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 for Poultry Exported to Russia

1. Purpose

This document provides the requirements for the Laboratory Approval Program (LAP) for Poultry Exported to Russia. This LAP is for laboratories seeking to perform confirmatory analysis of chemical residues and microorganisms in poultry and poultry products which are offered for certification by USDA Food Safety and Inspection Service (FSIS) for export to the Russian Federation. 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 poultry and poultry products for heavy metals, residues of pesticides and antibiotics, and/or microorganisms and all aspects of a laboratory's documented quality management system that apply to these analyses.

3. References

3.1 FSIS 2014. USDA FSIS – Export requirements for Russia. (http://www.fsis.usda.gov/wps/portal/fsis/topics/international-affairs/exporting-products/export-library-requirements-by-country/Russia).

3.2 EC 2002. Commission Decision of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results. Official Journal of the European Communities. L 221: 8-36.

3.3 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).

3.4 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).

3.5 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)

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

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

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 into the program at the following address:

Program Manager – LAP for Poultry Exported to Russia
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 signed statement from the laboratory director stating that the laboratory will analyze samples using only the methods accepted by AMS.

4.2.2 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.

4.2.3 Analysts' qualification, training procedures, and training records.

4.2.4 Method validation – The laboratory must submit method-relevant validation data and results including, but not limited to, method detection limit, method quantitation limit, linearity, accuracy, precision, sensitivity, selectivity, and recovery (See Section 11).

4.2.5 Proficiency test (PT) results – When required, the laboratory must participate in an external PT program, obtain a satisfactory status, and have the PT institutions send the PT reports directly to the PM.

4.2.5.1 For analysis of microorganisms – The laboratory testing for *Salmonella*, *Listeria monocytogenes* (*L. monocytogenes*) and Total Plate Count (TPC) must participate in a PT program administered either by the AOAC International or the American Proficiency Institute, obtain a satisfactory status, and have the PT reports sent to the PM.

4.2.5.2 For analysis of chemical residues – If a PT program becomes available, the laboratory may be required to participate in external PT program(s). Analyses of Certified References Materials (CRMs), where available and applicable, are required to be included in the validation process.

4.2.6 The program is user-fee supported and the laboratory must pay annual program fee(s) upon receipt of the billing. All fees must be received prior to admission to the program.

4.2.7 Declaration of analytical group(s) the laboratory wishes to analyze for this program. The laboratory may be admitted to test for any or all of the following groups: Heavy metals, Pesticides, Antibiotics (tetracyclines), *Salmonella*, *L. monocytogenes*, and TPC.

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

4.4 Issuance of Certificate/Acceptance Letter: AMS will provide a certificate and/or a letter of approval to the laboratory after it meets all program requirements.

5. Maintaining Program Status

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

5.2 Laboratories must participate in an LAP check sample program and/or external PT program when required, meet a satisfactory status, and have the PT institutions send the PT reports directly to the PM.

5.3 Laboratories must inform PM if their approved equipment/methods have been modified. The laboratory must perform method verification study again with acceptable results.

5.4 Upon analyst changes, the laboratory must inform the PM with the training record and the results of method verification study performed by the new analyst.

5.5 Laboratories must meet all program requirements. All method SOPs, method validation and verification data, and PT results **must be made available to the PM upon request**.

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 any one of 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 analytical requirements.
6.2.4 Failure to maintain an acceptable performance level as indicated by the results of PT and/or check samples.

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

7. Readmission

7.1 A laboratory removed from the program due to falsification of analytical results will be prohibited to reapply.

7.2 A laboratory removed from the program due to failure of analytical requirements must wait, at least six months, before it can re-submit a written request to the PM in order to initiate the admission process again.

8. Complaints

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 Director 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. Appeals

9.1 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 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 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 The program fee for participation is based on the number of groups for which a laboratory will be testing. Laboratories can analyze one group or a combination of several groups.

Number of Groups	1	2	3	4	5	6	7
1st Year Fee ($)	940	1,410	1,760	2,120	2,350	2,590	2,820
2nd Year and After ($)	800	1,200	1,500	1,800	2,000	2,200	2,400

List of groups: Pesticides, Heavy metals, Antibiotics (tetracyclines), *Salmonella*, *Listeria monocytogenes*, and Total Plate Count.

10.3 The yearly program fees are nonrefundable.

11. Technical Requirements

11.1 Chemical Analysis

11.1.1 Program Method Sensitivities: Following the "Export Requirements for Russia" (FSIS 2014), laboratories must be capable of analyzing chemical residues at or below those sensitivities.

