Advancing Peanut Industry Goals: The Value of Fit-for-Purpose Aflatoxin Testing

INTRODUCTION

The peanut businesses at every stage of the value chain, the ability to instill confidence in the safety and quality of their products frequently hinges on the strength of their aflatoxin test data. Information on aflatoxin levels plays a crucial role in far-reaching decisions by peanut industry members and other food safety stakeholders ranging from peanut growers, processors, and manufacturers to testing laboratories and government regulators. To ensure the accuracy and reliability of that information, the analytic tools and procedures that generate it must be specifically designed to simplify and resolve the intrinsic complexity of determining aflatoxin levels in peanuts.

Aflatoxin Regulations: The Public Health and Economic Implications of Compliance

One of the key factors complicating this task is the stringency of current aflatoxin regulations. The parts-per-billion (ppb) limits that prevail in more than 100 countries around the globe arose in response to substantial scientific evidence that aflatoxins pose serious risks to human and animal health. Susceptibility to those risks is greatest among humans and animals that are very young, undernourished, or immune-compromised and varies in mature animals depending on their species, gender, and breed. The most common form of aflatoxin, AFB_1, is of particular concern. Classified as a Group 1 carcinogen by the International Agency for Research on Cancer (IARC), AFB_1 is also linked to immune suppression and liver and kidney damage. Because these toxins can occur in any peanut-derived raw material or finished product – such as peanut hulls, peanut oil or peanut butter - the issues surrounding aflatoxin control affect every segment in the peanut industry value stream.

Despite international recognition of the need to limit aflatoxin concentrations in food and feed, the breadth and stringency of the restrictions imposed on peanut products in major peanut-growing regions and import markets vary considerably. (See Table 1.) Maximum allowable levels for peanuts intended for human consumption range downward from India's limit of 30 ppb of total aflatoxins (the sum of aflatoxins B_1, B_2, G_1, and G_2) to the EU's limit of 2 ppb for aflatoxin B_1. In addition to mandating the lowest limits, EU regulations incorporate the most detailed requirements, prescribing separate, somewhat less restrictive limits for total aflatoxins as well as for peanuts slated for processing. In the U.S., the FDA maintains an action level (AL)* of 20 ppb for total aflatoxins in peanuts marketed for human consumption; however, the industry voluntarily applies a limit of 15 ppb to these products. While separate standards for feedstuffs are in place in most developed nations, with some defining specific limits for peanut-derived ingredients, such regulations are rare in developing and transitional countries. These disparities reflect differences among nations in the many considerations that factor into regulatory philosophy, including economic and technology resources, trade concerns, risk perceptions, and patterns of food consumption.

**The level that allows for removal of noncompliant lots from the marketplace.*

Table 1: Aflatoxin regulations for peanut products in the four leading peanut-producing nations and import markets

Leading Peanut Producers*[1]	Food Limits (Total AFs)[2],[3]	Feed Limits (Total AFs)[4]	Largest Import Markets*[5],[6]	Food Limits (Total AFs/B_1)[7],[2]	Feed Limits (Total AFs)†[8]
China (41%)	20 ppb in peanut products for local consumption		Europe ($1.7 billion)	2 ppb of B_1 and 4 ppb of total aflatoxins in peanuts for direct consumption; 8 ppb of B_1 and 15 ppb of total aflatoxins in peanuts destined for sorting or other physical treatment or for use as a food ingredient	100 ppb in peanut-derived feed for breeding cattle and swine and mature poultry; 200 ppb for finishing swine (≥100 lb.); 300 ppb for finishing beef cattle
India (14%)	30 ppb		Indonesia ($345 million)	20 ppb	
Nigeria (7%)	20 ppb		Mexico ($176 million)	20 ppb	
United States (7%)	15 ppb (industry standard)/20 ppb FDA AL) in peanut products for direct human consumption	20 ppb in peanut-derived feed for dogs and cats and immature livestock; 100 pbb for breeding livestock and mature poultry; 200 ppb for finishing swine (≥100 lb.); 300 ppb for finishing beef cattle	Canada ($128 million)	15 ppb	

