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A Ready-To-Use Multi-Target Analytical System for GM Soy and Maize Detection for Enforcement Laboratories

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1. Introduction

Today GMO analysis has become an integral part in the development of new genetically modified plants, in subsequent breeding, seed production and verification programmes. GMO analysis is a key technology in export and import of agricultural commodity products, for ascertaining regulatory compliance of GMOs in different countries, for labelling requirements, and for product authenticity and traceability.

In Europe, in particular, GMO analysis is implemented in all Member States to fulfil legal requirements regarding GMOs and GMO-derived products, their release into the environment, cultivation, importation and their utilisation as food, food ingredients and animal feed (European Commission 2001, 2003a, 2003b). A key technical element for the authorisation of GMOs within the EU is the provision of an event-specific quantitative detection method - validated according to internationally accepted standards - to allow the control and monitoring of a particular GMO along the production, processing and the distribution chain.

In this context the European Commission Joint Research Centre (JRC) has the mission to provide scientific and technical support to EU policy development on GMO. The nominated European Union Reference Laboratory for GM Food and Feed (EURL-GMFF) at the JRC is working at the forefront of GMO analysis in Europe. Legally responsible for method validation under Regulation (EC) No 1829/2003 (European Commission 2003a), the EURL-GMFF works in close collaboration with the European Network of GMO Laboratories not only in method validation but also in the harmonisation of a number of topics spanning from sampling to method development, data analysis and measurement of uncertainty. In addition, in Europe, the EURL-GMFF plays a key role in the official food controls according to Regulation (EC) No 882/2004 (European Commission 2004a).

Still, the multi-factorial nature of GMO analysis, the diverse and often complex composition of the samples under examination, the increasing number of GM events and, finally, the

growing need for recognition of mutual analysis and data interpretation require further harmonisation and standardisation at the international level.

Testing needs in Europe, in particular, are constantly growing due to the steady rise of the number of GM events commercialised in various parts of the world (James, 2008). According to the latest ISAAA report (James 2008), 144 GM events, representing 24 crops, have so far received worldwide regulatory approval, and this number is still meant to rise (Stein and Rodríguez-Cerezo 2009).

Different analytical approaches have been developed for GMO identification and quantification: among all alternatives tested, real-time PCR (RTi-PCR) proved to be the most successful, accurate and powerful technique and accordingly, it is now the method of choice for GMO quantification (Miraglia et al. 2004). Due to its intrinsic specificity and to the fact that results are directly extrapolated from the instrument software, avoiding any post-PCR manipulations, RTi-PCR is also increasingly used for qualitative analysis of particular GMOs (Reiting et al. 2007).

The constant increase of different GMO invoked the necessity to screen for GMO presence in a way that post-screening analysis can be limited (James 2008). For this the matrix-approach has been considered most promising and a number of RTi-PCR platforms have been developed and some being used already on real-life samples. Here, we introduce two of these matrix-based approaches in more detail and discuss the possibility of incorporating them in a pre-spotted plate approach.

Finally, harmonisation of GMO analysis will largely depend on the availability of a common decision support system (DSS) to all stakeholders. Here, we will present some of our thoughts on key elements that should be present in such a DSS and discuss how the pre-spotted plate technology could sustain such approach.

2. A ready-to-use multi-target analytical system for GMO detection

Research conducted over the past years in the area of method development, optimisation and validation has provided a wide range of analytical tools already integrated on a routine basis in the GMO monitoring and verification programmes and for the above mentioned steps and applications. However, the conditions of post-marketing monitoring might be very complex.

The European market is not a closed system; as GMOs and derived products originating from non EU countries enter the Union, it can not be excluded that also unknown or GMOs unauthorised in the EU may be introduced and may be present on the EU market for a while before being detected, such as in the cases of Bt10 maize (Commission Decision 2005/317/EC; European Commission 2005a) and LLRICE 601 (Commission Decision 2006/578/EC; European Commission 2006). So far approximately 25 GM events were authorised for food and/or feed use in Europe (http://ec.europa.eu/food/dyna/gm_register/index_en.cfm) while the number of GM events commercialised in other parts of the world is much higher and it is constantly rising (James 2008). This asynchronous approval has resulted in a considerable increase in testing needs to identify authorised and unauthorised GMOs in food and feed samples. All these elements converge, from the practical point of view, on the need of high-throughput systems allowing the rapid and cheap screening of numerous samples for the monitoring and tracing of GMOs in the agricultural food and feed chain. In this context and in collaboration with the European Network of GMO Laboratories (ENGL;

<http://engl.jrc.ec.europa.eu/>) the European Commission JRC has deployed, tested and implemented a high-throughput detection system for the detection of GMOs (Querci et al. 2009) and has designed and explored test strategies that are the basis of a decision-making process to detect GMOs and to distinguish between approved and unapproved GMOs.

