Detection of genetically modified organisms in foods

Farid E. Ahmed

Legislation enacted worldwide to regulate the presence of genetically modified organisms (GMOs) in crops, foods and ingredients, necessitated the development of reliable and sensitive methods for GMO detection. In this article, protein- and DNA-based methods employing western blots, enzyme-linked immunosorbant assay, lateral flow strips, Southern blots, qualitative-, quantitative-, real-time- and limiting dilution-PCR methods, are discussed. Where information on modified gene sequences is not available, new approaches, such as near-infrared spectrometry, might tackle the problem of detection of non-approved genetically modified (GM) foods. The efficiency of screening, identification and confirmation strategies should be examined with respect to false-positive rates, disappearance of marker genes, increased use of specific regulator sequences and the increasing number of GM foods.

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Farid E. Ahmed
Dept of Radiation Oncology, Leo W. Jenkins Cancer Center, The Brody School of Medicine, LSB 014, East Carolina University, Greenville, NC 27858, USA.
e-mail: ahmedf@mail.ecu.edu

In recent years, agricultural enterprises in the USA, Canada and the European Union (EU) have developed new plant varieties by adopting modern biotechnology, including genetic transformation. In the USA, >40% of the corn, >50% of the cotton and >45% of soybean acres planted in 1999 have been genetically modified, and at least 60% of food products in US supermarkets contain genetically modified organisms (GMOs) [1]. Owing to public relation programs carried out by major grain biotech producers in North America in the early 1980s to mollify consumer concerns about the newly developed food biotechnology, the general public in that continent has shown less resistance to the introduction of genetically modified (GM) foods [2]. However, these modified foods have not gained worldwide acceptance because of unmollified consumer suspicion resulting from earlier food and environmental concerns, transparent regulatory oversight and mistrust in government bureaucracies, all factors which fueled debates about the environmental and public health safety issues of introduced genes (e.g. potential gene flow to other organisms, the destruction of agricultural diversity, allerginicity, antibiotic resistance and gastrointestinal problems [2,3]).

Other economical and ethical issues pertaining to intellectual property rights came into play [4], with the realization that inadvertent contamination of non-GM seeds with GMOs is likely. These factors induced countries, exemplified by those in the EU, to either restrict the import of bioengineered foods or to introduce legislation requiring mandatory labeling of GM foods and food ingredients that are no longer equivalent to their conventional counterparts [5,6], or foods or ingredients containing additives and flavorings that have been genetically modified or produced from GMOs [7]. EU regulations mandate labeling of food containing GMOs [5,8]. These regulations established a 1% threshold for contamination of unmodified foods with GM food products [4]. Norway and Switzerland, which are not members of the European Community, demand the labeling of GMOs in their food [9]. In the USA, recent legislation did not stipulate mandatory labeling of GM foods but has instead recommended a voluntary labeling of bioengineered foods and requested that companies must notify the US Food and Drug Administration (FDA) of their intent to market GM foods at least 120 days before launch [10].

Some of the major regulatory and scientific agencies in the world believe that GM crops pose no greater threat to human health than those posed by traditional crop breeding approaches [11,12]. Nevertheless, the countries that introduced mandatory-labeling legislation of GM foods have done so to give their consumers a choice in selecting the foods they feel comfortable with [13]. An agreement, the 'Cartagena Biosafety Protocol', puts into effect rules that govern the trade and transfer of GMOs across international borders (e.g. labeling of shipments of GM commodities), and allows governments to prohibit the import of GM food when there is concern over its safety [14]. Universal legislation makes it imperative that governments, the food industry, testing laboratories and crop producers develop ways to accurately quantitate GMOs in crops, foods and food ingredients to assure compliance with threshold levels of GM products. This article summarizes the various test methods, their potential and their limitations.

Types of GMO testing

GM products contain an additional trait encoded by an introduced gene(s), which generally produce an additional protein(s) that confers the trait of interest. Raw material (e.g. grains) and processed products (e.g. foods) derived from GM crops might thus be identified by testing for the presence of introduced DNA, or by detecting expressed novel proteins encoded by the genetic material. Both qualitative (i.e. those that give a yes/no answer) and quantitative methods are available [15].

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Laboratories carrying out these assays must be proficient in performing them.

Sampling of GM products for testing

Both sample size and sampling procedures are important issues for testing GMOs in raw material and food ingredients if one is to avoid problems of nonhomogeneity. The sampling plan should be performed in a manner that ensures a statistically representative sample, and the sample size must be sufficient to allow adequate sensitivity, because the statistical significance achievable with a small sample size is weak [16]. The raw sample (or seed) is ground into fine powder and mixed thoroughly. Extraction of either protein or DNA for subsequent testing follows. Although no sampling protocols have been developed that specifically address the challenges associated with detecting biotechnologically derived grains, government plans, such as those published by Grain Inspection, Packers and Stockyards Administration (GIPSA) of the US Dept of Agriculture, are adequate [17]. Ultimately, the optimum sampling strategy is a balance between sensitivity, cost and confidence. It would, however, be desirable if sampling plans were coordinated on a worldwide basis (e.g. through the Codex Alimentarius Commission of the UN) to ensure adequacy of testing [18].

