PCR method and factors that influence the quantification of genetically modified organisms in food

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ABSTRACT

The Brazilian government requires, for the marketing of genetically modified products, that producers and suppliers identify food containing more than 1% of transgenic components on the packaging. Quantitative real-time PCR (qPCR) is a reliable method for detecting and/or quantifying genetically modified organisms (GMOs) in food and animal feed samples. The data of the researched literature emphasize that an *in house* or collaborative validation study should be performed as part of the validation of the method, informing its performance. In addition, all measuring steps contributing to uncertainty, including sampling, homogeneity, DNA extraction, reference material, approximations and assumptions incorporated into the method and measurement procedure, experimental variations must be covered. This short review aimed to address the molecular methods and challenges faced by laboratories to correlate quantitative results and critical points that affect analytical quality of GMOs in food.

Keywords: GMOs legislation, GMOs labeling, transgenic components, food.

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INTRODUCTION

Although the subject of genetically modified organisms (GMOs) is still widely discussed in Brazil, it is currently the second largest producer of GMOs in the world, behind only to the United States (ISAAA, 2016). A study conducted by Céleres reported that the total area sown in Brazil in 2016/17 was 52.5 million hectares. The adoption of transgenic events reached 93.4% of the total cultivated area with the three crops (soybean, corn, cotton) representing 49.1 million hectares (Céleres, 2017).

Up to date, there are 525 approved events in 32 plant species worldwide, 146 genes that confer modifications related to herbicide tolerance, resistance to fungal and viral diseases, insect (coleoptera and lepidoptera), increased productivity, delayed senescence and softening of fruits, event related to rice allergy, oil/fatty acid, starch/carbohydrate modifications, biomass

increase, among others. In Brazil, there are 111 events approved for commercialization, one for beans, 23 for cotton, one for eucalyptus, 64 for corn, 19 for soybean and three for sugar cane (ISAAA, 2019).

Brazilian and multinational seed companies and public sector research institutions are working on the development of various biotech crops. There are a number of crops awaiting commercial approval, the most important of which are beans, sugar cane, potatoes, papaya, rice and citrus. With the exception of beans and sugarcane, most of these crops are in the early stages of development and approvals are expected within the next five years (ISAAA Briefs, 2017; ISAAA, 2016).

To provide new features to a plant or any other organism, a new DNA sequence is integrated into the genome. The "built-in" integrated DNA contains all the

information needed to express the desired new trait. The "construct" contains a promoter for gene transcription initiation, the coding region of the gene of interest that brings the new trait (e.g. herbicide resistance), a terminator that signals the end of gene transcription and a marker that helps to select plants or any other successfully transformed organism (Ahmad and Mukhtar, 2017; Parisi et al., 2016; Watson, 2019).

First-generation GM plants offer economic advantages in agriculture (herbicide tolerance, insect resistance, viral resistance) as well as environmental benefits (decreased use of herbicides, insecticides), which results in less pollution while facilitating manage the day to day life of the farmer. The second generation of GM plants offers consumer advantages such as improved food quality, increased vitamin content, etc.; the third generation provides better productivity, reduced pollutant treatment, drought and salt tolerance to withstand global warming and food shortages for the growing population, and improved biofuel quality (Buiatti et al., 2013; Qaim, 2016). Another category, still largely in the experimental phase, comprises GM plants capable of producing pharmaceuticals known as biopharmaceuticals, which include proteins, antibodies, antigens, vaccines, among others (Moon et al., 2020).

GMO legislation and labeling

The European Union (EU) introduced the first GMO labeling policies in 1997. As a result, in many countries GMO-containing food and feed labeling is mandatory and a limit on the content of products or ingredients has been set (Arujanan et al., 2018; Camara et al., 2013; Costa and Marin, 2011; Davison and Bertheau, 2007).

For the marketing of genetically modified products, the Brazilian government issued Decree No. 4,680 of 2003, which requires producers and suppliers to identify on their packaging foods containing more than 1% of transgenic components. According to the legislation, the Brazilian Ministry of Agriculture, Livestock and Supply (MAPA) is responsible for the supervision of products and activities that use GMOs and their derivatives intended for animal use, agriculture, livestock, agroindustry and the National Technical Biosafety Commission (CTNBio) to provide technical support and advice to Federal Government, mainly in activities involving the construction, experimentation, cultivation, handling, transportation, commercialization, consumption, storage, release and disposal of GMOs and derivatives (Brazil, 2003; Milavec et al., 2014).
Countries also have limits, with 3% in South Korea (Notification 2000-31/2000), 5% in Japan and Taiwan (Notification 1775/2000), 1% in Australia and New Zealand (Standard A18/2000) of content. On the other hand, in the United States, Canada, South Africa and Argentina, GMO labeling is voluntary (Branquinho et al., 2010).