** Listed in order of production share in 2018.*

** Listed in order of market value in 2018. The Netherlands, Germany, the U.K., and Poland account for $479M, $208M, $131M, and $95M, respectively of the total market value of European imports.[6]*

† Feed limits for peanut-derived ingredients set by U.K. food safety legislation prior to its exit from the EU. Feed limits set by EU apply to complete feed complementary feed rather specific ingredients and range from 5 to 20 ppb for AFB1.[9]

THE IMPORTANCE OF TESTING THAT MEETS THE TOUGHEST PERFORMANCE DEMANDS

For exporters from the four leading growing regions, the realities of today's international trade scene have raised the performance bar for aflatoxin testing far beyond the levels required for documenting compliance with domestic regulations. Currently the world's largest importer of peanuts, as well as its most strictly regulated trade region, Europe presents exporters with a highly alluring yet challenging market. In 2018, that market reached a value of €1.6 billion (euros)[5] ($1,731,200,000 USD) (see Table 1), derived mainly from peanuts sold as snacks and food ingredients.

Shipments from exporters seeking a meaningful share of these revenues may be randomly selected for aflatoxin testing on arrival at the border of the importing country. Those that test above the region's stringent legal limits are reported to the EU's Rapid Alert System for Food and Feed (RASFF) an information-sharing network that publicizes regulatory violations across all member states, effectively barring their entry into any EU nation.

Exporters in this situation have to either dispose of the noncompliant shipment at their own expense or redirect it to a market with less exacting regulations. The costs of the latter alternative include not only shipping charges as high as $4,000 per container but also the loss incurred from selling the products at a steep discount. Fees for warehousing the rejected consignment while the exporter searches for another buyer also figure into the tally. If the terms of the fallback deal require the exporter to pay for processing the peanuts to reduce their aflatoxin content, the sum total will increase accordingly. Failed inspections may entail long-term consequences as well. Future shipments from exporters cited by the RASFF often face increased scrutiny from regulators and may be viewed in a negative light by buyers. The fallout from the compliance failure lands on the import side, too, with buyers left scrambling for a new supplier.

Within this high-stakes trade arena, a solid market position requires a standardized approach to pre-export testing that lowers the limits of detection and measurement while raising the level of certainty attributed to the results.

THE NEED FOR A TEST PROCESS THAT SYSTEMATICALLY ADDRESSES SOURCES OF ERROR

To satisfy those requirements, the test process must account for not only the challenges of ultra-trace analysis, but also the complications presented by the makeup of the test sample. Peanuts comprise a variety of substances such as proteins and oils that can interfere with the accuracy of testing, increasing the chances of false positives and false negative results. Test samples that consist of materials that were improperly selected or prepared are even more likely to yield overestimates and underestimates of aflatoxin levels.

The significant potential for error that emerges from these testing complexities entails equally significant risks for buyers and sellers in every peanut industry sector:

False Positives	False Negatives
Sellers: Lose out on the true value of a compliant lot, including access to lucrative markets; pay for unnecessary aflatoxin-reduction treatments	**Sellers:** Lose trust of buyers; may face costly consequences if contaminated peanut products go to market and their consumption leads to negative health effects
Buyers: Reject clean lot, needlessly disrupting supply chain and the flow of goods required to meet customer demand	**Buyers:** Unknowingly blend contaminated peanuts with clean stock, damaging raw materials, potentially leading to the production of contaminated food or feed

Efforts to limit these risks figure prominently in the USDA-approved process of grading peanut lots intended for sale in the U.S. This methodical, end-to-end to process involves sampling peanut lots at designated points in the production chain in order to screen them in USDA-approved laboratories for compliance with industry-standard aflatoxin limits. USDA rules specify three possible options for noncompliant lots: (1) They may be destroyed; (2) reconditioned at the owner's expense to reduce aflatoxin content to an acceptable level; or (3) restricted to the manufacture of oil, feed ingredients, and industrial materials, excluding them from the much more profitable production of food items like snack peanuts and peanut butter.

