



## **Laboratory Approval Program – Aflatoxin Program Requirements**

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### **1. Purpose**

The purpose of this document is to outline the requirements for the Laboratory Approval Program for Analysis of Aflatoxins. The document describes the technical competency and quality management requirements that a laboratory must demonstrate to be a USDA-approved laboratory.

The Laboratory Approval Program is administered by the [Laboratory Approval Service \(LAS\) Branch](#). LAS is part of the [Agricultural Marketing Service \(AMS\)](#), [Science and Technology \(S&T\) Program](#), [Laboratory Approval and Testing Division \(LATD\)](#).

The LAS approves, or accredits, laboratories to perform testing services in support of domestic and international trade. At the request of industry, other Federal Agencies, or foreign governments, the LAS develops and administers programs to verify that the analysis of food and agricultural products meet country, and customer-specific requirements and that the testing of products marketed is conducted by qualified and approved laboratories.

### **2. Scope**

This Laboratory Approval Program (LAP) is for a laboratory seeking to obtain and maintain its status as a USDA-approved laboratory for the analysis of aflatoxins in almonds, pistachios, and/or peanuts based on the stipulations of the final market destination: U.S. domestic, export, and U.S. import.

- 2.1. Almonds for export to the European Union through the Pre-Export Certification program (PEC) of the Almond Board of California;
- 2.2. Pistachios for domestic and export markets, and import markets in accordance with 7 CFR 983 and 7 CFR §999.600, respectively;
- 2.3. Peanuts marketed domestically for human consumption, including imports, in accordance with 7 CFR 996.

This LAP verifies technical and quality control competencies of a laboratory for the analysis of aflatoxins. All aspects of a laboratory's quality management system (business processes) are applicable and are critical for ensuring the defensibility of the analytical results produced under the LAP.



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### 4. Glossary of Terms

AMS	Agricultural Marketing Service
AOAC	AOAC International
AOCS	American Oil Chemists’ Society
CFR	Code of Federal Regulations
CCV	Continuing Calibration Verification
CV	Coefficient of Variation
EU	European Union
FAPAS	Food Analysis Performance Assessment Scheme
FDA	Food and Drug Administration
FLD	Fluorescence Detector
HPLC	High Performance Liquid Chromatography
IAC	Immunoaffinity Column
ISO/IEC	International Organization for Standardization/ International Electrotechnical Commission.
LAP	Laboratory Approval Program
LAS	Laboratory Approval Service
LATD	Laboratory Approval and Testing Division
LOD	Limit of Detection
LOQ	Limit of Quantitation
PEC	Pre-Export Certification
PHRED	Photochemical Reactor Enhanced Detection



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PM	Program Manager
PT	Proficiency Test
S&T	Science & Technology Program
TLC	Thin Layer Chromatography
UPLC/UHPLC	Ultra (High) Performance Liquid Chromatography
USDA	United States Department of Agriculture

### 5. References

The following articles are referenced in this document. For the dated references, they only apply to the edition cited. For the undated references, the latest edition of the referenced document (including any amendments) applies.

#### 5.1. Methodology:

- 5.1.1. AOAC International, Official Methods 977.16, Sampling for Aflatoxins - Preparation for Sample Procedure.
- 5.1.2. AOAC International, Official Methods 991.31, Aflatoxins in Corn, Raw Peanuts, and Peanut Butter - Immunoaffinity Column (Aflatest) Method.
- 5.1.3. AOAC International, Official Methods 998.03, Aflatoxins in Peanuts - Alternative BF Method.
- 5.1.4. AOAC International, Official Methods 999.07, Aflatoxins B1 and Total Aflatoxins in Peanut Butter, Pistachio Paste, Fig Paste, and Paprika Powder – Immunoaffinity Column Liquid Chromatography with Post-Column Derivatization.
- 5.1.5. AOAC International, Official Methods 2005.08, Aflatoxins in Corn, Raw Peanuts, and Peanut Butter - Liquid Chromatography with Post-Column Photochemical Derivatization.

#### 5.2. U.S. Code of Federal Regulations (CFR)/ Inter-Agency Agreements:

- 5.2.1. [7 CFR Part 981 – Almonds grown in California.](#)
- 5.2.2. [7 CFR Part 983 – Pistachios grown in California, Arizona, and New Mexico.](#)
- 5.2.3. [7 CFR Part 996 – Minimum quality and handling standards for domestic and imported peanuts marketed in the United States.](#)
- 5.2.4. [7 CFR §999.600 – Specialty Crops; Import Regulations. Regulation governing the importation of pistachios.](#)
- 5.2.5. [Executive Order 13659 of February 19, 2014. Streamlining the Export/Import Process for America’s Businesses, Federal Register, Vol. 79, No. 37, Tuesday February 25, 2014.](#)



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5.2.6. [Memorandum of Understanding between AMS and FDA in inspecting, sampling, and testing peanuts, Brazil nuts, and pistachio nuts for aflatoxins.](#)