Pesticides	**Required Sensitivity (ppb)**
Aldrin	300
BHC	300
Chlordane	300
DDT and its metabolites	100
Dieldrin	300
Endrin	150
Heptachlor	200
Heptachlor epoxide	200
Hexachlorobenzene	500
Lindane (γ-HCH)	100
Methoxychlor	3000
Toxaphene	7000
Heavy Metals	**Required Sensitivity (ppb)**
Arsenic (As)	100
Cadmium (Cd)	50
Lead (Pb)	500
Mercury (Hg)	30
Antibiotics	**Required Sensitivity (ppb)**
Tetracycline	10

Chlortetracycline	10
Oxytetracycline	10

11.1.2 Analytical Methods

11.1.2.1 Pesticides: AOAC International Official Method 970.52, Organochlorine and Organophosphorus Pesticide Residues, or other latest AOAC methods.

11.1.2.2 Heavy Metals: USDA FSIS CLG-TM3. Determination of methods by ICP-MS and ICP-OES (Rev: 04, 09/30/2013) or equivalent.

11.1.2.3 Antibiotics: LC-MS/MS Methods

11.1.3 Criteria of Method Performance for Chemical Analysis

11.1.3.1 Calibration

11.1.3.1.1 Calibration range

Typical concentrations, after expressed as sample equivalent concentrations, are set at a minimum of 5 different levels below and above those required sensitivities. For example, if tetracycline is extracted from 5 g of meat and prepared in 5 mL of solution for analysis (dilution factor = 1), the calibration range should cover from ≤ 5 to ≥ 100 ppb. If 0.5 g of meat is digested and dissolved in 50 mL of solution for analysis of lead (dilution factor = 100), the calibration range should cover from ≤ 2.5 to ≥ 50 ppb.

11.1.3.1.2 Calibration matrix

Calibrators are prepared in three types of matrix: Type 1 – Standards in solvent/buffer; Type 2 – Standards fortified into control matrix extract (tissue extract); and Type 3 – Standards fortified into control matrix (tissue) and processed through the extraction procedure. These three linear-fitting results are evaluated. Type 1 and Type 3 slopes are within 100±30-40% of Type 2 slope. After a method is validated, Type 2 calibration is used for unknown samples, unless there is sufficient evidence to support using Type 1 calibration.

11.1.3.1.3 Internal Reference

Internal standard reference shall be used to correct matrix effect. Laboratories which choose not to apply internal standard reference must evaluate the interference caused by sample matrix effect. And laboratories must evaluate the significance of applying or not applying internal reference standard in calibration and in measuring real samples for reliable analysis. Evidence must be provided to the PM to indicate the insignificance if an internal reference standard is not used.

NOTE: *"Control matrix", "Negative control", or "Blank matrix", is the animal tissue which is confirmed to be drug residue free based the known history of animals which have not been exposed to regulated or forbidden drugs, 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.*

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

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

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

11.1.3.2.3 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%.

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

11.1.3.2.5 At least one precursor and two daughter ions shall be identified.

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

11.1.3.3.1 Establishing limit of detection (LOD)

- Analyze at least 12-20 blank samples (tissue extract from blank samples, n > 12).
- Convert the noise signal to concentration at the time window in which chemical residue is expected.
- Calculate the average (avg) of all those (noise) concentrations and the standard deviation (sd).
- Let P = 3 × avg.
- Let Q = avg + 1.64 × sd.
- LOD is the bigger value out of P and Q

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

11.1.3.4 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.

11.1.3.5 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

11.1.3.6 Precision

It is evaluated by fortifying drug standards in control matrix at 10 and 30 levels. 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

11.1.3.7 Recovery

Analytes are fortified to control matrix (tissue) at 10 and 30 levels. The recovery (= 100% × $C_{Measured}$ / $C_{Fortified}$) of fortified drug 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

11.2 Microbiological Analysis

Microbiological testing includes *Salmonella*, *L. monocytogenes*, and TPC in this LAP.

11.2.1 Test Method

11.2.1.1 Methods from the following resources may be used:

- AOAC International Official Methods of Analysis,
- US FDA Bacteriological Analytical Manual (BAM),
- USDA FSIS Microbiology Laboratory Guidebook (MLG), or
- Compendium of Methods for the Microbiological Examination of Foods

11.2.1.2 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 FDA BAM, the USDA FSIS MLG and International Standards Organization (ISO) all contain culture methods that are recognized reference culture methods.
A laboratory using other methods must conduct its specific validation of those methods against reference culture method(s) to validate inclusivity, exclusivity, sensitivity and the methods performance as established by collaborative study. 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. 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.]