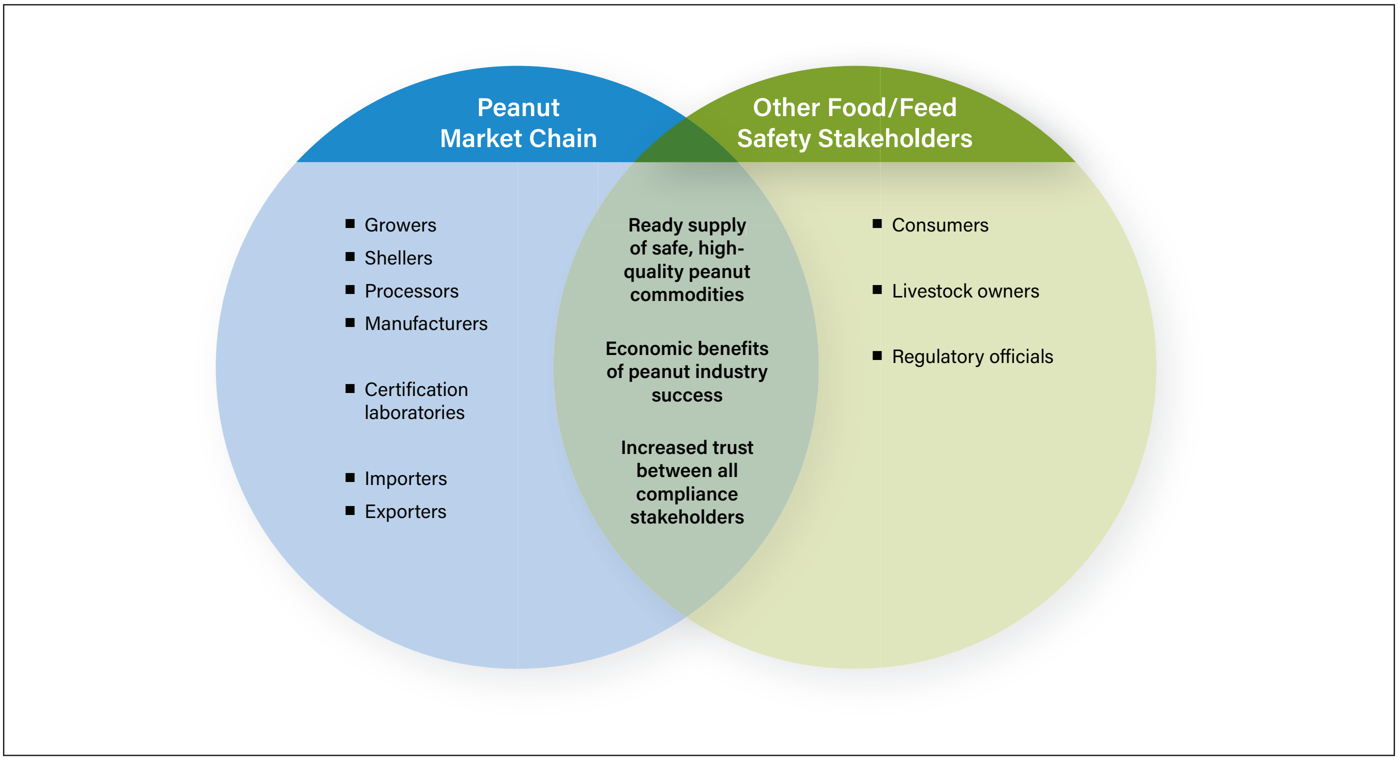
To ensure the integrity of the test process that determines the compliance status of peanut products, the USDA sets strict requirements for the laboratories that participate in its aflatoxin testing program. Chief among these is the use of USDA-approved test methods and sampling protocols. One of the most widely used of these approved test methods is AOAC Official Method 991.31.