5.3. Industry Requirements:

5.3.1. [Administrative Committee for Pistachios: Handler’s Guide, August 2017.](#)

5.3.2. [Almond Board of California, Pre-Export Checks \(PEC\) Program Manual, Version 9.0.](#)

5.4. Laboratory Approval Program:

5.4.1. LAP-PR.05, Laboratory Approval Program – General Policies and Procedures

5.4.2. LAP-PR.06, Laboratory Approval Program – Fees

5.5. Quality Assurance Standards:

5.5.1. ISO/IEC 17025. General requirements for the competence of testing and calibration laboratories (2005 or 2017).

5.5.2. AOAC International Guidelines for Laboratories Performing Microbiological and Chemical Analyses of Food, Dietary Supplements, and Pharmaceuticals. Prepared by the Analytical Laboratory Accreditation Criteria Committee of AOAC INTERNATIONAL, revised April 2015. Including Appendix A.

5.5.3. USDA AMS Laboratory Standards of Practice.

5.6. Country Specific Regulations (primarily for export):

These documents are amended frequently. The most current consolidated version can be found at <http://eur-lex.europa.eu/content/welcome/about.html>. The consolidated version includes all amendments and corrigendums to each original regulation.

5.6.1. Commission Regulation (EC) No. 401/2006 of 23 February 2006 laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs.

5.6.2. Commission Regulation (EC) No. 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs.

5.6.3. Commission Implementing Regulation (EU) 2015/949 of 19 June 2015 approving the pre-export checks carried out on certain food by certain third countries as regards the presence of certain mycotoxins.

5.7. Additional Guidance Documents:

5.7.1. [Eurachem Guides.](#)

5.7.2. [FDA-Chemical Methods 2015. Guidelines for the Validation of Chemical Methods for the FDA FVM Program, 2nd Edition.](#) US FDA. FDA Foods and Veterinary Medicine Science and Research Steering Committee, May 19, 2015.



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- 5.7.3. [FDA Compliance Policy Guides, Section 570.375 Aflatoxin in Peanuts and Peanut Products.](#)
- 5.7.4. [FDA Compliance Policy Guides, Section 570.500 Pistachio Nuts - Aflatoxin Adulteration.](#)
- 5.7.5. [FDA Compliance Program Guidance Manual, 7307.001, Chapter 07 – Molecular Biology and Natural Toxins. Mycotoxins in domestic and imported foods FY 15/16.](#)
- 5.7.6. Francis Jr., O. J. 1979. Sample Preparation of Some Shelled Tree Nuts and Peanuts in a Vertical Cutter-Mixer for Mycotoxin Analysis. JAOAC. 62(5):1182-5.
- 5.7.7. Good Laboratory and Clinical Practices, Techniques for the Quality Assurance Professional, edited by P.A. Carson and N.J. Dent, 1990.

### **6. Laboratory Approval Program Administrative Policy**

A laboratory seeking admission to the Laboratory Approval Program must fulfill the requirements and follow the process described in the LAP procedure, LAP-PR.05, Laboratory Approval Program - General Policies and Procedures. This procedure describes the process for application, assessment audits, acceptance, maintaining program status, suspension, withdrawal, dismissal, and appeals.

**Note:** A laboratory may request an exception to a program requirement by submitting a written request to the Program Manager (PM). The request should describe the exception and provide supporting validation records and documentation per LAP-PR.05 §25. The validation data must demonstrate that the variation provides the same or better performance than the original procedure. The PM reviews the request and supporting documentation to determine whether to grant approval. Official record of approval must be maintained and readily available.

This program is administered on an annual, calendar year, basis. The LAP procedure, LAP-PR.06, Laboratory Approval Program – Fees explains the fees for services.

The administrative procedures above are available on the [LAS website](#), or contact the LAS Aflatoxin Program Manager (PM) for the current version of the procedure.

### **7. Summary of General Program Requirements**

The laboratory must comply with all requirements set forth in this document in order to be compliant with the LAP. For a laboratory to maintain in good standing, each year it must:

- 7.1. meet all program requirements relevant to the scope of approval;
- 7.2. comply with mandatory laboratory practices based on the ISO/IEC 17025 standard (See §8);



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- 7.3. use test method(s) approved by AMS. The analytical and sample preparation methods must be validated prior to use and the validation data package must be available for review upon request. (See §10);
- 7.4. participate in quarterly external proficiency testing programs per analyte/matrix, as required and maintain satisfactory status. (See §15);
- 7.5. confirm quality of sample preparation grind process by conducting particle size tests (sieve tests) on ground sample matrix weekly, or with use (See §12);
- 7.6. communicate regularly with the PM to share vital information regarding the laboratory:
  - 7.6.1. send PT results within 30 days of receiving the PT report from the provider, and corrective action reports for unsatisfactory results;
  - 7.6.2. send summary of sieve test results quarterly;
  - 7.6.3. notify the PM when new analysts are hired, and provide record of training and competence evaluation;
  - 7.6.4. notify the PM of significant changes relevant to the laboratory’s approval status including legal, organizational, or ownership status; main policies and resources; change in key managerial personnel, contact persons, and signatories; significant change in location, equipment, facilities, and working environment; scope of approval, including the analytical testing method; or other matters that may affect the laboratory’s test results and/or its ability to meet the program requirements.

