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Detection of Central Nervous System Tissue on Meat and Carcass-Splitting Band Saw Blade Surfaces Using Modified Fluorescent Glial Fibrillary Acidic Protein Enzyme-Linked Immunosorbent Assay Sampling and Extraction Procedures

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ABSTRACT

This study was conducted to determine optimal buffer pH, extraction procedure, and temperature for detecting central nervous system (CNS) tissue on meat surfaces and on carcass-splitting band saw blades using swab sampling. Glial fibrillary acidic protein (GFAP) is restricted to CNS tissue and has been used as a marker for CNS tissue presence in meat products. Sample preparation, extraction procedure, and extraction temperature of glial fibrillary acidic protein fluorescent enzyme-linked immunosorbent assay (GFAP F-ELISA) were modified to detect CNS tissue on meat surfaces and on carcass-splitting band saw blades. Maximum GFAP recovery was observed with an extraction buffer pH of 7.4. Extracting samples at room temperature by vortexing for 30 s in 1 ml of extraction buffer (phosphate-buffered saline [pH 7.4] plus 0.05% sodium dodecyl sulfate) consistently provided detection of GFAP on meat surfaces contaminated with 500 μg of spinal cord suspension per 50 cm^2 and on carcass-splitting band saw blades contaminated with 20 μg of spinal cord suspension per 50 cm^2. Recovery of GFAP was not affected by storing samples overnight at 4°C. The current studies demonstrate the effectiveness of modified sampling procedures and preparations, sample extraction buffer pH, and extraction temperatures. These modifications introduced to the original F-ELISA sampling protocol result in a sensitive and repeatable assay for detection of CNS tissue on meat surfaces and on carcass-splitting band saw blades.

On 23 December 2003, the first case in the United States of bovine spongiform encephalopathy (BSE) was confirmed in a Washington state dairy cow *(12).* Current evidence suggests that BSE may cause variant Creutzfeldt-Jakob diseases (vCJD) in humans through consumption of central nervous system (CNS) tissue of cattle affected with BSE *(7).* BSE and vCJD are caused by an abnormal prion protein *(14).* Therefore, contamination of meat products with CNS tissue containing abnormal prion protein is a food safety concern *(16).* Inadvertent CNS tissue contamination of beef muscle products may result from the stunning of livestock *(2, 16, 18, 21),* from carcass contamination during processing *(5, 13),* or from the preparation of advanced meat recovery products from the vertebral column *(9, 20).* Schmidt et al. *(16)* reported that a fluorometric enzyme-linked immunosorbent assay (F-ELISA) that assays for the presence of glial fibrillary acidic protein (GFAP) may be used to detect CNS tissue in meat products. GFAP is highly restricted to the CNS *(3).*

Prendergast et al. *(13)* and Schmidt et al. *(16)* reported CNS tissue dissemination within animal carcasses from brain and spinal cord (SC) of cattle to meat and meat contact surfaces after captive bolt stunning and during carcass splitting. Prendergast et al. *(13)* confirmed that the carcass-splitting saw poses particular risk of cross-contamination of carcasses, equipment, surfaces, and employees by SC. Helps et al. *(5)* detected about 23 to 70 g of tissue debris remaining on the carcass-splitting saw with CNS tissue concentration ranging from 0.14 to 0.36 mg/g measured by F-ELISA *(5).* If an animal infected with BSE enters the slaughter line, tissue debris accumulated in carcass-splitting equipment may contaminate portions of the carcasses *(5).*

Agazzi et al. *(1)* reported the detection of 0.5% bovine brain using Ridascreen ELISA. Helps et al. *(6)* reported detection of GFAP on lateral and medial surface of each half of the split carcasses by synthetic sponges (100 by 100 by 10 mm^3). Swab samples were then transferred to a 150-ml container containing 20 ml phosphate-buffered saline (PBS) plus 0.5% Triton X-100 and were later used for ELISA assays. Hajmeer et al. *(4)* reported the detection of 0.025% SC tissue using commercial Ridascreen ELISA by swab sampling. Samples in this study were prepared by sterile Dacron fiber-tipped swabs. Ridascreen ELISA was not repeatable and sensitivity was 0.3% SC only, compared to 0.05% SC using F-ELISA. In addition, interassay CV for Ridascreen ELISA ranged from 15 to 26% as compared to 9.7 to 20% for F-ELISA *(8).*

