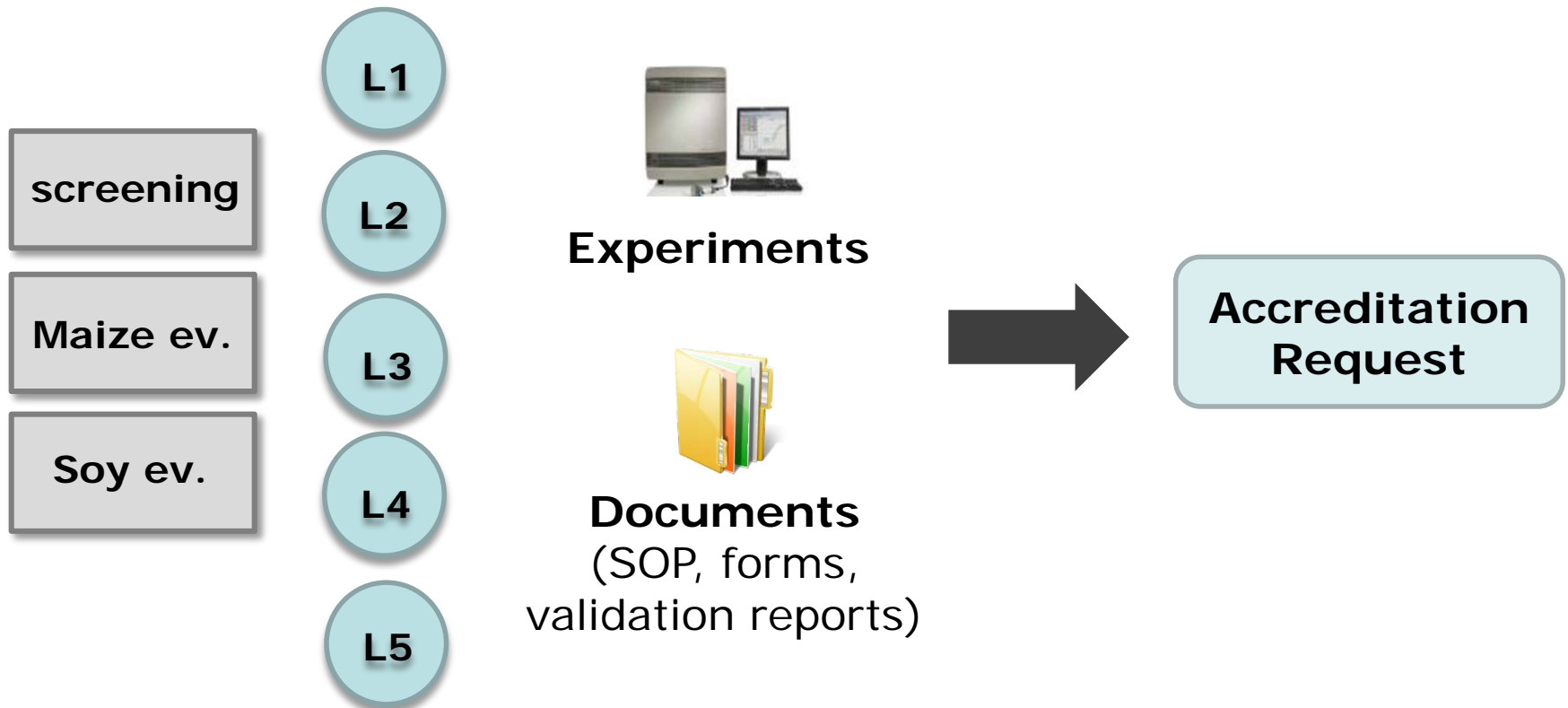
III. Feasibility study: Ready-to-use PCR plates for detection of Genetically Modified Organisms

Awarded and funded by the JRC Work Program "**Intellectual Property and Technology Transfer**" – 18 months period

Objective: Integration of PSPs in official testing laboratories for use in routine analyses.



Feasibility study proposed project layout



IV. Semi-quantitative use of ePSP

Eur Food Res Technol
DOI 10.1007/s00217-011-1615-5

ORIGINAL PAPER

Applicability of the “Real-Time PCR-Based Ready-to-Use Multi-Target Analytical System for GMO Detection” in processed maize matrices

Linda Kluga · Silvia Folloni · Marc Van den Bulcke ·
Guy Van den Eede · Maddalena Querci

