Good Manufacturing Guidelines for the
Removal of Spinal Cord During Slaughter Operations
And Sampling and Testing of Advanced Meat Recovery Product
For Glial Fibrillary Acidic Protein Analysis.

Introduction
Meat produced through the use of Advance Meat Recovery (AMR) systems must not contain spinal cord in order to meet the legal definition of “meat” as described in 9CFR§ 301.2(rr), industry standards and consumer expectations. FSIS Directive 7160.2 instructs inspection personal to verify that meat derived through AMR systems complies with the regulations by verifying the complete removal of the spinal cord from vertebral bone (neck and/or backbone) entering the system. It is therefore necessary that plants involved in the slaughter of livestock and/or the use of AMR systems establish a standard of zero spinal cord in the vertebral bone and develop sampling and testing protocols for the production of AMR products using a validated rapid testing method to assure compliance with these regulations.

The following Good Manufacturing Practices (GMPs) were developed for the purpose of identifying certain procedures that may be followed to improve process control for assuring the removal of spinal cord from vertebral bone and for developing sampling and testing protocols to assure that non-natural occurring levels of Glial Fibrillary Acidic Protein (GFAP), a naturally occurring protein specific to Central Nervous System tissue, are not present in AMR product.

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Glial Fibrillary Acidic Protein Rapid Testing Kits.
National Meat Association, in conjunction with the American Meat Institute, and Dr. Glenn Schmidt, Department of Animal Science, Colorado State University, conducted a Beta site analysis of the R-Biopharm Ridascreen® GFAP Test Kit on AMR products. The Beta site testing data clearly demonstrate that the levels found in whole muscle tissues. The testing also verify that the Biopharm rapid test Kits are a viable process control method for monitoring levels of Glial Fibrillary Acidic Protein (GFAP) in the manufacture of AMR products.

On October 4, 2001, industry representatives met with FSIS to present this data and to demonstrate that, in conjunction with the GMPs for spinal cord removal on the kill floor, the R-Biopharm rapid testing method for GFAP is a legitimate analytical method for validating the process controls for producing products from AMR systems.
Therefore, NMA recommends the use of the R-Biopharm Rapid Testing Kit as a means for validating AMR processes. NMA will provide updates as other testing kits become available.

**Good Manufacturing Practices (GMPs)**

**For the Removal of Spinal Cord During Slaughter**

**Objective:** To assure the removal of all spinal cord material present in the vertebral column after carcass splitting.

**Slaughter**

1. Remove spinal cord from the entire vertebral bone (butt bone to the end of the neck bone) before the final carcass wash during slaughter. Recommended methods for removing spinal cord include manual-hook or vacuum suction.

2. Establish monitoring and record keeping procedures on each carcass prior to entering the hot box. Correct any incomplete spinal cord removal or identify and segregate vertebral bones that may contain irremovable spinal cord for inedible disposal. Rework and remove any remaining spinal cord or properly identify and segregate all vertebral bones that may contain irremovable spinal cord for inedible disposal.

3. Establish a routine verification program on the slaughter floor and/or the hot box to verify the complete identification and segregation of all carcasses with incomplete spinal cord removal. Auditing program should include appropriate documentation, feedback and corrective actions.

**Fabrication**

1. Examine vertebral bones for spinal cord immediately prior to or during carcass disassembly. Remove any remaining spinal cord or properly identify and segregate all vertebral bones that may contain irremovable spinal cord for inedible disposal. (This step may be incorporated as a verification procedure of the aforementioned steps.)

2. Inspect vertebral bones prior to entering the AMR systems. Remove bones containing *any* spinal cord material for inedible disposal.

Establish a routine monitoring and record keeping procedures program for bones being processed through the AMR system. If spinal cord is identified, stop production immediately, discard all products to inedible back to last acceptable check, and wash, sanitize and inspect all associated equipment prior to resuming operations. Auditing program should include appropriate documentation, feedback and corrective actions.
Sampling Programs for the Collection of
AMR Products for Glial Fibrillary Acidic Protein Analysis.

Objective: This sampling procedure is intended to assure uniform sample collection for
detection of the presence of Glial fibrillary acidic proteins, utilizing R-Biopharm
Ridascreen® Risk Material test. *

Scope: To obtain a representative sample of AMR material by production period.

Safety:
1. The sample should not be taken directly from the rotating drum.
2. The material is often subjected to dry ice; therefore, the sample should be
taken wearing cotton gloves.
3. Use the appropriate tool to collect the sample. Do not sample with bare hands.
4. Wear safety glasses.

Supplies:
1. Ice Cream Scoop
2. Whirlpak Bags- Large (dimensions-61/2 inchesX 101/2 inches)
3. Sample label
4. Robot Coupe or Sample Grinder
5. Freezer

Procedure:
1. Wash and dry the scoop. It does not have to be sterile.
2. Collect, from the initial container below the Protecon drum, a 0.5lb
   (approximate) sample. Avoid collecting the sample directly from the combo.
3. Collect one sample of 0.5 lbs per production period
4. Place in a whirl-pack bag, seal the bag. Repeat, using the same sample bag,
   for each sampling period of the shift.
5. Wash and dry scoop before collecting the next period sample.
6. Store the sample at <40˚F, the same as you would a micro sample.
7. At the end of the shift, there should be three samples in the bag.
8. Seal the bag and knead the sample to mix the three sub samples.
9. Label with date and shift

Remit sample to the laboratory and process as follows:
1. Place sample in Robot coupe and mix for 15 seconds to create a homogenous
   paste.
   a. Robot coupe should be thoroughly cleaned between samples.
2. Place approximately 500 grams of sample homogenous paste into a sterile
   whirl-pak.
3. Label whirl pack with date, shift and plant identification
4. Freeze sample, prior to shipment if shipping to an outside laboratory
   a. If analyzed fresh, do so within 12-hours
   b. If sample is refrigerated, analyze within 2-weeks
   c. Laboratory Testing Procedures for Glial Fibrillary Acidic Protein Analysis of AMR Product.

