Laboratory Approval Program for Export of Meat and Poultry Products

1. Purpose

1.1 This 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 chemical residues, microorganisms, and parasites in meat and poultry products which are offered for certification by USDA Food Safety and Inspection Service (FSIS) for export to various countries.

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

This LAP may be used by laboratories that submit their testing program to LAS for approval, verification, and monitoring. It is limited to the analysis of chemical residues, microorganisms, and parasites in meat and poultry products 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 EC 2004. Document SANCO 2726 rev 4 (December 4, 2008) Guidelines for the implementation of Decision 2002/657/EC (https://ec.europa.eu/food/sites/food/files/safety/docs/cs_vet-med-residues_cons_2004-2726rev4_en.pdf).

3.4 EC 2004-41. Council Directive 2004/41/EC of the European Parliament and of the Council repealing certain Directives concerning food hygene and health conditions for the production and placing on the market of certain products of animal origin intended for human consumption and amending Coucil Directives 89/662/EEC and 92/118EEC and Council Decision 95/408/EC. April 21, 2004. Official Journal of the European Union, L 157: 33-44.

3.5 EC 2007. Community Reference Laboratories for Residues CRL guidance paper, December 7 2007: CRLs view on state of the art analytical methods for national residue control plans (https://ec.europa.eu/food/sites/food/files/safety/docs/cs_vet-med-residues_guideline_validation_screening_en.pdf).

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

3.7 FDA 2006. Mass spectrometry for confirmation of the identity of animal drug residues. Center for Veterinary Medicine (CVM), Guidance for Industry #118. (http://www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/UCM052658.pdf).

3.8 FDA 2011. Studies to evaluate the metabolism and residue kinetics of veterinary drugs in food-producing animals: Validation of analytical methods used in residue depletion studies. US FDA-VICH GL49. US FDA, Center for Veterinary Medicine, September 15, 2011. (http://www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/UCM207942.pdf).

3.9 FDA-Microbial Methods 2015. Guidelines for the Validation of Analytical Methods for the Detection of Microbial Pathogens in Foods and Feeds, 2nd Edition. US FDA, FDA Foods and Veterinary Medicine Science and Research Steering Committee, May 19, 2015. (http://www.fda.gov/downloads/ScienceResearch/FieldScience/UCM298730.pdf).

3.10 FDA-Chemical Methods 2015. Guidelines for the Validation of Chemical Methods for the FDA FVM Program, 2nd Edition. US FDA. FDA Foods and Veterinary Medicine Science and Research Steering Committee, May 19, 2015. (http://www.fda.gov/downloads/ScienceResearch/FieldScience/UCM273418.pdf).

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

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

3.13 USDA AMS Laboratory Standards of Practice.

3.14 USDA FSIS. Chemistry Laboratory Guidebook (CLG). http://www.fsis.usda.gov/wps/portal/fsis/topics/science/laboratories-and-procedures/guidebooks-and-methods/chemistry-laboratory-guidebook

3.15 USDA FSIS. Export Library. FSIS webpage "Export Library – Requirements by Country" providing the access point of requirements for meat, poultry and processed egg products (http://www.fsis.usda.gov/wps/portal/fsis/topics/international-affairs/exporting-products/export-library-requirements-by-country).

3.16 USDA FSIS. Microbiology Laboratory Guidebook (MLG). http://www.fsis.usda.gov/wps/portal/fsis/topics/science/laboratories-and-procedures/guidebooks-and-methods/microbiology-laboratory-guidebook/microbiology-laboratory-guidebook

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 for Export
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's

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 the 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 is 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) ISO/IEC 17025 accreditation status, i.e., an up-to-date copy of the accreditation scope;
*[**NOTE 1:** The applicant laboratory must be ISO/IEC 17025 accredited. The methodology used for the LAP must be on the scope of accreditation.*
***NOTE 2:** Laboratories providing trichinae analysis may choose to opt out of being ISO/IEC 17025 accreditation since these laboratories are onsite at an FSIS inspected facility.]*

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 Trichina analyst must complete the AMS training course, including lecture and laboratory, and successfully analyze an initial or second set of PT sample.

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

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

4.5 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.

4.5.1 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.

4.6 Performance of Initial Onsite Laboratory Audit: The PM will inform the applicant laboratory after the review has been completed and deemed acceptable.

4.6.1 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.]*

4.6.2 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.

4.6.3 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.