A first step was the development of an easy-to-use system for the detection of approved and unapproved GM events and derived food and feed products. The approach represents only one potential analytical alternative and is aimed at developing and providing a fast and handily ready-to-use multi-target system for the detection of (as many as possible) GM events approved and unapproved on the European market in a single experiment.

The selected strategy was formulated and based on a series of considerations summarized below:

1. EURL-GMFF experience and reliability of the data

Over the past years the JRC, through the activities conducted by the Molecular Biology and Genomics (MBG) Unit at the Institute for Health and Consumer Protection (IHCP), has developed a broad expertise in the different analytical aspects involved in qualitative and quantitative GMO analysis. Their recognised leading role in developing, optimising and validating analytical tests for the detection, identification and quantification of GMOs led to the establishment, within the MBG Unit, of the European Union Reference Laboratory for GM Food and Feed (EURL-GMFF) in the context of Regulation (EC) No 1829/2003.

Principal legal duties and tasks of the EURL-GMFF, as defined in Regulation (EC) No 1829/2003, are 1) testing and validation of detection methods for identification of the transformation event in the food or feed and 2) preparation, storage and distribution to national reference laboratories of the appropriate positive and negative control samples.

Detailed rules for the implementation of Regulation (EC) No 1829/2003, and in particular requisites to be followed by applicants when submitting a method of detection to the EURL-GMFF, as specified in Annex I of Regulation (EC) No 641/2004 (European Commission 2004b), include information about the method as such and about the method testing carried out by the applicant and demonstration that the method fulfils, among others, the following requirements:

being event-specific and by definition only recognizing one particular GMO or products derived thereof (and not recognizing any other GMO);

being applicable to food and feed samples and any control samples or reference materials.

Whereas the wide diffusion and adoption of the real-time PCR approach relies on its reliability for DNA quantification, the technique is also more and more frequently used for end-point analysis, for qualitative detection purposes, thanks to its increased intrinsic specificity and to the fact that it allows straight extrapolation of results directly from the instrument software avoiding analysis of PCR products by gel electrophoresis, a step that represents the main risk in terms of laboratory contamination.

2. Incorporation of EURL-validated event-specific methods into the detection platform

The approach followed for the development of the “Ready-To-Use Multi-Target Analytical System for GMO Detection” (Querci et al. 2009) is based on the detection of the different GM events by using event-specific methods. At the time of the start of the project, the EURL-GMFF - as an integral part of the EU approval process - had received for validation 39 dossiers containing molecular data and event-specific methods for the detection of the corresponding individual GM events (without considering 21 dossiers provided for the validation of methods for stacked GM lines) in 7 plant species.

From the methodological point of view, the approach is based on real-time PCR. Indeed, real-time PCR, in addition to the intrinsic specificity mentioned above, has the advantage of being a technique already commonly used in the EU and worldwide and adopted by most if not all (EU) GMO control laboratories. Choosing PCR as a technology guarantees the opportunity of immediate use and integration in the laboratories' working routine, avoiding the acquisition of new instrumentation or the implementation of new procedures and the need for technical formation.

3. A 96-well plate format for easy laboratory implementation

The selected format is in line with the aim to provide a rapid multi-target system (allowing the simultaneous detection of all targets in a single experiment) in a ready-to-use format, therefore reducing to the minimum the laboratory handling steps. The “Ready-To-Use Multi-Target Analytical System for GMO Detection” has been designed to be delivered in the format of pre-spotted plates containing, in lyophilized format, all primers and probes for the individual detection of all 39 single-insert GM events for which a method was submitted to the EURL-GMFF, and of the corresponding 7 plants species (maize, cotton, rice, oilseed rape, soybean, sugar beet and potato). As shown in Figure 1, to use system the operator just needs to perform few simple steps: extract the DNA from the sample, mix it with the TaqMan® Universal PCR Master Mix (Applied Biosystems), load the mixture on the plate, and start the time temperature programme. Results are then extrapolated directly from the ad-hoc software.

