Laboratory Approval Program – Mycotoxins

1 Purpose

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

The Laboratory Approval Program (LAP) 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 LAP is for a laboratory seeking to obtain and maintain its status as a USDA-approved laboratory for the analysis of mycotoxins in almonds, pistachios, and/or peanuts based on the stipulations of the final market destination: U.S. domestic, U.S. import, and export. This Program verifies technical and quality control competencies of a laboratory for the analysis of mycotoxins. All aspects of a laboratory's quality management system (business processes relative to the scope of approval) are applicable and are critical for ensuring the defensibility of the analytical results produced under the LAP.

2.1 Almonds for aflatoxins (B_1, B_2, G_1, G_2) for export to the European Union (EU) through the Pre-Export Certification program (PEC) of the Almond Board of California (ABC).

2.2 Peanuts for aflatoxins (B_1, B_2, G_1, G_2) marketed domestically for human consumption, including imports, in accordance with 7 CFR 996.

2.3 Peanuts for aflatoxins (B_1, B_2, G_1, G_2) for export to the EU.

2.4 Pistachios for aflatoxins (B_1, B_2, G_1, G_2) for domestic and import markets in accordance with 7 CFR 983 and 7 CFR §999.600, respectively.

2.5 Pistachios for aflatoxins (B_1, B_2, G_1, G_2) for export to the EU for the Administrative Committee for Pistachios' (ACP) Pistachio Export Aflatoxin Reporting (PEAR) program.

2.6 Pistachios for ochratoxin A (OTA) for export to the EU for the ACP PEAR program.

Laboratory Approval Program – Mycotoxins

3 Table of Contents

4 Glossary of Terms

ABC	Almond Board of California
ACP	Administrative Committee for Pistachios
AFL	Aflatoxin
AMS	Agricultural Marketing Service
AOAC	Association of Official Analytical Collaboration International
AOCS	American Oil Chemists' Society
APC	American Peanut Council
CFR	Code of Federal Regulations
CCV	Continuing Calibration Verification
CV	Coefficient of Variation

EC	European Commission
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
ICV	Independent Calibration Verification
ISO/IEC	International Organization for Standardization/International Electrotechnical Commission
LAP	Laboratory Approval Program
LAP-Mycotoxins	LAP for Analysis of Mycotoxins
LAS	Laboratory Approval Service
LATD	Laboratory Approval and Testing Division
LC	Liquid Chromatography (e.g., HPLC, UPLC)
LOQ	Limit of Quantitation
MOU	Memorandum of Understanding
OTA	Ochratoxin A
PEAR	Pistachio Export Aflatoxin Reporting Program
PEC	Pre-Export Certification
PHRED	Photochemical Reactor Enhanced Detection
PM	Program Manager
PT	Proficiency Testing
S&T	Science & Technology Program
TLC	Thin Layer Chromatography
UPLC	Ultra (High) Performance Liquid Chromatography
USDA	United States Department of Agriculture
VCM	Vertical Cutter Mill

5 References

5.1 The following are referenced in this document. Dated references apply to the edition cited and undated references apply to the latest edition published (including any amendments).

5.2 Laboratory Approval Program:

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

b) LAP-PR.06, Laboratory Approval Program – Fees.

c) USDA AMS LATD LAS Website, https://www.ams.usda.gov/services/lab-testing/lab-approval.

5.3 Quality Assurance Standards:

a) ISO/IEC 17025:2017. General requirements for the competence of testing and calibration laboratories.

b) USDA AMS Laboratory Standards of Practice.

5.4 USDA Requirements:

a) 7 CFR Part 981 – Almonds grown in California.

b) 7 CFR Part 983 – Pistachios grown in California, Arizona, and New Mexico.

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

d) 7 CFR §999.600 – Specialty Crops; Import Regulations. Regulation governing the importation of pistachios.

e) Milled Peanuts, Inspection Instructions. USDA MRP AMS SCP SCID. December 2020.

f) Pistachio Nuts In the Shell Shipping Point and Market Inspection Instructions. USDA AMS, February 2005.

g) Pistachio Nuts In the Shell Shipping Point and Market Inspection Instructions, Patch # 045, Testing Samples For Aflatoxin. USDA AMS, April 15, 2019.

h) Pistachio Nuts In the Shell Shipping Point and Market Inspection Instructions, Patch #066, Aflatoxin Sampling and Testing for Imported Raw and Blanched Pistachios. USDA AMS, April 28, 2021.

5.5 FDA Requirements:

a) MOU 225-19-031. Memorandum of Understanding between AMS and FDA in inspecting, sampling, and testing peanuts, Brazil nuts, and pistachio nuts for aflatoxins.

b) FDA Compliance Policy Guides, Section 570.375 Aflatoxin in Peanuts and Peanut Products.

c) FDA Compliance Policy Guides, Section 570.500 Pistachio Nuts - Aflatoxin Adulteration.

d) FDA Compliance Program Guidance Manual, 7307.001, Chapter 07 – Molecular Biology and Natural Toxins. Mycotoxins in domestic and imported foods FY 15/16.

e) Guidelines for the Validation of Chemical Methods in Food, Feed, Cosmetics, and Veterinary Products, 3rd Edition. US FDA. FDA Foods Program, October 2019.