Reference materials for GMO testing

Appropriate reference materials for positive and negative controls provide the basis for the validation of analytical procedures and for assessing the performance of methods and laboratories. Reference material should be independent of the analytical methods and should be focused on raw material or base ingredients rather than on finished foods. Each GMO requires specific reference material. Grains, altered DNA or expressed proteins have all been used as reference material. If grains are used, they should realistically mimic real-life test material (i.e. have matrix effects and consistency similar to test grain samples, be of consistent quality over a long period of time, and must account for specific sample homogeneity, GMO content and stability) [13]. In a few cases, both genomic and plasmid DNA have been used as reference material; the former being more realistic in terms of matrix effect, whereas the latter is easier to prepare in large quantities and might provide greater consistency. In contrast to protein detection methods, in which a single standard can be settled on relatively easy, DNA-based methods are better served through combinations of several positive controls. The availability of reference materials is currently limited owing to concerns over intellectual property rights and costs [4]. The Institute of Reference Materials and Measurements at the Joint Research Center in Geel, Belgium, offer a limited number of reference materials [through Fluka (Buchs, Switzerland)] for modified soya, corn and maximizer maize (MM).

Protein-based testing methods

Immunoassay technologies with antibodies are ideal for qualitative and quantitative detection of many types of proteins in complex matrices when the target analyte is known [19]. Both monoclonal (highly specific) and polyclonal (often more sensitive) antibodies can be used depending on the amounts needed and the specificity of the detection system (e.g. antibodies to whole protein or specific peptide sequences), depending on the particular application, time allotted for testing and cost. On the basis of typical concentrations of transgenic material in plant tissue (>10 μg per tissue), the detection limits of protein immunoassays can predict the presence of modified proteins in the range of 1% GMOs [20]. Immunoassays with antibodies attached to a solid phase have been used in two formats: a competitive assay in which the detector and analyte compete to bind with capture antibodies, or a two-site (double antibody sandwich) assay in which the analyte is sandwiched between the capture antibody and the detector antibody [21]; the latter assay is deemed preferable. Both western blot and enzyme-linked immunosorbant assay (ELISA) techniques have been used for the analysis of protein products of Monsanto's transgenic Roundup Ready soybean (RRS), which is resistant to the herbicide glyphosate and contains the gene encoding *Agrobacterium* spp. strain CP4-derived 5-enol-pyruvyl-shikimate-3-phosphate synthase (EPSPS) [22].

Western blot

The western blot is a highly specific method that provides qualitative results suitable for determining whether a sample contains the target protein below or above a predetermined threshold level [23], and is particularly useful for the analysis of insoluble protein [19]. Further, because electrophoretic separation of protein is carried out under denaturing conditions, any problems of solubilization, aggregation and coprecipitation of the target protein with adventitious proteins are eliminated [24]. This method, however, is considered more suited to research applications than to routine testing. The samples to be assayed are solubilized with detergents and reducing agents, and separated by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis. These components are transferred to a solid support (usually a nitrocellulose membrane), and binding immunoglobulin sites on the membrane are blocked by dried nonfat milk. The specific sites are then probed with antibodies (either a high-titer polyclonal antiserum or a mixture of monoclonal antibodies raised against the denatured antigenic epitopes). Finally, the bounded antibody is stained with Ponceau, silver nitrate or Coomassie, or a

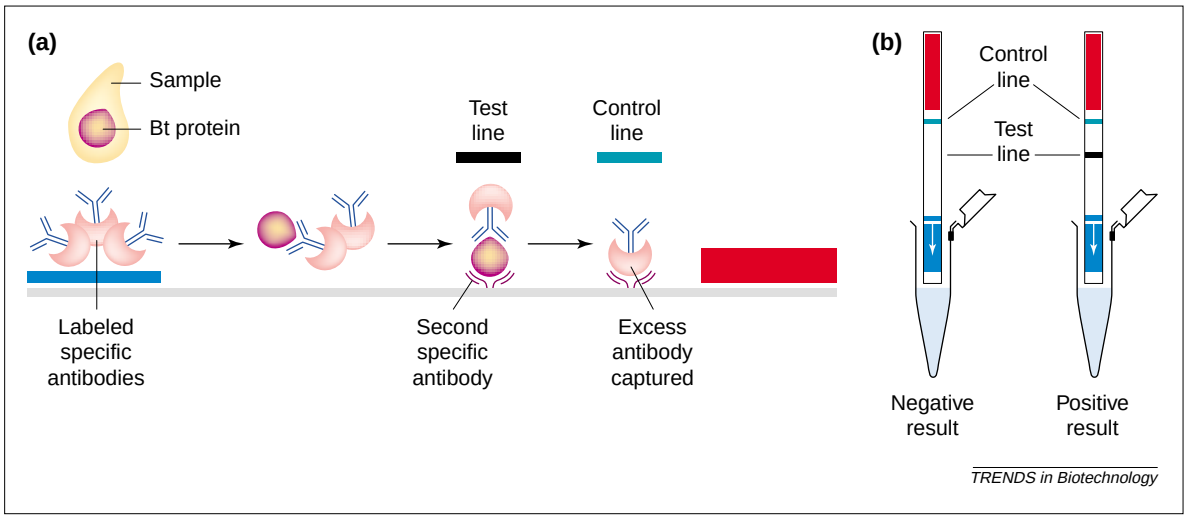

Fig. 1. Lateral flow strip assay format (courtesy of D. Layton). (a) Side view illustrating principles of the immunological test, and relative location of control and test lines on a nitrocellulose strip. (b) Vertical view of test strips dipped in an eppendorf containing genetically modified material, and showing both negative and positive test results. Abbreviation: Bt, *Bacillus thuringiensis*.

