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OPI: S&T/MD
PART 1 OF 2

PROCEDURES FOR COLLECTING AND SUBMITTING DOMESTIC SAMPLES FOR MICROBIOLOGICAL ANALYSIS

PART ONE--BASIC PROVISIONS

I. PURPOSE

This directive gives procedures for collecting, packaging, and mailing or shipping domestic samples to FSIS laboratories for microbiological analysis. The purpose of the directive is to provide procedures that will prevent environmental contamination and/or microbiological proliferation and maintain sample integrity.

II. CANCELLATIONS

MPI Guidelines for Laboratory Sampling
MPI Manual Subpart 23.B (23.10 - 23.13)

III. [RESERVED]

IV. REFERENCES

9 CFR 318.9 and 381.146, 9 CFR 318.300 and 381.300
FSIS Directive 7355.1, dated 2/4/88
FSIS Directive 8150.1, Rev. 1, dated 4/5/90
FSIS Directive 10,230.1, dated 10/14/87
FSIS Directive 10,240.1, Rev. 1, dated 8/30/90
FSIS Directive 10,600.1, dated 10/6/83
FSIS Directive 10,625.1, dated 2/26/86

V. ABBREVIATIONS and FORMS

The following appear in their shortened form in this directive:

IO	Inspection Operations
RO	Regional Offices
RP	Regulatory Programs
S&T	Science and Technology

FSIS Form 10,000-2 (formerly FSIS Form 6000-1), dated 7/88	Laboratory Report
FSIS Form 10,210-2 dated 2/91	Request for Sampling,
FSIS Form 7355-1	Sample Seal, dated 8/86
FSIS Form 10,600-1	Domestic Chemical Laboratory

Analysis, dated 11/89

VI. POLICY

Authorized FSIS program employees collect and mail or ship samples of meat and poultry to FSIS laboratories for analysis. The analyses are performed to: determine the presence of pathogenic/food poisoning and/or sanitary indicator microorganisms or their toxins for food safety and diagnostic purposes; determine animal species in raw or cooked products; determine the presence of antibiotic, sulfonamide, or other drug/chemical residues; and determine wholesomeness in accordance with the Federal Meat Inspection Act and the Poultry Products Inspection Act.

VII. RESPONSIBILITIES

FSIS personnel from IO, S&T, and RP are responsible for collecting and submitting microbiological monitoring and surveillance samples and other samples requested under special programs to FSIS laboratories. Since microbiological contamination may occur at any stage during the process of sample collection, mailing or shipment, and receipt at the laboratory, it is essential that the sample selection and handling procedures described are carefully followed to maintain sample integrity and identity.

PART TWO

COLLECTING SAMPLES FOR MICROBIOLOGICAL ANALYSIS

I. GENERAL INSTRUCTIONS FOR COLLECTING SAMPLES

A. Minimize External Contamination. Take samples in a manner that will minimize external contamination. Extraneous organisms from the environment may lead to erroneous analytical results. Where possible, use aseptic sampling techniques and sterilized equipment and supplies. Equipment and supplies are discussed below, in this part.

B. Take Samples in Specified Order. Take diagnostic microbiological specimens before histopathological specimens.

C. Apply Stringent Aseptic Techniques. Samples collected for microbiological analysis require more stringent aseptic techniques than sampling for residue analysis or species determination.

D. Separate Specimens or Tissue Samples. Do not pool specimens or different tissues as one "sample," whether from the same or different animals. Place each specimen or tissue sample in a separate, clear, plastic sample bag. Exceptions may be considered for special cases such as kidney samples from rabbits or poultry.

E. Package Raw and Processed Products in Correct Weight/Sizes. Submit samples from raw or processed products, appropriately labeled, in small packages weighing approximately 250 grams (about 1/2 pound) or conforming to the required weight as directed for sampling.

