United States Department of Agriculture
Food Safety and Inspection Service

FSIS Multi-Residue Methods for Veterinary Drugs

Experience Share for multi-residue analysis
APEC FSCF PTIN Proficiency Testing Workshop

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I. Background

II. Methods

- Multi-class residue method (MRM)
- Aminoglycosides (AMG)

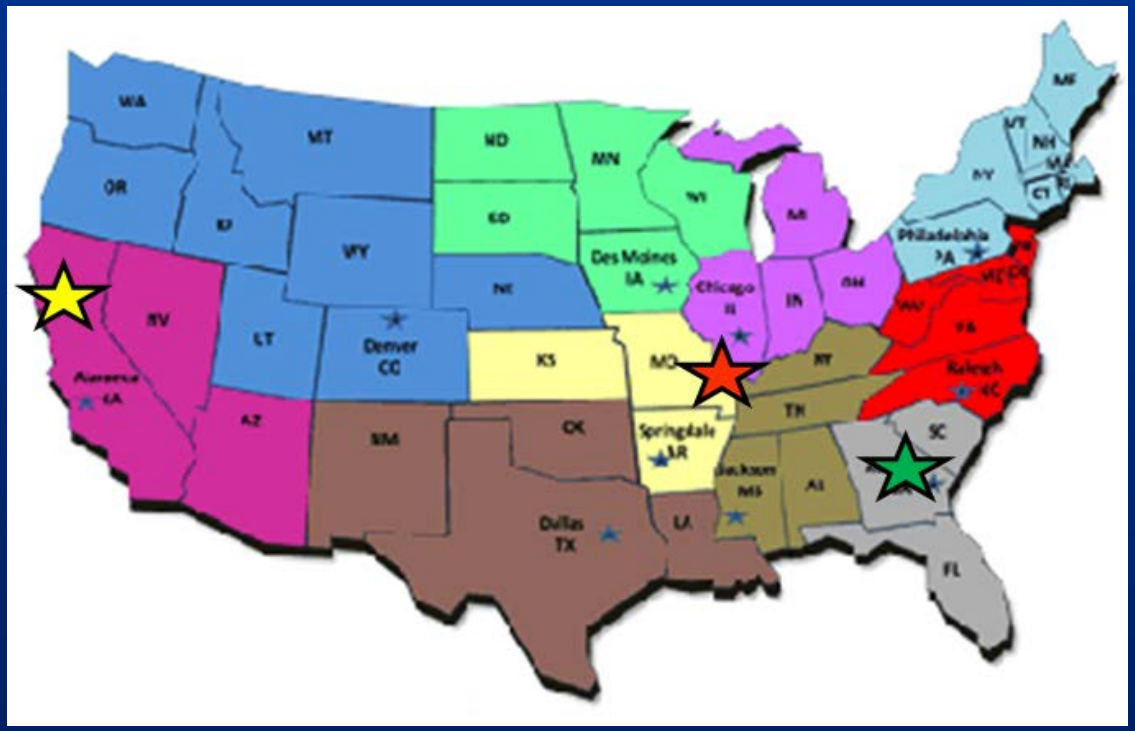
III. Maintaining Quality Control (QC)

- Batch Requirements
- Positive Controls

Mission

The Food Safety and Inspection Service (FSIS) is the public health agency in the U.S. Department of Agriculture responsible for ensuring that the nation's commercial supply of meat, poultry, and egg products is safe, wholesome, and correctly labeled and packaged.

FSIS Laboratory System



Western Laboratory
Alameda, CA

Midwestern Laboratory
(St. Louis, MO)

Eastern Laboratory and LQAS
(Athens, GA)

FSIS Laboratories

Analytical Chemistry Capabilities

Midwestern Laboratory

Inspector-generated residue analysis, primarily veterinary drugs

Western Laboratory and Eastern Laboratory

Scheduled chemistry analysis as described in the National Residue Program (NRP) – FSIS "Blue Book"

EL – Metals; Food chemistry

WL – Pesticides

All three labs are ISO 17025 and ALACC accredited

What is the role of LQAS?

- Ensures that Chemistry Laboratory Guidebook (CLG) methods are fit for purpose.
- Reviews and approves validation studies to implement new CLG methods or to revise existing CLG methods.

FSIS Primary Veterinary Drug Methods

- Screening and identification/confirmation of multiple residue classes (CLG-MRM1)
- Screening and identification/confirmation of aminoglycosides (CLG-AMG2)

- Both developed in USDA ARS (S. Lehotay research group); implemented in FSIS labs

FSIS Primary Veterinary Drug Methods

- How do FSIS laboratories use these methods?
  - Eastern, Western Labs – muscle screening for scheduled samples
  - Midwestern Lab – kidney, muscle screening of inspector-generated samples; identify screen-positives from all three labs
- Same methods are used for testing domestic and imported samples

CLG-MRM1

(multiple class residue method)

Summary

- Analyzes for 53 different veterinary drugs
- Extraction technique: dispersive solid-phase extraction (SPE)
- Instrumentation: UHPLC-MS-MS using a triple quadrupole mass spectrometer
- Applicable matrices: beef, pork, poultry, goat, and sheep muscle and kidney; horse muscle
  Consult method for specific applicability

CLG-MRM1

(multiple class residue method)

