Laboratory Approval Program for the Detection of Aflatoxin in Almonds, Peanuts, and Pistachio Nuts

1. Purpose

1.1 The Laboratory Approval Program (LAP) is intended to be used by a laboratory which plans to obtain an official approval of the Agricultural Marketing Service (AMS), Science and Technology (S&T) Program, Laboratory Approval and Testing Division (LATD), Laboratory Approval Service (LAS) on performing confirmatory analysis of aflatoxins in:

1.1.1 Almonds for export to the European Union through the Pre-Export Certification program of the Almond Board of California;

1.1.2 Pistachio nuts for domestic and export markets;

1.1.3 Peanuts marketed domestically for human consumption;

1.1.4 Peanuts and pistachio nuts imported into the United States.

1.2 This document provides the procedures and requirements used for the evaluation of the laboratory's technical competence and its quality management system.

2. Scope

The LAP may be used by laboratories that submit their analysis program to LAS for approval, verification, and monitoring. It is limited to the analysis of aflatoxins in almonds, peanuts, and pistachio nuts only and all aspects of a laboratory's documented quality management system that applies to this analysis.

3. References

The following articles are referenced in this document. To 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.

3.1 AOAC International Guidelines for Laboratories Performing Microbiological and Chemical Analyses of Food and Pharmaceuticals. Prepared by the Analytical Laboratory Accreditation Criteria Committee of AOAC INTERNATIONAL, revised March 2010.

3.2 AOAC International Official Method, Appendix E: Laboratory Quality Assurance

3.3 AOAC International, Official Method 991.31 and 998.03

3.4 AOAC International Official Method 977.16, Sampling for Aflatoxins.

3.5 7 CFR Part 983 – Pistachio nuts grown in California, Arizona, and New Mexico. http://www.ecfr.gov/cgi-bin/text-idx?SID=fc57d4f577dfe1a1b9f7e3f75676661d&mc=true&node=pt7.8.983&rgn=div5

3.6 7 CFR Part 996 – Minimum quality and handling standards for domestic and imported peanuts marketed in the United States. http://www.ecfr.gov/cgi-bin/text-idx?SID=fc57d4f577dfe1a1b9f7e3f75676661d&mc=true&node=pt7.8.996&rgn=div5

3.7 7 CFR §999.600 – Regulation governing the importation of pistachios. http://www.ecfr.gov/cgi-bin/text-idx?SID=fc57d4f577dfe1a1b9f7e3f75676661d&mc=true&node=pt7.8.999&rgn=div5#se7.8.999_1600

3.8 Eurachem Guides https://www.eurachem.org/index.php/publications/guides.

3.9 FDA guidelines for the validation of chemical methods for the FDA Foods Program. US FDA, FDA Foods Program Science and Research Steering Committee, March 22, 2012.

3.10 FDA compliance program guidance manual, Chapter 07 – molecular biology and natural toxins. Mycotoxins in domestic and imported foods FY 15/16. http://www.fda.gov/downloads/Food/ComplianceEnforcement/ucm073294.pdf

3.11 FDA Compliance Policy Guides, Section 570.375 Aflatoxin in Peanuts and Peanut Products http://www.fda.gov/ICECI/ComplianceManuals/CompliancePolicyGuidanceManual/ucm074598.htm.

3.12 FDA Compliance Policy Guides, Section 570.500 Pistachio Nuts - Aflatoxin Adulteration http://www.fda.gov/ICECI/ComplianceManuals/CompliancePolicyGuidanceManual/ucm074601.htm.

3.13 Good Laboratory and Clinical Practices, Techniques for the Quality Assurance Professional, edited by P.A. Carson and N.J. Dent, 1990.

3.14 ISO/IEC 17025:2005 General requirements for the competence of testing and calibration laboratories.

3.15 Memorandum of Understanding between AMS and FDA in inspecting, sampling, and testing peanuts, Brazil nuts, and pistachio nuts for aflatoxins.

3.16 Official Journal of the European Union, No. L 70, 9.3.2006, pp 12-34, 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.

3.17 Official Journal of the European Union, No. L 52, 3.3.2010, pp 32-43, Commission Regulation (EC) No. 178/2010 of 2 March 2010 Amending Regulation (EC) NO. 401/2006 as regards groundnuts (peanuts), other oilseeds, tree nuts, apricot kernels, liquorice and vegetable oil.