F. Label Contents of Each Plastic Bag.

G. Identify Large Bag Holding Smaller Individually Bagged and Labeled Samples from One Animal. Place all individually labeled and secured sample bags of tissues collected from a single animal into one large plastic bag and identify the outside bag with appropriate identification such as project name/number, owner, Retained tag number, or FSIS form number.

H. Avoid Conditions that Make Samples Unsuitable. Avoid conditions that may make samples unsuitable for microbiological analysis, such as:

1. Environmental contamination caused by failure to use aseptic technique in sample collection.

2. Sample specimen that is too small.

3. Sample submitted in formalin solution or formalin added.

4. Sample submitted in borax solution (unless instructed).

5. An incorrect number of animals/birds sampled.

6. Multiple tissue samples in the same plastic bag.

7. Samples thawed in transit.

8. Unbagged samples.

9. Unidentified samples.

10. Severely damaged cans received.

11. Hard swells that leaked or exploded in transit.

12. Cans submitted that have been previously opened and may have had product removed unless otherwise directed.

II. TECHNIQUES FOR COLLECTING SAMPLES

A. Use the techniques given below in this section when aseptic procedures are specified in section "III. Collection of Samples." Refer to section "IV. Equipment and Supplies" for materials available from FSIS laboratories for collecting diagnostic microbiology samples.

B. Sterilize Equipment and Instruments. When sterilized equipment and instruments are not available in the field, thoroughly wash equipment or instruments such as knives, scissors, spoons, and other non-expendable metal items and then sterilize them by one of the following methods.

1. Immerse sampling end of cleaned tool in 180 degrees F. water for 1 minute. Protect sampling end from contamination before use.

2. Immerse the sampling end or part of the item in sodium hypochlorite or other disinfecting solution for 1-2 minutes. Shake excess solution from utensil and protect sampling end from contamination. Normal disinfecting solutions: a 0.5 percent (500 ppm) sodium hypochlorite solution or a *** 1.5 AMENDMENT 1, 9/4/92 *** percent solution of household bleach (*** 2 AMENDMENT 1, 9/4/92 *** ounces per gallon of potable water). Note: This procedure is both effective and easily performed, using equipment available in any plant. A *** clean AMENDMENT 1, 9/4/92 *** bucket, sampling tools, household bleach, and hand washing facilities provide necessities for on-line sampling.

3. Flame the sampling end or part of the item with a propane torch, air cool, and protect from contamination before use. Caution: Excessive heating dulls knives and scissors.

III. COLLECTION OF SAMPLES

A. Specimens or Tissue Samples.

1. Diagnostic microbiological analysis.

a. Blood and serum for serology. Let blood stand for 2 hours at room temperature (or 4 hours in refrigerator) until the serum separates from the clot. Decant the serum into a separate sterile centrifuge tube. Freeze the tube or add Merthiolate to reach a final concentration of 1:10,000 and keep refrigerated.

b. Blood and serum for culture. Collect blood aseptically; label and freeze immediately.

c. Synovial fluid.

(1) Submit intact joint and surrounding muscle in a plastic bag; or

(2) Draw fluid from the joint using a sterile syringe and needle. Transfer to a sterile centrifuge tube and freeze.

d. Tissues. Tissue samples from suspected septicemic animals/birds should be approximately 2 x 1 x 1 inches in size (approximately 50 grams). Place each sample in a separate bag.

e. Gross lesions. When gross lesions are numerous, collect excised whole lesions or groups of lesions separately and submit for both diagnostic microbiological and pathological testing. Lesions submitted for microbiological analysis should be kept cold before and during shipment. When only one lesion is found, excise entire lesion and cut in half. Prior to shipment, chill one half for microbiological analysis and place the remaining half in buffered formalin solution for pathological examination.

f. Ship all diagnostic microbiological samples cold if overnight delivery is available.

g. Freeze all diagnostic microbiological tissue samples and ship frozen where overnight delivery is not available. Note: Do not freeze pathology samples; these are preserved by formalin.

