TECHNICAL REQUIREMENTS FOR THE ACCREDITATION OF GMO ANALYSIS PROCEDURES

CERINȚE TEHNICE PENTRU ACREDITAREA PROCEDURILOR DE ANALIZĂ OMG

SISEA C. R., D. PAMFIL,
IULIA FRANCESCA POP, IOANA VIRGINIA PETRICELE
University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca

Abstract. *Transgenic crops are spreading more rapidly than any other agricultural technology in history. However, different views on this subject had caused intense controversy. In order to ensure transparency and to meet consumers' needs, EU legislation established new policies such as labeling, traceability and post-market monitoring of GMO derived food and feed products. The implementation of these policies is based on molecular analyses performed by accredited laboratories. The main steps of an integrated protocol for qualitative and quantitative GMO analysis of plant-derived foodstuffs are presented and discussed. This protocol encompasses sampling and test sample preparation, DNA extraction, quantification of DNA extracts, qualitative analysis based on PCR and quantitative determination using real-time PCR approaches. The main features of ISO standards in this field are also covered, in order to facilitate the understanding of method accreditation process.*

Rezumat. *Plantele transgenice sunt considerate ca fiind tehnologia agricolă cu cea mai rapidă răspândire din istorie. Acest domeniu a generat însă numeroase controverse atât în rândul consumatorilor cât şi la nivelul decindeților şi al comunității academice. Pentru a spori încrederea consumatorilor, UE a adoptat politici mai transparente în acest domeniu, concretizate prin introducerea unor concepte ca etichetarea, trasabilitatea sau monitorizarea post-market a produselor derivate din OMG-uri, destinate consumului uman sau animal. Aplicarea acestor politici are la bază analize moleculare efectuate în laboratoare acreditate. Sunt descrise şi discutate etapele unui protocol integrat de analiză calitativă şi cantitativă a conținutului în OMG-uri al alimentelor derivate din plante. Un astfel de protocol cuprinde ca faze principale eşantionarea şi obținerea probei test, extracția ADN-ului, cuantificarea ADN-ului, analiza calitativă utilizând reacția PCR şi analiza cantitativă cu ajutorul tehnicii real-time PCR. Se fac trimiteri la standardele ISO în domeniul analizelor OMG, documente necesare pentru derularea şi finalizarea procesului de acreditare.*

The most important advantage of genetic engineering over traditional breeding programs is the possibility of creating cultivars with new characteristics that are not determined by genes present in the natural gene pool of the species to which the cultivar belongs, *i.e.* genes originating from unrelated organisms.

Since their introduction, transgenic crops are spreading more rapidly than any other agricultural technology in history (Raney, 2006). However, different views on

genetically modified organisms (GMOs), regarding their release into the environment, cultivation, importation and particularly, their utilization as food or feed ingredients, had caused intense controversy which raised the need for regulative approaches. No specific international regulatory systems in the field of GMOs are currently in place. However, several international organizations are involved in developing protocols for GMO analysis (Querci *et al*., 2004) in order to ensure safe use and to facilitate imports of this technology.

The use of GMOs is regulated in the European Union (EU) by a set of strict procedures laid down in several Directives and Regulations (*e.g.* Directive 2001/18/EC, Regulation (EC) 1829/2003, Regulation (EC) 1830/2003. The EU has identified traceability and labeling as two major issues in ensuring transparency and assisting consumers into making an informed choice (Querci *et al*., 2004). The implementation of the regulatory system is, in part, based on the availability of validated methods of analysis and subsequently on a harmonized application within the EU boundaries.

From a scientific point of view, at the European level, the Institute for Health and Consumer Protection (IHCP) of the Joint Research Centre (JRC) of the European Commission and the World Health Organization are the most active institutions in the field. The Biotechnology and GMOs Unit (B&GMOs) of the JRC's IHCP has the mandate to make sure that all methods are in place for the control of food samples (http://biotech.jrc.it/). These methods are to be used by specialized national control laboratories most of which are part of the European Network of GMO Laboratories (ENGL). In the context of EU food and feed regulation, the JRC assisted by ENGL acts as the Community Reference Laboratory (CRL) in the field of GMOs. The CRL has the mandate to evaluate and validate analytical methods, to ensure that they are fit for the purpose of regulatory compliance and to provide scientific and technical advice in case of disputes (http://gmo-crl.jrc.it/). Therefore, the validation and accreditation of analytical methods are important tools for ensuring proficiency.