Description of the system and short overview of the performance

Methods incorporated in the system include: event-specific methods for maize Bt11, NK603, GA21 (2 methods), MON863, 1507, T25, 59122, MON810, MIR604, Bt176, MON88017, LY038, 3272, MON89034, Bt10; oilseed rape T45, Ms8, Rf3, GT73, Rf1, Rf2, Ms1, Topas 19/2; cotton MON1445, MON88913, LLCotton25, MON 531, MON15985, 281-24-236 X 3006-210-23; soybean A2704-12, 40-3-2, MON89788, DP-356043; rice LLRICE62, LLRice601, Bt63; sugar beet H7-1; potato EH92-527-1 and a rice P35S::bar specific method; plus target taxon specific methods for the corresponding plant species. As detection of stacked GM lines is based on the use of event-specific methods developed for the parental GM events composing the stack, this system allows the detection of all stacks derived from the 39 single-insert GM events listed above.

Specificity of each of the 48 methods was assessed and confirmed by testing each wild-type plant species and each GM event, individually, against the whole set of methods. Sensitivity of the system was tested by individually loading, in each well, the corresponding wt DNA or GM at different concentrations. Sensitivity of all methods was confirmed to be at least 0.045% expressed as haploid genome equivalents in 100 ng DNA, in line with method specificities and in compliance with EU requirements for method LOD.

For transferability testing, pre-spotted plates were distributed to 31 EU control laboratories together with the blended DNA solution and a negative sample consisting of a 20 ng/μL herring sperm DNA solution. Data returned by all laboratories running the system on different platforms (7900HT Real-Time PCR System, 7300/7500 Real-Time PCR Systems, ABI PRISM® 7000/7700 SDS [Applied Biosystems], iCycler iQ Real-Time PCR Detection System [Bio-Rad, Hercules, CA]) showed high levels of reproducibility (EURL-GMFF, unpublished results). Experimental data indicate that this system is adequate for detecting several GM events in a single experiment at 0.045% (expressed in haploid genome copies), thus in full compliance with EU requirements for method performance.

Fig. 1. Four-step workflow for GMO analysis using the ready-to-use multi-target analytical system. 1) DNA is extracted from the sample to be analysed, 2) extracted DNA is added to the PCR master mix and the reaction mix is loaded on the plate, 3) RTi-PCR amplification, and 4) visualisation of the results using the ad-hoc instrument software.

3. Development of a RTi-PCR Ready-to use GM soy and maize detection tool

Considering the importance of GM soy and maize within the global seed commodity market, it was considered that such pre-spotted plates represent a flexible approach *per se* and can be an excellent starting point for a whole new set of applications. Indeed, applying the same principle, a crop-specific formulation was developed for the simultaneous detection and identification of 21 single-insert soybean and maize GM events and a number of stacked events derived from them (Figure 2). This formulation includes the event-specific methods also for 98140 maize, DP-356043 and DP-305423 soybean events, methods submitted for validation to the EURL-GMFF after the 48-method plates described above were formulated. As shown in Figure 2, the layout allows the analysis of 4 samples on a single plate. Also in this case system performance (specificity, efficiency, LOD, etc.) has been successfully confirmed by experimental testing (L. Kluga et al., in preparation).

Fig. 2. a) List of maize and soybean events detected by the soybean/maize ready-to-use multi-target analytical system and b) Overall plate design wherein the all set of test methods is spotted in quadruple.

This novel plate composition has been successfully applied on highly processed fractions, on low levels of GM targets and on airborne environmental samples (L. Kluga et al., S. Folloni et al., in preparation). The use of this set-up is demonstrated here for three particular legal EU situations (see Figure 3). A mixture of GM soy and maize was prepared to mimic the following situation: low level presence of an asynchronously authorized GM material in a GMO mixture, EU-authorized GM material present as a so-called 'botanical impurity (feed) or contamination (food)', and authorized GM material present at the labelling threshold (0.9%). In all cases, the ready-to-use pre-spotted plates correctly identified the presence of the different GMO present in the sample in a single analysis. In this way, the crop-specific set-up proved to represent a very valuable, cost-efficient tool for complying with the EU enforcement requirements (European Commission 2004a).