2. Antibiotic residues and species identification. Samples submitted for these purposes need not be taken aseptically; however, exercise caution to avoid contamination and commingling of tissues. Label, identify, package, and freeze all such raw tissue samples and processed products without delay. If samples are from dry or shelf-stable processed products, they may be shipped without refrigeration.

B. Product Samples.

1. Raw (unprocessed) muscle or organ tissues. Collect samples for microbiological analysis from various locations that are most likely to be indicative of maximum carcass contamination or abnormal conditions. Unless submitting a kidney, heart, or part of a liver, collect approximately 250 grams (1/2 pound) of tissues. Label and freeze the samples immediately.

2. Raw processed products. Collect samples for microbiological analysis aseptically from raw processed products like ground meat, patties (breaded or unbreaded), fine or coarse ground emulsions, or prepackaged products. Intact packages of prepackaged parts or products such as livers, gizzards, or pork sausages can be collected randomly. Collect approximately 250 grams (1/2 pound). Label and freeze samples immediately.

3. Special products. For categories "a" through "e," given below, collect 250-gram (about 1/2 pound) samples aseptically. If commercially packaged product is available, collect intact package(s). If product is not retail packaged or if sampling calls for multiple samples, collect multiple intact packages or open packages aseptically. Avoid contamination when collecting the required amount for diagnostic microbiology or for species identification.

Special products include but are not limited to:

a. Unheated, processed ready-to-eat products.
b. Fermented dry or semi-dry products.

c. "Keep refrigerated" products.

d. Heated products.

e. Cooked, ready-to-eat products for microbiological analysis or species identification.

C. Hermetically Sealed Product Samples. These directions cover canned

products as defined in 9 CFR 318.300 and 381.300. Special directions are given below for shelf stable and for perishable canned products.

1. Shelf stable canned food.

a. Before collecting and shipping, consult with the receiving laboratory for directions or for special mailing or shipping instructions. The laboratory supervisor or the laboratory staff will determine the number of cans to send.

b. Determine the number and kinds of abnormal cans available for examination. Hard swells may burst en route. If only one hard swell exists, package it with extra care in multiple sealed plastic bags. If more than one hard swell exists, select a can or cans that do not have buckled seams for submission to the laboratory. Cool to below 40 degrees F. but do not freeze. Ship in an insulated container with coolant. When possible, select different kinds of abnormal cans (springers, soft swells, and hard swells) for examination. However, the final decision on the kinds of cans and number to be submitted rests with the laboratory.

c. Collect 4-6 normal cans with the same code to send along with abnormal can(s).

d. Place each abnormal can in a separate plastic bag and wrap in paper.

e. Place in a strong corrugated box and pad cans with crumpled newspaper.

f. Pre-chill (do not freeze) and mail or ship cans so that they will arrive at the laboratory at refrigerated temperatures.

2. Perishable canned products.

a. Contact the receiving laboratory before shipping.

b. Place abnormal cans in separate plastic bags.

c. Refrigerate normal and abnormal cans overnight before shipping.

d. Send both normal and abnormal cans "Air Freight" or "Air Express" for delivery to the FSIS laboratory. Notify the laboratory as to expected time of sample arrival.

D. Entomology and Extraneous Material Samples.

1. To assure acceptable samples:

a. Collect a minimum of 250 gm (about 1/2 pound) of meat/poultry, spice or other products to be sampled.

b. Collect and include companion samples from the same production lot as the consumer complaint, if possible, when collecting

consumer complaint samples.

c. Identify each sample as to whether it is an index sample, a companion sample, or some other related sample.

d. Print or type "Extraneous Materials" on FSIS Form 10,000-2. Include exact nature of complaint/suspected contaminant (worm, hair, metal, and similar). Give description (color, size, condition, and so forth) and general location where found in relation to the product (surface, inside).