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

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

4.7 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.
*[**NOTE**: AMS will provide a certificate to the Trichinae analyst after she/he completes the training, see Section 4.3]*

4.8 The Official Listing of Approved Laboratories: The PM will list all USDA-approved laboratories 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) maintain ISO/IEC 17025 accreditation with specified testing methodology(ies) related to LAP;

c) 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.]*

d) participate in semiannual check sample and/or 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;

*[**NOTE**: Trichinae analyst must participate in a PT program administered by AMS. The PT samples will be provided by the USDA Agricultural Research Service.]*

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

f) 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;

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

h) resolve all nonconformities in a timely manner;

i) 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;

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

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

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

5.3.1 Onsite audit and nonconformities resolution processes, see the "Performance of Initial Onsite Laboratory Audit" subsection within Section 4.

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

5.4.1 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.

5.5 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 fee is based on the number of analyte groups the laboratory provides testing for as follows:

9.5.1 Chemical Residues

9.5.1.1 AB: Antibiotics: tetracyclines and chloramphenicol

9.5.1.2 BA: Beta agonists: ractopamine, clenbuterol, and zilpaterol

9.5.1.3 HM: Heavy metals: arsenic, lead, cadmium and mercury

9.5.1.4 PC: Pesticides: DDT, dieldrin, and lindane

9.5.1.5 RC: Resorcylic acid lactones: taleranol and zeranol

9.5.1.6 SR: Steroids: melengestrol acetate and trenbolone

9.5.2 Microorganisms

9.5.2.1 LM: *Listeria monocytogenes*

9.5.2.2 SM: *Salmonella*

9.5.2.3 TPC: Total plate count (microorganisms per gram or mL)

9.5.3 Parasites

9.5.3.1 TS: Trichinae: *Trichinella spiralis*

9.6 The program fees are as follows:

Number of Analytes/ Groups	Admission Fee $	Initial Yearly Fee $	Yearly Fee $	Trichinae Training Fee $	Trichinae PT Program per Analyst $
1	990	4000	2740	3320	1780
2	1540	4680	3110		
3	2030	5350	3550		
4	2530	6030	3980		
5	3020	6710	4410		
6	3510	7390	4840		
7	4000	8060	5270		
8	4500	8740	5700		
9	4990	9420	6130		
10	5480	10100	6560		

*[**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.]*

9.7 All fees are neither refundable nor prorated.

9.8 If international travel is required for an on-site audit, an additional fee will be charged to the laboratory.

10. Technical Requirements

10.1 Chemical Analysis

10.1.1 Methods

10.1.1.1 Participant laboratories must use LATD specified and/or LATD accepted methods.

10.1.1.2 If methods are not specified, LATD allows laboratories to use methods from different sources such as AOAC International Official Methods, US FDA methods, USDA FSIS methods, or US EPA methods. Laboratories may also adopt methods which are published/used by other national or international organizations.

10.1.1.3 If methods from the above sources are not available or not very relevant, laboratories can adapt methods from those sources into a laboratory method.

10.1.1.4 Methods from different sources must be relevant to the program in terms of matrices, concentration ranges, and methodology.

10.1.1.5 Semi-developed methods, in-house methods, proprietary methods without details being accessible by the PM, and similar methods are in general not permitted.

10.1.2 Standard Operating Procedures (SOP)

10.1.2.1 Laboratories must establish project-oriented and program-specific SOPs.

10.1.2.2 These SOPs must be operational under specific laboratory conditions (in terms of facilities and operator's qualification).

10.1.2.3 These SOPs must be reviewed and signed by quality managers, laboratory directors and/or by people with similar responsibilities.

10.1.2.4 These SOPs must be periodically reviewed (at least once every two years) and updated to the latest standards.

10.1.2.5 Manufacturer's Instrument Instructions/Manuals and similar documents are not accepted as substitutions for laboratory SOPs.

10.1.3 Analytes, Sensitivities, Method Detection Limits, and Specified Methods
Background: LATD manages this LAP-Export at the request of USDA FSIS so that establishments have means to ensure meat and poultry products comply with the FSIS Export Library. The Export Library declares sensitivities which are treated as maximum residue limits (MRL or MRLs) of analytes for export purpose. These sensitivities may be close to method detection limits (MDL or MDLs, such as 0.1 ppb of ractopamine in meat) or much higher than method detection limits of currently available methods (such as 500 ppb of lead in meat). Laboratories must be capable so that residues at or below these sensitivities are reliably detected and measured. Therefore, MDL, an indication of a laboratory's analytical capability, may be required to be lower than these sensitivities, or may be allowed to be close to these sensitivities, depending on sample matrices, residue concentration ranges, analytical challenges, other program requirements, etc.