Target	Ct							
	Labeling		Asynchr. autorisation		Botanical impurities			
	1	2	1	2	1	2	3	4
HMG			24.07	24.12	23.91	25.61	26.81	23.92
HMG			24.13	24.16	23.92	23.78	23.81	23.91
Bt11					41.85	44.22	40.39	39.18
NK603								
GA21								
MON863								
DAS1507			29.23	29.31				
T25								
DAS59122								
MON810			28.53	28.57	38.92	39.3	40.06	
MIR604								
MON88017								
LY038								
3272								
MON89034								
98140								
Lec	23.1	23.01	30.71	30.77	35.28	35.5	36.51	37.55
Lec	23.15	23.09	30.64	30.81	35.47	36.63	36.28	
A27024-12	33.15	32.72						
40-3-2	29	28.84	38.67	38.94	36.24	37.2	36.81	37.86
MON89788								
DP-356043								
DP-305423			32.32	32.48				
A5547-127								

Fig. 3. GM soy/maize RTi-PCR ready-to-use PCR analysis
(The respective GM materials present were 1) in case of the labelling requirement a mix of wild type soybean DNA supplied with 2% GTS40-3-2 DNA and 0.1% A2704-12 DNA; 2) in the case of the asynchronous authorisation a mix of wild type maize DNA supplied with 20% MON810 maize DNA, 5% TC1507 maize DNA and 0.5% DP-305423 soybean DNA and 3) in the case of botanical impurities WT maize spiked with traces of GTS40-3-2 soybean, Bt11 maize, and MON810 maize (spiking equals 1 GM kernel of each event in 10.000 kernels of wild type maize)

4. Quality assurance for in-house production of RTi-PCR ready-to-use GMO detection plates at the JRC-IHCP

The production of multi-target ready-to-use RTi-PCR plates represents a complex process requiring careful monitoring. The JRC aims at producing the necessary tools for determining the presence of GMO in-house. The foreseen PCR plate production line to support such tools will be managed by a Waters® pipetting robot station. The primers/probes will be purchased from accredited manufacturers. To establish the in-house QC system, it is proposed to apply initially a continuous sampling plan (CSP) approach, meaning that 100% of the produced plates within a single production round will be verified. In a later stage only a limited fraction of the produced plates within one production round will be tested. Hereby the assumption is made that the quality of the production over the lot is sufficiently guaranteed by the validation studies performed on the different sub-steps in the production process. The maximal number of plates verified as a full production would represent the guaranteed accepted number or *clearance number* 'I'.

In addition to the clearance number, it is also important to determine the so-called 'Average Outgoing Quality Limit' (AOQL). Any CSP has an AOQL depending on the fraction (designated 'f') of the lot tested and the clearance number I . The AOQL represents the maximum or worst possible defect rate or number of defective units for the average outgoing quality. Regardless of the incoming quality, the defect rate or number of defective units going to the customer should be no greater than the AOQL over an extended period of time. Individual lots might be worse than the AOQL but over the long run, the quality will not be worse than the AOQL. The AOQ curve and AOQL assume lots are 100% inspected, and is only applicable to this situation. They also assume the inspection is reasonably effective at removing defective units or defects (90% effective or more).

Fig. 4. Exemplary Model for an "Average Outgoing Quality Curve" (Acc = acceptance rate, AOQ indicated as blue line)

The AOQ curve initially increases because when more defective units/ defects are produced, more are released. As more and more lots are rejected, 100% inspections become more common and the AOQ curve starts to decrease as a result. The maximum value of the AOQ curve is called the Average Outgoing Quality Level (AOQL) (see figure 4 as an example).