5.6 Industry Requirements:

a) Administrative Committee for Pistachios: Handler's Guide.

b) Almond Board of California, Pre-Export Checks (PEC) Program Manual.

c) Pistachio Export Aflatoxin Reporting (PEAR) Program.

5.7 Methodology:

a) AOAC International, Official Methods 977.16, Sampling for Aflatoxins - Preparation for Sample Procedure.

b) AOAC International, Official Methods 991.31, Aflatoxins in Corn, Raw Peanuts, and Peanut Butter - Immunoaffinity Column (Aflatest) Method.

c) AOAC International, Official Methods 998.03, Aflatoxins in Peanuts - Alternative BF Method.

d) 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.

e) AOAC International, Official Methods 2005.08, Aflatoxins in Corn, Raw Peanuts, and Peanut Butter - Liquid Chromatography with Post-Column Photochemical Derivatization.

f) 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.

5.8 Country Specific Regulations:

a) EC 401/2006. 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.

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

c) EU 2015/949. Commission Implementing Regulation (EU) No. 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.

5.9 Additional Resources:

a) Eurachem Guides.

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

GENERAL REQUIREMENTS

6 Laboratory Approval Program Administrative Policy

6.1 A laboratory seeking admission to the LAP must fulfill the requirements and follow the process described in the program 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.

6.2 This program is administered on an annual, calendar year, basis.

6.3 The program procedure, *LAP-PR.06, Laboratory Approval Program – Fees,* explains the fees for services.

6.4 The administrative procedures above are available on the LAS website, or by contacting the LAS Mycotoxin Program Manager (PM) for the current version of the procedure.

7 Summary of General Program Requirements

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

a) meet all program requirements relevant to the scope of approval;

b) comply with mandatory laboratory practices based on the ISO/IEC 17025 standard (See §8);

c) use test method(s) approved by AMS. (See §9);

d) validate analytical and sample preparation methods prior to use and ensure the validation data package is available for review upon request (See §10);

e) evaluate analyst competency and maintain satisfactory status (See §11);

f) participate in proficiency testing (PT) programs and maintain satisfactory status (See §11);

g) submit sieve testing results to PM (See §14.3.h));

h) communicate regularly with the PM to share vital information regarding the laboratory and make all information relevant to the LAP available to PM upon request (See §5.2.a));

i) notify the PM within 30 days of significant changes relevant to the laboratory's approval status and/or ability to meet the Program's requirements including but not limited to: legal, organizational, or ownership status; laboratory policies, procedures, and resources; change in key managerial personnel, contact persons, and signatories;

and verification of adequate method performance after a change in location, equipment, facilities, and working environment. It is at the discretion of LAS whether an onsite visit/audit is required to evaluate any significant change that a laboratory undergoes (See §5.2.a));

j) be available for a biennial (every other year) re-assessment audit, have actual sample(s) ready to demonstrate its competency of performing the test method, and comply with requests for documents and records before and during the audit (See §5.2.a));

k) respond to each nonconformance found with a record of an investigation, root cause analysis, and correction or, if warranted due to time, a corrective action plan, within 30 calendar days of being reported (See §5.2.a));

l) resolve each nonconformance in a timely manner, whether identified by LAS auditor, another organization, or internally (See §5.2.a));

m) pay all program fees by the due date on the billing invoice (See §5.2.b)).

8 Mandatory Quality Assurance Practices

8.1 Implement quality assurance and quality control procedures to ensure a validated and qualified analysis, prove competence, and ensure defensible data. This Program does not require ISO 17025 accreditation; however, it is a common practice for testing laboratories to become ISO 17025 accredited.

8.2 LAS uses the ISO 17025 standard to evaluate all laboratory quality systems regardless of accreditation status. A nonconformance identified during a LAS assessment audit may be cited to the ISO 17025 standard (See §5.2.a)).

8.3 Maintain records for at least three years (See §5.2.a)).

9 Method Selection

9.1 Use analytical testing methods approved by AMS.

9.2 Use method fit for purpose based upon the needs of the customer and consistent with specified requirements.

9.3 Use methods as validated; for the purposes of this Program, consider methods, with collaborative studies, specifically those published by USDA, FDA, and AOCS, as validated methods (see §5.7).

9.4 Refer to sections below for approved methods and other technical requirements:

a) Almonds – AFL – Export to EU: See §18.

b) Peanuts – AFL – U.S. Domestic & U.S. Imports: See §19.

c) Peanuts – AFL – Export to EU: See §20.

d) Pistachios – AFL – U.S. Domestic & U.S. Imports: See §21.

e) Pistachios – AFL – Export to EU: See §22.

f) Pistachios – OTA – Export to EU: See §23.

10 Demonstration of Method Performance by Validation and Verification Evaluation

10.1 Demonstrate the sample test method(s) is competently and proficiently performed prior to using it for testing and reporting results by using a validation / verification process, where:

a) Validation is the process of demonstrating that a method is suitable for its intended purpose,

b) Verification is the process of demonstrating a validated method can be performed to the same level of performance determined during the validation, and

c) It is recognized that there are multiple ways to demonstrate performance; therefore, where the Program does not specify a specific protocol, consult with the Program Manager to determine a suitable validation / verification plan using U.S. and internationally recognized protocols.