It is at the discretion of LAS whether an onsite visit/audit, at the laboratory’s expense, is required to evaluate any significant change that a laboratory undergoes.
- 7.7. make all information relevant to the LAP available to PM upon request;
- 7.8. ensure LAS performs at a minimum a biennial (every other year) re-assessment audit during which the laboratory must have an actual sample ready to demonstrate its competency of performing the test method. Additionally, the laboratory must comply with requests for documents/records before and during the audit;
- 7.9. respond to each nonconformance found during an audit and documented in the audit report. The response must include an investigation and root cause analysis. Correction or corrective action must be planned and/or completed within 30 calendar days of receiving the report;
- 7.10. resolve each nonconformance in a timely manner, whether identified by a LAS auditor, another organization, or internally;
- 7.11. pay all program fees by the due date on the billing invoice.



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### **8. Mandatory Quality Assurance Practices**

The laboratory must implement quality assurance and quality control procedures to ensure a validated and qualified analysis, prove competence, and ensure defensible data and maintain records for at least three years. It is common practice for a testing laboratory to become ISO 17025 accredited. AMS laboratory approval for aflatoxin testing does not require ISO 17025 accreditation.

LAS uses the ISO 17025 standard to evaluate all laboratory quality systems regardless of accreditation status. Nonconformances identified during a LAS assessment audit may be cited to the ISO/IEC 17025 standard.

Given the primary objective of this program and vast differences in the size and business structure of program participants, LAS may elect to not emphasize certain ISO 17025:2005 clauses (e.g., 4.4 Review of requests, tenders and contracts, 4.5 Subcontracting of tests and calibrations, 4.6 Purchasing services and supplies).

### **9. Method Selection**

The laboratory must perform analytical preparation and testing methods based on AOAC methods 977.16, 991.31, 998.03, 999.07, and 2005.08.

#### **9.1. Sample Preparation**

9.1.1. Sample preparation procedure must be based on AOAC 977.16 for the sampling and preparation of aflatoxin samples and are required to achieve a minimum size reduction for ground samples. (See §12.)

9.1.2. Samples must be mixed after receipt in the laboratory and prior to grinding if the entire sample is not to be ground. This ensures the most representative sample of a lot is tested. The exception to this requirement is official USDA samples, including those received pre-ground. Note: Official USDA samples are mixed as part of the sampling process making additional mixing at the laboratory unnecessary.

#### **9.2. Extraction and Measurement**

9.2.1. Almond and Pistachio Export Program: B<sub>1</sub> and Total aflatoxin (B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, and G<sub>2</sub>) must be quantified using the following methods:

- a) Immunoaffinity column (IAC) cleanup method with HPLC/UPLC - FLD, AOAC 991.31, A-F and section H (iodine), 2005.08 (PHRED). Results should be analyzed to the nearest tenth ppb (i.e. 0.1 ppb) where ppb = 1 ng/g.
- b) Immunoaffinity column (IAC) cleanup method with HPLC/UPLC - FLD, AOAC 991.31, A-F and section H (iodine), 999.07 (Kobra Cell). Results should be analyzed to the nearest tenth ppb (i.e. 0.1 ppb) where ppb = 1 ng/g.



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9.2.2. Pistachio Domestic Program: Total aflatoxin may be tested using the following methods:

- a) Immunoaffinity column (IAC) cleanup method with direct fluorometry, AOAC 991.31, A-G. Results should be analyzed to the nearest tenth ppb (i.e. 0.1 ppb) where ppb = 1 ng/g.
- b) Immunoaffinity column (IAC) cleanup method with HPLC/UPLC - FLD, AOAC 991.31, A-F and section H (iodine), 2005.08 (PHRED). Results should be analyzed to the nearest tenth ppb (i.e. 0.1 ppb) where ppb = 1 ng/g.
- c) Immunoaffinity column (IAC) cleanup method with HPLC/UPLC - FLD, AOAC 991.31, A-F and section H (iodine), 999.07 (Kobra Cell). Results should be analyzed to the nearest tenth ppb (i.e. 0.1 ppb) where ppb = 1 ng/g.

9.2.3. Peanut Domestic Program: Total aflatoxin may be tested using the following methods:

- a) Immunoaffinity column (IAC) cleanup method with direct fluorometry, AOAC 991.31, A-G. Results should be analyzed to the nearest whole integer.
- b) Aflatoxins in Peanuts with Thin Layer Chromatography (TLC) analysis, designated as the alternative Best Foods (BF) method, AOAC 998.03. Results should be analyzed to the nearest whole integer.
- c) Immunoaffinity column (IAC) cleanup method with HPLC/UPLC - FLD, AOAC 991.31, A-F and section H (iodine), 2005.08 (PHRED). Results should be analyzed to the nearest tenth ppb (i.e. 0.1 ppb) where ppb = 1 ng/g.
- d) Immunoaffinity column (IAC) cleanup method with HPLC/UPLC - FLD, AOAC 991.31, A-F and section H (iodine), 999.07 (Kobra Cell). Results should be analyzed to the nearest tenth ppb (i.e. 0.1 ppb) where ppb = 1 ng/g.