3.18 USDA AMS Laboratory Standards of Practice.

4. Laboratory Approval Procedures

4.1 Initial Request for Admission: A laboratory seeking approval must send an email/letter to the Program Manager (PM) requesting admission to the program at the following address:

Program Manager – LAP Detection of Aflatoxin in Almonds, Peanuts, and Pistachio Nuts
Laboratory Approval & Testing Division
USDA, AMS, S&T
1400 Independence Ave. SW
Room 3533-S
Washington, D.C. 20250-0272
Telephone: (202) 690-0621
Email: LAS@ams.usda.gov

4.2 Submission of Required Information: After providing the initial request for admission, the applicant laboratory must submit an application package that includes laboratory information (Section 4.2.1) and required documentation (Section 4.2.2).

4.2.1 An applicant laboratory must provide the following information, but not limited to: the laboratory

a) legal name and physical address (number and street, city, state, and zip code);

b) ownership;

c) requested scope of approval;

d) authorized representative's name, title, phone number, and email address;
*[**NOTE**: Authorized representative is the laboratory's point of contact person who is responsible for (1) the information provided in the application package, (2) the commitment to condition of approval (Section 5.1), and (3) ensuring compliance with this LAP requirements.]*

e) staff designated to serve as Approved Signatories of test reports that reference USDA-approved laboratory listing their names, titles, phone numbers, and email addresses;

f) billing address, taxpayer identification number (Federal W-9 Form), and accounts payable person's contact information, i.e., name, phone number, and email address;

g) authorized representative's signature and date.
*[**NOTE**: By signing the application, the laboratory's authorized representative confirms that the application information are correct and commits the laboratory to fulfill the conditions for approval listed in **Section 5.1** of this requirements.]*

4.2.2 The applicant laboratory must provide the following documentation, but not limited to:

a) an organizational chart defining relationship that is relevant to the testing performance and the overall laboratory structure;

b) a general description of the laboratory, including its facilities, and operation;

c) conflict of interest statement;

d) an up-to-date copy of the accreditation scope — if the applicant laboratory is currently ISO/IEC 17025 accredited by other accreditation body;

e) quality manual and related management system documentation, including the latest internal and management review records;

f) standard operating procedures (SOPs), including the analytical methods used, quality assurance and quality control, instrument calibration, test results issuance, and equipment maintenance;

g) a list of all equipment, including records of in-house and external calibrations (i.e. equipment calibrations that your laboratory and an external company performs) and rental equipment, used to support the tests;

h) analysts' qualifications and training procedures/records;

i) both method validation and verification procedures and their data, see Section 10;

j) the latest proficiency testing (PT) report and any corrective action responses if an unsatisfactory result was observed;
*[**NOTE**: When required, laboratories must participate in an external ISO/IEC 17043 accredited PT program, where available and applicable, and obtain a satisfactory result.]*

4.3 The program is the user-fee supported and all laboratories must pay program fees (see Section 9) upon receiving of the billing invoice.

4.3.1 The admission fee must be received prior to advancing to the next step of approval process.

4.4 Review of Information Submitted: The PM will review the application package. She/he may request further information and/or ask for additional documents/records to facilitate the review.

4.4.1 The PM informs the applicant any nonconformities/discrepancies found. The laboratory must respond, in writing, addressing the nonconformities/discrepancies for further review prior to proceeding the next step of the approval process.

4.5 Performance of Initial Onsite Laboratory Audit: The PM will inform the applicant laboratory after the review (Sections 4.4) has been completed and deemed acceptable.

4.5.1 The AMS auditor contacts the laboratory to schedule a mutually agreeable date for the initial onsite audit.
*[**NOTE**: The initial yearly fee must be received before an onsite laboratory audit can be started.]*

4.5.2 During the audit, the auditor gathers objective evidence to verify the applicant laboratory's competence for the requested scope of approval. If any nonconformities found, the auditor will inform laboratory and document in the audit report.

4.5.3 The laboratory must respond in writing within 30 days upon receiving the final audit report addressing all documented nonconformities. The laboratory must supply evidence which clearly demonstrates that the actions taken have fully resolved and prevent the nonconformities.

4.5.3.1 If the laboratory's responses are found to be insufficient, LATD may request additional information.

4.5.4 If substantial nonconformities are cited, LATD may require an additional onsite audit with additional costs to the laboratory prior to granting approval.

4.6 Issuance of Acceptance Letter: AMS will provide a letter of approval to the laboratory after it meets all program requirements and the fees have been received.

4.7 The Official Listing of Approved Laboratories: The PM will list all USDA-approved laboratory on the official list and post on the LATD website.