secondary immunological reagent, such as protein A coupled to horseradish-peroxidase (HRP) or alkaline phosphatase [24]. The detection limits of western blots vary between 0.25% for seeds and 1% for toasted meal [13].

ELISA

ELISA assumes more than one format: a microwell plate (or strip) format, and a coated tube format. The antibody-coated microwells, with removable strips of 8–12 wells, are quantitative, highly sensitive, economical, provide high throughput and are ideal for quantitative high-volume laboratory analysis, provided the protein is not denatured. The typical run time for a plate assay is 90 min, and an optical plate reader determines concentration levels in the samples. Detection limits for CP4 EPSPS soybean protein was 0.25% for seeds and 1.4% for toasted meal [13]. The antibody-coated tube format is suited for field-testing, with typical run times ranging from 15–30 min, and tubes can be read either visually or by an optical tube reader; results are qualitative. Because there is no quantitative internal standard within the assay, no extra information can be obtained concerning the presence of GMOs at the ingredient level in food.

Lateral flow strip

A variation on ELISA, using strips rather than microtiter wells, led to development of lateral flow strip technology (Fig. 1a). Immobilized double antibodies, specific for the expressed protein, are coupled to a color reactant and incorporated into a nitrocellulose strip, which, when placed in a plastic eppendorf vial containing an extract from plant tissue harboring a transgenic protein, leads to an antibody sandwich with some of the antibody that is coupled to the color reagent. This colored sandwich flows to the other end of the strip through a porous membrane that contains two captured zones, one specific for the transgenic protein sandwich and another specific for untreated antibodies coupled to the color reagent. The presence of only one (control) line on the membrane indicates a negative sample, and the presence of two lines indicates a positive result (Fig. 1b). The lateral flow format gives results in 5–10 min, is economical, more amenable to point-of-sale application, and is suitable as an initial screening method early in the food chain. These strips have been developed commercially to detect endotoxins expressed by the bacterium *Bacillus thuringiensis* that protect against insects, as in CryI(Ab) in corn plants, seeds and grain, in addition to CP4 EPSPS protein in soybean, canola, cotton and sugar beet [23,25]. Commercially available lateral flow strips are currently limited to few biotechnology-derived protein-producing GM products, but strips that can simultaneously detect multiple proteins are being developed. In the near future, improvements in immunoassays are expected to occur via advances in antibody technology and improved instruments [13].

Other immunoassay formats

In addition to microplate ELISA and lateral flow devices, other immunoassay formats use magnetic particles as the solid support surface. The magnetic particles can be coated with the capture antibody and the reaction carried out in a test tube. The particles with bound reactants are separated from unbound reactants in solution by a magnet. Advantages of this format are superior kinetics because the particles are free to move in reaction solutions and increased precision owing to uniformity of the particles [19].

Advances are also being made in combining antibody methods with instrumental techniques. For example, in addition to the hyphenated methods, such as immunoassay-mass spectrometry, considerable advances in relative observation of antibody binding to target molecules using biosensors have been reported [19].

Optimization and validation assays for ELISA (and also for DNA-based methods) are important aspects of standardizing this technology for GMO detection. Assay validation for food analysis is complex considering the large diversity of food matrices. Factors affecting optimization include: (1) selection of parameters (e.g. quality of kits, methods to test modified proteins and incubation times); (2) selection of thresholds (e.g. limits for positive and negative tests); (3) tracking of controls (in-house controls versus commercial kits); and (4) the work environment [experience of the laboratory in performing tests, and potential contamination problems in the environment or by the individual(s) conducting the assays] [26]. Factors affecting validation include: (1) extraction efficiency, (2) accuracy of results, (3) precision and ability to distinguish between closely related values, (4) sensitivity, limit of detection, (5) specificity, (6) reproducibility, and (7) consistency and reliability of detection. Given the complexity of food matrices, a practical approach to validate results is to use standard reference materials with known concentrations of GMOs in a matrix similar to that of the test sample. A common understanding of performance characteristics is essential to reach defined performance standards [13].

A collaborative validation study in Europe, involving 38 laboratories in 13 EU member states and Switzerland, was conducted to test accuracy and precision of ELISA with monoclonal antibodies raised against CP4 EPSPS protein, and with a polyclonal antibody that conjugates with HRP for the detection of RRS in soybean flour [27]. The reference sample, which simulated an actual food matrix, contained 1.25% GMO. Results showed consistent detection on the basis of dry weight for samples containing ~0.35% GMOs.