### 9.3. Data Analysis

9.3.1. Significant Figures: Collect data to the appropriate significant figures for the methodology (see §9.2). Additionally, when two or more data points are calculated, the final value must not have more significant figures than the original data points.

9.3.2. Rounding Rule: If 4 and under, round down; and if 5 and over, round up. For example:

- a result of 9.4 ppb would be rounded to 9 ppb, whereas a result of 9.5 ppb would be rounded to 10 ppb for the final report; and
- a result of 4.24 ppb would be rounded to 4.2 ppb, whereas a result of 4.25 ppb would be rounded to 4.3 ppb.



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9.3.3. Correction for recovery (EU only): The analytical result must be corrected for recovery,

- unless the percent recovery is between 90-110%, additionally
- the regulation states that if the result is less than 50% of the maximum level or 5 times the maximum level it might not be reported.

§ 4.4.1(a), Annex II, (EC) No 401/2006, “Corrected for recovery, the level of recovery being indicated. The correction for recovery is not necessary in case the recovery rate is between 90-110 %.”

§ 4.4.1, Annex II, (EC) No 401/2006, “if the result of the analysis is significantly (>50%) lower than the maximum level or much higher than the maximum level (i.e., more than 5 times the maximum level), and on the condition that the appropriate quality procedures are applied and the analysis serves only the purpose of checking compliance with legal provisions, the analytical result might be reported without the correction for recovery and the reporting of the recovery rate and measurement uncertainty might be omitted in these cases.”

### 9.4. Method Variations

Variations or modifications to the AOAC methods require PM approval. If significant changes are proposed, a validation study may be required prior to method implementation. The validation data must demonstrate that the variation provides the same or better performance than the original procedure. The PM reviews the request and supporting documentation to determine whether to grant the proposed variation. Official record of approval for variations must be maintained and readily available.

## **10. Method Validation**

The laboratory must demonstrate it can competently and proficiently perform the selected method prior to using it for testing and reporting results. Demonstration of competency is carried out in accordance with the laboratory’s documented procedures (e.g., method validation) and as specified in this LAP to the extent necessary to meet the needs of the given application. The method must be used as validated.

Method validation and performance specifications are described in §10.1 for sample grinding methods, §10.2 for extraction and measurement methods, §10.3 for the data package, and §10.4 for export only requirements.

### 10.1. Method Validation of Sample Preparation (Homogeneity Test)

The laboratory must validate their sample preparation procedure for grinding samples to ensure a homogenous mixture is achieved for each analyte/matrix. The parameters such as the amount of time using a particular grinder and type of blade (e.g., smooth-edge, serrated, notch-edge) needed to achieve an adequate particle size to ensure a homogenous grind are



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defined and verified. Particle size is a performance control measurement of homogeneity (Francis, Jr., 1979).

A validation needs only be performed once unless significant changes to the grinding procedure are made.

10.1.1. Perform Homogeneity test: Start with an unground sample. Select one with either a known incurred natural aflatoxin contamination or spike the nuts (ideally in one location). Grind according to the laboratory's procedure. Analyze 10 representative subsamples individually through the entire method. Conduct particle size test (See §12.1) by passing a representative sample through a sieve. The percent pass through rate can then be used to periodically verify that the quality of grinding is maintained over time.

10.1.2. Evaluate results from the subsamples to determine accuracy and precision:

- a) Percent recovery of result must fall within acceptable range (See Table 1); and
- b) % CV must be  $\leq 20\%$ .

10.1.3. Prepare Method Validation Data Package to record procedure and test results demonstrating it is fit for use (See §10.3).

### 10.2. Method Validation of Extraction and Measurement

10.2.1. Validate each method used in the laboratory prior to its use to test and report sample results.

10.2.2. Validate a method when significant changes are made to the instrumentation and/or the facility that would impact testing. Minor changes can be verified by performing less rigorous testing of the method that demonstrates the changes have not impacted the quality of the testing.

### 10.3. Method Validation Data Package

10.3.1. The validation package must be sent to the PM for review and approval.

10.3.2. The validation data package must be readily available at the laboratory for as long as the method is utilized, plus three years after the last reported results.

10.3.3. The laboratory must prepare and maintain and auditable data package that includes at least the following:

- a) Cover Report: Explain what method and commodity is validated, details of how the validation was performed, performance capabilities, and a statement of whether the method is fit for use. ~ 1 page
- b) Table of Contents: List each section with page numbers.
- c) Test Method: Copy of the local procedure used to perform the test method.
- d) Statistical Analysis Summary: Summarize results of statistical analysis.