AOAC VALIDATION: THE BENEFITS OF TESTING GROUNDED IN RECOGNIZED SCIENTIFIC EXPERTISE

AOAC Official Method 991.31 is an application of the AflaTest immunoassay that its developer, VICAM, A Waters Business, specifically adapted to the challenges of accurately and reliably estimating the aflatoxin concentration of peanut products. The fruit of a collaboration between the company's scientific team and the AOAC International, this official method of analysis reflects the highest standards of technical excellence. An independent, not-for-profit scientific organization dedicated to fostering "worldwide confidence in analytical results," the AOAC reserves the "Official Method" designation for test methods that have been extensively vetted by unbiased experts in analytic chemistry. After an independent laboratory confirms that the method's performance characteristics meet the demands of its intended purpose, the AOAC runs a collaborative study to verify that the method delivers consistent results across a range of different laboratory conditions. This rigorous third-party validation process offers peanut industry stakeholders strong reassurance that the aflatoxin data obtained with the AflaTest method affords the degree of certainty required for its use in highly consequential decision-making.

Scientifically sound, actionable test results are the linchpin of aflatoxin surveillance outcomes that benefit domestic buyers and sellers, exporters, importers, and consumers alike:

- Earlier detection of contamination
- Fewer misclassified lots
- Smoother import and export processes
- Reduced risk of supply chain disruption
- Increased credibility with regulators, business partners, and customers
- Timely identification and elimination of potential sources of contamination at critical control points
- Improved INSIGHT into effectiveness of aflatoxin control efforts
- Safe, high-quality products



The Benefits of a Test Process That Generates Accurate Aflatoxin Data

HOW THE AFLATEST METHOD RESOLVES TESTING CHALLENGES

VICAM's AOAC-approved AflaTest method couples the company's high-performance immunoaffinity (IA) columns with fluorescence detection technology that capitalizes on the natural fluorescence of mycotoxins to detect and measure aflatoxins at levels as low as 2 ppb. The detection instrument paired with the columns can take the form of a portable fluorometer or an HPLC system with a fluorescence detector. The fluorometric version of the method requires no special training to deliver accurate results in less than 15 minutes. Its ease of use and affordability make this version a practical option for laboratories with limited resources as well as for in-house quality control inspectors who need rapid on-site access to reliable decision support data. The widespread adoption of the HPLC version in European reference laboratories defines it as the method of choice for achieving levels of sensitivity and precision that satisfy the performance criteria established by European authorities.

The company's global sales manager, Lanny Smith, calls AflaTest the gold standard for peanut industry testing. "It offers a full-scale safety system that extends from fast, accurate fluorometric testing across every critical control point to a definitive HPLC analysis of the aflatoxin levels in commodities destined for the most tightly regulated markets."

AflaTest IA columns help minimize the uncertainty entailed in aflatoxin testing by optimizing sample cleanup. The stationary beads inside the column are treated with ultra sensitive and selective monoclonal antibodies* that strongly bind with the aflatoxins in the sample, immobilizing them so that matrix impurities can be flushed with water. To complete the process, the user adds methanol to the column to detach the aflatoxins from the antibodies, collects the eluate, and mixes it with water. The high recovery rates achieved with this method, as well as its efficiency in removing matrix components that might interact with the antibodies in the column, minimize the risk of false positive and false negative test results. No additional steps are required to ensure the purity of the sample. The simplicity of this cleanup approach decreases the potential for human error and promotes fast workflows while maintaining the quality of test results.

**See text box.*

Monoclonal Antibody Technology: A Breakthrough in Testing Capabilities

The development of monoclonal antibody technology in the 1970s is widely recognized as a milestone in scientific progress toward ever more sensitive and specific diagnostic tests. Engineered in laboratories, monoclonal antibodies (MAbs) can only bind to a single site on the antigen of interest. This characteristic of MAbs optimizes their usefulness for the detection and purification of target analytes, making them powerful tools for identifying and isolating proteins, hormones, toxins, and other molecules of interest to a broad range of laboratory scientists, including biomedical researchers, drug developers, toxicologists, and analytic chemists. In 1986, VICAM secured one of the first patents for the use of monoclonal antibodies in an aflatoxin test kit, marking the development of its flagship AflaTest immunoassay as a significant innovation.

BUILDING SUCCESS INTO THE TEST PROCESS

To derive the full value of the AOAC-approved method, users should take care to observe the prescribed protocols. Shortcuts and workarounds, such as skipping steps or reusing the columns or other single-use items can compromise the accuracy, precision, and repeatability of the test results.