Objective: Qualitative analysis utilizing a sandwich enzyme immunoassay, for glial fibrillary acidic protein. These are cellular markers that are highly concentrated in central nervous tissue.

Scope: Monitor the presence of brain or spinal cord in advanced meat recovery products/muscle tissue utilizing a GFAP ELISA.

Safety:
1. Always follow the Chemical Hygiene plan and established laboratory safety procedures for handling materials, cleaning up spills and disposing of wastes.
2. Read and observe all precautionary measures and hazards noted in the MSDS for all chemicals and media used in this procedure.
3. Emphasis should be placed on disinfections of the bench-top both before and after working with samples or transferring.
4. Hands should be washed before and after handling sample, working with culture, handling chemicals, and before leaving the laboratory
5. Laboratory coat should be worn when working in the pathogen lab and should be removed before exiting.
6. The “STOP” solution is sulfuric acid. Follow all procedures for acid handling.

Waste Disposal:
Waste generated by this procedure should be placed in a biohazard bag and autoclaved.

Chemical/Reagent Components:
1. DASCREEN® Risk Material 10/5 Kit
   a. Kits must be within expiration dates on label
   b. Individual reagents should not be interchanged between kits of different lot number
   c. Reagents should not be diluted or adulterated
      i. Substrate/chromogen solution is sensitive to light
      ii. Blue coloration of the red substrate/chromogen solution is indication that reagent has deteriorated; reagent should not be used.
   d. Sample dilution buffer
      i. Provided at a 10-fold concentrate
1. Before use, the buffer must be diluted 1:10 with distilled water
   a. Crystals in the buffer concentrate can be dissolved in a waterbath at 37°C
   b. Dilution buffer acceptable up to expiration date on buffer label if stored at 2-8°C.
   e. Wash buffer
      i. Dissolve one pouch of buffer salt in 1 liter of distilled water
      ii. Prepared washing buffer expires after 4 weeks at 4°C
   f. Kit should be stored at 2-8°C: DO NOT FREEZE

2. Microtiter plate spectrophotometer, 450nm
3. Swabs, Fisher brand dacron fiber tipped cat # 14-9959-90
4. Pipettes
5. Test tubes- Falcon brand 12x75mm Fisher cat # 14959-2A
6. Repeator pipettor capable of dispensing 50-250uL

Sample Preparation:
1. Surface swabbing
   a. Pre-moisten swab by placing into the sample buffer
   b. Swab a representative area (4x4)
   d. Squeeze swab thoroughly into a test tube containing 1mlof sample buffer.
      The swab should run clean
   e. Proceed to test procedures

2. Homogenous sample (alternate sample methodology, however, for AMR Protecon material surface swabbing is acceptable methodology.
   a. Insert the swab into the homogenous sample several times
      i. If the sample is solid, swab the surface thoroughly while rotating the swab.
   b. Squeeze the swab thoroughly in a test tube containing 1 ml sample buffer.
      The swab should run clean.
   c. Proceed to test procedure.

Test Procedure
1. Bring all reagents and sample to room temperature (20-35°C) before use.
   a. Return all reagents to a 2-8°C refrigerator immediately after use.
2. Insert a sufficient number of wells into the microwell holder for all samples and standards.
   a. Record standard and sample positions. (Standards should be placed such that the lowest concentration is in well one, next well-2, etc., with the highest concentration in well-4.)
   b. No more than 4 strip with 8 wells should be analyzed at the same time (29 samples and 3 standards).
3. At all points through this procedure bubbles should be prevented from forming in the wells, through careful addition of samples, and reagents.
4. Add 50uL of standard or prepared samples to appropriate wells
5. Add one drop (50μL) of enzyme conjugate (red cap) to the bottom of each well
   a. Incubate for 10 minutes +/- one minute at room temperature (20-25°C).
   Avoid direct sunlight during all incubations, covering the plate is recommended.
6. Wash by pouring liquid out of the wells and tapping the holder upside down vigorously
   (three times in a row) against absorbent paper towel to ensure complete removal of liquid
   from wells.
7. Fill all the wells with 250μL of washing buffer.
8. Repeat step 5 and 6 two more times for a total of 3 washes.
9. Add two drops (100μL) of substance/chromogen to each well (white cap). Mix
    thoroughly and incubate for 5 minutes (+/- one minute) at room temperature, in the dark.
10. Add two drops (100μL of stop solution to each well (orange cap). Mix well.
    A. Plates must be read within 10 minutes of adding the stop solution

**Reading Plates:**

a. Utilizing a spectrometer, equipment with a 450nm filter, read the plate.
   i. The blank should be read against air.

**Interpretation of Results:**
1. The absorption value for the sample is compared with the absorption values obtained
   for the cut off.
   a. Negative results are obtained if absorption values for the sample are below absorption
      value for the cutoff.
   b. Positive result are achieved if the absorption value for the sample is higher than or
      equal to the cut off value.
   c. If r=0.98 (standard curve), the result of that run are invalid.
      i. Analysis must be re-run, standard at the sample tubes.
      ii. Verify control are the proper order in the wells as described in “sample
          Preparation 2. above.

**References:**
RIDASCREEN® Risk Material 10/5
R-Biopharm Inc. 7950 Old US 27 South, Marshall, MI 49068

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