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Include, as applicable:

- d-1) Linearity (linear regression equation,  $y = mx+b$  &  $R^2$ )
- d-2) Accuracy (% recovery)
- d-3) Precision (standard deviation)
- d-4) Measurement Uncertainty (summation of error around the quantitated value).
- d-5) Limit of Quantitation (LOQ) — The LOQ is defined as the mean of the measured content of blank samples ( $n \geq 10$ ) plus ten times the standard deviation of the mean.

$$LOQ = X_{Ave} + 10SD$$

(where  $X_{Ave}$  is the mean value of the matrix blank samples converted to ppb and SD is the standard deviation of the blank samples).

- d-6) Limit of Detection (LOD) — The LOD is defined as the mean of the measured content of blank samples ( $n \geq 10$ ) plus three times the standard deviation of the mean.

$$LOD = X_{Ave} + 3SD$$

(where  $X_{Ave}$  is the mean value of the matrix blank samples converted to ppb and SD is the standard deviation of the blank samples).

- e) Data: Summarize the raw data from the instrument. Show calculations that supply statistical analysis summary. Include equations for calculations.
- f) Instrument Data: Provide instrument printouts of method parameters, acquisition parameters, processing parameters, the sequence, calibration curve and table, and each chromatogram.
- g) Traceability Records: Solution preparation (chemicals, measurement equipment).

### 10.4. Method validation (export only)

Method validations, export only, must comply with (EC) No. 401/2006 of 23 February 2006 Annex II Part 4 (i.e., Part 4.2, Part 4.3.1.1(a), Part 4.3.1.1(i), Part 4.3.2.3 and subparts, Part 4.3.2.4, Part 4.3.2.6, Part 4.3.2.7, and Part 4.3.2.8), as relevant.



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### **11. Quality Controls**

The laboratory must utilize controls to demonstrate testing is performed correctly and factors that could negatively impact the results are mitigated. If any quality control does not perform as expected, immediate action must be taken prior to any samples being tested and or sample results reported.

- 11.1. Quality controls must be run daily with use.
- 11.2. The minimum quality controls required for each method are listed below. Any additional quality controls the laboratory chooses to run are acceptable.
  - a) Immunoaffinity column (IAC) cleanup method with direct fluorometry, AOAC 991.31, A-G, requires Matrix Spike (positive control), and Reagent Blank (negative control).
  - b) Aflatoxins in Peanuts with Thin Layer Chromatography (TLC) analysis, designated as the alternative Best Foods (BF) method, AOAC 998.03, requires Matrix Spike (positive control) and Matrix Blank (negative control) and/or Reagent Blank (negative control).
  - c) Immunoaffinity column (IAC) cleanup method with HPLC/UPLC - FLD, AOAC 991.31, A-F, H and 2005.08, requires Matrix Spike (positive control), Matrix Blank (negative control) and/or Reagent Blank (negative control), and CCV (continuing calibration verification).
- 11.3. LAS interpretation of each quality control and its purpose is defined. Note: It is not a requirement for the laboratory to use exactly the same terms for each type of control as long as they use the correct control for the correct purpose.
  - a) Matrix Blank (negative control): known negative nut sample that has gone through the entire solvent extraction procedure.
  - b) Matrix Spike (positive control): matrix blank with a known quantity of aflatoxin added, purchased positive nut sample with known aflatoxin concentration, or nut sample with incurred, natural aflatoxin in known concentration. This sample must go through extraction. It is utilized to evaluate percent recovery for each commodity.
  - c) Known Positive (positive control): previously ground and tested sample that is known to be positive.
  - d) Reagent Blank (negative control): sample made up of only the reagents used in testing the samples that goes through extraction. Usage of a matrix blank would make this type of QC optional.
  - e) Continuing Calibration Verification (CCV): Solution of known concentration, typically at or near the midpoint of the calibration curve. Use to ensure the instrument remains stable throughout the sequence and determine repeatability.



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At a minimum this is to be analyzed at the beginning of a batch, the end of the batch, and throughout the batch with a recommended frequency of every 10 ( $\pm$  2) injections.

- f) Independent Calibration Verification (ICV) – Solution of known concentration that is from a different manufacturer, or same manufacturer but different lot, or a separate preparation from the solutions used to calibrate. Used to evaluate the accuracy of reference material and/or accuracy of preparation techniques. It is a best practice to incorporate this control into your quality system processes.

### **12. Quality Measures**

The laboratory must evaluate the quality controls to identify acceptability of data, trends, and potential problems. If any of the quality controls do not perform as expected, immediate action must be taken prior to samples being tested and/or reported.

- 12.1. Vertical Cutter Mill (VCM) Sieve Test: This measure helps to 1) ensure an adequate particle size reduction is achieved for homogenous distribution of contaminated nuts, and 2) provide a monitoring system to assess grinding conditions over time. A lower than normal pass through rate may indicate the need for sharpening blades, increasing grind time, grinder maintenance/repair, etc.

