American Crop Protection Association

Methods for Detection of GMO Grain in Commerce

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Prepared by Kimberly Magin, Charles Mihaliak, Ph.D., Larry Somerville Ph.D., Laura Privalle, Ph.D., Stacy Charlton, and Leah Porter, Ph.D. for the Diagnostic Testing Work Group of the American Crop Protection Association

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Executive Summary

There are two scientific methods in general commercial use today for detecting genetic modification in field crops such as soybeans, corn, cotton and others. One method involves testing for specific proteins that have been incorporated through biotechnology into a crop, the other is based on the detection of DNA sequences inserted in the crop. Each method has two specific techniques: for proteins, ELISA (Enzyme Linked Immunosorbant Assay) and Lateral Flow Strip; PCR (Polymerase Chain Reaction) for DNA.

A number of companies have developed and are now selling kits for use by grain elevator operators and others to identify genetic modification in soybeans, corn, cotton and other crops. It is important to understand that these kits are designed to detect *specific proteins or specific DNA sequences in specific crop varieties.*

Currently, there is no single, rapid or inexpensive test to verify whether a crop or crop sample is free of genetic modification, or "GMO-free." Before purchasing a commercial test kit, it is best to know the specific crop to be tested, for example, an insect resistant corn variety.

The following chart summarizes the methods to test for genetic modification, their cost, duration, ease-of-use and expected results.

METHOD	TESTS FOR	COST/SAMPLE ($U.S.)	DURATION	EASE OF USE	RESULTS
ELISA	Protein	$75-100[a]	2-4 days[d]	Moderate; requires familiarity with laboratory practices and some specialized equipment	Quantitative measurement of protein levels
Lateral Flow Strip	Protein	$8-10[b]	10-20 minutes	Little training and no sophisticated lab equipment required;	Qualitative measurement of presence of absence of target protein analyte
PCR	DNA	$200-600[ac]	5-14 days[d]	Difficult; requires specialized equipment and training	Qualitative/semi-quantitative measure ment of levels of target DNA sequence

[a] *Estimated cost of analysis performed by a commercial testing laboratory*

[b] *Estimate cost of material to perform on-site analysis, excluding labor*

[c] *Prices for PCR tests vary widely and depend on the nature of the test being performed (e.g., screening versus quantitative assessment)*

[d] *Estimate of time required to have samples analyzed by a commercial testing laboratory*

Purpose of this document

This document describes currently available methods for detection of genetically modified (GM) DNA and novel protein expression in genetically modified crops and ingredients derived from these crops. Additionally, the inherent advantages and limitations will be addressed for each method.

Background

Genetically modified (GM) crops currently available in the marketplace have passed rigorous safety assessments in numerous countries around the world, and have been deemed substantially equivalent to conventional grain. However, some food and feed manufacturers choose to use non-GMO commodities to meet the demands of certain niche markets for non-GMO products, and require testing for compliance with the EU mandatory labeling laws.

GM products differ from their non-genetically modified counterpart in that in addition to the tens of thousands of genes and proteins regularly found in the plant, there is an additional trait encoded by an introduced gene(s). The gene(s) is made of DNA and is able to produce an additional protein(s) that confers the trait of interest. The amount of DNA inserted is extremely small compared to the size of the host plant DNA. Typically, the introduced gene(s) come from species that do not generally exchange genetic material with the crop species in question. These genes confer agronomically beneficial traits such as herbicide tolerance or insect protection. In the case of most GM plants on the market today, the novel DNA encodes information used by the plant cells to construct a novel protein such as an enzyme. It is the novel protein that is actually directly involved in effecting the novel trait of the GM plant. Grain derived from genetically modified (GM) crops may be identified by testing for the presence of the introduced DNA (DNA-based testing) or by detection of the expressed novel protein (protein-based testing) encoded by the introduced genetic material.