ELISA is the method of choice to screen for a particular GMO in raw material, semiprocessed foods and processed ingredients, provided the expressed protein is not degraded and can be detected. However, because ELISA has lower detection power than PCR methods, it is less sensitive for testing finished food products with many ingredients, especially if the threshold for detection is low. Although protein-based tests are practical and effective, some GM products do not express a detectable level of protein [28].

DNA-based testing methods

DNA detection methods for GM foods rely on the complementarity of two strands of DNA double helix that hybridize in a sequence-specific manner. The DNA that has been engineered into a crop consists of several elements that govern its functioning. They are typically a promoter sequence, structural gene and a stop sequence for the gene. Although several techniques are available, two are commonly used: Southern blot and particularly PCR analyses [24,28]. Microarray-based technology for detecting gene expression is currently under development [29].

Southern blot

The method involves fixing isolated sample DNA onto nitrocellulose or nylon membranes, probing with double-stranded (ds)-labeled nucleic acid probes specific to the GMO, and detecting hybridization radiographically, fluoremetrically or by chemiluminescence. Earlier probes were labeled with ^{32}P. However, nonradioactive fluorescein-labeled DNA [30], digoxigenin-, or biotin-labeled DNA probes [31], with sensitivity equal to ^{32}P probes, were recently used, obviating the need for radioactivity in the testing laboratory. These nonradioactive probes reduced detection to <1 h, as opposed to 24 h labeling required by ^{32}P. However, because only one probe is used, and no amplification is carried out, this method is considered less sensitive than PCR, which employs DNA of two primers.

Recently, an alternative Southern blot technology has been attempted with near infrared (NIR) fluorescent dyes (emitting at ~700 and 800 nm) coupled to a carbodiimide-reactive group and attached directly to DNA in a 5 min reaction. The signals for both dyes are detected simultaneously (limit in the low zeptomolar range) by two detectors of an infrared imager, something not yet possible with conventional radioactive or chemiluminescent detection techniques [32].

Qualitative PCR

PCR also exploits the specificity of DNA polymerase to allow the selective amplification of specific DNA segments occurring at low frequency in a complex mixture of other DNA sequences [28]. In a standard PCR test, two pairs of primers are used: (1) forward, sense or 5′→3′, and (2) reverse, antisense or 3′→5′. These primers are designed to hybridize on opposite strands of the sequence of interest, and through a series of repetitive cycles, of 2–3 thermal steps, amplify the sequence between the primers millions of times. Amplified pieces can be subjected to agarose gel electrophoresis to separate amplified DNA according to size, although other separation methods, such as high performance liquid chromatography (HPLC) and capillary electrophoresis (CE), have been used [28,33]. Several food ingredients (e.g. soy, wheat, canola, potatoes, rice, maize, celery and tomatoes) have been analyzed using PCR. A crucial rate-limiting step is DNA extraction and purification. Currently, several methods of extraction are available, with two widely used: (1) the CTAB method, based on incubating food sample in the presence of the detergent cetyltrimethylammonium bromide [34], and (2) the Wizard method, employing DNA-binding silica resins (Promega Corp., Madison, WI) [35]. Both methods produce satisfactory DNA isolation

without unacceptable DNA degradation and are cost effective. Factors such as excessive heat, nuclease activity and low pH (quite common in food processing) contribute to DNA degradation. This is most likely for products with long shelf lives, such as prepared meatballs in tomato sauce and beefburgers. Compounds present in foods (e.g. proteins, fats, polysaccharides, polyphenols, cocoa extracts and caramelized sugar) can inhibit DNA polymerase [36], and data suggest that the critical minimum average DNA size for successful PCR analysis is ~400 bp [37].

Most currently available GMOs in the EU contain any of three genetic elements: the cauliflower mosaic virus (CaMV) 35S promoter, the nopalin synthase (NOS) terminator, or the kanamycin-resistance marker gene (nptII) and others (Table 1). These elements also occur naturally in some plants and soil microorganisms, and can thus be detected using PCR, giving false-positive results. If the PCR assay gives a positive result, product-specific PCR methods that have been developed for a range of different GM foods can be carried out. These methods exploit a set of primer pairs that spans the boundary of two adjacent genetic elements (e.g. promoters, target genes and terminators), or that are specific for detection of the altered target gene sequence. Detection limits are in the range of 20 pg to 10 ng of target DNA and 0.0001–1% of the mass fraction of GMOs [13].

Different methods can be used to confirm the PCR results: (1) specific cleavage of the amplified product by restriction endonuclease digestion [38]; (2) hybridization with a DNA probe specific for the target sequence [30]; (3) direct sequencing of the PCR product [24]; and (4) nested PCR [39], in which two sets of primer pairs bind specifically to the amplified target sequence (Fig. 2e).

A collaborative validation study involving 29 laboratories in 13 countries employing qualitative PCR to determine 35S promoter and NOS terminator from GM soybeans, maize and other processed components showed that samples containing 2% RRS or MM were unequivocally identified by all laboratories, and correct classification was achieved by analysing the CaMV 35S promoter in samples containing 0.5% GM soybeans. The method for the detection of NOS terminator was less sensitive, giving higher false-negative results (3 and 7 at the 0.5 and 0.1% GMO, respectively, compared with 0 and 5 for the CaMV promoter). Because of the large size of the maize genome, this qualitative PCR method was somewhat less sensitive for the detection of transgenic maize in raw material (i.e. the number of false negatives for 35S promoter in maize at the 0.1% GMO was 14) [38].