- 12.1.1. Frequency: Perform at a minimum weekly or with use. Record and submit surveillance data to the PM quarterly.

- 12.1.2. Technical requirement for adequate grind: Follow AOAC 977.16, Sampling for Aflatoxins, Preparation for Sample Procedure. Summary: “Aim at maximum practical size reduction and thoroughness of mixing to achieve effective distribution of contaminated portions...To achieve this degree of size reduction, nut must be ground to pass No. 20 sieve.

Note: A laboratory may request an exception or variation to AOAC 977.16 method by submitting a request and validation data. The validation data must demonstrate that the variation provides the same or better performance than the original procedure.

- 12.1.3. Procedure: Place ~50-100g of ground sample in No. 20 sieve that has been weighed. Work the sample through the sieve with warm water (tap) being careful not to lose sample. Dry the sieve and remaining material in an oven at ~100 – 105°C or air-dry until there is no more change in weight. The drying process should not cook or burn the material. Weigh and calculate % pass through.

- 12.2. Percent Recovery: Compare expected concentration to actual measured concentration to evaluate extraction performance. The equation for calculating percent recovery is shown below.



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$$\% Recovery = \frac{[aflatoxin\ recovered]}{[aflatoxin\ expected]} * 100$$

12.2.1. The performance criteria are defined in Table 1.

Table 1. Performance criteria for aflatoxin.

Criteria	Concentration Range	Acceptable Range
Blanks	All	Negligible
Recovery– Aflatoxins B <sub>1</sub> , B <sub>2</sub> , G <sub>1</sub> , G <sub>2</sub>	< 1.0 µg/kg	50 – 120 %
	1 – 10 µg/kg	70 – 110 %
	> 10 µg/kg	80 – 110 %

[NOTE: Values apply to both B<sub>1</sub> and total aflatoxins.]

12.3. Control Charting: The purpose of control charting is to track performance over time and serve as an indicator if something in the analytical process is out of control and needs investigation or correction.

12.3.1. Plot % recovery results of each matrix spike analyzed. Plot for the method regardless of analyst or instrument used to evaluate overall performance of the method. Acceptable % recovery range is determined by the concentration of the spike (See Table 1). All data points must be within the acceptable range. If they are not, then an investigation needs to be conducted.

Note: Additional, optional, control charting methods can be used to evaluate other variables of the testing procedure. For example, a plot by analyst evaluates an individual analyst’s performance compared to other analysts. Or, a plot by instrument evaluates individual instrument performance, and can help identify instrument problems.

12.4. Coefficient of Variation (CV): This value is a measure of the spread that describes the amount of variability relative to the mean expected concentration (or peak area). Comparing continuing calibration verification (CCV) sample results throughout a sequence can demonstrate the repeatability or stability of the instrument/method.

$$\% CV = \frac{Standard\ Deviation\ of\ CCV\ results}{Expected\ concentration\ (or\ average\ of\ same\ two\ data\ points)} * 100$$

12.4.1. % CV must not be greater than 20% between any two CCV data points during a single sequence run. If % CV is greater than 20%, the sample, run, and instrument must be evaluated.

12.5. Calibration Curve: The calibration curve is made up of standards at various known concentrations which enable the quantification of unknown samples.

12.5.1. The same type of curve used during method validation must be used.

12.5.2. For HPLC/UPLC, the calibration curve must have a minimum of 4 points.

12.5.3. The R<sup>2</sup> value for each curve must be ≥0.995.



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- 12.5.4. The calibration curve should be linear and must never be forced through zero as that would create a bias that would favor the low end of the calibration range.
- 12.5.5. The calibration range is to bracket the range of reported result. Results outside of the calibration range may be diluted and re-analyzed so the result falls within the range. It is not acceptable to extrapolate the concentration.
- 12.5.6. The calibration curve must be low enough to accurately report at the LOQ level set by the lab or industry. A low calibration standard at the LOQ level or the blank run with the calibration can be used as a calibration point creating an acceptable range.

### **13. Country Specific Requirements (Export Only)**

This section includes additional applicable EU Regulation requirements, where not found in other related sections of the document and must be followed for all export samples.

- 13.1. Protecting sample from daylight: See (EC) No. 401/2006 of 23 February 2006, Annex II 1.1
- 13.2. Calculating and reporting shell/kernel ratio of whole nuts: See (EC) No. 401/2006 of 23 February 2006, Annex II 1.2  
In shell pistachio ratio is accepted as 50/50 (FDA CPGM 7307.001).
- 13.3. Method validation: See (EC) No. 401/2006 of 23 February 2006 Annex II Part 4 (i.e., Part 4.2, Part 4.3.1.1(a), Part 4.3.1.1(i), Part 4.3.2.3 and subparts, Part 4.3.2.4, Part 4.3.2.6, Part 4.3.2.7, and Part 4.3.2.8), as relevant.
- 13.4. Accuracy and Precision Criteria and Equations: See (EC) No. 401/2006 of 23 February 2006 Annex II 4.3.1.1 (a) and (i).

