United States Department of Agriculture | Agricultural Marketing Service | Science and Technology Program | Laboratory Approval and Testing Division | Room 3533-S 1400 Independence Ave., SW Washington, DC 20250-0272

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

1. Purpose

1.1 This document provides the requirements for the Laboratory Approval Program (LAP) for Aflatoxin in Almonds, Peanuts, and Pistachio Nuts. This LAP is for laboratories seeking to perform confirmatory analysis of aflatoxin 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.

1.2 It also provides the procedures and requirements used for the objective evaluation of a laboratory's analysis program submitted for approval and monitored by the Agricultural Marketing Service (AMS), Science and Technology (S&T) Program, Laboratory Approval and Testing Division (LATD), Laboratory Approval Service (LAS).

2. Scope

This 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 aflatoxin in almonds, peanuts, pistachio nuts, and all aspects of a laboratory's documented quality management system that apply to this analysis.

3. References

3.1 Official Journal of the European Communities, No. L 70, 9.3.2006, pp 12-34, Commission Regulation (EC) No. 401/2006 0f 23 February 2006 for the detection of Aflatoxin in Almonds (export) Program.

3.2 Official Journal of the European Communities, 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.

3.3 7 CFR Part 996. Minimum quality and handling standards for domestic and imported peanuts marketed in the United States.

3.4 Memorandum of Understanding for Aflatoxin Testing of Domestic Peanuts (FDA and USDA).

3.5 AOAC International, Official Method 991.31 and 998.03

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

3.7 AOAC International Official Method 977.16, Sampling for Aflatoxins

3.8 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.

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

3.10 FDA 2012. 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. http://www.fda.gov/downloads/ScienceResearch/FieldScience/UCM298730.pdf

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 Aflatoxins 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 into the program, the applicant laboratory must address all program requirements; including fees (see Section 10). The following requirements must be provided to the PM for review:

4.2.1 A letter from the management providing information as to:

4.2.1.1 Corporate entity, name, address, phone number, email address, and legal status;

4.2.1.2 A clearly defined scope for approval;

4.2.1.3 The name(s), title(s) and contact information of laboratory staff designated to serve as Approved Signatory(ies) of test reports/certificates of analysis (COA) that reference USDA-approved laboratory;

4.2.1.4 Conflict of interest statement; and

4.2.1.5 Signature of an authorized representative of the applicant laboratory.

4.2.2 Analysts' qualifications and laboratory training procedures.

4.2.3 Standard operating procedures (SOPs) – They include, but not limited to, the analytical method used, quality assurance and quality control, instrument calibration, test results issuance, and equipment maintenance.

4.2.4 A description of the physical condition of the laboratory and the capabilities in terms of major and critical equipment/instruments for the analysis.

4.2.5 An organization chart showing the overall laboratory structure. Additional documents detailing the relevant person's (quality manager and analysts at a minimum) laboratory functions, qualifications and training procedures/records.

4.2.6 Method Validation – Laboratories must submit method validation data and results including, but not limited to, uncertainty of measurement, method detection limit, method quantitation limit, linearity, accuracy, precision, sensitivity, and recovery (See Section 11). The laboratory's method validation process shall also incorporate the use of Standard Reference Materials.

4.2.7 Proficiency test (PT) results - Laboratories must participate in an external PT program, obtain a satisfactory status on quarterly PT samples, and send the PT sample reports to the PM. If the PT results indicate unsatisfactory performance, reports of corrective actions must be sent to the PM.

4.2.8 The program is user-fee supported and all laboratories must pay annual program fees upon receipt of the billing invoice. All fees must be received prior to admission to the program.

4.2.8.1 For billing purpose, the laboratory needs to provide Federal W-9 Form showing the laboratory's taxpayer identification number, or Federal W-8BEN Form (Certificate of Foreign Status of Beneficial Owner for United States Tax Withholding) showing the "Foreign tax identifying number" of a foreign laboratory. The laboratory also needs to supply the contact person(s) of account payable.

4.3 Review of Information Submitted: The PM will review the required information and communicate any concerns or deficiencies. Laboratories must respond, in writing, to the concern or deficiency.

4.4 Required Sample Testing: 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).

4.4.1 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.

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

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

4.4.4 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

4.5 Initial Onsite Laboratory Review: A laboratory audit will be conducted by LATD personnel after required sample testing results have been analyzed successfully.

4.6 Issuance of Acceptance Letter: AMS will provide a letter/email of approval to the laboratory after it meets all program requirements.

5. Maintaining Program Status

5.1 The LAP is managed on a yearly basis (January – December).

5.2 Laboratories must participate in an external PT sample program administered by an ISO 17043 accredited PT institution.

5.2.1 Laboratories must participate in an external proficiency program that provides quarterly PT samples. If a laboratory performs aflatoxin analysis on more than one matrix under this program, participation in two PT programs is required.

5.2.1.1 As soon as reports are received from the PT sample provider, the laboratory must share the reports of results with the PM.