In Switzerland, studies of different PCR systems for the detection of GMOs, organized by a subcommission of the Swiss Food Manual, showed that the amplification of qualitative PCR systems can lead to interlaboratory differences of at least a factor of ten [9].

Table 1. Plant species and genetic elements included in the GeneScan™ GMO Chip[a,b]

DNA	For the detection of
from plants	Corn
	Canola
	Soy
	Rice
	CaMV
from GMOs	CaMV 35S-promoter
	bar gene (BASTA resistance for Sh)
	Gene encoding phosphinothricine-acetyltransferase from Sv
	Nos-Terminator
	Bt-Xtra™ corn
	Bt11 corn
	Maximizer™ Bt176 corn
	MON 810/809/802 corn
	Roundup Ready™ soy

[a]Abbreviations: Bt, *Bacillus thuringiensis*; CaMV, cauliflower mosaic virus; GMO, genetically modified organism; Sh, *Streptomyces hygroscopicus*; Sv, *Streptomyces viridochromogens*. [b]Modified from [40].

Recently, GeneScan Europe has introduced a test kit for the detection of GMOs in food products, which allows a multiplex PCR for the specific detection of DNA sequences from plant species and GM traits [40]. The procedure starts with the isolation and purification of DNA from the sample. Then, specific DNA sequences from both plant species and GM traits (Table 1) are amplified by two separate multiplex PCR reactions, products of both reactions mixed, and single-stranded (ss) DNA is created by digestion with an exonuclease. After mixing with hybridization buffer, the sample is spread on the chip and amplified sequences that will hybridize with cDNA probes covalently bound in the chip are stained with the fluorescent dye Cy5 and analyzed by a biochip reader (e.g. Biodetect 654™). The detection limit for the GMO Chip Kit is in the range of 250 copies of each of the target DNA sequences in the PCR.

Quantitative end-point PCR

A crucial aspect of analysis of GMOs in food is quantitation, because maximum limits of GMOs in foods are the basis for labeling in the EU [8,41]. Therefore, more quantitative PCR approaches were needed. PCR was shown to be quantitative if an internal DNA standard was coamplified with target DNA [42]. In systems such as the quantitative-competitive (QC)-PCR method (Fig. 3), the presence of PCR inhibitors will be noticed immediately because the amplification of both internal standard and target DNA will be simultaneously affected [43]. QC-PCR consists of four steps: (1) coamplification of standard- and target-DNA in the same reaction tube; (2) separation of the products by an