5. Maintaining Program Status

5.1 Conditions for Approval: To maintain its approval, a laboratory must agree in writing, see the note of Section 4.2.1 g), to comply with the following LAP conditions for approval:

a) meet all program requirements;

b) use test method(s) approved by AMS;
*[**NOTE**: Any changes prior to implementing in the laboratory must notify and send verification results to the PM. Significant changes to an approved test method, a validation study may be required by the PM.]*

c) participate in quarterly external ISO/IEC 17043 accredited PT programs per analyte/matrix, as required, and meet satisfactory status
- the laboratory must send the PT reports with any corrective action responses, if any unsatisfactory results were observed, to the PM within 30 days of receipt of the report;
- overtime, every analyst performing the method(s) must participate in the check sample/PT program and submit the results with analyst name to PM;

d) make all information relevant to the LAP available to PM upon request;

e) during an onsite laboratory audit the laboratory must have an actual sample ready to demonstrate its testing competency and allow access to documents/records related to the LAP;

f) upon analyst changes the laboratory must inform PM with the training record and the results of method verification study performed by the new analyst;

g) resolve all nonconformities in a timely manner;

h) notify the PM within 30 days any significant changes relevant to its approval, status, or operation relating to
- legal, organizational, or ownership status;
- main policies and resources;
- organization, top management, or key personnel including the contact person, approved signatories, and analysts;
- location, equipment, facilities, and working environment, where significant;
- scope of approval;
- other matters that may affect the laboratory's test results and/or its ability to meet the program requirements;

i) pay all program fees by the due date on the billing invoice.

5.2 The laboratory must conform to 7 CFR Part 996 for peanuts, 7 CFR Part 981 for almonds, 7 CFR Part 983 for pistachio nuts or EU Council Directive 401/2006 of 23 February 2006 of the European Communities for the export of almonds, peanuts (ground nuts), and pistachio nuts.

5.3 Homogeneity studies: Laboratories are required to conduct homogeneity studies of dry grinds of all commodities on, at least, a quarterly basis using AOAC Official Method 977.16 "Sampling for Aflatoxins".

5.3.1 All laboratories are required to achieve a degree of size reduction, the dry ground nuts sample must pass through U.S. Standard.

a) No. 20 sieve for ground almonds and peanuts,

b) No. 10 sieve for ground shelled pistachio kernels, and

c) No. 5 sieve for ground in-shell pistachio nuts.

5.3.2 Copies of raw analytical data and statistical calculations of percent coefficient of variation (COV) must be provided to the PM in a timely manner throughout the year.

5.3.3 Laboratories are required to take corrective action if the COV is greater than or equal to:

a) 15 % for almonds and peanuts, and

b) 21% for pistachio nuts.

5.4 The LAP is managed on a calendar year (January – December).

5.5 Following initial approval, LATD will conduct an onsite laboratory audit during the first year of approval and every two years thereafter.

5.5.1 Onsite audit and nonconformities resolution processes, see Sections 4.5.

5.6 Renewal of Approval: Each approved laboratory receives a renewal notification email before the expiration date (the 31st of December) to start the renewal process.

5.6.1 The PM will send a renewal letter to the laboratory after it meets all program requirements with the yearly fee received. Then, the status of laboratory will be updated into the official listing of approved laboratories and posted on LATD website.

5.7 At any time, if there is concern about a laboratory's ability to meet program requirements, AMS may conduct an onsite audit of the laboratory at the laboratory's expense.

6. Removal from the Program

6.1 Voluntary Removal: A laboratory may voluntarily remove itself from the program at any time by submitting a written request to the PM.

6.2 Involuntary Removal or scope reduction: A laboratory may be involuntarily removed or scope reduced from the approval program. The PM informs the laboratory through an email/letter with one of the following reasons, but not limited to:

6.2.1 Falsification of analytical results.

6.2.2 Failure to use methods and procedures approved by AMS.

6.2.3 Failure to meet technical requirements.

6.2.4 Failure to maintain an acceptable performance level as indicated by the PT results and/or check samples; i.e., three unsatisfactory analyses in four consecutive sets of PT/or check samples, regardless of the approval year.

6.2.5 Persistently failed to perform corrective actions in a timely manner and/or satisfactory responses.

*[**NOTE**: The yearly fee will not be refunded nor prorated, regardless of voluntary removal or involuntary removal/scope reduction.]*

7. Readmission to the Program

7.1 A laboratory removed from the program due to falsification of analytical results may not reapply for approval into the program.

7.2 A laboratory involuntary removed from the program must wait, at least, for six months before it can reapply for approval (see Section 4).