Samples used in F-ELISA assay are homogenized in 10 volumes of 1% sodium dodecyl sulfate (SDS) at 85 to 90°C *(17).* This sampling method is used for ground beef or advance meat recovery samples. GFAP from samples

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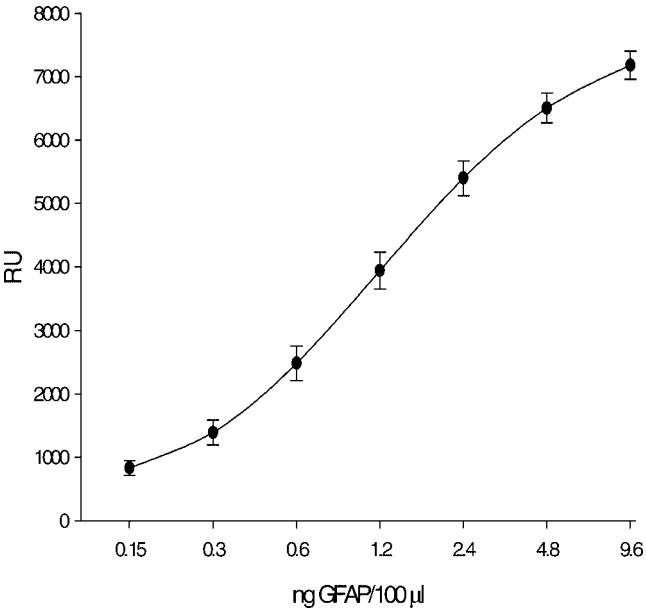

FIGURE 1. *F-ELISA GFAP standard curves developed from data collected on seven different days (*n = 7*). Each point is the mean of duplicate determination.*

collected on meat surfaces and on carcass-splitting band saw surfaces using swabs could not be effectively recovered using this procedure. The objective of this study was to develop a simple extraction procedure to detect CNS tissue on meat surfaces and on carcass-splitting band saw blade samples.

MATERIALS AND METHODS

Preparation of standards for ELISA. Serial dilutions of bovine GFAP standards (American Research Products, Boston, Mass.) were prepared in PBS (Pierce Chemical Co., Rockford, Ill.) containing 0.5% Triton X-100 (Bio-Rad, Hercules, Calif.). Final concentrations of each standard solution were 0.15, 0.30, 0.60, 1.2, 2.4, 4.8, and 9.6 ng/100 μl. To each ELISA plate (Immulon-2, Dynex Technologies, Chantilly, Va.), a blank (PBS plus 0.5% Triton X-100) and each of the seven standards were added to the first 16 wells of the plate in duplicate. All samples were analyzed in duplicate.

Fluorescent GFAP ELISA reagents and assay procedure. The microplate was coated with polyclonal rabbit anti-GFAP (Dako Corporation, Carpinteria, Calif.), incubated at 37°C for 1 h, washed, and then blocked with 5% nonfat dry milk. After addition of standards and samples, the microplate was incubated for 1 h at room temperature, washed, and incubated for 1 h at room temperature with anti-porcine GFAP (Chemicon International, Inc., Temecula, Calif.). The microplate was incubated with peroxidase-labeled goat anti-mouse immunoglobulin G for 45 min and then with Quantablu Fluorogenic peroxidase for 30 min, and the resulting fluorescence was read at 460 nm (360 nm excitation) using a Bio-Tek FLX800B plate reader (Biotek Instruments, Winooski, Vt.) *(17).*

SC suspension preparation. Bovine SC samples ($n = 7$) collected from a commercial cattle packing plant were thoroughly minced. The minced SC tissue was diluted 1:10 (10 g of SC tissue plus 90 ml of distilled water). Diluted samples were homogenized (Virtis Co., Inc., Gardiner, N.Y.) for 40 s (2 × 20-s bursts) and stored at −20°C in polypropylene tubes measuring 12 by 75 mm. This SC suspension was used throughout the study.