Extensive legislation has been introduced in the EU, including GMO detection, traceability and labeling to support this view (Davison and Ammann, 2017). There are different approaches adopted in different countries, for example, Japan, China, South Korea and other countries are divided between the EU and the United States. The US explanation for not supporting GM food labeling is that the US Department of Health and Human Services (FDA) federal agency considers that there is no scientific evidence that GM foods are nutritionally different from conventional non-GMO (Carter and Gruère, 2003; Gruère and Rao, 2007), that is, the new variety derived from genetic modification must have a level of safety similar to the conventional variety that gave rise to it.

Regardless of the voluntary or mandatory labeling of GMO products, the definition of the label in use should be clear to consumers. In the absence of regulations on labeling of GMO voluntary, there is also no requirement for product validation and therefore no form of consumer protection. Labeling is essential to food because it is through labels that consumers are informed of what they are buying. Brazilian law is based on the labeling of Codex Alimentarius – the main international body responsible for setting standards for food safety and labeling. According to this body, label is "any inscription, caption or image, or descriptive or graphic matter, written, printed, embossed, lithographed or collected on the food packaging". It is through the label that products can be traced, being an essential means for consumer health (Costa and Marin, 2011).

In 2003, when transgenic soybeans were released for planting and marketing, entering the food chain of Brazilians, the controversy that concerns consumer rights arises: the guarantee of having access to information on the label of the food they consume. By Decree No. 4,680/2003, labeling has been extended to all packaged foods, in bulk or *in natura*, including foods of animal origin, containing more than 1% transgenics in their composition. It also requires the identification of the gene donor species by one of the words: "(transgenic (product name))" or "contains (transgenic ingredient name or ingredients)" or "product produced from transgenic (product name)" (Brazil, 2003; Costa and Marin, 2011).

The Brazilian National Health Surveillance Agency (ANVISA) is the federal agency responsible for overseeing the production and marketing of Brazilian foods, in addition to regulating their labeling. Among the products that are subjected to sanitary inspection by ANVISA, the products with the possibility of health risk obtained by genetic engineering deserve special mention (Brazil, 1999). The growth of the surface area planted with GM crops, with the consequent release of these crops for the environment and for commercialization, has raised questions about the safety of these products, especially in the transgene sequences, including the source or source organism of the DNA fragments used, their nucleotide sequences, identifying the coding

regions, the presence of transcriptional regulatory elements and the possible occurrence of transposition elements (Nascimento and Lajolo, 2018).

According to Costa and Marin (2011), in Brazil, specific legislation exists and is in force, however, there is no control over its application in marketed products. Another problem is the lack of information of the Brazilian population that knows little about GMOs and is unaware of the existence of legislation that gives the opportunity to choose the purchase of products containing or not GMOs.

In many countries and regions, consumers have expressed concerns about gene technology, have required adequate information, or the labeling of foods derived from GMOs. In order to provide information to consumers and to facilitate international trade, reliable analyze that can benchmark GMO content of products are needed, especially within their limits. To ensure the reliability of such analytical data, there is a need for accurate methods that must be validated, verified in the laboratory and used together with appropriate controls (Milavec et al., 2014).

The present short review aimed to address the Polymerase Chain Reaction (PCR) method and the challenges faced by laboratories to correlate quantitative results on critical points that affect analytical quality and impact on uncertainty, as well as the GMO limits provided by legislation and the accuracy of the molecular method.

MATERIALS AND METHODS

Articles were consulted, referring to GMO issues, legislation, labeling, molecular methods and sources of uncertainty. The search was performed on the electronic platforms PubMed, Google Scholar, Science Direct, among others.

Molecular methods and limits of quantification

All GMO detection methods, considered by the European Network of GMO Laboratories (ENGL) that meet EU regulations and their performance criteria, use PCR. Quantitative real-timePCR (qPCR) is a reliable method for detecting and/or quantifying GMOs in food and feed samples because its purpose is genetic information. Deoxyribonucleic acid (DNA) is a stable molecule and qPCR is highly sensitive, accurate, safe and capable of detecting a wide range of GMO events. In addition, the reaction product is analyzed directly in the reaction tube, significantly reducing the risk of contamination (Salihah et al., 2016). The biggest challenge for this GMO detection and quantification methodology is the large number and complexity of new approved events, which leads to the need to detect combined targets (Biotecon, 2015; Datukishvili et al., 2015).