2. To facilitate identification and verification of extraneous material:

a. Do not use staples on the sample bag. Do not attach foreign objects to any type of surface with adhesive tape.

b. Separate and package objects and other small sample pieces in a plastic specimen jar, test tube, or pill box. Secure lid with adhesive tape.

c. If object is not separated from product, clearly mark packaging to show suspect area.

d. Package dead insects, worms, and similar extraneous material in a plastic vial, test tube, or jar with 70 percent ethyl or isopropyl alcohol (rubbing alcohol). Secure lid with adhesive tape.

e. Package live insects, worms, or similar material in a plastic vial, test tube, or jar without alcohol. Include small amount of food material. Avoid freezing. Ship refrigerated.

E. Refer to Special Sampling Directives. Take special samples, as directed by RO, IO, and S&T, to comply with FSIS Directives such as the following:

1. Microbiological Monitoring Program: Sampling, Testing Procedures and Actions for Listeria monocytogenes and Salmonella, FSIS Directive 10,240.1, Rev. 1.

2. Procedures for Evidentiary Samples, FSIS Directive 10,625.1.

3. Any other pertinent directives or notices.

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PART 2 OF 2

PROCEDURES FOR COLLECTING AND SUBMITTING DOMESTIC SAMPLES FOR MICROBIOLOGICAL ANALYSIS

IV. EQUIPMENT AND SUPPLIES

A. Obtaining Equipment and Supplies. Materials necessary for collecting and submitting diagnostic microbiology samples are available from FSIS laboratories.

B. List of Equipment and Supplies. Supplies designated with an asterisk (*) will be provided for specific program initiated samples as needed.

1. Sterile "whirl-pack" type bags, or equivalent bags, for collecting up to approximately 16 ounces of sample (tissue, powder, or liquid).

2. "Zip lock bags" are heavy gauge and are suitable for samples that cannot be placed in the "whirl-pack" type bags (turkey rolls, beef rolls, and so forth).

3. Mailing/shipping containers. Insulated mailing/ shipping containers.

*4. Forceps. Regular sterilized or sterile, disposable, plastic forceps.

*5. Gloves. Sterile, disposable, plastic gloves for collecting and transferring samples from production line to sample container.

*6. Swabs. Sterile swabs for diagnostic and other specialized sampling purposes.

*7. Tongue depressors. Sterile tongue depressors for diagnostic microbiology and other specialized sampling purposes.

*8. Centrifuge tubes. Sterile, disposable, plastic centrifuge tubes (50 ml).

*9. Scissors. Sterile scissors set.

*10. Scalpels. Sterile disposable scalpels.

*11. Syringes. Sterile disposable syringes.

*12. Hypodermic needles. Sterile and disposable.

C. Use of "Whirl-Pack" Type Bags for Samples.

1. Tear off the perforated strip completely.

2. Pull the paper side tabs to open the bag slightly; push the end tabs to open fully.

3. Fill the bag with about 7-8 ounces (approximately half full).

4. Squeeze to expel all air, pull the tape-covered wire together, and whirl the bag three times over the wire.

5. Lock shut and label. Fold the ends of the tape flat against themselves to seal the bag.

6. Place the sealed "whirlpack" type bag into a regular bag; expel the air and firmly close with rubber band.

7. Freeze, wrap in newspaper, and place in an insulated shipping container with frozen coolant to maintain temperature during shipment.

V. FORMS

A. FSIS Forms.

Applicable form(s) named in Part One, section "V," of this directive must be filled out and submitted to the FSIS laboratory with the sample being sent for microbiological analysis.

1. Fill out the appropriate laboratory sample form(s) completely. For example, on FSIS Form 10,000-2 provide information that will fully answer two questions: "What prompted sampling?" and "How, when, and where was sample obtained?" Completed forms aid the microbiologist in selecting appropriate tests and interpreting results.

2. Indicate whether product from which sample was taken is being retained pending analysis. This information will let the laboratory know that analysis and reporting need to be expedited.