Analyte Classes

- Beta-lactams/cephalosporins
  - Amoxicillin
  - Ampicillin
  - Cefazolin
  - Cloxacillin
  - DCCD (Ceftiofur)
  - Dicloxacillin
  - Naficillin
  - Oxacillin
  - Penicillin G
- Fluoroquinolones
  - Ciprofloxacin
  - Danofloxacin
  - Desethylene Ciprofloxacin
  - Difloxacin
  - Enrofloxacin
  - Norfloxacin
  - Sarafloxacin

CLG-MRM1

(multiple class residue method)

Analyte Classes (continued)

- Hormones
  - Prednisone
  - Zearalanol
  - Melengestrol Acetate
- Phenicols
  - Chloramphenicol
  - Florfenicol
- Macrolides
  - Clindamycin
  - Erythromycin A
  - Gamithromycin
  - Lincomycin
  - Pirlimycin
  - Tilmicosin
  - Tylosin
  - Tulathromycin A

CLG-MRM1

(multiple class residue method)

Analyte Classes (continued)

- Sulfonamides
  - Sulfachloropyridazine
  - Sulfadiazine
  - Sulfadimethoxine
  - Sulfadoxine
  - Sulfaethoxypyridazine
  - Sulfamerazine
  - Sulfamethazine
  - Sulfamethizole
  - Sulfamethoxazole
  - Sulfamethoxypyridazine
  - Sulfanitran
  - Sulfapyridine
  - Sulfaquinoxaline
  - Sulfathiazole

CLG-MRM1

(multiple class residue method)

Analyte Classes (continued)

Tetracyclines

- Chlortetracycline
- Oxytetracycline
- Tetracycline

Carbadox

- 2-QCA (metabolite)

Analgesics/ Anti-Inflammatory

- Flunixin
- Phenylbutazone
- Oxyphenylbutazone

Beta-Agonists

- Cimaterol
- Ractopamine
- Salbutamol

More analytes will be added based on USDA FSIS needs

CLG-AMG2

Screening and Confirmation of Aminoglycosides

- 9 different aminoglycosides
- Extraction: disposable pipette extraction (DPX) with weak-cation exchange sorbent
- Choice of Instrumentation:
  - UHPLC-MS-MS with ion-pair chromatography
  - HPLC-MS-MS with normal phase (HILIC) chromatography

CLG-AMG2

Screening and Confirmation of Aminoglycosides

- Matrices: beef, pork, poultry kidney; beef and pork liver; and beef, pork, poultry, horse muscle
- Analytes:
  - Amikacin
  - Apramycin
  - Dihydrostreptomycin
  - Gentamicin
  - Hygromycin B
  - Kanamycin
  - Neomycin B
  - Spectinomycin
  - Streptomycin

Additional Multi-Residue Methods

- Avermectins (3 analytes)
- Pesticides (88 analytes)
- Heavy Metals (17 analytes)
- Hormones (4 analytes)

Maintaining Quality Control

Batch Requirements

- Screening Set for CLG-MRM1
  - External Standard(s) (optional)
  - Matrix matched standard
  - Recovery(ies) (positive controls)
  - Intralaboratory check sample (as needed)
  - Tissue blank (negative control)
  - Samples
  - External standard, matrix matched standard, or recovery

Maintaining Quality Control

Positive Control Requirements

Analytes in positive control must meet criteria:

- Retention time match against a reference
- All monitored ions must be present… (screening)
- …and with acceptable ion ratios (confirmation)

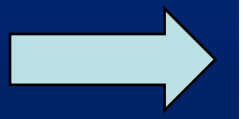
Each method provides specific acceptability requirements for positive and negative controls

Methods Available Online

- Chemistry Laboratory Guidebook (CLG)
  Link:
  http://www.fsis.usda.gov/wps/portal/fsis/topics/science/laboratories-and-procedures/guidebooks-and-methods/chemistry-laboratory-guidebook/chemistry-laboratory-guidebook
- Multi-class Residue Method (CLG-MRM1)
  Link:
  http://www.fsis.usda.gov/wps/wcm/connect/b9d45c8b-74d4-4e99-8eda-5453812eb237/CLG-MRM1.pdf?MOD=AJPERES
- Aminoglycosides Method (CLG-AMG2)
  Link:
  http://www.fsis.usda.gov/wps/wcm/connect/c7d1fc07-6359-4d64-959b-1931596bef9a/CLG-AMG2.pdf?MOD=AJPERES

FSIS Method Updates

sign up for automatic updates



Chemistry Laboratory Guidebook

This **Chemistry Laboratory Guidebook** contains test methods used by FSIS Laboratories to support the Agency's inspection program, ensuring that meat, poultry, and egg products are safe, wholesome and accurately labeled.

The Guidebook contains methods for the analysis of food composition, food additives, nutrients, veterinary drug and pesticide residues. Methods are designed to provide analysts with written documentation to facilitate training, performance, quality assessment, and interpretation of data.

The contents of this Guidebook are continuously revised and updated. Future updates will include other methods previously published in the printed version of the Guidebook, which is no longer available for distribution.

Receive email notification when the Chemistry Laboratory Guidebook is updated.

Method Number	Method Title	Effective Date
FOOD CHEMISTRY		
F01	Moisture Determination (PDF Only)	Aug 10, 2009
F02	Protein Determination by Combustion (PDF Only)	Jul 27, 2009
F03	Determination of Fat (PDF Only)	Aug 10, 2009
F04	Determination of Salt (PDF Only)	Jul 27, 2009

Questions?