### **14. Critical Equipment & Reagents**

- 14.1. Equipment and instrumentation considered critical to the analytical method must be calibrated or verified before being put into service and there after calibrated/verified regularly while in service. Calibration refers to checking the measurements of a device and adjusting the device if corrections are needed. Verification refers to checking that a device's measurement remains within a determined acceptable range, adjustments should not be needed. If a device is found to be outside tolerance at verification, it may need to be calibrated. Records must be kept.
  - 14.1.1. Grinding Equipment: Grinding equipment must be in proper working order. Records of blade sharpening and other maintenance must be retained.
  - 14.1.2. Balances & Scales: Balances and scales must be verified daily when in use with working weights that represent the entire weight range used by the laboratory. An



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annual calibration must be performed by an accredited organization or internally with the same requirements being met.

- 14.1.3. Reference & Working Weights: Reference weights must be calibrated every 5 years. Working weights must be verified against reference weights annually.
- 14.1.4. Analytical Instruments: All analytical instrumentation must be kept in good working order. Records of significant maintenance and repair must be kept.
- 14.1.5. Volumetric Devices: Volumetric delivery devices must be verified regularly. Records of the verification must be retained.
  - a) Mechanical pipets, micropipettors, mechanical burets, and bottle-top dispensers must be verified at least every 6 months for accuracy and precision using a gravimetric or colorimetric method.
  - b) Positive displacement syringes must be verified for accuracy upon receipt (a manufacturer's certificate of accuracy may be accepted).
  - c) Plastic graduated cylinders must be verified for accuracy and precision upon receipt (prior to use) and every five years for accuracy using a gravimetric method. Additional verification should occur if damage is visible.
  - d) Volumetric non-class A glassware must be verified for accuracy and precision using a gravimetric method upon receipt (prior to use).
- 14.2. Critical reagents must be stored according to the manufacturer's instructions and must not be used past the designated expiration date. Critical reagents include, but not limited to, IAC columns, aflatoxin standard, iodine, and any other reagents with specific storage instructions from the manufacturer.

### **15. Proficiency Testing**

- 15.1. The laboratory must participate in at least quarterly proficiency testing. The PT program must be relevant to the analyte/matrix tested in the laboratory.
- 15.2. The PT program must be administered by an external third party program such as AOCS, FAPAS, or similar. The third party program should be ISO 17043 accredited where possible.

The EU requires the FAPAS proficiency test for a laboratory testing samples for export.
- 15.3. Every analyst responsible for performing the method(s) must participate in the PT program and submit the results with the analyst name and PT ID to the PM. Analysts can rotate performing the same PT as long as each analyst is evaluated at least once in a two-year span.



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- 15.4. The laboratory must send the results of proficiency tests (PT reports) within 30 days of the receipt of the report.
- 15.5. If any unsatisfactory results are observed, the laboratory must initiate and follow their corrective action process. The corrective action response records must be sent to the PM within 30 days of receipt of the report.
- 15.6. Unsatisfactory PT results are defined by reported z-scores as follows:
  - $2 \leq |z| \leq 3$ , should be evaluated in the context of other scores obtained in the same test and other PTs over time. A score in this range must be investigated if there are repeated similar values in that test or over several tests.
  - $|z| \geq 3$ , must trigger an immediate corrective action investigation on the part of the laboratory to establish root cause.

### **16. Official Certificate of Analysis/Report**

Test reports must satisfy the ISO 17025 §5.10 standard for reporting the results in addition to the customer requirements and the following commodity specific requirements.

#### 16.1. Pistachio and/or almond export to the EU

##### 16.1.1. Sample lot number.

##### 16.1.2. In order to comply with the (EC) 401/2006 regulation, the aflatoxin analysis certificates/reports must include:

- a) Analytical method used,
- b) Limit of quantification,
- c) Percent recovery for B<sub>1</sub> and total aflatoxin,
- d) Analytical result (See §9.3.3),
- e) A statement about correction for recovery,
- f) Measurement uncertainty presented as the result  $\pm$  uncertainty (i.e.,  $10.4 \pm 2.1$   $\mu\text{g}/\text{kg}$ ).

§ 4.4.1(b), Annex II, (EC) No 401/2006, “As  $x \pm U$  whereby x is the analytical result and U is the expanded measurement uncertainty, using a coverage factor of 2 which gives a level of confidence of approximately 95%.”

##### 16.1.3. If a statement is to be made concerning compliance with the (EC) No. 401/2006, use the statement:



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“Sample analysis was conducted in compliance with Annex II of Regulation (EC) No. 401/2006 for aflatoxin in [insert nut].”

16.1.4. If reporting results for in shell nuts use the statement (as applicable):

- a) “In shell pistachio results are reported on a kernel basis for a 50/50 shell/kernel ratio.”
- b) “In shell [insert nut type] are reported on a kernel basis for a [insert X/Y] shell/kernel ratio.”