5.2.1.2 The laboratory must meet expected performance standards of the PT program regardless of type of program. *[**Note:** Discussions with the PM regarding PT sample failures or statistical outliers should be initiated after the first consecutive unsatisfactory analyses. Discussions may be initiated by the approved laboratory or the PM.]*
5.2.1.3 Three unsatisfactory analyses in four consecutive set of PT samples, regardless of the approval year, will result in the involuntary removal of the laboratory from the program. *[**Note:** Once an approved laboratory reaches this status in the LAP, the PM will notify AMS, Fruit and Vegetable Program (F&V), the Almond Board of California (ABC), the Pistachio Administrative Committee (PAC), and the Peanut Standards Board (PSB) of the laboratory's status, so that F&V, the ABC, the PAC, and the PSB can notify clients using this approved laboratory at the same time as the laboratory.]*
5.2.1.4 The client(s) that are using a laboratory that has three unsatisfactory consecutive PT samples results will be required to submit samples to another USDA Approved Laboratory for Aflatoxin Analysis until the problem is resolved. In the interim, laboratories with statistical outliers need to initiate discussion with the PM and an on-site audit may follow. *[**Note**: If an onsite review is necessary, in addition to the yearly review, the laboratory will be responsible for the additional travel expenses.]*

5.3 The laboratory must conform to 7 CFR Part 996 for peanuts, 7 CFR Part 981 for almonds, and 7 CFR §983.150 for pistachio nuts in the domestic program or EU Council Directive 401/2006 0f 23 February 2006 of the European Communities for the export of almonds , peanuts (ground nuts), and pistachio nuts.

5.4 Laboratories that test for aflatoxins in almonds, peanuts, and pistachio nuts are required to perform homogeneity studies of dry grinds of all three commodities subsamples on, at least, a quarterly basis using the written instructions provided in the AOAC International, Official Method 977.16, "Sampling for Aflatoxins." In this method of sampling and preparing samples for aflatoxins analysis, laboratories are required to achieve a degree of size reduction, such that nuts must be ground to pass No. 20 sieve for ground almonds and peanuts, U.S. Standard No. 5 sieve for ground in-shell pistachio nuts, and U.S. Standard No. 10 sieve for ground shelled Pistachio kernels.

5.4.1 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.
5.4.2 Laboratories are required to take corrective action if the COV is greater than or equal to 15 percent for almonds and peanuts, and 21% for pistachio nuts.

5.5 The laboratory must inform the PM if it's approved equipment or methods have been modified. The laboratory must perform method verification study again with acceptable results including the uncertainty of measurement.

5.6 Upon analyst changes, the laboratory must inform the PM and provide the training record and the results of method verification study performed by the new analyst including the uncertainty measurement. Method verification study for the new analysts must be comparable to other analysts performing the same method, or results must be comparable to method validation performance data.

***NOTES**: 1) Only with the PM's approval, laboratories may change their operations. Those operations include but are not limited to: location, SOP's, equipment, instruments, and analysts. Any change may require the laboratory to verify or validate the analytical method. 2) The approved laboratory must inform the PM if its name, physical location, or contact information has changed. 3) Not informing the PM of these changes may lead to immediate removal from this program which would require the laboratory to re-apply in order to participate in the program.*

5.7 The laboratory must meet all program requirements. All method SOPs, method validation and verification data, and PT results must be made available to PM upon request.

5.8 The laboratory must pass announced or unannounced onsite audits at least every two years.

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

***NOTE**: The Laboratory can be suspended for failing to send the required written responses to USDA or failing to implement corrective actions for nonconformance items within the agreed upon timeframe of an onsite audit.*

5.9 The export pistachios and almond aflatoxin certificates/reports issued by the laboratory must include:

5.9.1 The statement that "Results are not corrected for recovery or expanded measurement of uncertainty" and

5.9.2 The percent level of daily determined recoveries for total aflatoxins and individual aflatoxin B_1.

5.10 Official peanut aflatoxin analysis certificate issued by the laboratory must include all of the following information (5.11.1 to 5.11.3):

5.10.1 One of the following statements may be used for the methodology as approved:

5.10.1.1 An immunoaffinity column with direct fluorometry method of analysis;

5.10.1.2 Water slurry method with thin-layer chromatography (TLC) analysis designated as the alternative Best Foods (BF) method of analysis; or

5.10.1.3 An immunoaffinity column cleanup with high performance liquid chromatography (HPLC) method.

5.10.2 "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".

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

5.11 The laboratory must pay the yearly program fee on time. On time is defined as payment must be received within 30 days from the date on the invoice or billing document.

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: A laboratory may be involuntarily removed from the approval program with/for any one of following reasons, but not limited to:

6.2.1 Falsification of analytical results or aflatoxin certificates.

6.2.2 Failure to maintain an acceptable performance level as indicated by PT sample results.

6.2.3 Failure to accept and implement recommendations made by the PM to improve performance and/or eliminate bias in laboratory results.

***NOTE**: The yearly program fee will not be refunded (whole or prorated), regardless of voluntary removal or involuntary removal.*

7. Readmission to the Program

7.1 A laboratory removed from the program due to falsification of analytical results or aflatoxin certificates will be prohibited to reapply for approval in the program.