7.3 A laboratory voluntary removed from the program may reapply for approval (see Section 4).

8. Appeals

8.1 Within 30 days of receiving of the letter for involuntarily removed from the program, the laboratory may file a written appeal to the LAS Branch Chief with supporting evidence as to why the laboratory should not be removed from the program. Within 30 days of receipt of the written appeal, the Branch Chief shall make a final determination and take an action, as deemed appropriate, with respect to the removal. The contact information is as follows:

Laboratory Approval Service, Branch Chief
Laboratory Approval & Testing Division
USDA, AMS, S&T
1400 Independence Ave. SW
Room 3533-S
Washington, D.C. 20250-0272
Email: LAS@ams.usda.gov

8.2 If the appeal to the LAS Branch Chief cannot be resolved to the satisfaction of a laboratory, an appeal, in writing, may be filed with the LATD Director. Within 90 days of receipt of the written appeal with supporting evidences, the LATD Director shall make a determination and take an action, as deemed appropriate, with respect to the removal. The contact information is as follows:

Kerry R. Smith, Ph.D., Director
Laboratory Approval & Testing Division
USDA, AMS, S&T
1400 Independence Ave. SW
Room 3533-S
Washington, D.C. 20250-0272
Telephone: (202) 690-4089
Email: KerryR.Smith@ams.usda.gov

9. Fee Schedule

9.1 LATD sets the program fees (e.g. admission fee, initial yearly fee, and yearly fee) for each program based on the time required by LATD personnel to perform the desk audit (documents review), onsite audit (travel to and from audit site and audit time), and associated administrative activities. Program fees are reviewed annually and adjusted as necessary to ensure that the fees are adequate to cover the cost of providing the service.

9.2 The admission fee covers the application activities.
*[**NOTE**: If the initial onsite audit cannot be started within 365 days (starting from the date of the applicant submission of required information) due to the applicant's delinquency, the applicant laboratory needs to pay the admission fee again to complete the application process.]*

9.3 The initial yearly fee payment is required prior to an initial onsite audit.
*[**NOTE**: The initial yearly fee covers the first calendar year, regardless when your laboratory was accepted into the program.]*

9.4 The yearly fee payment is required to continue the status of approval into next year from January to December.

9.5 The program fees are as follows:

Number of Commodities Approved to Analyze	Admission Fee $	Initial Yearly Fee $	Yearly Fee $
1	2710	5690	5620
2	3270	6640	8270
3	4070	7850	10860

*[**NOTE**: All fees must be paid by the due date on the billing invoice or interest and penalty charges will be assessed in accordance with the Code of Federal Regulations.]*

9.6 All fees are neither refundable nor prorated.

10. Technical Requirements

10.1 Analytical Methods

10.1.1 Peanut Domestic Program – Total aflatoxin in peanuts may be tested using following methods:

a) The immunoaffinity column (IAC) cleanup method with HPLC method (AOAC Official Method 991.31, A-F, H);
b) The Water Slurry method with thin-layer chromatography (TLC) analysis, designated as the alternative Best Foods (BF) method (AOAC Official Method 998.03); or
c) The IAC with direct fluorometry method (AOAC Official Method 991.31, A-G).

10.1.2 Pistachio Domestic Program – Total aflatoxin in pistachios may be tested using following methods:

a) The immunoaffinity column (IAC) cleanup with HPLC method (AOAC Official Method 991.31, A-F, H); or
b) The IAC with direct fluorometry method (AOAC Official Method 991.31, A-G).

10.1.3 Almond and Pistachio Export Program — Total aflatoxin and B1, B2, G1, and G2 in almond and pistachio samples must be quantified by an AMS-approved confirmatory method (e.g., HPLC): The IAC cleanup with HPLC method (AOAC Official Method 991.31, A-F, H).

10.2 Sample Preparation

10.2.1 Laboratories must follow the AOAC International, Official Method 977.16, "Sampling for Aflatoxin" for the sampling and sample preparation.

10.2.2 For aflatoxin analysis, laboratories are required to achieve a size reduction, if dry grind is used.

10.3 Method Validation Parameters

10.3.1 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).
*[**NOTE**: LODs should be determined for both aflatoxin B1and total aflatoxins by using HPLC. Determination of total aflatoxins by using the fluorometry method must be confirmed by HPLC.]*

10.3.2 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.
$LOQ = X_{Ave} + 10SD$.
*[**NOTE**: LOQs should be determined for both aflatoxin B1and total aflatoxins by using HPLC. Determination of total aflatoxins by using the fluorometry method must be confirmed by HPLC.]*

10.3.3 Percent Recovery and Repeatability ─ The recovery and repeatability study is to be conducted on three groups of samples, one group spiked at ½ tolerance, a second group at tolerance, and the third group at 2 x tolerance. Each group shall consist of five samples of program appropriate nuts, and all analyses are to be started and completed on the same day.

a) The sample weight is 50 grams in-shell pistachios.

b) The sample weight is 150 grams almonds

c) The sample weight is 196 grams of slurry peanuts (1100g of peanut meal blended with 1600 mL water)

10.3.3.1 All duplicate results are to be averaged; calculate the mean, standard deviation, relative standard deviation (RSDr) for all samples; and the percent aflatoxin recovered for each sample.