Validated methods are listed on the Community Reference Laboratory (CRL) website and primarily use the TaqMan hydrolysis probe. Although qPCR multiplex has the potential for better economy by simultaneous processing of multiple primers, it is difficult to optimize given the overlap in spectra of the different fluorophores that are used in the TaqMan or Minor Groove Binder (MGB) probes. In addition, multiple qualitative PCR reactions are useful for initial sample screening prior to identification and quantification of present GMOs (Davison and Bertheau, 2007).

Most food-approved GMOs have common sequences, such as the 35S promoter (p-35S) from the Cauliflower Mosaic Virus (VMCF) and the terminator (t-NOS) sequence of the Nopaline Synthase gene, isolated from *Agrobacterium tumefaciens*, among other events, that can be detected by qPCR (Bawa and Anilakumar, 2013). P-35S is present in approximately 75% of commercial GMOs, which greatly facilitates the detection of these organisms. The main disadvantage of this method is the occurrence of false positive results, as these common sequences also occur naturally in some soil plants and microorganisms or even in false-negative results as new GM plants appear and have new events and regulatory elements such as p-35S or t-NOS are no longer detected (Biotecon, 2015; Jain et al., 2018).

GMO quantification determines the relative content of GMOs, calculated as the ratio between the total number of GMO-specific target copies (e.g. Roundup Ready 2 Yield soybeans) and the total number of copies of target plant species (e.g. lecithin), which is calculated and expressed as a percentage (% GMO soybeans of total soybean content). The copy number is calculated with the help of respective standard curves, which are measured in parallel (Biotecon, 2015; ISO 21570, 2005).

The need for adequate reference standards is therefore twofold: first, as qualitative PCR standards required acting as positive controls for identifying authorized or unauthorized GM events; second, as quantitative standards required to construct standard curves for GM DNA (target) and total DNA of the plant. Certified Reference Materials (CRM) should be used and, if these are not available, positive control sample can be used as reference material (Chaouachi et al., 2013).

Although specific DNA sequences can be detected by hybridization, regulatory authorities have accepted variations of the PCR technique. This makes DNA-based methods advantageous because of their specificity and sensitivity. While PCR is the dominant DNA technology, alternatives such as isothermal amplification (Li et al., 2018; Singh et al., 2017), direct detection of genomic DNA by electrochemical sensors (Wang et al., 2016), analysis of complementary DNA (cDNA) using microarray have also been proposed (Mahgoub and Nollet, 2019; Turkec et al., 2016). These high throughput methods have been developed, but are not yet found in service provider laboratories. Normally routine labs are using combinations of one or more oligoplex PCRs followed by multiplexing, amplified DNA identification or custom

plates for multiple PCRssimultaneously (Datukishvili et al., 2015; Holst-Jensen, 2009).

Selecting analytical method can be challenging because there are several options of commercially available methods and the best choice for one laboratory or situation is not necessarily the best for another. Reducing expenses and time is often a priority. In this regard, the European Network of GMO Laboratories (ENGL) has prepared a guide to methods that are adopted by the European Community Commission, which is based on quantitative methods using PCR technology. These and other related documents on the EU-RL GMFF website (http://gmo-crl.jrc.ec.europa.eu/) may be useful for analytical laboratories in selecting, comparing and validating methods. Harmonization of methods is often desirable because it facilitates transparency and comparison of results between laboratories.

However, one may also have reasons for choosing non-harmonized methods, such as cost, specificity, convenience, new information, availability of reference material, some parameters of choice. The lack of correlation between the results obtained by different laboratories may be caused by differences between methods, including specificity, sensitivity, conversion factor, analyte recovery, among others. In addition, the conversion factor is always derived from the reference material used and does not necessarily reflect the nature of the reference material, such as whether it is derived from a hemizygous GMO (only one allele present rather than two in one diploid organism), whereas the sample is derived from a homozygous GMO (alleles present on the homologous chromosomes are the same), so the conversion factor will produce an estimated concentration-based mass that is approximately twice the actual concentration-based mass. Therefore, cloned DNA is an alternative for use as reference material (Holst-Jensen, 2009; Wu et al., 2016).