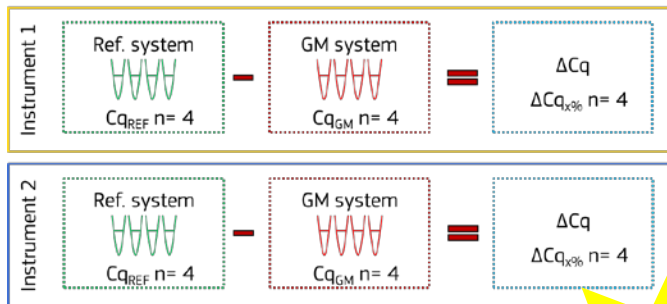
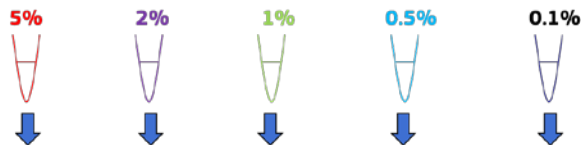
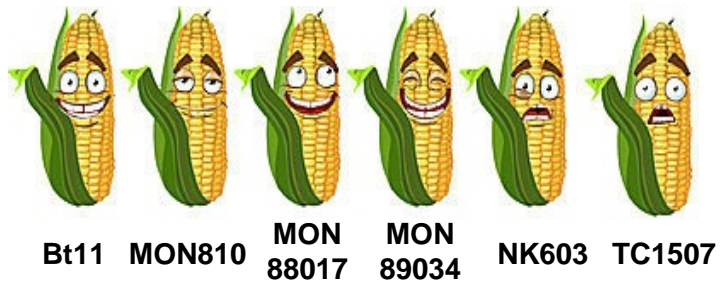
Table 5 Theoretic correlation between the ΔC_t , the dilution factor and the GM%

ΔC_t	Dilution	GM%
1	2	50.00
2	4	25.00
3	8	12.50
4	16	6.25
5	32	3.13
6	64	1.56
7	128	0.78
8	256	0.39
9	512	0.20
10	1024	0.10
11	2048	0.05
12	4096	0.02
13	8192	0.01
14	16384	0.01

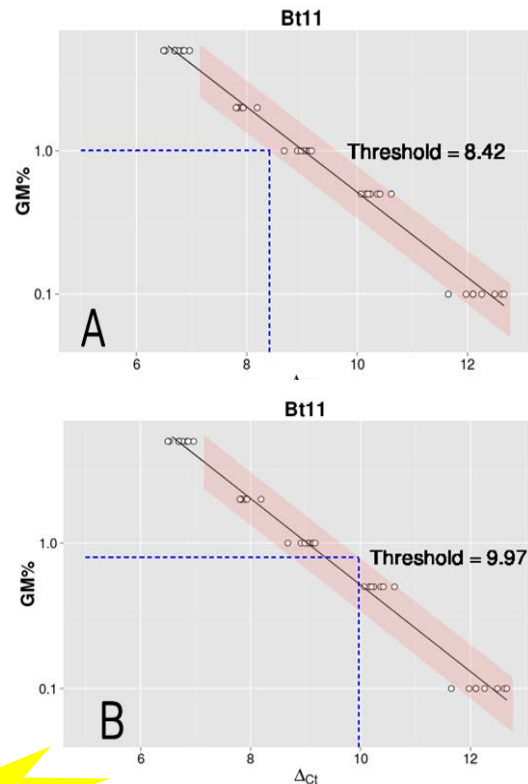


$$\Delta Cq = Cq_{GM} - Cq_{Txn} \approx \%GM$$

Estimation of ΔCq Thresholds



**40
 ΔCq**

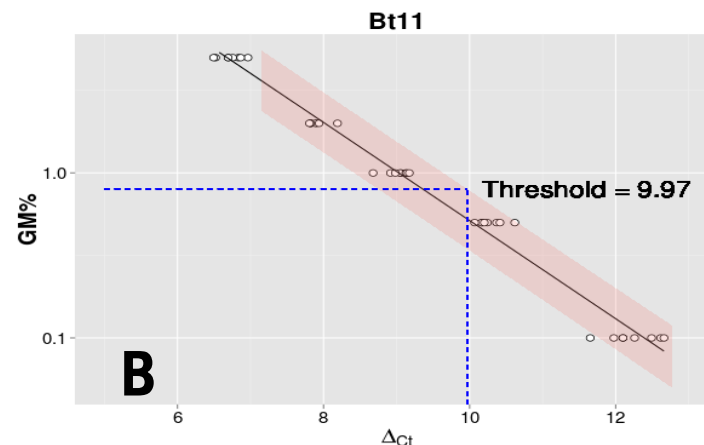
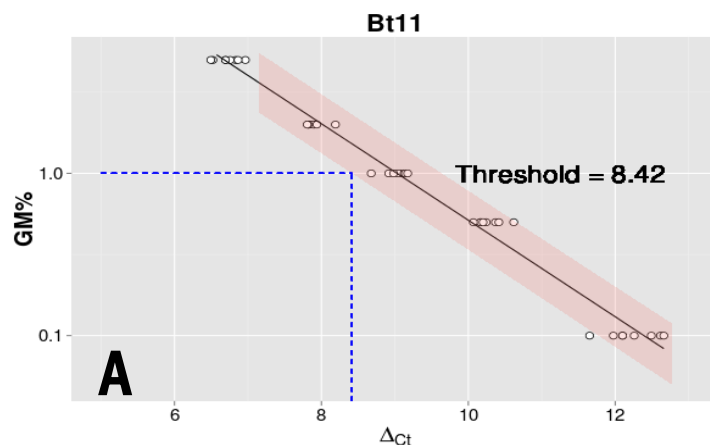


Above

?

