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Meat Science 65 (2003) 757–763

MEAT SCIENCE

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Spinal cord tissue detection in comminuted beef: comparison of two immunological methods

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Received 31 July 2002; received in revised form 7 October 2002; accepted 7 October 2002

Abstract

Two commercial immunological kits for detection of central nervous system (CNS) tissue in beef were compared: ScheBo® Brai*no*stic™, based on CNS-specific antigen (neuron specific enolase) detection, and Ridascreen® Risk Material 10/5 test, an enzyme immunoassay for glial fibrillary acidic protein. Spinal cord (SC) was added to batches of choice, select, and utility grades of ground fresh beef shoulder clod to yield 0.0, 0.0125, 0.025, 0.05, 0.1, 0.2, 0.4, 0.8, and 1.6% SC in meat. Sensitivity and specificity in detecting SC in fresh and frozen samples were determined. Both Brai*no*stic™ and Ridascreen® kits detected SC at claimed levels: 0.25% and 0.11%, respectively. The Ridascreen® test consistently detected SC at 0.025%, below its claimed sensitivity level, expressed for brain and SC combined. The Ridascreen® test was ~10× more sensitive, easier, faster to run and less expensive than the Brai*no*stic™. Overall, quality grade had no influence on SC detection in fresh or frozen meat.

Keywords: Beef; Brai*no*stic™ test; Detection of neural tissue; Ridascreen® test; Spinal cord

1. Introduction

Contamination of beef with central nervous system tissue (CNST) has raised food safety concerns because of potential transmission of bovine spongiform encephalopathy (BSE) to humans. Introducing CNST (i.e. brain and spinal cord materials) to the edible portion of the carcass is suspected of increasing the risk of human infection resulting in new variant Creutzfeldt-Jakob disease (vCJD). Contamination of edible meat with CNST may result from the method of stunning employed (Bauer, Garland, & Edwards, 1996; Garland, Bauer, & Bailer, 1996; Schmidt, Hossner, Yemm, & Gould, 1999; Schmidt, Hossner, Yemm, Gould, & O'Callaghan, 1999) or during meat preparation and fabrication processes. Stunning may result in the transport of brain tissue via the blood stream to the edible part of a carcass, and processing practices such as advanced meat recovery (AMR) systems might introduce CNST to the product (BSEEB, 1999; USDA, 1998). In an effort to prevent CNST from being introduced into the human food chain, the European Commission (EC) in 1997 endorsed a proposal to regulate the use of specified risk materials (SRM) such as brain and spinal cord presenting a BSE hazard, and decided that SRM must not to be used in food effective 1 October, 2000 (MAFF, 2001).

In 1995, the United States Department of Agriculture's (USDA) definition of meat was amended to include products prepared via AMR systems (USDA, 2002). Under that directive, meat derived via AMR is considered comparable in texture and composition to meat trimmings and manually deboned products, and no special labeling is required. Additionally, products produced using such systems cannot contain spinal cord (SC) tissue because it falls outside the definition of meat. Recently, USDA issued a revision to their earlier directive under which inspectors at establishments using AMR systems will be instructed to take routine regulatory samples to verify that spinal cord is not present in AMR products (USDA, 2002). This is intended to prevent misbranding of meat products under the Food Safety and Inspection Service regulations.

To assure that meat and meat products are not being adulterated with SC or other SRM, effective methods

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0309-1740/03/$ - see front matter © 2003 Elsevier Science Ltd. All rights reserved.
doi:10.1016/S0309-1740(02)00278-4

are needed for the detection of CNST in those products. This project aimed to compare two commercial methods that are currently in use, particularly in Europe, for the detection of CNST in meat and meat products. The first method, using the ScheBo® Brai*no*stic™ kit (ScheBo® Biotech, Giessen, Germany), is based on immunological detection of CNS-specific antigen (neuron specific enolase; NSE) using a monoclonal antibody by a Western blotting procedure (ScheBo® Biotech). The second method, using the Ridascreen® risk material kit (R-biopharm AG, Darmstadt, Germany)[1] is an enzyme immunoassay for the semi-quantitative analysis of risk material (CNS; brain and spinal cord) in processed meat and meat products. In this method, detection of the CNS material is based on an assay for the glial fibrillary acidic protein (GFAP), a cellular marker restricted to CNS (R-biopharm, Inc.).

Each method was evaluated individually for its effectiveness and sensitivity for the recovery of CNST before the kits were introduced on the market (Lücker et al., 1999; Schmidt, Hossner, Yemm, Gould, & O'Callaghan, 1999). However, no direct comparison of the two kits is available, and the publications do not exactly describe the methods as eventually performed using the commercial kits. In this study, the methods were evaluated and compared under similar conditions. This will provide researchers, food industry, and regulatory authorities that are testing products for SRM with better information regarding the strengths and weaknesses of each kit or procedure.

2. Materials and methods

2.1. Meat selection and preparation

Fresh non-cured beef shoulder clod (trimmed; North American Meat Processors Association, #114C), was obtained from a local commercial establishment and transported to the Food Safety Laboratory, University of California Davis (UCD). Three quality grades of beef (choice, select, and utility) were individually purchased and used during this study. Fresh spinal cord was obtained from the UCD Meat Laboratory after slaughter of animals at that facility. Only spinal cord (SC) was used as the CNST in this project; one spinal cord was used per experimental run.