TABLE 1. *Mean and SD of GFAP extracted and recovered with 1% SDS or phosphate-buffered saline (PBS; pH 7.4) at different extraction temperatures*

Treatment	Temp	GFAP (ng/well)		GFAP (ng/mg [wet weight])	Mean GFAP recovery compared to 1% SDS at 95°C
		Mean	SD		
1% SDS	95°C	3.20	0.27	6,391	100
PBS, pH 7.4	RT[a]	2.08	0.33	4,155	65
	37°C	2.39	0.32	4,774	75
	50°C	2.37	0.54	4,741	74
	95°C	2.54	0.31	5,089	80

[a] RT, room temperature (22 to 25°C).

GFAP extraction with 1% SDS. Schmidt et al. *(16, 17)* reported that 0.1% SC tissue could be detected by homogenizing samples with 1% SDS at 85 to 90°C and that SDS should not exceed 0.08% in the GFAP ELISA. To avoid SDS interference in GFAP ELISA, samples homogenized in 1% SDS were diluted at least 12-fold prior to GFAP ELISA. SC dilutions demonstrated dose response parallel to the GFAP standard curve at very low tissue concentrations *(16).* Hence, we compared GFAP detection levels in each experiment to GFAP levels extracted with 1% SDS.

Study 1: modifications of GFAP extraction procedures. Several sampling protocols and extraction procedures were tested using different buffers at different pH values and temperatures to detect CNS tissue on the surface of meat and on carcass-splitting band saw blades using a swab sampling.

Homogenized SC suspension was diluted to 1 mg (wet weight) per ml in PBS (pH 6.0 or 7.4) and in 50 mM phosphate buffer (PB; pH 7.4 or 8.0). To all buffers, 0.05% SDS (Sigma Chemical Co., St. Louis, Mo.) was added after determining the pH. Two replicates of SC suspension were extracted at four different temperatures (room temperature [22 to 25°C], 37, 50, and 95°C) by vortexing for 30 s. SC suspensions were further diluted in PBS (pH 7.4) plus 0.5% Triton X-100 and stored overnight at 4°C. One hundred microliters of these overnight samples was assayed using the F-ELISA procedure described above. Immediately after extraction, all samples were placed on ice.

Five different SC suspensions (25, 50, 100, 500, and 1,000 μg [wet weight] per ml) were prepared in PBS (pH 7.4) and extracted at 50°C by vortexing for 30 s in PBS (pH 7.4) plus 0.05% SDS. After extraction, SC suspensions were further diluted in PBS (pH 7.4) plus 0.5% Triton X-100 and stored overnight at 4°C. One hundred microliters of these samples was used, in duplicate, in the F-ELISA assay to develop a dose-response curve for GFAP detection.

Study 2, part A: development of a swab-sampling protocol for meat and carcass-splitting band saw blade surfaces. Three different SC suspensions (500, 1,000, and 2,000 μg [wet weight] per ml) were prepared in PBS (pH 7.4). One milliliter of each SC suspension was spread onto surfaces of beef steaks (10 by 5 cm) with a siliconized sigmacote glass rod (Sigma) and left at room temperature for 20 min. Meat surfaces were then swabbed with either dry ($n = 12$) or premoistened ($n = 4$) Dacron swabs (Fisher Scientific, Houston, Tex.). Swab samples were collected using 10 horizontal motions and 20 vertical motions (back and

TABLE 2. *Mean and SD of GFAP recovered from meat surfaces contaminated with 500, 1,000, and 2,000 μg of SC per 50 cm² utilizing moistened (with distilled water) Dacron-tipped swabs incubated to 50°C (moist, inc.), dry Dacron-tipped swabs incubated to 50°C (dry, inc.), dry Dacron-tipped swabs extracted in preincubated (50°C) extraction buffer (dry, pre-inc.), or dry Dacron-tipped swabs extracted in room temperature extraction buffer (dry, RT)*