It is the aim to set up a production line guaranteeing the highest acceptable level of accuracy in a lot. To date, a reasonable size of a lot productions at the JRC would be about 20 plates per run (required $I = 20$), representing 20×96 wells = 1920 RTi-PCR analysis. At a 99% AOQL, 19 errors on 20 plates would still be the maximal accepted number of errors, thus one error/plate (calculated as follows: $1920 - (0.99 \times 1920) = 19$). At a 99,5% AOQL, 10 errors would be acceptable, while at a 99,9% AOQL only 2 errors on 20 plates would be accepted. An initial experiment at the JRC with 12 plates demonstrated that no errors occurred at the production line, suggesting that an AOQL close to 99.9% might be technically feasible. In these analyses, considering that the RTi-plates are not uniform but comprise various PCR methods on the same plate, already an estimation of potential cross-contamination during the reagents plate loading by the robot station was assessed. For this, a specific plate set-up was tested wherein 4 different methods were inter-challenged by adding the targets of any of the other methods as

the substrate. No cross-contamination in any of the wells could be determined, demonstrating that the mechanical handling at the robot station is highly accurate.

Taking into account the above findings, the fraction size to be tested, the so-called sampling fraction 'f', can be relatively small. Thus, one full 96-well plate would be tested in cross-challenge modus per lot of 20 plates, wherein any failure at any measurement point would result in the rejection of the entire lot ($n=20$) for commercial release.

5. Towards a ready-to-use GM soy and maize screening system

Next to event-specific identification, efficient GMO screening approaches are a major request from enforcement laboratories. Screening methods are recognized as useful tools for the rapid and reliable reduction of test samples by direct identification of negative samples, which do not need to be further analyzed. Screening methods are traditionally developed by designing primers targeting the most common sequences used in transformation, and are meant to detect the widest range of GM crops (lines) without requirements of precise identification.

The EC was the world first to validate screening methods for the detection of GMO in raw and processed food samples in the years 1999 and 2000. At that time screening tests, based on the detection of the 35S promoter and the NOS terminator (tNOS) – regulatory sequences globally used in the development of GMO – were considered excellent initial targets to assay irrespective of modification type and, depending on the outcome of the 35S/NOS results, were followed by verification of positives with other rounds of PCR targeting specific transgenic elements or constructs for confirmation and identification purposes.

Over the years the situation varied as new events – containing a wider range of constructs building blocks – were introduced into the market. It is already known that several second-generation biotech products do not have the 35S nor the tNOS sequence. At the present situation in a wide range – globally applicable – screening system the number/type of PCR tests have to be adjusted and include primers also for new constructs elements. In response to the increasing needs in GMO testing, the JRC together with the Scientific Institute of Public Health (IPH, Belgium), a member of the European Network of GMO Laboratories, are developing a ready-to-use tool that includes a wide range of screening methods allowing the detection of any commercial, either approved or unapproved GMO present in the sample. This so-called CoSYPS strategy developed at IPH applies a matrix-based approach which provides guidance on the nature of the GMO present in a product based on the detection of various analytical targets present in commercialized GMOs, both approved and not approved in the EU. The following genetic targets are included today in CoSYPS: Large Subunit of Rubisco gene, Lectin, Alcohol Dehydrogenase, Cruciferin A, CaMV 35S promoter, Agrobacterium Nopaline Synthase terminator, EPSPS-CP4, CryIAb, PAT/pat, PAT/bar (Barbau-Piednoir et al. 2009, Van den Bulcke et al. 2010).

CoSYPS applies SYBR®GREEN real-time PCR methods. SYBR®GREEN is a fluorescent molecule which has a very low fluorescence in the absence of double stranded DNA and a very high fluorescence in the presence of double-stranded DNA. During real-time PCR, SYBR®GREEN binds to any double-stranded DNA which is considered to be advantageous in screening approaches as to extend the detection of closely related targets (Querci et al. 2010). It is expected that the SYBR®GREEN methods will easily be amendable to the pre-spotting technology as in this case only two instead of three oligonucleotides are to be included per assay. The development of ready-to-use CoSYPS screening plates is considered a very complementary tool to the already developed event-specific plates developed at the JRC.

6. Web-based tool for harmonising GMO analysis in Europe

In a final stage, it is envisaged that harmonized GMO analysis will be greatly enhanced when a common decision support system is available and applied. Preferentially, the DSS should consist of a web application for GMO detection and allow for the on-line organisation of a customized GMO-screening setup by applying validated Q-PCR methods and Certified Reference Materials (CRM) within a matrix-based approach.