To comply with the EU legislation operators involved in GMO detection need to answer three sequential questions: detection, identification and quantification of GM content. GMO detection is based on analyses performed by specialized GMO testing laboratories. These laboratories have special facilities and work programs to include analyses which comply with the world wide good laboratory practices, requirements and acts in the field of biotechnologies, also covering GMOs. The main topics that are generally considered important for the correct functioning of a GMO testing laboratory are: laboratory set-up and implementation, how to avoid contamination, forward-flow system, good laboratory practice, method selection, proficiency testing, method validation and accreditation (EC, 2005).

DISCUSSIONS

There are three types of methods that can be used to identify GMOs as such or contained in food and feed: phenotype identification, DNA-based and protein-based methods. Protein-based methods are using the antigen-antibody interaction and include lateral flow strips for rapid field identification, and ELISA. However,

DNA-based techniques are more often/frequently used in GMO analysis. In this case, qualitative analysis is usually based on PCR, while quantitative measurement is archived by real-time PCR, technique considered to be the most powerful tool for quantitative nucleic acids analysis (Kubista *et al*., 2006).

Providing correct, that is reliable and repeatable, analytical results is the most important factor in satisfying customers. High quality results are produced when the testing laboratory is able to work in a proper and acceptable way, following existing international standards, choosing analytical methods that are officially validated, if available, or at least demonstrated to give reliable and repeatable results (EC, 2005).

According to the EU legislation analytical methods used for food and feed control purposes shall be validated before their use by control laboratories (ENGL document). Method validation is therefore regarded as an important parameter for assessing technical performances of GMO testing laboratories. Performance criteria include: specificity, sensitivity, limit of detection (LOD), limit of quantification (LOQ), accuracy, trueness, precision, repeatability, reproducibility, dynamic range, applicability and measurement uncertainty (ENGL, 2005; ISO 24276:2006; Žel *et al*., 2006). More information on how to define or calculate these parameters are available in ISO standards 24276 and 5725. Literature on performance requirements for analytical methods for GMO testing is also available on the ENGL web page (http://engl.jrc.it/).

Accreditation of the laboratory is overall highly advisable. Among different quality assurance tools, accreditation is the most detailed and valuable one (EC, 2005). ISO defines accreditation as a procedure by which an authoritative body gives formal recognition that a laboratory operates a quality system, is technically competent, and is able to generate technically valid results. This does not guarantee that a given analytical result is correct, but it does establish standards that must be met and a framework approach to detect non-conformities when they occur (EC, 2005). The official ISO standard defining the general requirements for the competence of testing and calibration laboratories is ISO standard 17025. Requirements to comply in with this standard include: demonstrated technical competence of laboratory personnel, use of well defined test methodology, use of CRMs and participation to proficiency testing schemes, equipment management and calibration, records management and provision of adequate test reports including traceability (ISO/IEC 17025:2005). In addition to the need of compliance to the general requirements for the competence of testing and calibration laboratories according to ISO standard 17025, is worth knowing that specific documents defining the criteria and analytical protocols for the detection and quantification of GMOs have been elaborated. ISO standard 24276 serves as horizontal document for GMO testing laboratories, while ISO standards 21568, 21569, 21570, 21571, 21571 and 21572 deal with different analytical steps of detecting, identifying and quantifying GMOs.

The following indications are considered to be crucial when testing samples with the PCR/real-time PCR technique. However, the concepts can be adapted to fit other

procedures, such as ELISA and microarrays. The two most important requirements regarding the working area are the presence of separated areas and the implementation of a unidirectional work flow. Ideally, for each specific step of the procedure a physically separated area is needed and each of these areas is to be fitted with dedicated equipment (EC, 2005; Žel et al., 2006).

An important factor for testing laboratories is contamination avoidance (EC, 2005; Žel et al., 2006). It is generally believed that the main contamination sources in a GMO testing laboratory are: cross-contamination, aerosols, dust. Precautions should apply to rooms, equipment, working methods, personnel. The opportunity of contamination to occur will be highly reduced by establishing a unidirectional workflow (Žel et al., 2006). Good laboratory practice for contamination avoidance also include: changing lab coats every time a separated area is entered; the same applies to gloves; using sterile and aerosol resistant tips; routine cleaning and decontamination (before and after work) of laboratory working areas and equipment (Žel et al., 2006). An additional suggestion for the GMO testing laboratories is to install specific air flow systems for key areas (EC, 2005; Žel et al., 2006). The system has the provide positive/negative pressure, depending on the situation, in order to keep avoid spreading of contaminants.

