FEATURE

- The regulations defining GMO ingredient thresholds and the required testing methodologies to detect them vary worldwide.
- Both DNA- and protein-based technologies are utilized to evaluate GM materials. Protein assays are typically applied during the early stages along the pathway. This paper focuses on a protein analysis methodology that utilizes an antibody to interact with a specific antigen (GM protein) to yield a discernible result.
- Because many regulations do not take into consideration the limitations of the current analytical technologies, it is often difficult to obtain consistent results across different laboratories.
- Reliable reference materials and standardized testing procedures resulting from validated methods will enable the industry to better rely on results obtained from different laboratories.

Detecting and Quantifying GMOs in Grain Using Protein Methods

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A number of fundamental aspects must be considered when applying immunoassays (protein-based methodology) to the detection of genetically modified organisms (GMOs) in grain. Many factors such as genetics and the environment, processing, and biological and statistical errors affect the end result. The most commonly used methodology includes lateral flow devices (LFD) and enzyme-linked immunosorbent assays (ELISA). The need for regulations that consider the critical aspects involved in the accurate detection of GMOs is a necessity to ensure reliable results across different laboratories and to create stability in the global marketplace. Recently, a consortium of experts (1) dedicated to the goal of achieving analytical excellence in the field of biotechnology published a manuscript in the *Journal of AOAC International* (2) that details the use of protein-based GMO test methods for the agricultural biotechnology industry. The document highlights the many areas to which attention must be paid in order to produce reliable test results.

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The following article provides an introduction to the topic and a summary of some of the salient issues.

Advances in molecular biotechnology techniques have made it possible for agricultural scientists to quickly improve crop characteristics, such as yield, pest resistance, and herbicide tolerance, through the direct manipulation of the plant's genome. The products of this technology are commonly known as genetically modified organisms or GMOs.

For the four key global crops (i.e., corn, soy, cotton, and canola), the adoption rate for GM varieties is nearly 30%. However, GMOs haven't been uniformly accepted and that has led to mandatory labeling requirements in many countries for foods that contain more than a specific percentage of genetically modified material (%GM). Unfortunately, the regulations defining ingredient thresholds and the required testing methodologies to detect them vary worldwide.

Both DNA- and protein-based technologies are utilized to evaluate GM materials. However, protein assays, such as LFD and ELISA, are typically applied during the early stages along the pathway, including during product development in the seed, grain, food, and feed supply chain, and at the farm level. While DNA-based technology has recently been reviewed in detail (3), this paper focuses on a protein analysis methodology that utilizes an antibody to interact with a specific antigen (GM protein) to yield a discernible result.

Protein expression is dependent upon a number of factors, both genetic and environmental. One of the genetic factors affecting the amount of protein present in corn is whether the protein expression is derived from a homozygous or heterozygous set of alleles. If two copies of the gene are present, homozygous expression can lead to a twofold difference in the protein quantity expressed compared to heterozygous expression, where only a single copy of the gene is present. Additionally, environmental factors such as climate and soil composition can also affect the protein expression in a plant. However, the authors of this manuscript explain that protein tests can accommodate this genetic and environmental variability and provide highly accurate, semi-quantitative results on raw materials. This allows grain handlers to comply with regulatory thresholds cost-effectively and with a high degree of confidence.

Processing to create oil, starch, corn syrup, and feed products impacts the quantity and structure of the protein. While protein tests are typically applied to minimally processed food, they can also be applied to processed food matrices if changes in protein quantity and structure are adequately accounted for in the methodology. However, the authors stress that methods for a given processed food matrix need to be validated.

Dependable results throughout different stages of food processing are achieved by carefully considering a number of factors, such as properly evaluating the method's capabilities and limitations, analyzing food matrices and their influences on results on a case-by-case basis, ensuring that the analytical method is used as directed, and working with regulators to ensure that subsequent regulations reflect these factors.

Because many regulations do not take into consideration the limitations of the current analytical technologies, it is often difficult to obtain consistent results across different laboratories, as little room remains to accommodate a reasonable degree of statistical error. As a result, the authors suggest that it is challenging to establish a standardized testing procedure for the detection of GM proteins in grains. In fact, while many countries set their thresholds for unapproved GM events at levels where proteins may occur in only trace amounts, others have gone so far as to enforce a zero-tolerance policy. Unfortunately, the only way to ensure a 100% absence of GMO materials in a batch is to test the entire batch of material. Not only would this be a massive undertaking, but doing so would also destroy the shipment. This is not a viable option. These factors not only complicate product compliance protocols, but also have enormous implications for the global grains market.

"Method validation" is a well-known scientific process whereby a method undergoes a thorough examination of its capabilities using clearly defined protocols. Validated methods have been incorporated in several standards of analysis for different analytical fields and are adopted worldwide. Globally, there are numerous organizations that regulate GMO technology. Two examples in the United States are the Environmental Protection Agency (EPA), which regulates the insecticides expressed by GM plants, and the U.S. Department of Agriculture–Grain Inspection, Packers, and Stockyards Administration (USDA/GIPSA), which offers a proficiency program to enable GMO testing laboratories to assess their in-house capabilities and a performance testing program to validate methodology claims. Furthermore, there are a number of standards developed by organizations such as ISO, IUPAC, AOAC International, and the Clinical and Laboratory Standards Institute that provide protocols for method validation. For general considerations of GM testing methodology, Codex has established guidelines and ISO and CEN have established specific international standards.

To combat the inconsistencies found between laboratory results due to a lack of standardized testing procedures, method validation provides a key to creating a more reliable system.

Reference materials play a crucial role at many different points in the testing process. They are most commonly used in assay development, validation, diagnostic troubleshooting, and in the routine application of assays. The manuscript authors explain that positive reference materials help establish the accuracy, precision, sensitivity, LOD, and the false negative rate in quantitative assay validations. Negative reference materials are very important in determining the false positive rates and the specificity of the test method. The manuscript explores the different qualities of available reference standards and discusses when each is applicable. There are a number of organizations throughout the United States, Europe, and Japan that provide these standards for testing purposes. Due to the broad impact of reference materials at many different points, it is vital that they be useful and reliable.

Reliable reference materials and standardized testing procedures resulting from validated methods will enable the industry to better rely on results obtained from different laboratories. Improvements in this area will also enable those testing for GM proteins throughout the food and feed supply chain to gain additional confidence in the results.

Though often overlooked, sampling procedures can also have a dramatic impact on accuracy. While one can estimate the approximate deviation of a sample from the lot, it is only useful when the sample being tested is representative of the lot tested. Additionally, a measurement device can typically accommodate only a small quantity of a much larger lot, so attention must be paid to design appropriate sampling plans that minimize errors and maximize the accuracy of results.

Besides elaborating on the issues described above, the authors also discuss biological and analytical sources of error, matrix effects in assay development and application, and the qualitative and quantitative method validation criteria as well as explain the different levels at which a method can be validated. This manuscript offers the reader the opportunity to understand the extent to which the issues surrounding biotechnology and the accompanying protein-based methodology impact the many different stakeholders along the way and confers the knowledge that when used according to specifications, immunoassays have been repeatedly shown to be fast, reliable, and economic test methods in the field of agricultural biotechnology.

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