The residues are divided into groups. Each group may contain several analytes (Note: µg/g = µg/mL = ppm, µg/kg = µg/L = ppb).

10.1.3.1 Antibiotics

10.1.3.1.1 Methods: LC-MS/MS

10.1.3.1.2 Matrix: Liver, muscle

10.1.3.1.3 List of analytes

Analyte	Sensitivity (ppb)	MDL (ppb)
Tetracycline	10	≤ 10
Chlortetracycline	10	≤ 10
Oxytetracycline	10	≤ 10
Chloramphenicol	10	≤ 10

10.1.3.2 Beta agonists

10.1.3.2.1 Methods: LC-MS/MS

10.1.3.2.2 Matrix: Liver, muscle

10.1.3.2.3 List of analytes

Analyte	Sensitivity (ppb)	MDL (ppb)
Ractopamine	liver 0.2, muscle 0.1	≤ 0.1
Clenbuterol	liver 0.2, muscle 0.1	≤ 0.1
Zilpaterol	liver 1, muscle 1	≤ 0.5

10.1.3.3 Heavy Metals

10.1.3.3.1 Methods: USDA FSIS CLG-TM3. Determination of metals by ICP-MS and ICP-OES (Rev: 04, 09/30/2013) or equivalent

10.1.3.3.2 Matrix: Muscle

10.1.3.3.3 List of analytes

Analyte	Sensitivity (ppb)	MDL (ppb)
Arsenic (As)	100	≤ 10
Cadmium (Cd)	50	≤ 10
Lead (Pb)	500	≤ 25
Mercury (Hg)	30	≤ 20

10.1.3.4 Pesticides

10.1.3.4.1 Methods: USDA FSIS CLG-CHC3. Determination of chlorinated hydrocarbons (CHCs) and chlorinated organophosphate hydrocarbons (COPs) with gel permeation

chromatography (GPC) (Rev: 04, 06/01/2010) or equivalent. AOAC International Official Method 970.52, Organochlorine and organophosphorus pesticide residues or equivalent

10.1.3.4.2 Matrix: Fat

10.1.3.4.3 List of analytes

Analyte	Sensitivity (ppb)	MDL (ppb)
DDT and its metabolites	100	≤ 50
Dieldrin	300	≤ 50
Lindane (γ-HCH)	100	≤ 50

10.1.3.5 Resorcylic acid lactones

10.1.3.5.1 Methods: LC-MS/MS

10.1.3.5.2 Matrix: Liver, muscle, urine

10.1.3.5.3 List of analytes

Analyte	Sensitivity (ppb)	MDL (ppb)
Taleranol	1	≤ 1
Zeranol	1	≤ 1

10.1.3.6 Steroids

10.1.3.6.1 Methods: LC-MS/MS

10.1.3.6.2 Matrix: Liver, muscle, urine

10.1.3.6.3 List of analytes

Analyte	Sensitivity (ppb)	MDL (ppb)
Melengestrol acetate	Liver 5, muscle 1	≤ 1
Trenbolone	Liver 5, muscle 1	≤ 1

10.1.3.7 Other analytes not listed above:
AMS approved laboratories may analyze other infrequent residues on the condition that the analytical methods are on the scope of the laboratory's ISO/IEC 17025 accreditation.

10.1.4 Calibration

10.1.4.1 Calibration range: Typical concentrations are set at a minimum of 5 different levels below and above the sensitivities. The calibration range, expressed as analytes in sample, should cover from ≤ 50% of MDL to ≥ 2-10 × sensitivity.

10.1.4.2 Matrix effect: Laboratory must evaluate the specific and non-specific interferences caused by sample matrix, with respect to clean solvent/buffer. Laboratory must evaluate the effect of applying internal reference standard, matrix-matching, internal standard addition, application of stable isotopes, etc., for relieving or eliminating matrix effect. Evidence must be provided to the PM to indicate the matrix effect is corrected to a satisfactory degree.