7.2 A laboratory involuntary removed from the program must wait at least one year before it can resubmit a written request to the PM in order to initiate the admission process again.

7.**3** A laboratory that has voluntarily removed itself from the program may not reapply until the beginning (January) of the following year.

7.3.1 If the laboratory has not been participating in the program for up to one year, it has to pay the yearly fee in full and is required to have an onsite laboratory review.

7.3.2 If the laboratory has not been participating in the program for more than a year, it has to resubmit a written request to the PM in order to initiate the admission process again.

8. Complaints

8.1 All complaints should be brought to the attention of the PM for timely resolution. If the complaint cannot be resolved by the PM to the satisfaction of the complainant, the complaint may be brought to the attention of the Director of the LATD. The contact information for the current Director is as follows:

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

9. Appeals

9.1 Within 30 days a laboratory that has been involuntarily removed from the program may file a written appeal to the Deputy Administrator of the S&T Program 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 Deputy Administrator shall make a determination and take an action, as deemed appropriate, with respect to the removal. The name, address, and telephone number of the current Deputy Administrator are as follows:

Ruihong Guo, Ph.D., Deputy Administrator
USDA, AMS, Science and Technology
1400 Independence Ave. SW, Room 3543-S
Washington, D.C. 20250-0270
Telephone: (202) 720-8556
Email: Ruihong.Guo@ams.usda.gov

9.2 If the appeal to the Deputy Administrator of the S&T Program cannot be resolved to the satisfaction of a laboratory, an appeal, in writing, may be filed with the Administrator of AMS. Within 90 days of receipt of the written appeal with supporting evidences, the Administrator shall make a determination and take an action, as deemed appropriate, with respect to the removal. The name, address, and telephone number of the current Administrator are as follows:

Anne Alonzo, Administrator
USDA, Agricultural Marketing Service
1400 Independence Ave. SW, Room 3071-S
Washington, D.C. 20250-0201
Telephone: (202) 720-4276

10. Fee Schedule

10.1 LATD sets the program fee for each program based on administrative costs. Program fees are reviewed yearly to determine if they are adequate compared with USDA operational costs.
10.2 For each commodity (almonds, peanuts, or pistachio nuts), the Aflatoxin Fee Schedule is as follows:

# of Commodities Approved to Analyze	Admission Fee	Initial Yearly Fee	Yearly Fee
1	2601	5402	5480
2	3035	6052	8081
3	3468	6702	10393

10.3 The admission and yearly fees are nonrefundable.

11. Technical Requirements

11.1 Analytical Methods

11.1.1 Peanut Domestic Program – Total aflatoxin in peanuts can be tested using following methods:

11.1.1.1 An immunoaffinity column (IAC) cleanup with HPLC method (AOAC Official Method 991.31, A-F, H);
11.1.1.2 Water slurry method with thin-layer chromatography (TLC) analysis, designated as the alternative Best Foods (BF) method (AOAC Official Method 998.03); or
11.1.1.3 An IAC with direct fluorometry method (AOAC Official Method 991.31, A-G).

11.1.2 Pistachio Domestic Program – Total aflatoxin in pistachios can be tested using following methods:

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

11.1.3 Almond and Pistachio Export Program ─ Total aflatoxin and B_1, B_2, G_1, and G_2 in almond and pistachio samples must be quantified by an AMS-approved confirmatory method (e.g., HPLC).
11.1.3.1 An IAC cleanup with HPLC method (AOAC Official Method 991.31, A-F, H).

11.2 Method Validation Parameters
11.2.1 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, and determined total aflatoxins by using the fluorometry method must also be confirmed by HPLC.*

11.2.2 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 and determined total aflatoxins by using the fluorometry method must also be confirmed by HPLC.*

11.2.3 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.
11.2.3.1 The sample weight is 50 grams **in-shell** pistachios.
11.2.3.2 The sample weight is 150 grams almonds
11.2.3.3 The sample weight is 196 grams of slurry (1100g of peanut meal blended with 1600 mL water)
11.2.3.4 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 should perform the analysis also. It should be noted that the 15 points of data for each analyst can be isolated and used as part of their documented training.*

11.3 Data Acceptability

11.3.1 Percent Recovery is determined by calculating the percent recovery (aflatoxin B1 and/or total aflatoxins) for each of the sample results generated in section 11.2.3. Suitability of results is determined by comparison of recoveries to the recommended values in the table below are based on concentration range.

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 B1 and total aflatoxins.*

11.3.2 Repeatability is determined by calculating the relative standard deviation (RSD) for each of the sample results generated in section 11.2.3. 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.

11.3.3 RSD_R is calculated from the ½ tolerance, tolerance, and 2 x tolerance levels used in section 11.2.3. 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.*

11.3.4 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.

11.3.5 Sample preparation

11.3.5.1 For aflatoxin analysis, during dry grind, laboratories are required to achieve a size reduction, such that nuts must be ground to pass through a No. 20 sieve.

11.3.5.2 Laboratories must follow the AOAC International, Official Method 977.16, "Sampling for Aflatoxin" for the sampling and preparing of samples.