	GFAP (ng/well)					
	500 μg of SC/50 cm²		1,000 μg of SC/50 cm²		2,000 μg of SC/50 cm²	
Treatment	Mean	SD	Mean	SD	Mean	SD
Dacron swab (moist, inc.)	0.34	0.12	0.76	0.23	2.12	1.14
Dacron swab (dry, inc.)	0.48	0.18	0.87	0.18	1.53	0.53
Dacron swab (dry, pre-inc.)	0.39	0.13	0.80	0.36	1.81	0.79
Dacron swab (dry, RT)	0.43	0.16	1.01	0.34	2.43	1.03

forth counting as 2 motions) with gentle fingertip pressure being applied to the swab. Following sampling, moistened swabs were vigorously dipped several times and squeezed against the wall of polypropylene tubes (12 by 75 mm) containing 1 ml of extraction and incubated until the extraction buffer reached 50°C (135 s), vortexed for 30 s, 5 μl of Triton X-100 was added, and samples were vortexed again for 5 s (moist, incubated). One hundred microliters of the extraction was used, in duplicate, in the F-ELISA procedure. Dry swabs were extracted using one of three extraction procedures ($n = 4$). In the first extraction procedure, swabs were vigorously dipped several times and squeezed against the wall of polypropylene tubes containing 1 ml of extraction and incubated until the extraction buffer reached 50°C (135 s), vortexed for 30 s, 5 μl of Triton X-100 was added and samples were vortexed again for 5 s (dry, incubated). In the second extraction procedure, swabs were vigorously dipped several times and squeezed against the wall of polypropylene tubes containing 1 ml of preincubated (50°C) extraction buffer, vortexed for 30 s, 5 μl of Triton X-100 was added and samples were vortexed again for 5 s (dry, preincubated). Finally, in the third procedure, swabs were vigorously dipped several times and squeezed against the wall of polypropylene tubes containing 1 ml of room temperature extraction buffer, vortexed for 30 s, 5 μl of Triton X-100 was added and samples were vortexed again for 5 s (dry, room temperature). One hundred microliters of each extraction was used, in duplicate, in the F-ELISA procedure.

Study 2, part B. One milliliter of a 1,000-μg (wet weight)-per-ml SC suspension was spread onto surfaces of beef steaks (10 by 5 cm) with a siliconized sigmacote glass rod (Sigma) and left at room temperature for 20 min. Meat surfaces were then swabbed as previously described with either dry ($n = 3$) or premoistened ($n = 3$) cotton swabs (Unilever, Greenwich, Conn.). Following sampling, swabs were vigorously dipped several times and squeezed against the wall of polypropylene tubes (12 by 75 mm) containing 1 ml of extraction buffer at room temperature, vortexed for 30 s, 5 μl of Triton X-100 was added, and samples were vortexed again for 5 s. One hundred microliters of each extraction was used, in duplicate, in the F-ELISA procedure.

TABLE 3. *Mean and SD of GFAP recovered from meat surfaces contaminated with 1,000 μg of SC per 50 cm² utilizing either moistened (with distilled water) or dry cotton-tipped swabs extracted in room temperature extraction buffer*

	GFAP (ng/well)	
Treatment	Mean	SD
Dry swab	0.45	0.11
Moist swab	0.39	0.14

Study 2, part C. Two different SC suspensions (20 and 50 μg [wet weight] per 500 μl), prepared in PBS (pH 7.4), were spread on 50 cm² (1.8 by 35 cm) carcass-splitting band saw blades ($n = 2$) with a siliconized glass rod and allowed to stand at room temperature for 20 min. After 20 min, SC suspension on band saw blade surfaces were swabbed as previously described with Dacron swabs. Subsequently, sampling swabs were vigorously dipped several times and squeezed against the wall of polypropylene tubes (12 by 75 mm) containing 1 ml of extraction buffer at room temperature, vortexed for 30 s, 5 μl of Triton X-100 was added, and samples were vortexed again for 5 s. One hundred microliters of each extraction was used, in duplicate, in the F-ELISA procedure.