It is important for the laboratory to report detection limits (LD) [lowest amount or concentration of analyte in a sample, which may be detected but not necessarily quantified] and/or limit of quantification (LQ) [lowest amount or concentration of analyte in a sample, which can be quantified with an acceptable level of accuracy and precision] (INMETRO, 2009), together with the result of the analysis. This is important to distinguish between detection and quantitation limit for the sample subjected to analysis. For example, a processed product may contain very little analyte compared to an unprocessed ingredient, therefore, the sample LD/LQ may be 100 times lower than the method LD/LQ, which ultimately influences the outcome (Holst-Jensen, 2009).

Sources of uncertainty and critical points

It is critical that the laboratory ensure that the technical records for each activity contain the results, the report (e.g. test or calibration certificate) and sufficient information to facilitate identification of factors affecting the measurement result and its uncertainty, including those from sampling, as well as allowing the activity to be repeated under the same conditions as the originals (ISO 17025, 2017).

Measurement uncertainty is a parameter associated with the result of a measurement that characterizes the dispersion of values that can reasonably be attributed to the measurand. Phenomena that contribute to uncertainty and thus to the fact that the result of a measurement cannot be characterized by a single value are called sources of uncertainty. The Brazilian Normative NIT-DICLA-021 describes several possible sources of uncertainty in a measurement by highlighting sampling, homogeneity, inaccurate values of measurement standards and reference materials, approximations and assumptions incorporated into the measurement method and procedure, variations in experimental conditions, among others. Measurement uncertainty provides a quantitative estimate of a test result and is therefore a central element of a quality system for calibration and/or testing laboratories (INMETRO, 2010a,b).

Suggested by Europe, the modular approach proposes ways to independently develop and validate analytical modules and calculate the measurement uncertainty of the various combined elements, such as DNA extraction, reference genes, PCR testing and calibration. Standardization of different simple detection methods and their use in duplex or multiplex reactions is necessary to improve time and cost efficiency for use in analytical laboratories. In addition to using appropriate sampling plans to minimize sampling and measurement uncertainty, it can lead to variations within and between laboratories participating in validations. Sample dilutions also reduce measurement uncertainty (Davison and Bertheau, 2007; Holst-Jensen and Berdal, 2004).

According to Weighardt (2007) and in the review published by Gryson (2010), the factors that influence the applicability and reliability of qPCR analysis in processed samples are: 1) DNA degradation, caused by temperature and pH, depletes the amplifiable DNA sequences of the sample. This can influence the detection and quantitation limits of a method as fewer intact target sequences are available as a result of DNA fragmentation; 2) in qPCR, two measurements are taken, one directed to a sequence of GM event-specific markers and a second segmentation to a species-specific reference sequence, allowing detection of all genomes. Relative GMO content is obtained by dividing the value of the specific GMO measurement with the value of the reference sequence. Event-specific and species-specific amplification products generally show significant differences in base length and/or composition of the target sequences. This is not a problem as long as quantification is performed on DNA samples with levels of degradation similar to those of certified reference

materials used as standard; 3) DNA extraction from processed foods often represents a compromise between achieving high DNA yield and maintaining PCR inhibitors at low concentrations. Under such conditions, choosing the most appropriate DNA extraction methodology for a given matrix becomes crucial. Several extraction methods have been developed or are under development, but it is not always possible to extract DNA of the required quality and purity, making qPCR quantification difficult; and 4) particle size homogeneity in a product can be a serious issue. DNA extracted from large-scale particles is underrepresented relative to DNA extracted from smaller particles. As a result, GMO content may be exceeded or underestimated because of different particle size distributions. Food products usually contain different components derived from the same species, such as soy flour, lecithin and oil. In practice, all components that derive from a species are considered a single ingredient. Having an ingredient with components that represent different levels of DNA degradation makes accurate quantitative analysis virtually impossible.

All of these questions suggest caution when analyzing processed foods and feed for GMO content. In practice, the GMO labeling structure depends on the analysis of raw materials before processing and then their traceability along the production chain to the final product (Dabbene et al., 2014). Even so, the analysis of the raw materials must be adjusted to avoid errors, for example the effect of food batch heterogeneity.

From sample preparation to data evaluation, several steps can influence the analytical result. To use the measurement uncertainty estimate properly, it is important to have a replica, two PCR reactions and one DNA sample. In addition, the calibration curve provides a single measurement result for the laboratory with close threshold cycle (Ct) values. It is also essential to insert an internal quality control (analytical standard from reference material) to ensure that the performance of the analytical process remains effectively unchanged (Trapman et al., 2009).