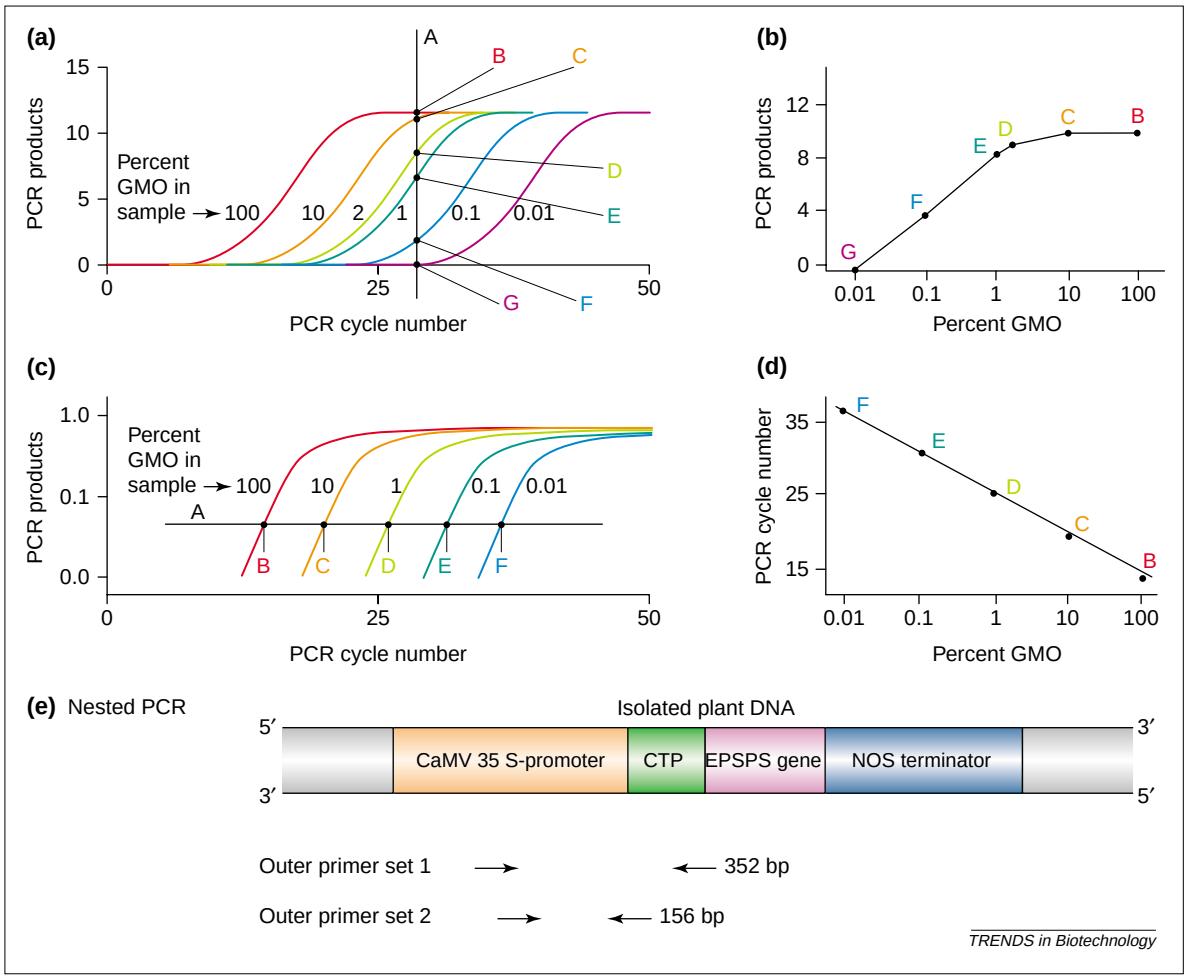

Fig. 2. Differences between traditional PCR (a,b) and real-time PCR (c,d) (courtesy of J. Fagan). The concentration of products present at point A (a,c) are plotted as a function of percent GMO present in the initial sample, demonstrating that proportionality between DNA concentration and PCR products (dynamic range) is limited for conventional PCR (b). By contrast, there is a linear relationship between PCR products and DNA concentration during exponential phase of real-time PCR. (e) Schematic illustration of nested-PCR method using two sets of primers selected to bridge the 35S promoter sequence and the petunia-derived CTP sequence. A two-step approach – an outer primer set followed by an inner primer set – will improve selectivity and sensitivity of the reaction. Detection limits of the available DNA were between 0.01 and 0.1% depending on the type of product examined (soybean meal, protein, lecithin, oil or several processed products) and the size of test portion [53]. Abbreviations: CaMV, cauliflower mosaic virus; CTP, cells transit peptide; EPSPS, 5-enol-pyruvyl-shikimate-3-phosphate synthase; NOS, nopalin synthase.

appropriate method, such as agarose gel electrophoresis and staining the gel by ethidium bromide; (3) analysis of the gel densitometrically; and (4) estimation of the relative amounts of target and standard DNA by regression analysis. At the equivalence point [Fig. 3b(iv)], the starting concentration of internal standard and target are equal [i.e. the regression coefficient is >0.99 and the slope of the regression line ≈ 1; Fig. 3d(iv)] [44]. In the QC-PCR, the competition between the amplification of internal standard DNA and target DNA generally leads to loss of detection sensitivity. Nevertheless, the Swiss example allows as little as 0.1% GMO DNA to be detected [43], which is within the threshold limits specified by the European Novel Food Regulations [5].

Twelve European control laboratories attempted to validate QC-PCR. Each of eight coded samples that contained GMOs was processed twice and compared with external standards containing 0.5 and 2% RRS. No false-negative results were found among 246 determinations for six samples containing RRS, and no false-positive results for the negative control sample were reported. Interlaboratory differences were smaller for QC-PCR than for qualitative PCR, and were mainly caused by insufficient sample homogenization. Furthermore, the calibration of QC-PCR can be controlled by certified reference material available commercially (Fluka, Buchs, Switzerland) [41,45].

Quantitative real-time PCR

To circumvent some of the problems of conventional quantitative end-point PCR, a real-time Q-PCR was introduced [46]. In theory, production of PCR products should proceed exponentially. However, in practice it reaches a plateau between 30 and 40 cycles because certain reaction components become limiting [28]. In conventional PCR, products of the reaction are measured at a single point in the reaction profile. Plotting the concentration of