Study 3: effect of storage conditions on GFAP detection. Two different SC suspensions (100 and 200 μg [wet weight] per ml) were prepared in PBS (pH 7.4). Fifty microliters of each SC suspension ($n = 2$) was added directly to a Puritan cotton swab (WASSCO, Poway, Calif.). Swabs were then either immediately assayed or stored overnight dry at 4°C in polypropylene tubes (12 by 75 mm) or stored overnight at 4°C with 1 ml extraction buffer. After extraction, Triton X-100 (5 μl) was added to all samples, and vortexed for 5 s. One hundred microliters of these samples was used in F-ELISA assay.

Statistical analyses. Data from all studies was analyzed using the general linear models procedures of SAS *(15).* Two-sample Student's *t* tests were performed for calculating the difference between treatment means. The α level was set at 0.05 throughout the study.

RESULTS AND DISCUSSION

The sensitivity of the ELISA assays was defined as the lowest value detected that was different from zero. This was calculated as the mean relative fluorescent units ± 2 standard deviations *(10).* Although it is possible to detect very low concentrations that differ from zero, the imprecision of such measurements will be so high that they are of little practical value *(11).* So for reporting results useful in practical conditions, Spencer et al. *(19)* reported functional sensitivity, the lowest concentration at which an assay can report practically useful results, e.g., the concentration at which the interassay coefficient of variation is ≤20%. Based on those observations, the sensitivity of F-ELISA was determined as 0.3 ng of GFAP per well (Fig. 1). The

TABLE 4. *Mean and SD of GFAP detected on carcass-splitting band saw blade surfaces contaminated with 20 or 50 μg of SC per 50 cm²*

GFAP (ng/well)			
20 μg of SC/50 cm²		50 μg of SC/50 cm²	
Mean	SD	Mean	SD
3.22	0.42	4.01	0.55

interassay coefficient of variation for seven GFAP standard curves ranged from 3 to 13% for all standards.

Study 1: modifications of GFAP extraction procedures. Mean GFAP recovery (%) from SC suspension using different buffers (PBS or PB) at different pH values (6.0, 7.4, and 8.0) at different temperatures (room temperature, 37, 50, and 95°C) ranged from 54 to 85% compared to 1% SDS at 95°C. Maximum GFAP recovery was observed at pH 7.4 (data not shown). Mean GFAP recovery using extraction buffer PBS (pH 7.4) plus 0.05% SDS was 65 to 80% compared to 1% SDS at 95°C (Table 1). Based on the results from this experiment, PBS (pH 7.4) plus 0.05% SDS was used as extraction buffer for further studies as there was no difference ($P > 0.05$) in GFAP recovery compared to other buffers at similar temperature. Also PBS (pH 7.4) buffer was easier to prepare and adjust compared to the other extraction buffers. Mean GFAP recovery with PBS (pH 7.4) at 95°C was 20% lower compared to GFAP recovered using 1% SDS at 95°C ($P < 0.05$). Mean GFAP recovery from SC suspensions at room temperature and 50°C with PBS (pH 7.4) compared to mean GFAP recovery with 1% SDS at 95°C was 35 and 26% lower, respectively ($P < 0.05$; Table 1).

Study 2, part A: development of a swab sampling protocol for meat and carcass-splitting band saw blade surfaces. No differences ($P > .05$) in GFAP recovery were observed between dry or moistened Dacron swab samples (Table 2). Dry swabs extracted at room temperature had numerically higher mean GFAP recovery compared to other samples collected from surfaces contaminated with either 1,000 or 2,000 μg of SC per 50 cm².

Study 2, part B. Recovery of GFAP from meat surfaces contaminated with 1,000 μg SC per 50 cm² using a cotton-tipped swab was approximately half of that obtained from Dacron swab samples (Table 3). All swab methods tested in this study were able to detect GFAP, however, samples collected using dry Dacron swabs and extracted at room temperature were the simple and sensitive method.