Types of GMO Testing

GM trait testing typically involves analysis of either the introduced DNA or protein:

- Currently, the most common DNA-based detection method in use today is Polymerase Chain Reaction (PCR). This sensitive DNA method can detect specific sequence(s) within the introduced DNA. While this method provides a reliable qualitative assessment of introduced DNA sequence(s), it does not provide an easily performed quantitative determination of percent GM grain in a given sample.

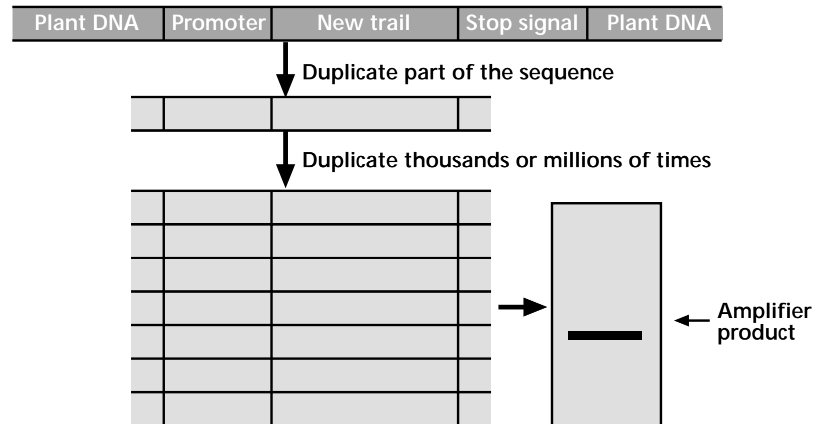
- Protein-based testing is most commonly via an Enzyme Linked Immunosorbant Assay (ELISA), or by immobilized antibody detection technology such as lateral flow strips. Both of these methods detect specific proteins, but only the ELISA, in conjunction with a standard using the protein of interest, can provide quantitation of the protein.

DNA and Protein Detection Methods: An Overview

DNA-based: DNA test methods rely on the complementary specificity of the two strands that form the double helix of double stranded DNA. The two complementary DNA strands anneal or hybridize in a sequence specific manner; this specificity is exploited in the detection process.

Polymerase Chain Reaction (PCR): PCR also exploits the specificity of DNA strands combined with the synthetic enzyme that catalyzes DNA synthesis, known as DNA polymerase. PCR allows the selective amplification of specific segments of DNA occurring at low frequency in a complex mixture of other DNA sequences. In PCR, the small complimentary DNA pieces are referred to as primers and are used in pairs. These primers are designed to hybridize on opposite strands of the gene of interest, such that through a series of repetitive cycles a specific DNA polymerase replicates and amplifies the sequence between the primers **(Figure1).** These amplified pieces are subjected to electrophoresis so their size and presence can be detected.