16.1.5. Interpretation of results by the laboratory does not generally occur. The commodity’s destination is often decided based on the aflatoxin results. It is the responsibility of the handler (processor) not the laboratory, to determine which limits apply to their product. If requested by the handler to include an interpretation statement on the analytical report of the maximum limits set by Regulation (EC) No. 1881/2006, the following types of statements are to be used:

- a) “result exceeds the limit of 12.0 µg/kg B<sub>1</sub> and/or 15.0 µg/kg sum of B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub> for pistachios to be subjected to sorting, or other physical treatment, before human consumption or use as an ingredient in foodstuffs (§2.1.2 of Annex II Regulation 1881/2006)”
- b) “result exceeds the limit of 8.0 µg/kg B<sub>1</sub> and/or 10.0 µg/kg sum of B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub> for pistachios intended for direct human consumption or use as an ingredient in foodstuffs (§2.1.6 of Annex II Regulation 1881/2006)”
- c) “result exceeds the limit of 15 ppb total aflatoxin for domestic human consumption (CFR 983.150).”

16.2. Domestic or imported pistachios

The aflatoxin analysis certificates/reports must include:

16.2.1. the statement, “USDA or USDA-approved laboratory to test for total aflatoxin content in samples for domestic and imported pistachios marketed in the United States”;

16.2.2. one of the following statements may be used for the methodology as approved. Include AOAC method number or internal SOP number, where applicable:

- a) an immunoaffinity column with direct fluorometry method of analysis (AOAC 991.31),
- b) an immunoaffinity column cleanup with high performance liquid chromatography (HPLC) method.



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### 16.3. Peanut aflatoxin analysis certificate

The aflatoxin analysis certificates/reports must include all of the following information:

- 16.3.1. the statement, “The designation of aflatoxin negative is defined as the average analytical result of 15 parts per billion (ppb) or less aflatoxin, and applies to product distributed within the United States under 7 CFR Part 996. Results are reported as whole integers; therefore, further calculations must be rounded to the nearest whole integer for proper interpretation.”
- 16.3.2. the statement, “USDA or USDA-approved laboratory to test for total aflatoxin content in samples for domestic and imported peanuts marketed in the United States”;
- 16.3.3. one of the following statements may be used for the methodology as approved. Include AOAC method number or internal SOP number, where applicable:
  - a) an immunoaffinity column with direct fluorometry method of analysis (AOAC 991.31),
  - b) water slurry method with thin-layer chromatography (TLC) analysis designated as the alternative Best Foods (BF) method of analysis (AOAC 998.03), or
  - c) an immunoaffinity column cleanup with high performance liquid chromatography (HPLC) method.

### 16.4. Imported peanut and pistachio reporting

As per the Executive Order 13659, a laboratory that tests imported peanuts and pistachios must participate in the International Trade Data System (ITDS). Participation includes the regular submission of test results to the ITDS.



## Laboratory Approval Program – Aflatoxin Program Requirements

### 17. Revision History

New Rev.	Description of Change	Prepared by
02/03/15	Laboratory Approval Program Requirements for the Detection of Aflatoxins in Almonds, Peanuts, and Pistachio Nuts.	Program Manager
12/20/16	Laboratory Approval Program Requirements for the Detection of Aflatoxins in Almonds, Peanuts, and Pistachio Nuts	Isaac Sterling, Program Manager
10/10/17	LAP-Aflatoxin Program Requirements Clarify requirements and remove the LAS procedures that are represented in other internal documents. A line by line description of changes has been compiled and filed with the program records. Inclusion of relevant EU requirements for pistachios and almonds.	Lauren Shoemaker, Program Manager
01/19/18	LAP-Aflatoxin Program Requirements Consolidated and incorporated comments from laboratory review of 10/10/17 draft copy and comments from 2017 EU Audit Report. Included language to address comments and concerns expressed by industry and laboratory representatives.	Lauren Shoemaker, Program Manager
4/26/18	Draft version implemented for 2018 calendar year.	Lauren Shoemaker, Program Manager
11/15/18	LAP-Aflatoxin Program Requirements §1, 7.9, 10.1: Minor editing, no change to requirement. §5.5.1: (2005 or 2017). §7.2 and §7.3 switched. §8 and 10.3 clarified record retention time. §9. The exception was reworded to be “official USDA samples, including pre-ground.” §9.2.1: Added B1 where it was missing. §9.1, 9.2.1-3, 10.1.2, 10.2 10.3, 11, and 12: re-organized into additional subsections for clarification purposes. §10: reorganized informational text, no requirement changes. §15.5.4: added calibration curve should be linear for clarification purposes. §14 added reagents and §14.2. §14.1.2: clarified language about weights used to calibrate/verify balance. §15.3 added analyst PT ID. §16.1.1 added. §16.1.3: changed “pistachios” to “[insert nut]”	Lauren Shoemaker, Program Manager

### 18. Review Approvals

Program Manager – Aflatoxin  
(Author)

Branch Chief  
(Approver)