10.2 Demonstrate that variations or modifications to a validated method (e.g., different matrix, different analyte, etc.) are fit-for-purpose.

10.3 Prepare a Method Validation Data Package to record the procedure has been demonstrated as fit for use.

a) Consolidate pertinent information into an integrated and auditable data package to support the objective's conclusion that includes at least the following: cover statement containing the conclusion of the validation / verification process, test method procedure, relevant statistics (i.e., linearity, accuracy, precision, measurement uncertainty, and limit of quantitation), and traceable data (raw and summarized).

b) Send validation / verification package to the PM for review and approval.

c) Ensure validation / verification data package is readily available at the laboratory for as long as the method is utilized, plus three years after the last reported results.

d) Ensure official record of approval for method is maintained and readily available.

10.4 Validate the sample preparation procedure for grinding samples to achieve an adequate particle size and homogenous grind for each analyte/matrix.

a) Determine optimal parameters to achieve a homogeneous grind, including type of grinder, type of blade (e.g., smooth-edge, serrated, notch-edge), and minimum time to grind.

b) Select an unground sample with either a known incurred natural mycotoxins contamination or spike the nuts (ideally in one location).

c) Grind sample using optimized parameters.

d) Analyze at least 10 representative subsamples to determine if the resulting grind is homogeneous.

e) Evaluate results from the subsamples. Acceptance criteria for a homogeneous grind is percent recovery as found in Table 1; and Coefficient of Variation (CV) ≤ 20%.

f) Establish sieve test acceptance criteria for maintaining vertical cutter mill equipment by performing a particle size tests using subsamples of the grind passed through a No. 20 sieve (see §14.3).

g) Document, in a procedure, the final parameters validated including type of grinder, type of blade, minimum grind time, and sieve test acceptance criteria.

h) For significant changes made to the grinding procedure perform validation to demonstrate the changes result in a homogeneous grind and re-establish the sieve test acceptance criteria.

i) An exception or variation to AOAC 977.16 method may be considered by submitting a request and validation data that demonstrates the variation provides the same or better performance than the original procedure.

10.5 Demonstrate the sample testing method(s) can be competently and proficiently performed prior to using it for testing and reporting results.

a) Demonstrate performance criteria of the reference method (e.g., AOAC) are met or exceeded (e.g., linearity, accuracy, precision, measurement uncertainty, limit of quantitation (lowest concentration that can be reliably detected and measured with accuracy and precision).

b) Demonstrate performance criteria outlined in EC 401/2006 of 23 February 2006 Annex II Part 4 are met, as relevant to scope of approval including export to EU (e.g., parts 4.2, 4.3, 4.4, and 4.5) (see §5.8.a)).

11 Analyst Competency and Proficiency Testing

11.1 Analyst Competency:

a) Ensure new analyst responsible for performing test method(s) demonstrates competency and provide record of training and competence evaluation to the PM.

b) Ensure every analyst responsible for performing the method(s) participates in the PT program at least once in a two-year span.

11.2 Laboratory Analytical Competency:

a) Participate in a PT program on a quarterly basis.

b) Use a PT program relevant to the approved method in terms of analyte, concentration range, and matrix on the scope of approval—administered by an external third-party program such as AOCS, FAPAS, or similar (with preference to ISO 17043 accredited provider).

c) Participate in the FAPAS PT program when scope of approval includes export to EU.

d) Participate in PT Program and at the frequency specified by the Program Manager when a quarterly PT program is unavailable.

e) Review PT report and initiate the corrective action process when unsatisfactory results are observed. Unsatisfactory PT results and action to take are defined as follows:

$\|z\| \geq 3$	Initiate immediate corrective action investigation on the part of the laboratory to establish root cause.
$2 \leq \|z\| \leq 3$	Evaluate in the context of other scores obtained in the same test and other PTs over time. Investigate to determine the root cause and take action as needed.

11.3 Submit to the PM:

a) Record of new analyst training and demonstration of competency.

b) Copy of the PT report with analyst name, and PT ID, within 30 days of receipt.

c) Record of corrective action response for unsatisfactory results, within 30 days of receiving the PT report.

*****

The following sections include requirements organized by scope; and are in addition to the requirements outlined above.

TECHNICAL REQUIREMENTS (GENERAL)

12 Performance Criteria

Table 1. Performance criteria for aflatoxins (B_1, B_2, G_1, G_2).

Criterion	Concentration Range	Recommended Value	Maximum permitted Value
Blanks*	All	Negligible	
Recovery* Aflatoxins B_1, B_2, G_1, G_2	< 1.0 ug/kg	50 to 120%	
	1-10 ug/kg	70 to 110%	
	> 10 ug/kg	80 to 110%	
Reproducibility* RSD	All	As derived from Horwitz Equation	2 x value derived from Horwitz Equation
Repeatability* RSD_r	May be calculated as 0.66 times Reproducibility RSD_R at the concentration of interest		
Measurement Uncertainty** Fit-for-use	Certainty of 95% at target concentration		

Calculation	Concentration Range	Equation
Horwitz Equations†	$1.2\ x\ 10^{-7} \leq C \leq 0.138$	$RSD_R = 2^{(1-0.5 \log C)}$
	$C < 1.2\ x\ 10^{-7}$	$RSD_R = 22\%$

Note: Values apply to B_1 and sum of $B_1 + B_2 + G_1 + G_2$
* EC 401/2006, Annex II, 4.3.1.1(a)
** EC 401/2006, Annex II, 4.2.2.2
† EC 401/2006, Annex II, 4.3.1.1(i)

Table 2. Performance criteria for ochratoxin A.