Calibration of equipment, such as pipettes and real-time PCR instruments, is also of great importance in order to ensure repeatability and reproducibility of analytical methods.

Another important aspect is monitoring analytical performance over time. Valid and reliable measurements depend on the regular use of reference materials (EC, 2005). Availability of reference materials is crucial in case of quantitative analysis where it is necessary to construct a calibration curve at known GM concentration. Certified Reference Materials (CRMs) should be used on a regular basis in order to have a good quality assurance system. In Europe CRMs suitable for GMO detection and quantification are produced by the JRC Institute for Reference Materials and Measurements (IRMM).

Aside from reference materials, any analytical procedure requires the use of suitable controls (EC, 2005; Žel et al., 2006). The types of controls that should be used in a GMO analysis can be found in ISO standard 24276.

A general procedural flowchart for GMO analysis starts with sample preparation, followed by the extraction and purification of the analyte (DNA or protein), detection of the analyte, interpretation of collected data and reporting of results.

Sampling is a very important and complex step within the process of GMO analysis. However, this task is usually carried out by specialized personnel employed by national authorities. Some practical considerations on the sampling process are available in EN/TS ISO 21568:2005.

Prior to DNA isolation some of the samples preparation (*e.g.* milling, reduction of size) is carried out in a dedicated area. The enemy in this specific case is the production of dust and precautions to keep this area clean and to avoid contamination are crucial at this stage (EC, 2005). The purpose of a proper sample preparation is to ensure that the test portion is a homogenous representation of the whole laboratory sample.

A large variety of DNA extraction and purification methods are available and used in GMO analysis: CTAB-based extraction protocols (Somma, 2004), Wizard® Magnetic DNA Purification System for Food (Promega), DNeasy Plant Mini Kit and QIAamp DNA Stool Mini Kit (Qiagen), MagNA Pure LC DNA Isolation Kit I (Roche) etc. These methods differ in terms of principle, ease of use, cost per sample, efficiency etc. Each laboratory should select and implement an extraction method according to its own needs and resources. General consideration and examples of extraction methods for GMO analysis and manual extraction protocols are also provided in EN ISO 21571:2005. One important consideration is that each unknown sample should be extracted at least in duplicate (EC, 2005). Validated methods for DNA extraction are also available on the CRL web page (http://gmo-crl.jrc.it/).

DNA quantification is important in order to assess the characteristics of the extracted DNA. It is usually achieved by spectrophotometer measurement or by agarose gel electrophoresis. The second method offers indications on the molecular weight of extracted DNA. The first option provides information about the concentration and the purity of the extract important in determining the LOD and LOQ of analytical methods. Examples of methods for DNA quantification can be found in EN ISO 21571:2005, Annex B.

From a technical point of view, PCR and real-time PCR in the filed of GMO analysis are covered by ISO 21569 and ISO 21570, respectively. These documents provide information on technical aspects of these methods as well as examples of analysis methods for screening, taxon, construct and event identification and characterization. Validated methods for quantification of different GM events are also available on the CRL web-site (http://gmo-crl.jrc.it/).

In the case of qualitative PCR each extraction should be analyzed in duplicates, while for real-time PCR amplifications must be carried out in at least in triplicate (European Communities, 2005). For quantitative PCR four to six values are needed to construct the standard curve (EN ISO 21571:2005).

US Environmental Protection Agency also provides guidelines on quality assurance and control for laboratories (EPA, 2004).

Results should be reported according to the precise guidelines detailed in the international standards. In case of accredited laboratories, ISO standards 21569 and 21570 provide the guidelines for correct and comprehensive reporting of results.

CONCLUSIONS

We can summarize that there are three critical factors for ensuring reliable analyses: the competence of the operators (ensured by proper training), the application of good analytical methods (ensured by the validation process which verifies that the method, already optimized can be successfully implemented while maintaining its features) and the overall quality of the laboratory (ensured by the implementation of a quality system such as accreditation and by regular participation to proficiency testing schemes).

There are also some issues that are not yet properly solved or fully understood in order to be implemented in routine analysis of GMOs. These include: proper sampling of bulk commodities; quantification of stacked events; increase of GM events; lack of adequate CRMs for all tested matrices; biological diversity of a particular GMO, *i.e.* zygosity or ploidy; conversion of the measurement units; new emerging methods for analysis, such as microarrays or other non-DNA-based assays; cost-efficiency of analyses.

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