Figure 1. Diagram of the new trait and the PCR reaction



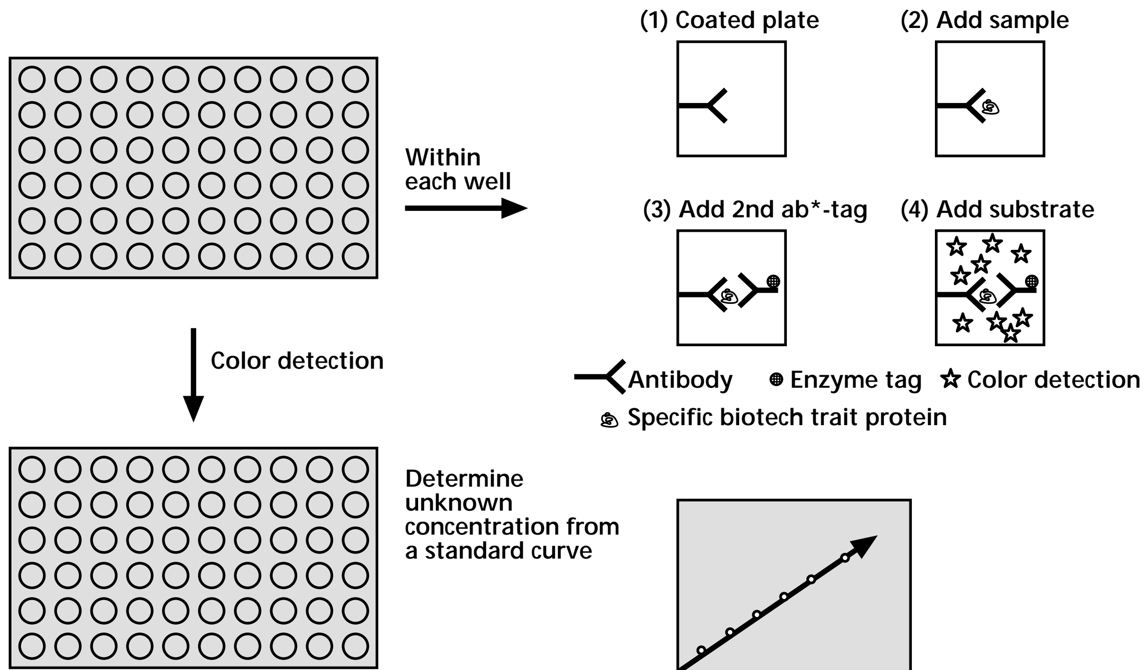
The DNA which has been introduced into the crop consists of three elements, the promoter, the gene of interest and the stop signal for the gene. PCR can be used to detect any of these elements, but care must be exercised to focus on the gene of interest if the other elements are endogenous to the plant. The promoter regulates when and in what plant part the gene of interest is expressed. The stop signal is a signal found at the end of the gene of interest. PCR uses small complementary sequences of DNA as primers to bind to the targeted DNA sequence. These primers are designed to hybridize on opposite strands of the DNA such that through a series of cycles a specific DNA polymerase replicates and amplifies the sequence between the primers. The resulting amplified products are then separated by an agarose gel using electrophoresis, which separates products based on their size and charge. The gel is stained with a fluorescent dye (ethidium bromide). Detection is performed by viewing the gel under ultraviolet light to view the fluorescent dyed DNA, which appears as a band on the gel. Tests can usually be completed in one to three days, depending on the difficulty and requirements of the test.

PCR is a laboratory - based technique, requiring a trained staff and specialized equipment. Some of the key characteristics of PCR diagnostics are as follows:

- Can be extremely sensitive, capable of detecting one or a few copies of the gene within an entire organisms' genetic material, or genome. As a result of this high sensitivity, very low levels of inadvertent contamination can result in false positives. Therefore, great care must be taken to prevent cross contamination.
- Require little reagent development time compared to immunological assays (primer synthesis vs. antibody production).
- Nearly all required reagents are commercially available and can be readily accessed from a number of sources. However, some of these require a license for use in diagnostic applications.
- Sample analysis time requires approximately one day and can take 1-3 days or longer to receive results from a testing lab. Per sample costs can range from less $100 to greater than $300.
- PCR methods have not been as broadly validated as ELISAs, but appear to be the current method of choice in Europe.
- PCR is sometimes capable of discriminating between different types of genetic modification (also referred to as transgenic events) if properly developed. Diagnostic methods for identifying specific transgenic events require additional development time and validation efforts.

Protein-based methods: The common protein based test methods use antibodies specific for the protein of interest. ELISAs detect or measure the amount of the protein of interest in a sample that may contain numerous other dissimilar proteins. ELISAs use one antibody to bind the specific protein, a second antibody to amplify detection (optional), and an antibody-conjugated to an enzyme whose product generates a color that can be easily visualized and quantified based on comparison of a standard curve of the protein of interest **(Figure 2).** Non quantitative 96-well formatted procedures are also options.