Level ug/kg*	RSD_r %*	RSD_R %*	Recovery %*	Measurement Uncertainty**
< 1	≤ 40	≤ 60	50 to 110	Certainty of 95% at target concentration
≥ 1	≤ 20	≤ 30	70 to 110	

* EC 401/2006, Annex II, 4.3.1.1.b
** EC 401/2006, Annex II, 4.4.1

13 Quality Controls

13.1 Use controls, daily with use, to demonstrate testing is performed correctly and factors that could negatively impact the results are mitigated.

13.2 Take immediate action prior to continuing testing and/or reporting results when any quality control does not perform as expected.

13.3 Include minimum quality controls:

a) Matrix spike, matrix blank and/or reagent blank, calibration standard, and

b) For LC-FLD, or similar method, continued calibration verification (CCV).

13.4 LAS' interpretation of each quality control and its purpose is defined below. It is not a requirement for the laboratory to use the same term for each type of control as long as the correct control is used for the correct purpose.

a) Matrix Blank: Substance that closely matches the samples being analyzed with regard to matrix components. Ideally, the matrix blank does not contain the analyte(s) of interest but is subjected to all sample processing operations including all reagents used to test the samples. Use to determine the absence of significant interference due to matrix, reagents, and equipment used in the analysis.

b) Matrix Spike: Aliquot of a sample prepared by adding a known amount of analyte(s) to a specified amount of matrix and subjected to the entire procedure. Use to establish if the method is appropriate for the analysis of a specific analyte(s) in a particular matrix.

c) Incurred Sample: Sample that contains the analyte(s) of interest, which were not derived from laboratory fortification.

d) Reagent Blank: Sample containing only the reagents taken through the entire procedure. Optional if a matrix blank is used.

e) Calibration Standard: Solution containing known amount or concentration of analyte. Without further indication, calibration standard(s) is usually prepared by adding the analyte(s) of interest in neat solvent/solution. A "matrix-matched calibration standard(s)" is prepared by adding analyte(s) of interest in matrix blank. Use to establish a reference for identification and/or quantification for measuring/detection system.

f) Continuing Calibration Verification (CCV): Solution of known concentration, typically at or near the midpoint of the calibration curve. Use to evaluate instrument stability throughout the sequence and repeatability.

g) 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. Use to evaluate the accuracy of reference material and/or accuracy of preparation techniques. Optional; however, inclusion is a best practice.

14 Quality Measures

14.1 Evaluate quality controls to identify acceptability of data, trends, and potential problems.

14.2 Take immediate action prior to continuing testing and/or reporting results when any quality measure not performing as expected.

14.3 Vertical Cutter Mill (VCM) Sieve Test: Use to monitor VCM equipment (i.e., blade sharpness). Perform at a minimum frequency of weekly or with use.

a) Use a No. 20 sieve.

b) Place ~50 - 100g of ground sample in sieve that has been weighed.

c) Work the sample through the sieve with warm water (tap) being careful not to lose sample, as needed.

d) Dry the sieve and remaining material in an oven at ~100 – 105ºC or air-dry until there is no more change in weight. Avoid cooking or burning the material.

e) Weigh and calculate % pass through.

f) Record result and evaluate against acceptance criteria defined in sample preparation procedure.

g) Monitor trending decrease of % pass through to facilitate maintaining acceptable VCM performance.

h) Submit sieve test data to the PM quarterly.

14.4 Calibration Curve: Use to quantify concentration of unknown samples.

a) Use the same type of curve used during method validation.

b) Ensure a minimum of 4 points is used for LC.

c) Ensure R^2 value for each curve must be ≥0.995.

d) Ensure regression equation is not forced through zero.

e) Ensure calibration range brackets the range of result reported and includes the LOQ for the method. 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.

14.5 Continuing Calibration Verification (CCV): Use to demonstrate repeatability and stability of the instrument/method (e.g., calibration curve).

a) Collect CCV data points at a minimum, at the beginning of a batch, the end of the batch, and throughout the batch with a recommended frequency of every 10 (± 2) injections.

b) Calculate percent recovery of CCV data point (see §14.7).

c) Evaluate against the acceptance criteria where percent recovery must within 15% of expected concentration.

14.6 Coefficient of Variation (CV): Use to demonstrate the repeatability.

a) Calculate coefficient of variation (CV) for a group of data points using equation:

$$\%\ CV = \frac{Standard\ Deviation}{Average} * 100$$

b) Evaluate % CV during validation of sample preparation procedure (see §10.4); and during validation of sample testing method (see §10.5).

14.7 Percent recovery: Use to compare expected concentration to actual measured concentration to evaluate method performance.

a) Calculate percent recovery using equation:

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

b) Evaluate recovery of a matrix spike for aflatoxins against performance criteria defined in Table 1 (see §12).

c) Evaluate recovery of a matrix spike for ochratoxin A against performance criteria defined in Table 2 (see §12).