3. Record suspected disease and/or causes of disease (etiological agents) on sample collection form when diagnostic microbiological samples are submitted.

4. Enclose applicable laboratory sample form(s) in a separate plastic bag to avoid soiling of form(s).

5. Affix required FSIS form(s) to samples sent for microbiological analysis. Mail/ship form(s) along with the samples to the appropriate FSIS

laboratory. Address as shown in the Meat and Poultry Inspection Directory.

6. When applicable, attach a copy of the label for the processed product.

B. Problems Related to Use of Required Forms.

1. Not filled out properly or completely.

a. Information not legible.

b. Multi-samples listed on one form instead of one livestock or one 6- or 30-bird sample per form.

2. Soiled from improper packing in containers.

3. Laboratory report form(s) mailed or shipped to laboratory separate from sample and lost en route.

PART THREE

SUBMITTING SAMPLES FOR MICROBIOLOGICAL ANALYSIS

I. PACKAGING AND FREEZING INSTRUCTIONS

A. General Instructions. For purposes of this directive, samples that are "mailed" are conveyed under public authority from one post office to another. Samples that are "shipped" are conveyed under private authority in any form of conveyance, sometimes under contract. Observe postal regulations and/or carrier or contract carrier terms and conditions. The integrity of the sample must be maintained whether mailing or shipping samples. Follow these steps:

1. Place each specimen or tissue sample in a strong, separate plastic bag. Twist the bag at the top; fold the twist over; secure with a rubber band; and identify. Place the individually bagged and identified sample(s) from one animal into a second plastic bag and again close with a rubber band. This doublebagging will (1) prevent leakage and a transfer of residue or microorganisms from tissue to tissue and (2) aid in ensuring sample integrity.

2. Chill or hard freeze most samples immediately after collection. Place frozen samples in prechilled insulated shipping containers with frozen coolant canisters prior to mailing or shipping. Note: Various coolant canisters and mailing/shipping containers are stocked in Regional Offices and/or in FSIS laboratories.

3. Wrap doublebagged samples in newspaper. Fill empty spaces of mailing or shipping container with crumpled newspaper to protect both sample and insulated shipping container during transit.

4. Affix a "perishable" label.

5. Mail or ship sample(s) at the appropriate time during the week after consulting with postal authorities or with shipper so that sample(s) arrive at the required temperature.

B. Prevent Problems with Packaging and Freezing.

1. Assure that a clear identification label has been placed on both individual samples and larger bags containing more than one sample from the same animal before placing in mailing or shipping carton.

2. Follow instructions when packing containers.

3. Use proper container for frozen or refrigerated sample(s), that is, a mailing or shipping container intended for sending frozen or refrigerated samples.

4. Do not remove frozen coolant canisters from containers to make more room for samples.

5. Pack sample(s) properly in container so that container will not break when sample(s) is frozen.

6. Prechill container properly.

7. Address mailing/shipping container properly; place return address on flip card or reverse flap.

8. Contact RO or an FSIS laboratory to obtain containers in a timely manner.

9. Do not thaw or refreeze tissue samples before they are submitted to the laboratory. Thawed and/or refrozen samples cannot be analyzed for most residues or for microbiological purposes.

C. Maintain Sample Integrity.

1. FSIS Directive 7355.1--Use of Sample Seals for Program Samples and other Applications--gives requirements for sample seals and security of samples.

2. FSIS Directive 8150.1--Sample Collection and Integrity, gives requirements for Compliance Division, RP, samples.

II. MAILING AND SHIPPING INSTRUCTIONS

A. General Instructions. General directions for mailing and/or shipping

are given in FSIS Directive 10,600.1--Sample Shipment Procedures. Give laboratory prior notification as to how samples(s) are being sent, if hand delivered or sent other than by mail or approved contract carrier.