On the other hand, validation of both qualitative and quantitative methods can also be performed *in house*. Hübner et al. (2001) emphasized that for the evaluation of results obtained through quantitative PCR, validation data regarding sensitivity, specificity, precision, accuracy and reproducibility should be available. Without these validation parameters, it is difficult to detect and establish GMO values for enforcement purposes.

In addition to these parameters as a form of validation, there are minimum performance requirements for GMO analytical methods that have been elaborated by EURL (ENGL, 2015). They consider that DNA extraction should not involve the use of hazardous chemicals such as phenol or mercaptoethanol; if possible, alternative solutions should be used. The average DNA concentration should be greater than 40 ng/µl, the sample DNA should not have PCR inhibitors, and the slope of the inhibition curve should be in the range of -3.1

to -3.6, which corresponds to an amplification efficiency of 110 to 90%, being 100% of -3.32. In cases of specific samples where it is difficult to extract genomic DNA such as processed foods, animal feed, refined oils, lecithin, an inclination of the inhibition curve between -4.1 and -3.1 is accepted. Individual values of the linear correlation coefficient (R^2) of the standard curve shall be greater than or equal to 0.98 and with at least five dilutions in the calibration curve, which shall cover all variation. The limit of quantification must be less than or equal to the smallest amount or concentration including variation (e.g. 0.09% or 50 copies) and the detection limit must have a confidence level of 95% with lower false-negative results or equal to 5%.

Laboratories using quantitative PCR can only measure target DNA in copy number. These copies are measured by serial Certified Reference Material (CRM) dilutions through qPCR and these values are used to construct a standard curve to determine the number of copies in routine samples. Although sufficient information from CRM normally available for mass/mass transformation to relative number of copies (e.g. zygosity, tissue ploidy, parent GMO origin, material DNA extractability, etc.), there is a lack of information about the composition of these samples tested and the influencing the GMO copy number (Wu et al., 2019).

Chaouachi et al. (2013) also point out that the chance of detecting a single copy of the haploid genome is different for each species, since PCR amplification efficiency is influenced by the total amount of DNA present in the reaction. For example, for GM soy, a GM copy is 0.001%, while for GM corn this value is 0.003%. LD and LQ are always related to the genome size of the species under study, but also to the amount of DNA used and, therefore, in the analyzed portion and the original sample size. Genome-related factors such as ploidy and zygosity levels have become irrelevant based on the use of haploid genome copy number for the expression of GMO content.

Reliable results between laboratories depend on method comparisons, validation and harmonization. Uncertainty in the measured value can lead to challenges, particularly when large lots or loads are involved. The concepts of LD and LQ are important and, in practice, the detection limit (LD) in a qPCR reaction is about five copies, while the absolute limit of quantification (LQ) is approximately 100 copies (Davison and Bertheau, 2007; Žel et al., 2012).

Therefore, there is a need for a global harmonization document that gathers all information for the validation of GMO methods in order to avoid disagreement of results when different plant materials in a different geographical area are used (Holst-Jensen et al., 2006).

Molecular method accuracy and result analysis

For GMOs to achieve worldwide approval and

commercialization, accurate and reliable diagnostic methods are required to evaluate transgenic content. Conventional methods, such as PCR and enzyme linked immunosorbent assay (ELISA), are routinely employed for DNA and protein based quantitation, respectively. Although these techniques are considered significant and productive, more technologies that allow detection and/or quantification are needed, since the production of more complex GMOs is increasing every day (Cankar et al., 2006; Salisu et al., 2017).

In Brazil, there are still few publications on detection and/or quantification of transgenic material present in processed foods (Greiner and Konietzny, 2008; Greiner et al., 2005). In addition, few of these have been labeled as "contains transgenic". A study conducted by Branquinho et al. (2010) analyzed 240 samples of soybean and 25 corn products from 2004 to 2007. The researchers concluded that all samples were positive for Roundup Ready® soybeans and quantitative analysis revealed that GMO content ranged from 0.05 to 1% in 43 (63.2%) samples and more than 1% in 25 (36.8%) samples. They concluded that there is a need for a food product-monitoring program by the Brazilian regulatory authorities, as several samples exceeded the limit provided for in Decree No. 4,680.

Qualitative testing it is common to differentiate between authorized and unauthorized material, to identify safe or potentially unsafe material, or to certify material purity. Quantitative testing may be used for labeling compliance control or agreed limits with respect to the GMO. In addition, they can play a role in safety assessment, risk management of GMOs by providing a means of traceability and, if necessary, collecting GM material by providing data for characterization of the GMO itself and environmental samples (Holst-Jensen, 2009).