14.8 Control Charting: Use 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.

a) Plot percent recovery results of each matrix spike analyzed.

b) Plot for the method regardless of analyst or instrument used to evaluate overall performance of the method.

c) Evaluate whether results are acceptable using acceptance criteria, Table 1 (AFL) or Table 2 (OTA). All data points must be within the acceptable range.

d) Additional, optional, control charting methods can be used to evaluate other variables of the testing procedure. For example, plotting by analyst or by instrument can show individual performance when more than one is used.

15 Data Analysis

15.1 Significant Figures: Collect data to the appropriate significant figures for the methodology (see §18-23). Additionally, when two or more data points are calculated, the final value must not have more significant figures than the original data points.

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

a) 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

b) 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.

15.3 Correction for recovery (export only): Correct the analytical result for recovery,

a) unless the percent recovery is between 90-110%, additionally

b) 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 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 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."

16 Critical Equipment & Reagents

16.1 Calibrate or verify equipment and instrumentation considered critical to the analytical method before putting into service and thereafter calibrate / verify regularly while in service.

a) Calibration refers to checking the measurements of a device and adjusting the device if corrections are needed.

b) Verification refers to checking that a device's measurement remains within a determined acceptable range, adjustments should not be needed.

16.2 When a device is found to be outside tolerance at verification, investigate and correct issue before using.

16.3 Maintain records of calibration, intermediate checks, performance verification, maintenance, and significant repair.

16.4 Grinding Equipment: Keep in good working order. Monitor performance using VCM sieve test (See §14.3). Record blade sharpening.

16.5 Balances & Scales:

a) Daily when in use: Verify with working weights that fall within the weight range used by the laboratory.

b) Annual calibration: Performed by an accredited organization or internally with the same requirements being met.

16.6 Reference & Working Weights:

a) Reference weights: Verify every 5 years.

b) Working weights: Verify against reference weights annually.

16.7 Analytical Instruments: Keep in good working order.

16.8 Volumetric Devices:

a) Mechanical pipets, micropipettors, mechanical burets, and bottle-top dispensers: Verify at least every 6 months for accuracy and precision using a gravimetric or colorimetric method.

b) Positive displacement syringes: Verify for accuracy upon receipt (a manufacturer's certificate of accuracy may be accepted).

c) Plastic graduated cylinders: Verify 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: Verify for accuracy and precision using a gravimetric method upon receipt (prior to use).

16.9 Critical reagents and consumables: Store according to the manufacturer's instructions and do not use past the designated expiration date.

17 Official Certificate of Analysis/Report

17.1 Meet the ISO 17025 standard for reporting results.

17.2 Meet commodity specific requirements for reporting results.

17.3 Meet customer's requirements for reporting results.

*****

The following sections include requirements organized by scope; and are in addition to the requirements outlined above.

TECHNICAL REQUIREMENTS (SCOPE SPECIFIC)

18 Almond – AFL – Export to EU

18.1 Applies to analysis for aflatoxins (B_1, B_2, G_1, and G_2).

18.2 Sample Preparation Method:

a) Protect sample from daylight (See §5.8.a), Annex II 1.1).

b) Use a sample preparation procedure based on AOAC 977.16 to achieve a minimum size reduction for ground samples (See §10 and §24).

c) Ensure sample is representative of the incoming sample prior to grinding. Mixing may occur at 1) the processing facility as part of the sampling process (See §5.6.b), Product Sample Selection and Analysis); or 2) at the laboratory (See §5.8.a), Annex I, D.2.4). When the incoming sample is split prior to grinding at the laboratory, ensure the sub-samples are representative of the incoming sample.

18.3 Test Method:

a) IAC with LC-FLD, Iodine: AOAC 991.31, A-F, H

b) IAC with LC-FLD, Kobra Cell: AOAC 991.31, A-F, H / AOAC 999.07

c) IAC with LC-FLD, PHRED: AOAC 991.31, A-F, H / AOAC 2005.08

18.4 Data Analysis:

a) Analyze to the nearest tenth ppb (i.e. 0.1 ppb) where ppb = 1 ng/g.

b) Calculate and report shell/kernel ratio of whole nuts (See §5.8.a), Annex II 1.2), if applicable.

18.5 Official Certificate of Analysis / Test Report:

18.5.1 Include all the following information:

a) Sample lot number.

b) Analytical method used (see §5.8.a)).

c) Limit of quantification (see §5.8.a)).

d) Percent recovery for B_1 and total aflatoxin (see §5.8.a)).

e) Analytical result (see §5.8.a)).

f) A statement about correction for recovery (see §5.8.a)).

g) Measurement uncertainty presented as the result ± uncertainty (i.e., 10.4 ± 2.1 µg/kg),

§ 4.4.1(b), Annex II, EC 401/2006, "As x ± 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%."

18.5.2 If a statement is made concerning compliance with the EC 401/2006, use the statement:

"Sample analysis was conducted in compliance with Annex II of Regulation (EC) No. 401/2006 for aflatoxin in almonds."

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

"In shell almonds are reported on a kernel basis for a [insert X/Y] shell/kernel ratio."

18.5.4 If requested by the handler to include an interpretation statement on the analytical report of the maximum limits set by Regulation EC 1881/2006, use the following types of statements.