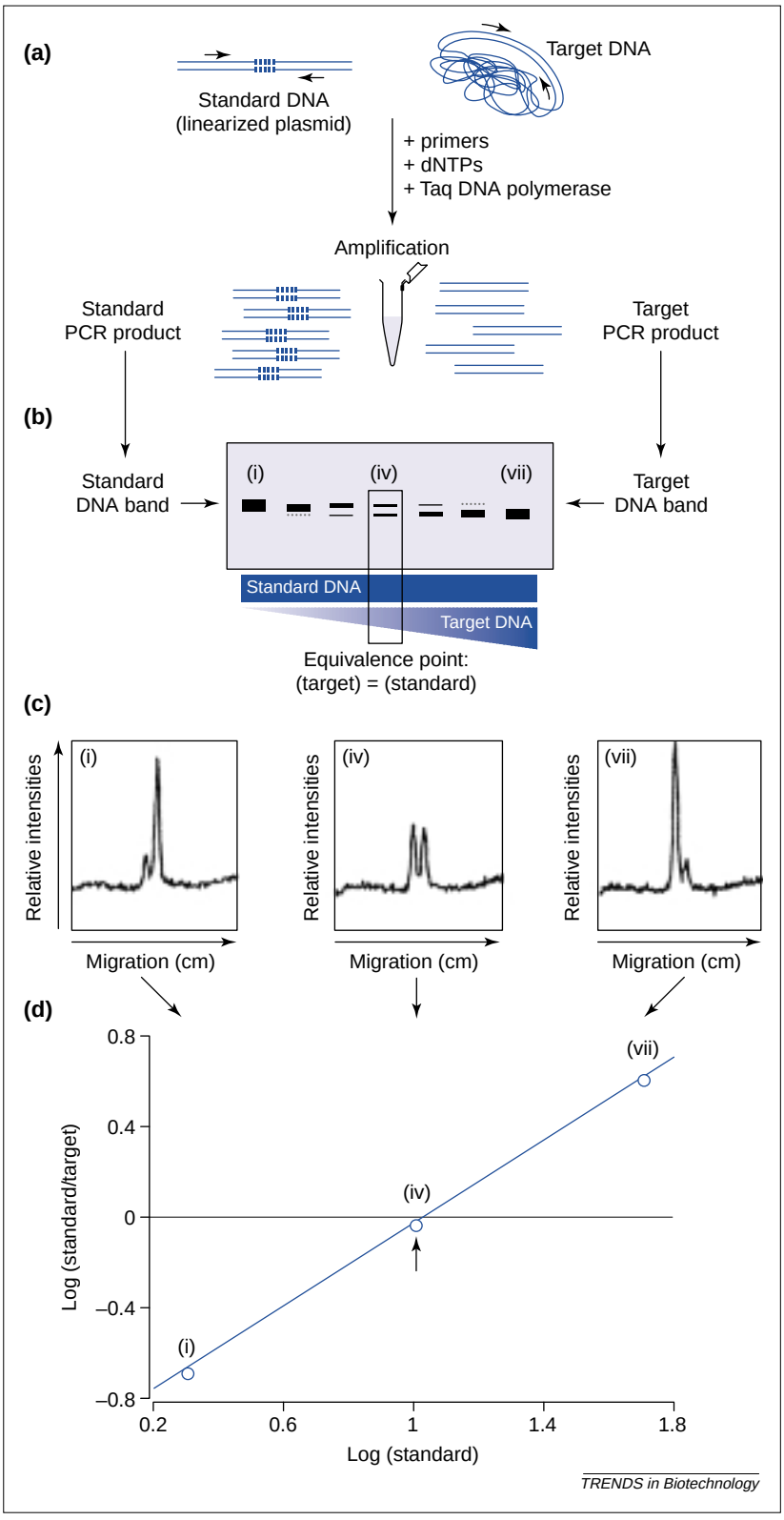

Fig. 3. Schematic illustration of milestones carried out in a Swiss QC-PCR. Standard and target DNAs are coamplified in the same reaction tube (a). Following the PCR, the products are separated by gel electrophoresis (b), which distinguishes the standard DNA from the amplified target by the size of the product. Gels stained with ethidium bromide. At the equivalent point (iv), the starting concentrations of internal standard and target are equivalent. Densitometric analysis of the various bands (c) can be used to calculate the linear regression (d). Reproduced, with permission, from [45].

products present at this point as a function of the initial amount of DNA present in each of those reactions shows that proportionality between DNA concentration (dynamic range) and PCR products occurs over a limited range of DNA concentrations (Fig. 2), leading to loss of precision in quantitation. However, it has been shown empirically that the concentration of DNA in real-time PCR reaction is proportional to PCR cycle number during the exponential phase of PCR [47]. Therefore, if the number of cycles it takes for a sample to reach the same point in its exponential growth curve is known (Fig. 2c), its precise initial DNA (then GMO) content can be determined (Fig. 2d). Real-time PCR also allows for detection of low copy DNA number [47]. Several commercially available real-time PCR thermal cyclers automate the analytical procedure and allow cycle-by-cycle monitoring of reaction kinetics, permitting calculation of the target sequence concentration. Several formats are used to estimate the amount of PCR product: (1) the ds-DNA-binding dye SYBR Green I; (2) hybridization probes or fluorescence resonance energy transfer (FRET) probes; (3) hydrolysis probes (TaqMan® technology); and (4) molecular beacons [47]. These systems also permit differentiation between specific- and nonspecific PCR products (such as primer-dimer) by the probe hybridization or by melt curve analysis of PCR products, because nonspecific products tend to melt at a much lower temperature than do the longer specific products [47].

A total of 179 food products containing GM RRS (e.g. baby food and diet products, soy drinks and desserts, tofu and tofu products, cereals, noodles, fats, oils and condiments) were analyzed by TaqMan®. The method proved to be sensitive. Amplifiable soy DNA could not, however, be detected in fats, oils and condiments. The genetic modification of RRS was detected in 34 samples, eight of which contained >1% RRS [48].

ABI Prism 7700, employing TaqMan® in a relative quantitative PCR, detected 2 pg of MM and RRS per gram of starting sample in 3h after DNA extraction [49].

Including a reference housekeeping gene as an internal standard, which will amplify in parallel with the gene of interest, provides quantitation in PCR-based assays [47].