One aspect of testing that warrants particular attention to this caveat is the collection and processing of sample materials. The USDA certification program requires the use of representative sampling and proper sample preparation techniques. The integration of these best practices into the test process is essential for achieving optimal results with the AflaTest method. To deviate from the approved protocols is to set up the entire process for failure. A landmark study by internationally known sampling expert Thomas Whitaker quantified this risk: More than 80 percent of the variability of aflatoxin testing stems from missteps in process of creating test samples.[10]

Nancy Zabe Collette, VICAM's Technical Services and Applications Manager, stressed that successful testing starts with the full awareness that aflatoxin levels can vary greatly across different samples from the same lot. This variability reflects the tendency of aflatoxins to occur in isolated "hot spots." In practice, this distribution pattern means that total amount of aflatoxin of a 20 metric ton lot may be confined to one or two peanut kernels. That amount can be substantial, ranging as high as several hundred thousand to 1 million ppb.[11] Consequently, sampling just a few small areas of a lot could potentially result in wildly skewed estimates of its aflatoxin content.

"To get an accurate picture of the lot," Collette explained, "you need to obtain your test sample from a large aggregate sample that's been ground and mixed together so that any contaminated material is evenly spread throughout the subsample." In static lots, the aggregate sample should be assembled by collecting multiple incremental samples from all areas of the lot, using a manual or mechanical probe that extends from top to bottom to draw a representative cross-section of kernels. A diverter-type sampler should be used to take incremental samples at regular intervals from a moving stream. All procedures and equipment used should promote random sampling to ensure an unbiased test process.

In *Good Manufacturing Practices and Industry Best Practice for Peanut Manufacturers*, the American Peanut Council recommends Thomas Whitaker's "Sampling Methods to Measure and Grade Factors of Peanuts" as an authoritative source of detailed information on representative sampling, including suitable equipment, sample weights, and probe patterns. A pdf of the chapter is available at https://apresinc.com/wp-content/uploads/2015/12/APS-Chapter-14.pdf.

OPTIMIZING THE TEST SAMPLE

Preparation: The initial stage of AflaTest column cleanup calls for combining a ground 25 gram sample and 5 grams of salt with a methanol:water solution in a blender and blending at high speed for 2 minutes. The USDA guidelines for certification laboratories emphasize the importance of minimizing the particle size of the kernels with a suitable grinding mill, such as a vertical cutter mill (VCM), and thoroughly homogenizing the sample mixture to ensure that any aflatoxins present are uniformly distributed throughout the sample. The extracted sample is then filtered and diluted and then filtered again to remove insoluble particles that could interfere with HPLC performance and damage the system's components. The user should perform the chromatography procedure immediately after collecting the filtrate from the second filtration in the glass syringe barrel that will serve as the sample reservoir for the column. The procedure is outlined in the following diagram.

Sample Extraction

Blend

Place 25 g ground sample and 5 g salt in blender jar and add 125 mL methanol:water (70:30).

Cover jar and blend at high speed for 2 minutes.

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Filter 1

Filter through a funnel lined with fluted filter paper into a clean vessel.

↓

Dilute

Pipette or pour 15 mL extract into clean vessel.

Dilute with 30 mL purified water.

Mix well.