Note: Interpretation of results by the laboratory does not generally occur. The commodity's destination is often decided based on the test results. It is the responsibility of the handler (processor) not the laboratory, to determine which limits apply to their product.

a) "result exceeds the limit of 12.0 µg/kg aflatoxin B_1 and/or 15.0 µg/kg sum of aflatoxin B_1, B_2, G_1 and G_2 for almonds 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 EC 1881/2006)."

b) "result exceeds the limit of 8.0 µg/kg aflatoxin B_1 and/or 10.0 µg/kg sum of aflatoxin B_1, B_2, G_1 and G_2 for almonds intended for direct human consumption or use as an ingredient in foodstuffs (§2.1.6 of Annex II Regulation EC 1881/2006)."

Laboratory Approval Program – Mycotoxins

19 Peanut – AFL – U.S. Domestic & U.S. Imports

19.1 Applies to analysis for aflatoxins (B_1, B_2, G_1, and G_2).

19.2 Sample Preparation Method:

a) Visually inspect to verify incoming sample bag (unground) is marked with weight on the positive lot identification (PLI) tag (See §5.4.e), Sample Weight) and it is not less than 48lbs.

b) Use sample preparation procedure based on AOAC 977.16 to achieve a minimum size reduction for ground samples (See §10 and §24).

c) Ensure sample is representative of the incoming sample prior to grinding.

Note: Samples are mixed prior to grinding at the processing facility as part of the USDA Federal/State Inspection Service sampling process (See §5.4.e), Sampling).

19.3 Test Method:

a)	IAC with direct fluorometry:	AOAC 991.31, A-G
b)	TLC:	AOAC 998.03
c)	IAC with LC-FLD, Iodine:	AOAC 991.31, A-F, H
d)	IAC with LC-FLD, Kobra Cell:	AOAC 991.31, A-F, H / AOAC 999.07
e)	IAC with LC-FLD, PHRED:	AOAC 991.31, A-F, H / AOAC 2005.08

19.4 Data Analysis:

19.4.1 Analyze to the nearest:

a)	IAC with direct fluorometry:	whole integer
b)	TLC:	whole integer
c)	IAC with LC-FLD:	tenth ppb (i.e. 0.1 ppb) where ppb = 1 ng/g

19.5 Official Certificate of Analysis / Test Report:

19.5.1 Include 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."

19.5.2 Include 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."

19.5.3 Include one of the following statements for the methodology as approved, to include AOAC method number or internal procedure number, where applicable:

a) 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).

c) Immunoaffinity column cleanup with high performance liquid chromatography (LC) method.

19.6 Reporting to USDA:

a) Submit records of sample analysis to AMS Specialty Crops Inspection Division (sciinspectionoperations@usda.gov) in the format requested.

b) Submit records of sample analysis to AMS Specialty Crops Market Development Division (8Eimports@usda.gov and complianceinfo@usda.gov) in the format requested.

20 Peanut – AFL – Export to EU

20.1 Applies to analysis for aflatoxins (B_1, B_2, G_1, and G_2).

20.2 Sample Preparation Method:

a) Visually inspect to verify incoming unground sample has the weight recorded on the positive lot identification (PLI) tag and it is not less than 48lbs (See §5.4.e), Sample Weight).

b) Protect sample from daylight (See §5.8.a), Annex II 1.1).

c) Use sample preparation procedure based on AOAC 977.16 to achieve a minimum size reduction for ground samples (See §10 and §24).

d) Ensure the sub-samples are representative of the incoming sample when the incoming sample is split prior to grinding at the laboratory.

Note: Samples are mixed prior to grinding at the processing facility as part of the USDA Federal/State Inspection Service sampling process (See §5.4.e), Sampling).

20.3 Test Method:

a) IAC with LC-FLD, Iodine:	AOAC 991.31, A-F, H
b) IAC with LC-FLD, Kobra Cell:	AOAC 991.31, A-F, H / AOAC 999.07
c) IAC with LC-FLD, PHRED:	AOAC 991.31, A-F, H / AOAC 2005.08

20.4 Data Analysis:

a) Analyze to the nearest tenth ppb (i.e. 0.1 ppb) where ppb = 1 ng/g.

b) Calculate and report shell/kernel ratio of whole nuts (See §5.8.a), Annex II 1.2), if applicable.

20.5 Official Certificate of Analysis / Test Report:

20.5.1 Include all the following information:

a) Sample lot number (positive lot identification, PLI).

b) Analytical method used (see §5.8.a)).

c) Limit of quantification (see §5.8.a)).

d) Percent recovery for B_1 and total aflatoxin (see §5.8.a)).

e) Analytical result (see §5.8.a)).

f) A statement about correction for recovery (see §5.8.a)).

g) Measurement uncertainty presented as the result ± uncertainty (i.e., 10.4 ± 2.1 µg/kg),

§ 4.4.1(b) Annex II, EC 401/2006, "As x ± 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%."

h) State whether the sheller is a signatory of the APC MOU.

20.5.2 If a statement is made concerning compliance with the EC 401/2006, use the statement:

"Sample analysis was conducted in compliance with Annex II of Regulation EC No. 401/2006 for aflatoxin in peanuts."