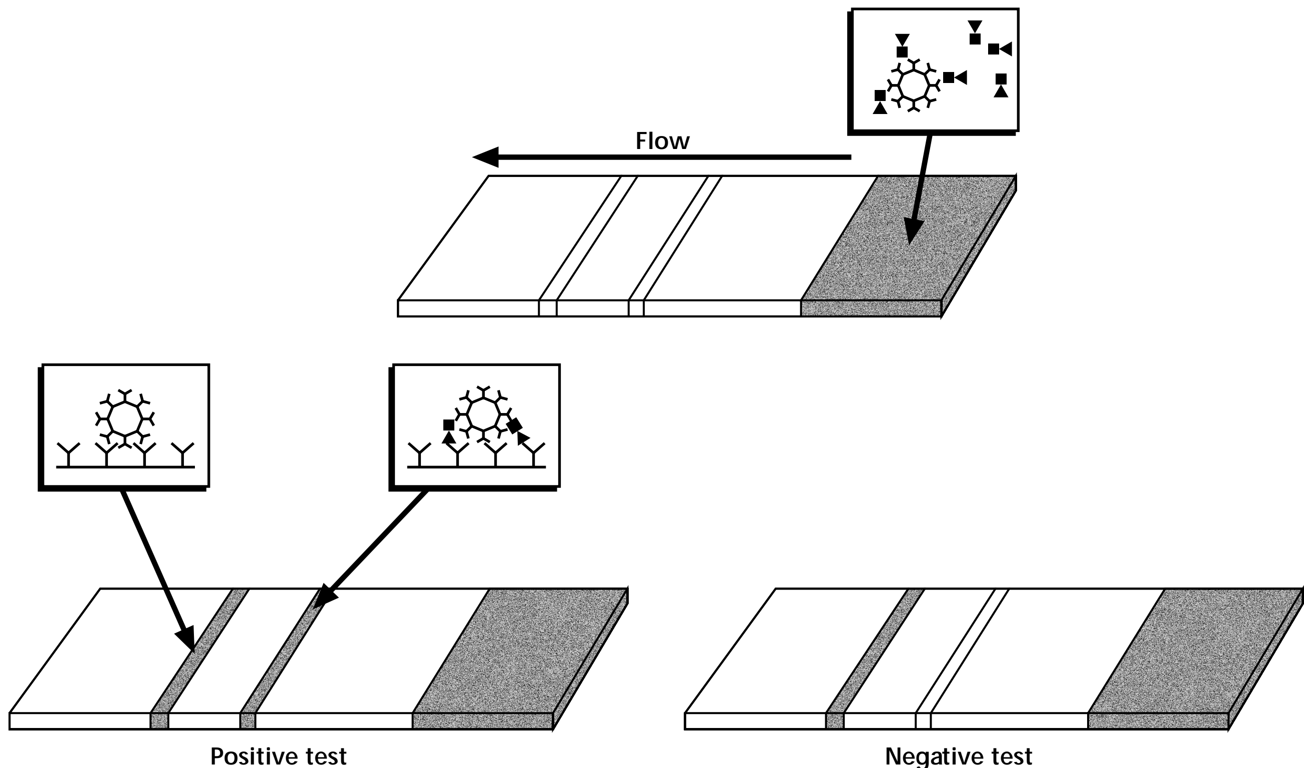
Figure 2. Schematic of an ELISA.



In an ELISA, antibodies specific for the genetically modified (GM or biotech trait) protein of interest are added and bound to each well of the plastic ELISA plate. After a wash step to remove unbound antibody, sample extracts containing the protein of interest, standards of known concentrations and controls are added, followed by the addition of a second antibody specific for the biotech protein trait linked to a color tag for detection. To increase the sensitivity of the ELISA, an untagged antibody specific to the protein of interest, but generated in a different animal than the primary antibody may be used. Incubation times are critical and washing between all steps is necessary. If the specific protein of interest is present, it will be bound to the first antibody and the second antibody/tag. A substrate solution is added to develop the plate. A colored product is formed when the protein of interest is present. The plate may be visually inspected for color intensity or read using a spectrophotometer to generate exact color intensity readings. The amount of the protein of interest can be determined by estimation against a standard curve. The time to perform the test is 2 - 8 hours, including sample preparation time.

As an alternative to plate or lab based methods, field portable methods are also available. An example is the lateral flow strips, which use a combination of immobilized antibody to capture the protein of interest and a conjugated antibody that is easily detected. **(Figure 3).** This method is qualitative only.