Such platform would thus be driven by the availability of concise information on validated PCR methods, Certified Reference Materials and a suitable Decision Support System. The central unit within the system could be the Central Core DNA Sequence Information System (CCSIS; <http://mbg.jrc.ec.europa.eu/home/bioinformatics/ccsis.htm>). CCSIS represents the most up-to-date GMO sequence Dbase to date. The data formats of the Dbase are compatible with bioinformatics tools and are accessible to other IT applications. As the key principle in GMO screening analysis is a matrix-based approach, a matrix-generating emulation tool should be incorporated. Such tool should allow to present the target/GMO combinations in a format that allows on the one hand to determine the optimal set of screening methods to be used for a particular application, and on the other hand to provide a means to mathematically 'tag' the GMO that could be present in a sample. The latter has been elegantly demonstrated by the IPH through the use of prime numbers as denominators for each of the screening methods (Van den Bulcke et al. 2010). The former has been developed in a prototype format as the so-called GMOTrack utility that allows defining optimal sets of screening methods based on sample information and a cost function (<http://kt.ijs.si/software/GMOTrack/>).

Both the above modules would feed information for the development of a dual set of complementary pre-spotted plates, one comprising GM-screening elements (the CoSYPS approach), a second one containing the event-specific GMO targets (the ready-to-use-event-specific-pre-spotted plates). The outcome of the analytical results using both sets of plates should then be integrated into an overall decision on GMO presence by transferring all raw data from the PCR devices straight into an analytical result interpretative module. The output of this IT program would indicate which GMO are present (albeit only at qualitative/semi-quantitative levels). In a later stage of development, it may be envisaged to include alarming the user of the presence of unassigned elements in the screening or unauthorized GMO at the event-specific level.

As indicated above, such Decision Support System (DSS) would preferentially be made available through a secure web application managed by a dedicated host server under secure transfer protocols. The need of a common DSS has been already recognized in other GMO research programs (such as the EC 7th Framework CoExtra project; <http://www.coextra.eu/>) and it is considered most valuable to integrate the already developed modules into a common DSS.

7. Conclusions

The 'real-time PCR based ready-to-use multi-target analytical system' developed by the Molecular Biology and Genomics Unit is considered a very suitable approach for the purpose of detection of several GM events in a single experiment. Given the flexible production setup, ready-to-use plates can be a very useful tool for detection of authorised and unauthorised GM events on the Food & Feed market. The selected methodology and format allows a straightforward implementation of the system since real-time PCR using the

96-well plate format is a technique commonly applied in the EU and worldwide. Moreover, the ready-to-use format can be easily extended to other PCR technologies such as SYBR®GREEN, LUX™, or Plexor™. The lyophilized state of the reagents and the primers/probes offers the advantage of long-term storage of the plates for up to one year. In addition, the use of these plates will greatly economize on technical preparation of the PCR analysis and on the storage of all reagents.

The event-specific system was shown already to provide an opportunity to allow testing for all EU-regulated genetically modified plants with minimal experimental handling required. Extending the ready-to-use format to screening applications will also facilitate this part in the GMO analysis. It is considered very advantageous to integrate both the screening and the event-specific analysis into the same platform. As event-specific GMO detection methods are considered the only type of method allowing the univocal identification of a GMO, a concerted interpretation of both screening and identification data is considered essential for accurate interpretations of the experimental results using e.g. differential PCR statistical analysis tools (Cankar et al., 2008).

The usefulness of the pre-spotted plates will largely be defined by the flexibility of production of different formats according to the customers need and the legal environment covered by enforcement measures. The JRC approach described here is shown to be a possible option for such platform and may even be applicable to a broader field than the detection of genetically modified organisms (e.g. pathogens, allergens, tissue-typing, genetic testing...).

Any of the above listed advantages will however be largely dependent on the availability of a common, accurate and updated decision support system (DSS). Such DSS should be based on sound documentation on the GMO, include facilities to develop optimal screening/identification approaches in line with up-to-date information, and allow for interpretation of the analytical results in line with accepted standards. It is considered that a web-based application would herein the most appropriate utility to harmonize GMO analysis throughout the GMO Community.