Below

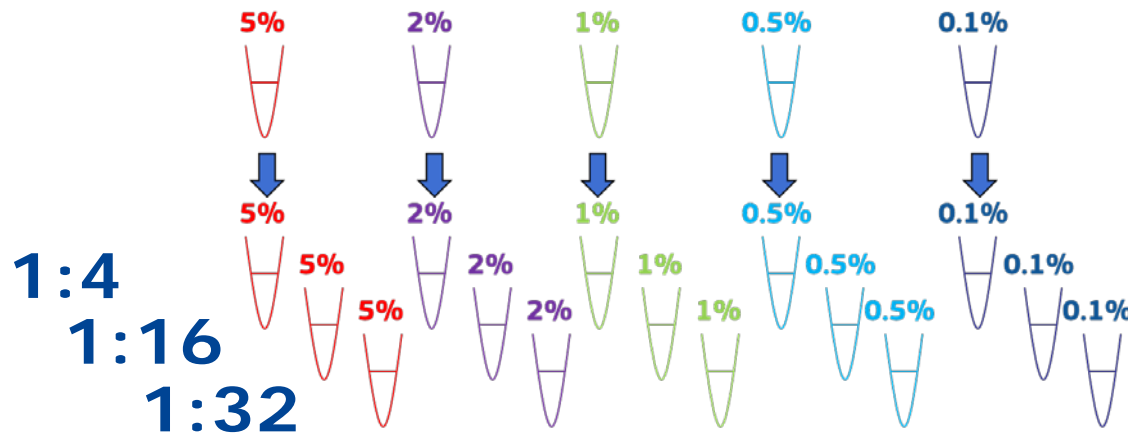
Estimation of ΔCq Thresholds



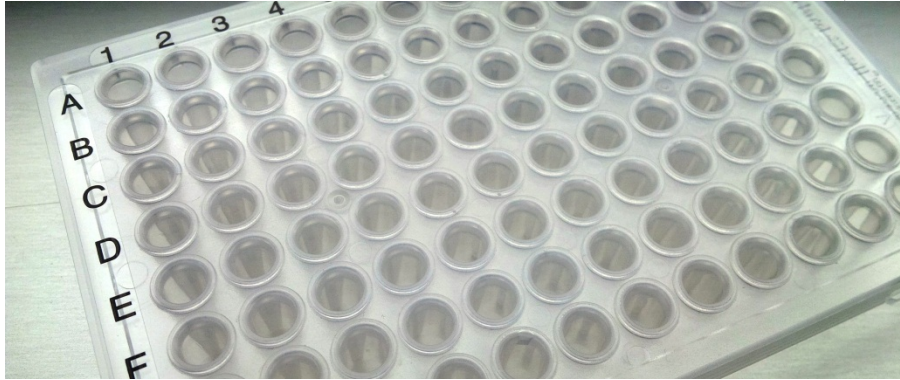
n. GM events	GM level	MON810		Bt11		MON88017		MON89034		NK603		TC1507	
		Op.1	Op.2	Op.1	Op.2	Op.1	Op.2	Op.1	Op.2	Op.1	Op.2	Op.1	Op.2
1	1.0%	7.56	7.74	8.42	7.62	6.61	6.79	7.91	7.63	8.36	8.22	6.66	6.90
	0.8%	9.11	9.34	9.97	9.92	8.36	7.78	9.44	9.24	9.73	10.38	8.35	8.35
2	0.5%	8.55	8.81	9.43	8.79	7.65	7.79	9.01	8.71	9.34	9.28	7.67	7.93
	0.4%	10.10	10.42	10.98	11.10	9.40	8.78	10.54	10.33	10.71	11.44	9.36	9.38

Assessment of ΔC_q Thresholds

Simulated complex-matrix samples



	Operator 1			Operator 2		
GM%	<0.9%	Quant.	>0.9%	<0.9%	Quant.	>0.9%
0.1	99.1	0.9	0.0	97.3	2.7	0.0
0.5	58.2	41.1	0.7	55.3	44.7	0.0
1	10.5	83.2	6.3	11.2	83.2	5.6
2	0.0	17.5	82.5	0.0	25.9	74.1
5	0.0	0.7	99.3	0.0	0.0	100.0



Thank you for your attention!

JRC-EURL-GMFF-PSPP
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<http://europa.eu/expo2015/sylvia-s-lab>