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

"In shell peanut are reported on a kernel basis for a [insert X/Y] shell/kernel ratio."

20.5.4 If requested by the handler to include an interpretation statement on the analytical report of the maximum limits set by Regulation EC 1881/2006, use the following types of statements.

Note: Interpretation of results by the laboratory does not generally occur. The commodity's destination is often decided based on the test results. It is the responsibility of the handler (processor) not the laboratory, to determine which limits apply to their product.

a) "result exceeds the limit of 8.0 µg/kg aflatoxin B_1 and/or 15.0 µg/kg sum of aflatoxin B_1, B_2, G_1 and G_2 for peanuts to be subjected to sorting, or other physical treatment, before human consumption or use as an ingredient in foodstuffs with the exception of peanuts for crushing for refined vegetable oil production (§2.1.1 of Annex II Regulation EC 1881/2006)."

b) "result exceeds the limit of 2.0 µg/kg aflatoxin B_1 and/or 4.0 µg/kg sum of aflatoxin B_1, B_2, G_1 and G_2 for peanuts intended for direct human consumption or use as an ingredient in foodstuffs with the exception of crude vegetable oils destined for refining and refined vegetable oils (§2.1.5 of Annex II Regulation EC 1881/2006)."

Laboratory Approval Program – Mycotoxins

21 Pistachio – AFL – U.S. Domestic & U.S. Imports

21.1 Applies to analysis for aflatoxins (B_1, B_2, G_1, and G_2).

21.2 Sample Preparation Method:

a) Use sample preparation procedure based on AOAC 977.16 to achieve a minimum size reduction for ground samples (See §10 and §24).

b) Ensure sample is representative of the incoming sample prior to grinding. Mixing may occur at 1) the processing facility as part of the sampling process (See §5.4.g) and 5.4.h)); or 2) at the laboratory.

21.3 Test Method:

a) IAC with direct fluorometry:	AOAC 991.31, A-G
b) IAC with LC-FLD, Iodine:	AOAC 991.31, A-F, H
c) IAC with LC-FLD, Kobra Cell:	AOAC 991.31, A-F, H / AOAC 999.07
d) IAC with LC-FLD, PHRED:	AOAC 991.31, A-F, H / AOAC 2005.08

21.4 Data Analysis:

a) Analyze to the nearest tenth ppb (i.e. 0.1 ppb) where ppb = 1 ng/g.

21.5 Official Certificate of Analysis / Test Report:

21.5.1 Include 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."

21.5.2 Include one of the following statements for the methodology used, to include AOAC method number or internal procedure number, where applicable:

a) Immunoaffinity column with direct fluorometry method of analysis (AOAC 991.31).

b) Immunoaffinity column cleanup with high performance liquid chromatography (LC) method.

21.5.3 If requested by the handler to include an interpretation statement on the analytical report of the maximum, use the following types of statements.

Note: Interpretation of results by the laboratory does not generally occur. The commodity's destination is often decided based on the test results. It is the responsibility of the handler (processor) not the laboratory, to determine which limits apply to their product.

a) "result exceeds the limit of 15 ppb total aflatoxin for domestic human consumption (7 CFR 983.150)."

21.6 Reporting to USDA:

a) Submit records of sample analysis to AMS Specialty Crops Inspection Division (sciinspectionoperations@usda.gov) in the format requested.

b) Submit records of sample analysis to AMS Specialty Crops Market Development Division (8Eimports@usda.gov and complianceinfo@usda.gov) in the format requested.

22 Pistachio – AFL – Export to EU

22.1 Applies to analysis for aflatoxins (B_1, B_2, G_1, and G_2).

22.2 Sample Preparation Method:

a) Protect sample from daylight (See §5.8.a) Annex II 1.1).

b) Use sample preparation procedure based on AOAC 977.16 to achieve a minimum size reduction for ground samples (See §10 and §24).

c) Ensure sample is representative of the incoming sample prior to grinding. Mixing may occur at 1) the processing facility as part of the sampling process (See §5.6.c), Creating and Sampling the Lot); or 2) at the laboratory (See §5.8.a), Annex I, D.2.4). When the incoming sample is split prior to grinding at the laboratory, ensure the sub-samples are representative of the incoming sample.

22.3 Test Method:

a) IAC with LC-FLD, Iodine: AOAC 991.31, A-F, H

b) IAC with LC-FLD, Kobra Cell: AOAC 991.31, A-F, H / AOAC 999.07

c) IAC with LC-FLD, PHRED: AOAC 991.31, A-F, H / AOAC 2005.08

22.4 Data Analysis:

a) Analyze to the nearest tenth ppb (i.e. 0.1 ppb) where ppb = 1 ng/g.

b) Calculate and report shell/kernel ratio of whole nuts (See §5.8.a), Annex II 1.2), if applicable.

c) In shell pistachio ratio is accepted as 50/50 (see §5.5.d)).

22.5 Official Certificate of Analysis / Test Report:

22.5.1 Include all the following information:

a) Sample lot number.

b) Analytical method used (see §5.8.a)).

c) Limit of quantification (see §5.8.a)).

d) Percent recovery for B_1 and total aflatoxin (see §5.8.a)).

e) Analytical result (see §5.8.a)).

f) A statement about correction for recovery (see §5.8.a)).

g) Measurement uncertainty presented as the result ± uncertainty (i.e., 10.4 ± 2.1 µg/kg).