10.1.4.3 Calibration matrix: Calibrators are prepared in a minimum of two types of matrix: Type 1 – Standards in clean solvent/buffer; and Type 2 – Standards fortified into control matrix extract (tissue extract). The two responses (as slopes of their calibration lines) are within 100±30% of each other, in general. Laboratory must decide the most optimum calibration for unknown samples

*[**NOTE:** "Control matrix", "Negative control", or "Blank matrix", is the animal tissue which is confirmed to be chemical residue free based the known history of animals which have not been exposed to regulated or forbidden drug/chemicals, or based on previous measurements. "Control matrix extract" is the extracted solution after the control matrix is processed through the whole procedure but the measuring process. "Solvent/buffer" is the solution which is used to prepare the control matrix extract right before the measuring process.]*

10.1.5 Selectivity (Specificity) required in LC-MS/MS analysis, when applicable.

10.1.5.1 Deuterated standards shall be used as an internal reference standard when applicable.

10.1.5.2 Chromatographic separation: The drug residue retention time of a sample shall match that of calibration standard within an accepted window.

10.1.5.3 The retention time ratio of drug residue to deuterated standard of a sample shall correspond to that of calibration standard at a tolerance of ±2.5%.

10.1.5.4 The relative intensities of fragment ions of a sample shall match that of the calibration standard.

10.1.5.5 At least one precursor and two daughter ions shall be identified

10.1.6 Sensitivity (in terms of limit of detection and limit of quantitation)

10.1.6.1 Establishing limit of detection (LOD)

10.1.6.1.1 Analyze at least 12-20 blank samples (tissue extract from blank samples, n > 12).

10.1.6.1.2 Convert the noise signal to concentration at the time window in which drug residue is expected.

10.1.6.1.3 Calculate the average (avg) of all those (noise) concentrations and the standard deviation (sd).

10.1.6.1.4 Let P = 3 × avg.

10.1.6.1.5 Let Q = avg + 1.64 × sd.

10.1.6.1.6 LOD is the greater value out of P and Q.

10.1.6.2 Limit of quantitation (LOQ): LOQ is the mean plus 10 standard deviations of the above mentioned measurements

10.1.7 Sensitivity (in terms of method detection limit and method quantitation limit) The above LOD and LOQ should be converted to method detection limit (MDL) and method quantitation limit (MQL), accordingly, such as by applying proper dilution factors. These MDL and MQL shall be expressed as drug residue in tissue.

10.1.8 Accuracy – It is the closeness (trueness) of measured concentration to confirmed concentration of an analyte in a certified reference material (CRM) containing incurred analyte. Trueness may be calculated as bias (= 100% × ($C_{Measured}$ - $C_{Certified}$)/ $C_{Certified}$)) and the requirements are listed below

Concentration (ppb)	Range of Trueness (%) (n > 6)
≤ 1 (e.g. 0.3)	-50 - +20
1 - 10 (e.g. 3)	-40 - +10
10 - 100	-30 - +10
> 100	-20 - +10

*[**NOTE:** According to Commission Decision 2002/657/EC (EC 2002), when no such CRM is available, it is acceptable that trueness of measurements is assessed through recovery of additions of known amounts of the analyte(s) to a blank matrix. Data corrected with the mean recovery are acceptable only when they fall within the ranges shown above.*

When trueness is expressed and calculated as recovery (100% × $C_{Measured}$ / $C_{Certified}$), the requirement is given below in the "Recovery" section.]

10.1.9 Precision – It is evaluated by fortifying chemical standards in control matrix at 1× and 3× the sensitivity levels as given in Section 11.3 (0.5 and 3 ppb if the required sensitivity of 11.3 is 0.1ppb). The acceptable precision (coefficient of variation, CV = 100% × one standard deviation / mean of repeated measurements) is listed below

Analyte concentration (ppb)	Within-run precision (Repeatability) (%CV) (n > 10)	Between-run precision (Reproducibility) (%CV) (n > 10)
≤1	30	45
1 - 10	25	32
10 - 100	20	23
100-1000	15	20
> 1000	10	16

10.1.10 Recovery
Analytes are fortified to control matrix (tissue) at 1× and 3× the sensitivity levels as given in Section 11.3 (0.5 and 3 ppb if the required sensitivity of 11.3 is 0.1ppb). The recovery (= 100% × $C_{Measured}$ / $C_{Fortified}$) of fortified chemical standards meets the following requirements.