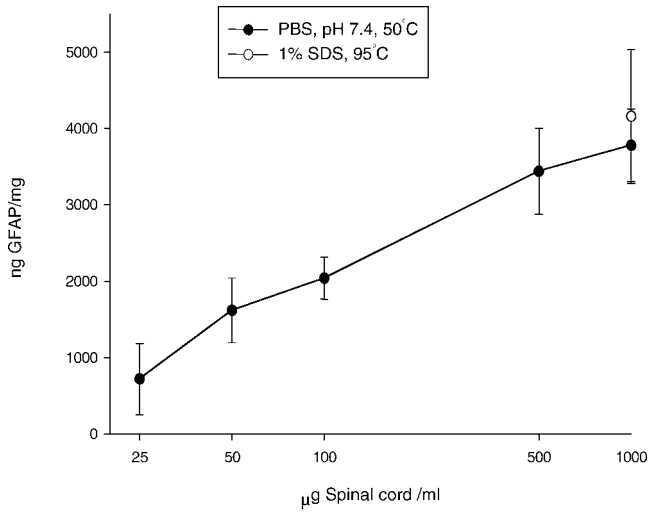

FIGURE 2. *F-ELISA GFAP dose response curve using PBS (pH 7.4) plus 0.05% SDS as the extraction buffer.*

Study 2, part C. CNS tissue detection on carcass-splitting band saw blade surfaces was also examined using 20 and 50 μg of SC suspension spread over a 50-cm² surface area of the carcass-splitting band saw blade. As shown in Table 4, GFAP was detectable at both blade contamination levels. Recovery of GFAP increased with increasing amounts of SC suspension and showed good linear response with increasing amounts of SC suspension (Fig. 2).

Triton X-100 was an essential component for dilution buffer as GFAP recovery was very low when Triton X-100 was not present in the assay buffer (data not shown). These results demonstrate an increased sensitivity of the swab sampling method when 5 μl of 100% Triton X-100 is added to the extraction buffer and vortexed for 5 s. This sampling, extraction, and dilution process will detect CNS tissue levels as low as 500 μg of SC per 50 cm² on meat surfaces and 20 μg of SC per 50 cm² on carcass-splitting band saw blades.

Study 3: effect of storage conditions on GFAP detection. To examine the effect of storage time on GFAP

TABLE 5. *Mean and SD of GFAP detected from spinal cord (SC) suspensions (5 and 10 μg of SC [wet weight] per 50 μl) added directly to cotton swab*[a]

	GFAP (ng/well)			
	5 μg of SC		10 μg of SC	
Treatment	Mean	SD	Mean	SD
Swab stored overnight	2.85	0.40	4.44	0.06
Swab stored overnight in 1 ml of extraction buffer	2.83	0.07	3.70	0.12
Swab assayed immediately after extraction	3.33	0.22	3.79	0.21

[a] Swabs were stored overnight prior to extraction dry, in 1 ml of extraction buffer (PBS [pH 7.4] plus 0.05% SDS), or immediately extracted in 1 ml of extraction buffer (PBS [pH 7.4] plus 0.05% SDS) and assayed.

detection, 5 and 10 μg of SC in suspension were added directly to swabs in duplicate. No significant differences ($P > 0.05$) in GFAP recovery were observed with any of the storage methods evaluated (samples stored dry overnight at 4°C, samples stored with 1 ml extraction buffer overnight at 4°C, or samples assayed immediately). As GFAP recovery was not affected by storing dry swab samples overnight at 4°C, swab samples can be shipped or stored overnight without affecting GFAP detection or recovery (Table 5).

Helps et al. *(5)* reported that current band saw and washing regimes are inadequate for preventing tissue debris buildup in the splitting saw. Existing methods for detection of GFAP on meat surfaces and on carcass-splitting band saw surfaces are not sensitive and repeatable. Modifications introduced to the original F-ELISA can be used to detect CNS tissue on meat surfaces and on carcass-splitting band saw blades, to monitor the efficacy of washing regimes, and to evaluate the efficacy of carcass splitting by different methods.

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