§ 4.4.1(b), Annex II, EC 401/2006, "As x ± 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%."

22.5.2 If a statement is made concerning compliance with the EC 401/2006, use the statement:

"Sample analysis was conducted in compliance with Annex II of Regulation EC No. 401/2006 for aflatoxin in pistachio."

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

"In shell pistachio results are reported on a kernel basis for a 50/50 shell/kernel ratio."

22.5.4 If requested by the handler to include an interpretation statement on the analytical report of the maximum limits set by Regulation EC 1881/2006, use the following types of statements.

Note: Interpretation of results by the laboratory does not generally occur. The commodity's destination is often decided based on the test results. It is the responsibility of the handler (processor) not the laboratory, to determine which limits apply to their product.

a) "result exceeds the limit of 12.0 µg/kg aflatoxin B_1 and/or aflatoxin 15.0 µg/kg sum of B_1, B_2, G_1 and G_2 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 EC 1881/2006)."

b) "result exceeds the limit of 8.0 µg/kg aflatoxin B_1 and/or 10.0 µg/kg sum of aflatoxin B_1, B_2, G_1 and G_2 for pistachios intended for direct human consumption or use as an ingredient in foodstuffs (§2.1.6 of Annex II Regulation EC 1881/2006)."

23 Pistachio – OTA – Export to EU

23.1 Applies to analysis of ochratoxin A.

23.2 Sample Preparation Method:

a) Protect sample from daylight (See §5.8.a), Annex II 1.1).

b) Use sample preparation procedure based on AOAC 977.16 to achieve a minimum size reduction for ground samples (See §10 and §24).

c) Ensure sample is representative of the incoming sample prior to grinding. Mixing may occur at 1) the processing facility as part of the sampling process (See §5.6.c), Creating and Sampling the Lot); or 2) at the laboratory (See §5.8.a), Annex I, D.2.4). When the incoming sample is split prior to grinding at the laboratory, ensure the sub-samples are representative of the incoming sample.

23.3 Test Method:

a) Use method fit for purpose and that meets the validation / verification requirements outlined in §10.

23.4 Data Analysis:

a) Analyze to the nearest tenth ppb (i.e. 0.1 ppb) where ppb = 1 ng/g.

b) Calculate and report shell/kernel ratio of whole nuts (See §5.8.a), Annex II 1.2), if applicable.

c) In shell pistachio ratio is accepted as 50/50 (see §5.5.d)).

23.5 Official Certificate of Analysis / Test Report:

23.5.1 Include all the following information:

a) Sample lot number.

b) Analytical method used (see §5.8.a)).

c) Limit of quantification (see §5.8.a)).

d) Percent recovery for OTA (see §5.8.a)).

e) Analytical result (see §5.8.a)).

f) A statement about correction for recovery (see §5.8.a)).

g) Measurement uncertainty presented as the result ± uncertainty (i.e., 10.4 ± 2.1 µg/kg).

§ 4.4.1(b), Annex II, EC 401/2006, "As x ± 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%."

23.5.2 If a statement is made concerning compliance with the EC 401/2006, use the statement:

"Sample analysis was conducted in compliance with Annex II of Regulation (EC) No. 401/2006 for ochratoxin in pistachio."

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

"In shell pistachio results are reported on a kernel basis for a 50/50 shell/kernel ratio."

23.5.4 If requested by the handler to include an interpretation statement on the analytical report of the maximum limits set by Regulation EC 1881/2006, use the following types of statements.

Note: Interpretation of results by the laboratory does not generally occur. The commodity's destination is often decided based on the test results. It is the responsibility of the handler (processor) not the laboratory, to determine which limits apply to their product.

a) "result exceeds the limit of 10 µg/kg ochratoxin for pistachios to be subjected to sorting, or other physical treatment, before human consumption or use as an ingredient in foodstuffs (Annex II Regulation EC 1881/2006)."

b) "result exceeds the limit of 5 µg/kg ochratoxin for pistachios intended for direct human consumption or use as an ingredient in foodstuffs (Annex II Regulation EC 1881/2006)."

24 Explanations for Technical Requirements

24.1 For sample grinding:

a) Particle size is a performance control measurement of homogeneity (See §5.7.f)).

b) Adequate grind is described in AOAC 977.16 and summarized as: "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.

25 Revision History

New Rev.	Description of Change	Prepared by
08/12/22	Original. This document is the product of rebranding LAP-Aflatoxins to incorporate additional mycotoxins into the Program's scope.	Grace Vaillant Branch Chief Heath McClure Program Manager
02/08/23	Added §20.5.1.h) "State whether the sheller is a signatory of the APC MOU" because the requirement was added to the APC MOU for 2023.	Grace Vaillant Branch Chief

26 Review / Approvals

HEATH MCCLURE
Digitally signed by HEATH MCCLURE
Date: 2023.02.08 07:43:10 -05'00'

Heath McClure
Program Manager - Aflatoxin
Reviewer

GRACE VAILLANT
Digitally signed by GRACE VAILLANT
Date: 2023.02.07 16:13:22 -05'00'

Grace Vaillant
Branch Chief
Approver