Since no method is 100% accurate, it is therefore essential that the analytical report provide the uncertainty and limitations associated with the test result. This information must be present in a way that is perceived and interpreted correctly by the customer. Responsibilities for laboratory analysis include appropriate choice of methods, validation, identification of potential sources of error in test reports, interpretation of results, communication with the client to explain what the results mean unambiguously and should include all information agreed upon the client and those required by the method used (Holst-Jensen, 2009; Holst-Jensen et al., 2003; ISO 17025, 2017).

DISCUSSION AND FINAL CONSIDERATIONS

While GM crops are gaining global attention, their proper approval and commercialization requires accurate and reliable diagnostic methods to evaluate transgenic content. Time-consuming conventional PCR and ELISA-based methodologies have been replaced by ever faster and more suitable technologies for detecting GMOs.

Therefore, recent approaches such as microarray, digital PCR and next generation sequencing are promising due to their accuracy and detection of transgenic contents (Cankar et al., 2006; Holst-Jensen, 2009; Salisu et al., 2017). Many companies that sell GMO detection and/or quantification kits already customize multiple targets on a single PCR reaction plate, which facilitates quantitative detection in a single run.

New technologies such as digital PCR with the ability to accurately quantify the number of targets and improve sequencing technology, with the generation of large amounts of data in single experiments, are expected to find the appropriate place for detection and quantification. Properly combining multi-target detection systems from multiple GMOs in a single sample should be the method of choice for the increasing number of GMOs, along with the possibility for simultaneous quantification. Although PCR is currently the method of choice for the detection, identification and quantification of GMOs, there are still challenges that can be highlighted, such as determination of qPCR efficiency, possible sequence mismatches, and characteristics of taxon-specific genes and appropriate units of measurement, which are potential sources of measurement uncertainty (Dong et al., 2015; Fraiture et al., 2015).

Biotechnology companies in this emerging market expect consumers to be attracted to new products, the so-called GMO 2.0. The newer technology, genes editing method "CRISPR", may pose health risks, and some of the genetic material used, such as double-stranded RNA, may affect gene expression in human cells in ways that have not yet been investigated (Wilbie et al., 2019; Unniyampurath et al., 2016).

The first generation of GMOs was promoted to reduce the use of pesticides in agriculture. However, data from the International Agency for Research on Cancer (IARC) show that GMO cultures increased the use of glyphosate-based herbicide Roundup, which is a potential human carcinogen, in addition to causing long-term liver damage even at low doses.

For the scientific community remains unclear how these new technologies will evolve once launched in the environment; how they can interact with their ecosystems; and whether permanent changes in other organisms or ecosystems can result. Although some authors suggest that gene editing techniques are more accurate than first generation genetic engineering technologies (Knott and Doudna, 2018; Whelan and Lema, 2015). CRISPR technology is likely to be used to produce more herbicide-tolerant GMOs, which perpetuate the toxic wake of increased chemical dependency in agriculture, further away from healthy food systems. GMO 2.0 foods can also affect millions of small sustainable farmers worldwide, whose sustenance depend on cultivating the valuable natural crops that will

be replaced and thereby alter an entire food chain.

To overcome these problems and deal with the increase in the number of GMO events, the laboratory must meet the requirements of ISO 17025 (2017), and it is essential that it has the means and criteria to demonstrate through validation that methods lead to results reliable and appropriate to the intended quality.

As GMO market approval is under strict regulation, concern should also be focused on contamination by unauthorized GMOs rather than common GM food allergies. Transgenic plants can spread their genes to conventional crops through cross-pollination. To avoid such occurrences, there are guidelines stipulating that GM plants must be physically segregated from nearby plants by a damping zone sized in proportion to the distance traveled by pollen; however, it should be noted that not all producers comply with these guidelines, and that some pollen can travel long distances. Thereby, the EU Plant Scientific Committee states that contamination is inevitable and therefore the consequences of contamination must be considered before market approval.

Because different individuals may be allergic to different foods, the greatest risk of food allergy comes from involuntary ingestion of the allergen. Therefore, it will be helpful to consumers know the exact composition of their food, whether it is transgenic or not. Mandatory labeling of transgenic ingredients, which is required by law in Brazil, can help consumers identify potential allergens and facilitate the recall process if necessary. A comprehensive assessment for market approval, food safety surveillance and proper labeling can minimize the health risks of food allergies.

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