Exhaustive limiting dilution PCR method

This method is based on: (1) optimization of the PCR so that amplification of an endogenous control gene will take place in an all-or-nothing fashion, derived from the terminal plateau phase of the PCR; and (2) the premise that one or more targets in the reaction mixture (e.g. GMO) will give a positive result. Accurate quantitation is achieved by performing multiple replicates at serial dilutions of the material(s) to be assayed. At the limit of dilution, where some end points are positive and some are negative, the number of targets present can be calculated using Poisson statistics from the

Table 2. Summary of methods that specifically detect rDNA products produced by GM foods[a–c]

Parameter	Protein-based			DNA-based			
	Western blot	ELISA	Lateral flow strip	Southern blot	Qualitative PCR[d]	QC-PCR and limiting dilution	Real-time PCR
Ease of use	Difficult	Moderate	Simple	Difficult	Difficult	Difficult	Difficult
Needs special equipment	Yes	Yes	No	Yes	Yes	Yes	Yes
Sensitivity	High	High	High	Moderate	Very high	High	High
Duration[e]	2 d	30–90 min	10 min	6 h[f]	1.5 d	2 d	1 d
Cost/sample (US$)	150	5	2	150	250	350	450
Gives quantitative results	No	Yes[g]	No	No	No	Yes	Yes[h]
Suitable for field test	No	Yes[g]	Yes	No	No	No	No
Employed mainly in	Academic labs	Test facility	Field testing	Academic labs	Test facility	Test facility	Test facility

[a]Abbreviations: ELISA, enzyme-linked immunosorbant assay; GM, genetically modified; QC-PCR, quantitative-competitive PCR; rDNA, recombinant deoxyribonucleic acid. [b]Modified from [52]. [c]Near infra-red detects structural changes (not DNA or protein), is fast (<1 min) and inexpensive (~US$1); [d]Including nested PCR and GMO Chip; [e]Excluding time allotted for sample preparation; [f]When nonradioactive probes are used; otherwise 30 h with ^{32}P-labeled probes; [g]As in the antibody-coated tube format; [h]With high precision.

proportion of negative end points [50]. An advantage of this method is that coamplification of added reporter DNA is not required. However, caution should be exercised because of potential contamination of PCR reactions owing to various dilutions and manipulations [50].

NIR spectroscopy

NIR transmittance spectroscopy has been used by grain handlers in elevators in most of the world for nondestructive analysis of whole grains for the prediction of moisture, protein, oil, fiber and starch. Recently, the technique has been used in attempts to distinguish RRS from conventional soybean [51]. In this study, spectral scans were taken from three Infratec 1220 spectrometers where whole-grain samples flow through a fixed path length. Locally Weighed Regression using a database of ~8000 samples was 93% accurate for distinguishing RRS from unmodified soy. The advantages of this technique are: (1) it is fast (<1 min), (2) sample preparation is not necessary because it uses whole kernels (~300 g), which are dropped into measurement cells or flow through the system, and (3) it is therefore cheap. The major disadvantage is that it does not identify compounds, thereby necessitating a large set of samples to generate spectra. This calibration dataset is then used to predict the GMO event. Thus, this method cannot be more accurate than the reference method used to build the model. Moreover, a calibration needs to be developed for each GMO to be predicted. Furthermore, although NIR is sensitive to major organic compounds (e.g. vibration overtones of C–H, O–H and N–H), its accuracy is limited. For example, with respect to GMOs, it does not detect a change in DNA or a single protein, but much larger unknown structural changes, such as those linked to the parietal portion of the seed (e.g. lignin or cellulose) that are introduced by the presence of the new DNA. The procedures for detecting GMOs in foods described here are presented in Table 2.

Conclusions

To respond to EU regulations, a tiered approach might be employed, starting with qualitative PCR for GMO detection. If no GMOs are detected with a validated qualitative method, the product(s) would be evaluated for the presence of protein. If no protein is detected, the product is presumed not detectable. If the qualitative PCR shows a positive result, the product is considered as 'non-approved GMO', and a validated Q-PCR is used to detect the level of GMO. If the GMO level is above an established threshold, the product is labeled as 'non-approved GMO', but if below the threshold, the product need not be labeled [13]. The high sensitivity and specificity of Q-PCR methods and their flexibility with different food matrices make them suitable for detecting GMOs at low thresholds in various foods.

The greatest uncertainty of using DNA-based assays, as for protein-based methods, is that not all products derived from GM foods (e.g. refined oil) contain enough DNA. In addition, heating and other processes associated with food production can degrade DNA. Similarly, if GMO is expressed on a relative basis (i.e. % GMO), it is important to know whether the estimate is to be based on total DNA from all sources, or on the basis of analyzed product DNA. This approach known as 'genetic equivalence', which is sound and pragmatic, was correlated with results of studies where GMO content was expressed as a percentage of mass [13]. Q-PCR might best be applied at the early stages in the food production chain. Using the genome equivalent approach to assess the GMO content of food ingredients and tracking the ingredients used should allow for accurate estimation of GMOs. This approach is also consistent with current EU food-labeling regulations that focus on ingredients, and is also applicable to finished products containing more than one GMO-derived ingredient [13].

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