8. References

- Barbau-Piednoir E, Lievens A, Mbongolo-Mbella G, Roosens N, Sneyers M, Casi AL, Van den Bulcke M (2009) SYBR®Green qPCR screening methods for the presence of "35S promoter" and "NOS terminator" elements in food and feed products. *European Food Research and Technology* 230 (3) p. 383-393
- Cankar K, Chauvensy-Ancel V, Fortabat MN, Gruden K, Kobilinsky A, Zel J, Bertheau Y. (2008) Detection of non-authorized genetically modified organisms using differential quantitative polymerase chain reaction: application to 35S in maize. *Anal Biochem.* 376(2) p. 189-199
- European Commission (1997) Regulation (EC) No 258/97 of the European Parliament and of the Council of 27 January 1997 concerning novel foods and novel food ingredients. *Official Journal L* 043, p. 1-7
- European Commission (2001) Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC. *Official Journal L* 106, p. 1-37

- European Commission (2003a) Regulation (EC) No. 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed. Off. J. Eur. Union L 268, p. 1-23
- European Commission (2003b) Regulation (EC) No 1830/2003 of the European Parliament and of the Council of 22 September 2003 concerning the traceability and labelling of genetically modified organisms and the traceability of food and feed products produced from genetically modified organisms and amending Directive 2001/18/EC. Off. J. Eur. Union L 268, p. 24-28
- European Commission (2004a) Regulation (EC) No 882/2004 of the European Parliament and of the Council of 29 April 2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules. Off. J. Eur. Union L 191, p. 1-52
- European Commission (2004b) Commission Regulation (EC) No. 641/2004 of 6 April 2004 on detailed rules for the implementation of Regulation (EC) No. 1829/2003 of the European Parliament and of the Council as regards the application for the authorisation of new genetically modified food and feed, the notification of existing products and adventitious or technically unavoidable presence of genetically modified material which has benefited from a favourable risk evaluation. Off. J. Eur. Union L 102, p. 14-25
- European Commission (2005a) Commission Decision of 18 April 2005 on emergency measures regarding the non-authorised genetically modified organism Bt10 in maize products (2005/317/EC). Off. J. Eur. Union L 101, p. 14-16
- European Commission (2005b) Guidance documents on the website of the Community Reference Laboratory (2009) <http://gmo-crl.jrc.ec.europa.eu/guidancedocs.htm>
- European Commission (2006) Commission Decision of 23 August 2006 on emergency measures regarding the non-authorised genetically modified organism LL RICE 601 in rice products (2006/578/EC). Off. J. Eur. Union L 2, p. 308-310
- James C (2008) Global Status of Commercialized Biotech/GM Crops (2008) ISAAA Brief No. 39. ISAAA: Ithaca, NY
- Miraglia M, Berdal KG, Brera C, Corbisier P, Holst-Jensen A, Kok E, Marvin HJP, Schimmel H, Rentsch J, van Rie JP, Zagon J (2004) Detection and traceability of genetically modified organisms in the food production chain. Food and Chemical Toxicology 42, p. 1157-1180
- Querci M, Foti N, Bogni A, Kluga L, Broll H, Van den Eede G (2009) Real-time PCR based ready-to-use multi-target analytical system for GMO detection. Food Analytical Methods 2, p. 325-336
- Querci M, Van den Bulcke M, Žel J, Van den Eede G, Broll H (2010) New approaches in GMO detection. Analytical and Bioanalytical Chemistry, Vol. 396 (6) p. 1991-2002
- Reiting R, Broll H, Weiblinger H, Grohmann L (2007) Collaborative study of a T-nos real-time PCR method for screening of genetically modified organisms in food products. Journal für Verbraucherschutz und Lebensmittelsicherheit 2, p. 116-121
- Stein AJ, Rodríguez-Cerezo E (2009) The global pipeline of new GM crops: implications of asynchronous approval for international trade. JRC Technical Report EUR 23486 EN. Office for Official Publications of the European Communities, Luxembourg
- Van den Bulcke M, Lievens A, Barbau-Piednoir E, MbongoloMbella G, Roosens N, Sneyers M, Capi AL. (2010) A theoretical introduction to "combinatory SYBR®Green qPCR screening", a matrix-based approach for the detection of materials derived from genetically modified plants. Anal Bioanal Chem. 396(6) p. 2113-2123

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