**Figure 3. Schematic of lateral flow strip qualitative test.
Two Site Double Antibody Format**



A lateral flow strip test is made of a nitrocellulose strip to which a capture antibody is bound. A detection antibody linked to gold or latex particle is also applied and dried on the strip. A sample extract containing the protein of interest is added to the top of the strip. There is an absorbent pad at the opposite end of the strip, which draws the sample to the end of the strip. The capture antibody is dried near the end close to the absorbent pad. As the sample and the detection antibody move toward the other end of the strip, the protein of interest is bound by both the capture antibody and the detection antibody. A colored band will form where the detection antibody accumulates. The presence of two bands indicates a positive test for the protein of interest. A single band indicates that the test was performed correctly, but that there was no protein of interest present in the sample. Each strip can only test a single sample and establish presence or absence of the protein of interest. This test takes approximately 5-15 minutes to perform.

Similar to PCR, conducting ELISAs requires trained personnel and specialized equipment. Key characteristics of ELISA evaluations include:

- Less sensitive than PCR, therefore, less susceptible than PCR to 'false positives' caused by minor levels of contamination.
- High up-front costs for assay development and generation of antibodies and protein standards.
- Low per sample cost once reagents are developed.
- Cannot discriminate different expression patterns and modes among different transgenic events that express similar protein characteristics.
- Protein based methods require significant lead-time for reagent and method development. Protein-based testing provides a practical and effective testing process when a detectable protein is produced. However, there are genetically modified products that do not express a detectable level of protein.

Sampling of Genetically Modified Products

Sample preparation for both DNA-based and protein-based methods is critical for detection and/or quantification. It is important to know the limitations of each procedure as well as the question (e.g., qualitative or quantitative) of interest. Both the sample size and sampling procedures dramatically impact the conclusions that may be drawn from any of these testing methods.

Validation

Method validation is critical to ensure that the test detects the specific introduced DNA or protein and only these components. Ideally, each method should be performed in numerous laboratories to demonstrate reproducible, sensitive and specific results. Currently, only an ELISA and PCR method for Roundup Ready®, soybean and a PCR for Maximizer corn (BT176) have been validated for commercial testing of grain. This validation was performed by the European Union's Joint Research Centre. Also, a PCR method for Bt11 has been developed at the request of the Swiss authorities at the 'Kantonales Laboratorium' in Basel-Stadt. Validation of both DNA and protein based methods, as well as sampling techniques, need to be performed for other genetically modified plant products.

Finally, neither DNA nor protein-based methods are able to definitively establish that a grain shipment is GM-free. Multiple tests (and sampling) would have to be performed and a certification program followed to assure that the planted seed was not derived from a genetically modified source, was grown under conditions to minimize contamination by GM-pollen, grain harvested and transported in vehicles free from GM grain. Since inadvertent contamination of non-GM seed is likely, threshold levels of genetically modified products in non-genetically modified samples must be established to enable reasonable implementation of these testing methods. However, this requires that methods applied must be quantitative and be able to assure compliance with these yet to be established threshold values.

Available Tests and Current Uses

Life science and seed companies developing genetically modified plant products develop and utilize these test methods in their own laboratories during 1) new trait development, 2) characterization and regulatory safety assessments for submission to global regulatory agencies, and 3) during the breeding and seed quality programs needed for commercial production.

Commercially, most diagnostic PCR and ELISA testing is being conducted by seed improvement laboratories and other private and university laboratories around the world. The application of lateral flow strips or other field based testing of whole grain is relatively new, and kits have recently become commercially available by diagnostic kit manufacturers.

Additional References

Summary Report of a Workshop on Detection Methods for Novel Foods derived from Genetically Modified Organisms, International Life Sciences Institute (ILSI) Europe Report Series, April 1999

NGFA White Paper On Agricultural Biotechnology, National Grain and Feed Association, October 1999.

1156 Fifteenth Street, N.W.
Suite 400
Washington D.C. 20005
Ph: 202.296.1585 Fax: 202.463.0474
www.acpa.org