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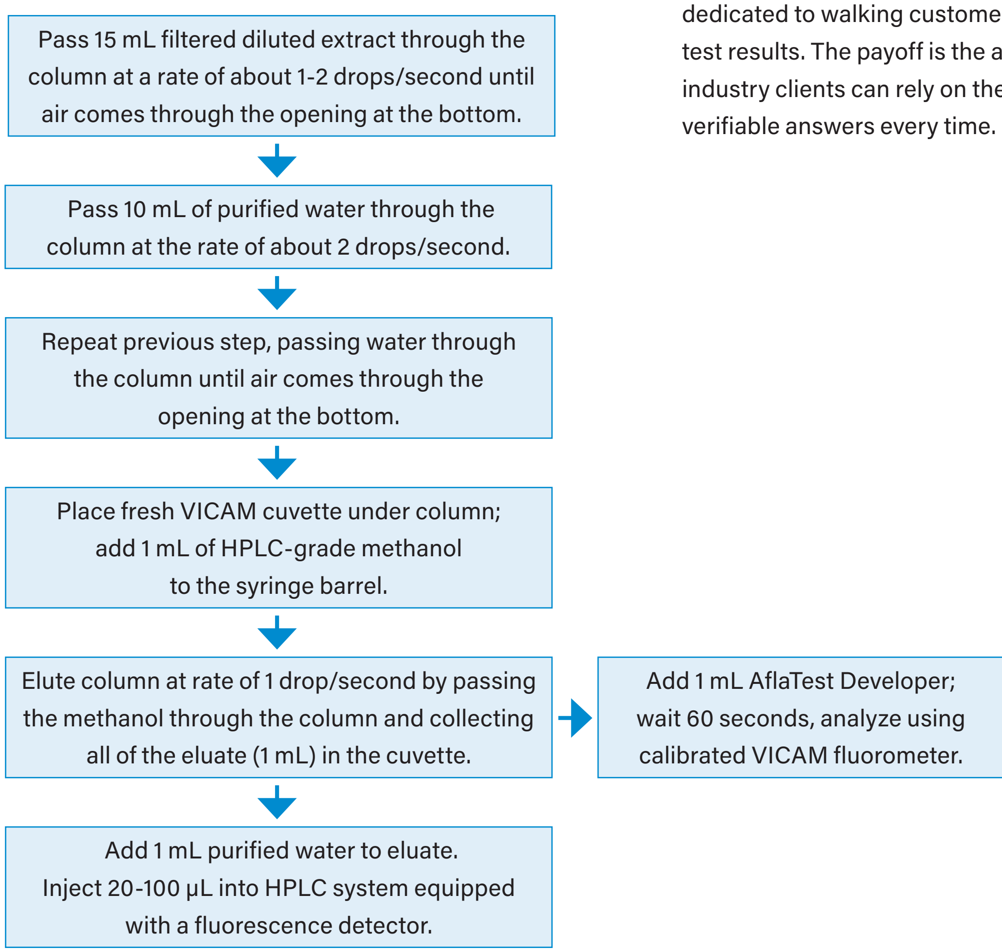
Filter 2

Filter through glass microfiber filter into a clean syringe barrel.

Note: Careful attention to the instructions at every phase of testing is a fundamental requirement for a smooth process and a successful outcome. This requirement includes using the recommended equipment and reagents (i.e., HPLC-grade methanol and purified, deionized, or distilled water). Each of the recommended items offers performance benefits. For instance, the fluted filter paper and a microfiber filter optimize recovery of aflatoxins and promote efficient removal of particulate matter that could block the HPLC column and cause unnecessary peaks in the data. Likewise the use of high-purity reagents helps ensure uninterrupted HPLC performance and precise test results. Attention to the cleanliness of the equipment is equally vital. Because background fluorescence from contaminated equipment can affect test results, all test materials and equipment must be kept free of dust, fingerprints, fibers, and other autofluorescent contaminants. The use of a fresh VICAM cuvette to collect eluate is also essential. The disposable cuvettes should never be washed or reused.

Purification: To purify and concentrate the filtered and diluted extract for HPLC analysis, the user passes a 15 mL portion through the AflaTest column. The extract is pushed into the column from a syringe barrel attached to a pump stand by applying air pressure from an aquarium pump. The column is then washed with water and eluted with methanol. The user should take care to maintain the proper flow rate during these steps in order to optimize extraction efficiency. The following diagram outlines the procedure.

Sample Cleanup



THE VALUE OF CUSTOMER EDUCATION

VICAM provides additional details on the proper use of AflaTest columns in its AflaTest HPLC Instruction Manual, including information on preparation of extraction solutions, shelf life, storage conditions, pump stand assembly, and HPLC setup. The instruction manual for VICAM's AOAC-validated AflaTest fluorometric method provides a similar level of detail, including step-by-step directions for calibrating the fluorometer. The company's investment in customer education extends to on-site product and equipment training as well as phone access to an expert technical services team dedicated to walking customers through the steps to optimal test results. The payoff is the assurance that VICAM's peanut industry clients can rely on the AflaTest method for credible, verifiable answers every time.

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