Fortified concentration (ppb)	Recovery (%) (n > 6)
≤ 1	50 - 120
1 - 10	60 - 110
10 - 100	70 - 110
> 100	80 - 110

10.1.11 Extension of Sample matrix
A method validated on one sample matrix (e.g. pork muscle) is extended to other sample matrix (e.g. turkey muscle) in the following general ways. Six sets for each species/matrix combination (e.g. heavy metals in turkey muscle); each set consist of six samples: two blanks, two samples at sensitivity level, one sample at 2× sensitivity level, and one sample at 4 × sensitivity level. Two sets of samples are analyzed in one day and six sets of samples are analyzed in three different days. The results must meet the above specifications.

10.2 Microbiological Analysis

10.2.1 Microorganisms, Sensitivity, and Methods

10.2.1.1 *Salmonella*

10.2.1.1.1 Methods: AOAC International Official Method, US FDA methods, and/or USDA FSIS methods that specifies: All Foods; Raw, highly contaminated foods; or Poultry; and tests for all *Salmonella* (both motile and non-motile).

10.2.1.1.2 Sensitivity: 0 (negative in 25 gram of tested sample).

10.2.1.2 *Listeria monocytogenes*

10.2.1.2.1 Methods: AOAC International Official Method, US FDA Bacteriological Analytical Manual (BAM), and/or USDA FSIS method may be used.

10.2.1.2.2 Sensitivity: 0 (negative in 25 gram of tested sample).

10.2.1.3 Total Plate Count

10.2.1.3.1 Methods: AOAC International Official Methods (Petrifilm Plate count Method), US FDA methods, USDA FSIS methods, and/or Compendium of Methods for the Microbiological Examination of Foods.

10.2.1.3.2 Sensitivity: 10 CFU/g (colony forming units/g).

10.2.2 Other Methods
Other methods (such as rapid screening methods) used to test for pathogenic microorganisms must have been tested against a reference cultural method.

*[**NOTE:** The reference method is defined as that method by which the performance of an alternate method is measured or evaluated. Validation studies must include comparison to a recognized reference method to demonstrate equivalence or increased performance, the significance of which must be determined statistically. For bacterial analytes, reference methods are generally culture-based and result in a pure isolate. The AOAC International Official Methods, the US Food and Drug Administration, Bacteriological Analytical Manual (BAM), the USDA, Food Safety and Inspection Service, Microbiology Laboratory Guidebook (MLG) and International Standards Organization (ISO) all contain culture methods that are recognized reference culture methods.*

A laboratory using other methods must conduct a specific validation of those methods against reference culture method (s) to validate inclusivity, exclusivity, sensitivity and the methods performance as established by collaborative study. When new rapid methods based on DNA test technology are used, method accuracy, precision, repeatability, and reproducibility should also be considered.

The validation should challenge the methods ability to detect the pathogen of interest in samples inoculated with low and high levels concentrations of the target organism, as-well-as samples inoculated with competitive levels of other organisms including the test organism. One should also consider samples that are naturally contaminated as well as samples inoculated with concentrations of competing organism at levels higher than that of the target organism. The results from these samples should demonstrate very low or no false positive/false negative rates.

Any samples tested positive by those other methods must be confirmed by reference culture methods. Cultural confirmation includes the use of biochemical and serological tests to demonstrate that the other method did properly detect the targeted test organism.]

10.3 Trichinae Analysis

10.3.1 Location: The laboratory must be located onsite where the product is processed.

10.3.2 Analyst Training: Analysts must be trained by AMS to receive certification.

10.3.2.1 The training will consist of both lecture (including test method) and laboratory.

10.3.2.2 The training will occur onsite at the laboratory.

10.3.2.3 Upon completion of the training, the analysts must successfully analyze an initial or second set of initial proficiency samples in his/her own laboratory to complete his/her certification.

10.3.3 The company/establishment and laboratory must conform to the Council Directive 77/96/EEC of 21 December 1976 on the examination of *Trichinae* (*Trichinella Spiralis*) upon importation from third countries of fresh meat derived from domestic swine.

10.3.4 Test Method

10.3.4.1 The following acid digestion methods are accepted into this program: Magnetic Stirrer Method for Pooled Sample Digestion (Magnetic Stirrer method) and Mechanically Assisted Pooled Sample Digestion Method, Sedimentation Technique (Stomacher method).

10.3.4.2 The laboratory can use either one or both of the above methods to conduct the analysis.

*[**NOTE**: The Magnetic Stirrer method is included in the Trichinella Analyst Training Manual, which is provided in the training session.]*

11. Official Analysis Certificate/Report

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