***NOTES**: 1) With three groups of samples and five samples in each group analyzed in duplicate, there are a total of 30 samples. Fifteen results are to be used in the statistical analysis since duplicate results are to be averaged. 2) For those laboratories with more than one analyst, repeat the procedure for recovery and repeatability on a different day using a different analyst. If the laboratory only has one analyst, the analysis should be repeated on a different day by the analyst, and the 30 data points from the two days used for the statistical analysis. If there are more analysts, they must perform the analysis also. The 15 data points for each analyst can be isolated and used as part of their documented training.*

10.4 Data Acceptability

10.4.1 Percent Recovery is determined by calculating the percent recovery (aflatoxin B1 and/or total aflatoxins) for each of the sample results generated in Section 10.3.3. Suitability of results is determined by comparison of recoveries to the recommended values in the table below, based on the concentration ranges.

Criteria	Concentration Range	Recommended Value
Blanks	All	Negligible
Recovery– Aflatoxins B_1, B_2, G_1, G_2	< 1.0 µg/kg	50 – 120 %
	1 – 10 µg/kg	70 – 110 %
	> 10 µg/kg	80 – 110 %

*[**NOTE**: Values apply to both B_1 and total aflatoxins.]*

10.4.2 Repeatability is determined by calculating the relative standard deviation (RSD) for each of the sample results generated in Section 10.3.3. Suitability of results is determined by

comparison to the expected or predicted result calculated from the Horwitz equation, $PRSD_R = 2C^{(-0.15)}$. In the equation C is the mass fraction of the concentration of analyte.

10.4.3 RSD_R is calculated from the ½ tolerance, tolerance, and 2 x tolerance levels used in Section 10.3.3. An assessment of the acceptability of the precision found can be made by calculating the ratio of the precision found to that of the predicted RSD, called HORRAT, see the equation: HORRAT = (found) RSDr / (predicted) $PRSD_R$.
*[**NOTE**: Values ≤ 1.3 are generally considered acceptable for single-laboratory validation studies.]*

10.4.4 Linearity of calibration curve ─ It is determined by preparing standard solutions at five concentration levels, including ½ tolerance, tolerance, and 2 x tolerance. Five levels are required to detect curvature in the plotted data. The standards should be prepared and analyzed at least three times. Acceptability of linearity data is judged by examining the correlation coefficient and y-intercept of the linear regression line for the response versus concentration plot. A correlation coefficient of > 0.99 is generally considered as evidence of acceptable fit of the data to the regression line.

10.5 Technical Competence

10.5.1 The laboratory must demonstrate its ability to detect and quantify aflatoxin in almonds, peanuts, and pistachio nuts by testing a set of 10 samples spiked with aflatoxins in the range of 1 to 30 parts per billion (ppb).

10.5.2 Samples are to be analyzed in two sets of 5 samples analyzed on two different days, and if possible, each set of 5 samples analyzed by two different analysts.

10.5.3 The laboratory must submit all information on how the samples were prepared, spiked, the spiking concentrations, how they were run, operating parameters of the instrument, and the chromatograms to the PM for review.

10.5.4 Naturally contaminated samples may be used in lieu of spiked samples.

10.5.5 In order to be acceptable, each test result provided for these samples must meet the following criteria:

Acceptable Range of Recovery	Aflatoxin Levels ppb of Spiked or Incurred Samples
50-120%	< 1 ppb
70-110%	1 to 10 ppb
80-110%	> 10 ppb

11. Official Analysis Certificate/Report

11.1 An example of official analysis certificate/report must sent to the PM for review.

11.2 For pistachio and/or almond export, the aflatoxin analysis certificates/reports must include:

a) "Results are not corrected for recovery or expanded measurement of uncertainty" and

b) the percent level of daily determined recoveries for total aflatoxins and individual aflatoxin B1.

11.3 Official peanut aflatoxin analysis certificate must include all of the following information:

a) one of the following statements may be used for the methodology as approved
- an immunoaffinity column with direct fluorometry method of analysis,
- water slurry method with thin-layer chromatography (TLC) analysis designated as the alternative Best Foods (BF) method of analysis, or
- an immunoaffinity column cleanup with high performance liquid chromatography (HPLC) method;

b) "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"; and

c) "USDA laboratory approved to test for total aflatoxin content in samples for domestic and imported peanuts marketed in the United States".