1. Perishable product/tissue sample: Proceed as follows:

a. Obtain a temperature controlled mailing/shipping container from RO. Freeze coolant containers for 8-10 hours in a 0 degree F. to 10 degrees F. freezer. Caution: Do not freeze below -10 degrees F.

b. Sample should not be larger than available space in mailing/shipping container.

c. Samples that were taken and frozen immediately must be maintained in a frozen state until they reach the laboratory.

d. Place coolant canisters in mailing/ shipping container and bagged sample between coolant canisters. If more space is needed, use another shipping container rather than trying to force too much into one.

2. Dry Product. Do not freeze dry product(s) such as non-fat dry milk, breading mix, eggs, and spices. To submit, place unfrozen dry product in suitable, strong container and send to appropriate FSIS laboratory by regular mail.

3. Canned Product.

a. Pack cans in such a way as to prevent damage during mailing/shipping.

b. Shelf stable canned product. Submit several normal and abnormal cans. Contact laboratory before submitting hard swells. Chill abnormal cans in refrigerator (not freezer) overnight. Wrap each chilled abnormal can in a plastic bag, then in paper, and ship by any suitable means. Send normal cans without refrigeration.

c. Keep refrigerated canned product. Submit several normal and abnormal cans. Contact laboratory before submitting hard swells. Maintain abnormal cans in refrigerator (not freezer) overnight. Wrap each chilled normal and abnormal can in separate plastic bags, then in paper, and ship by any suitable means.

B. Specific Instructions for Mailing Samples. Samples that are "mailed" are conveyed under public authority from one post office to another. Postal regulations must be followed. Protect the integrity of the sample in the following ways:

1. Use the proper mailing address with the heading, "Microbiology."

2. Call any contract or other carrier being used to convey mail to the post office. Make arrangements for pickup by them or delivery to them.

3. Samples should be mailed preferably either on Monday or Tuesday to assure arrival at the laboratory during working hours.

4. Make sure samples are picked up by or delivered to contract or other carriers, as arranged. Note: For direct delivery, samples sent through carriers must be sent to a street address and not to a post office box.

5. Take the following precautionary measures:

a. Take the sealed package to the post office at a time determined previously through communication with postal authorities. Do not allow the package to sit in the post office for 24 hours prior to the time it is sent to the laboratory. Place a note on the outside of the box when the box contains dry ice. Follow postal (and other carrier) regulations.

b. Check and replace worn pre-addressed labels (mailing "franks") prior to shipment.

c. Remove old mailing instructions from carton.

d. Do not use soiled boxes for mailing samples to the laboratory. Postal employees have authority to destroy these or refuse acceptance of shipment.

e. Mail samples by first class mail, overnight mail, or air express.

f. Do not use improper mailing "frank."

C. Specific Instructions for Shipping Samples. For purposes of this directive, samples that are "shipped" are conveyed under private authority in any form of conveyance. When contract carriers are used, their rules and regulations must be observed. In addition, protect the integrity of the sample by adhering to the following:

1. Protect packages from tearing. Double bag and wrap packages of samples in newspaper.

2. Freeze samples. Hard freeze sample and coolant canisters prior to shipping.

3. Fill empty spaces of the shipping container with crumpled newspaper to protect both sample and shipping container during transit.

4. Pack large samples in boxes. Pack samples too large for refrigerated shipping containers in double-walled or corrugated boxes (preferably styrofoam lined) with 15-25 pounds of dry ice. Samples and dry ice should be placed in a large tightly closed plastic bag. Note: Plastic bag retards sublimation of dry ice by creating back pressure on the sublimation process.

5. Protect sample bags shipped with dry ice. When shipped with dry ice, plastic bags often become brittle and break during transit. Follow these instructions:

a. Double bag each sample and identify.

b. Hard freeze overnight.

c. Wrap each bagged sample with 3 or 4 sheets of clean newspaper.

d. Place dry ice in container; place crumpled newspaper next to the dry ice.

e. Wrap all samples together with 3 or 4 sheets of newspaper and place in container.

f. Add more crumpled newspaper and more dry ice if needed.

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