Three different quality grades of beef were evaluated to account for marbling. Marbling is a term denoting fat interspersed with the lean meat, whereby it cannot be removed by trimming, as can external fat. In this study, choice meat represents that with the most marbling, followed by select and then utility. Marbling is normally of interest because it enhances flavor, juiciness and tenderness of the meat; however, in the context of this project we aimed to determine whether grade level, and its inherent fat or marbling, had an effect on the sensitivity of the test. Lücker et al. (1999) suggested that reduced fat content in the system tested increased sensitivity of the test.

Upon receipt, the meat was ground through a 3/8 then 3/16-inch (0.95 and 0.48 cm, respectively) plate using a Kitchen Aid® grinder (Kitchen Aid Portable Appliances, St. Joseph, MI), and portioned into 1-kg units in preparation for mixing with the SC. The SC was homogenized and minced using a Toastmaster Chopster (Toastmaster Inc., Boonville, MO) and split into two equal portions; one was stored refrigerated (4–5 °C) and the other under frozen conditions (−20 °C). Pre-determined amounts of SC (refrigerated and frozen) were individually added to the meat, mixed using a Kitchen Aid® mixer, and homogenized using a Cuisinart™ food processor (Cuisinart®, East Windsor, NJ) to yield final concentrations of 0, 0.0125, 0.025, 0.05, 0.1, 0.2, 0.4, 0.8, and 1.6% (w/w) SC in the meat. Samples then were removed from each batch and tested for SC material using the ScheBo® Brai*no*stic™ and the Ridascreen® risk material kits per manufacturers' instructions. Five samples were tested per SC concentration per meat grade; each experiment was repeated three times.

2.2. Testing protocols

In each experimental run, tests using the ScheBo Brai*no*stic™ and the Ridascreen® risk material kits were conducted in parallel and on the same day. For both kits and per test, samples were obtained from the same batch of meat.

2.2.1. ScheBo Brainostic™ (Western blot) kit

Samples (0.1 g each) from the 0, 0.1, 0.2, 0.4, 0.8, and 1.6% SC-in-meat batches were individually removed, placed into 2-ml extraction tubes (Q-Biogene; Matrix E, Carlsbad, CA) containing 1 ml extraction buffer, and homogenized using a Q-Biogene Fastprep® homogenizer at 5.5 m/s for 45 s. This was followed by centrifugation of samples at 13,000×*g* for 5 min to allow extraction of the neuron-specific enolase (NSE) protein. Centrifugation separated the sample in the tube into three layers; solid (on the bottom), liquid (in the middle, which contained the extract of interest), and fat (on the top). With a micro-pipette, 200 μl of extract was removed from the middle phase without contamination of the sample by the fat or solid phase. NSE detection in meat was then performed via sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

Samples were prepared for SDS-PAGE by individually removing 15 μl of each extract into a safe-lock tube, and adding 30 μl of sample buffer to the extract to yield

[1] Trade names are used in this paper only to assure clarity of communication.

a total of 45 μl. The negative (standard 1) and positive (standards 2–4) controls were prepared in a similar manner. The solution in each tube was mixed and incubated for 5 min at 95 °C. Ready-to-use molecular-weight standard (20 μl) was included in the set. Following incubation, the tubes were mixed thoroughly and allowed to cool to room temperature (20–25 °C). Then, samples were loaded on pre-cast gels (10% SDS-polyacrylamide) assembled in a Bio-Rad Criterion™ (Bio-Rad Laboratories, Inc., Hercules, CA) chamber containing running buffer. The gel was run for 10 min at 100 V and for 1.5 h at 140 V, electro-transferred to the blotting membrane at 1.25 mA/cm^2 for 60–90 min as described by the manufacturer (Bio-Rad), and immunostained. Following staining, the membrane was washed in a sufficient volume of tap water, dried between paper towels, scanned, and analyzed per manufacturer's instructions.

2.2.2. Ridascreen® Risk Material 10/5 kit

Samples were prepared for this analysis by dipping a Dacron® fiber-tipped, sterile swab (Fisher Scientific, Houston, TX) five times into the sample while rotating the swab. The swab was removed, and squeezed in a test tube containing 1 ml sample buffer. A 100-μl sample was removed from each tube and dropped per assay well. A sufficient number of antibody-coated wells was inserted into the micro-well holder to accommodate the number of samples and all standards tested (four standards, containing 0, 0.11, 0.22, and 0.55% CNST composed of brain and SC, were provided with the Ridascreen® assay). Enzyme conjugate (50 μl) was added to each well containing sample, and the plate was incubated for 10 min at room temperature (20–25 °C). After incubation, the liquid was poured out to empty the wells, and the micro-well holder tapped upside down thoroughly against absorbent paper to completely remove the liquid added earlier from the wells. The wells were washed with 250 μL washing buffer and emptied as described earlier. A 100 μl volume of substrate/chromogen was added to each well, mixed thoroughly, and the plate incubated for 5 min at room temperature in the dark. The reaction was stopped by adding 100 μl stop solution to each well. Color intensity or optical density (absorbance) was determined by photometric evaluation using a microtiter spectrophotometer (SpectraMAX 340, Molecular Devices Corporation, Sunnyvale, CA) with a filter at 450 nm. Results were interpreted as indicated in the Ridascreen® risk material 10/5 kit information booklet.

3. Results and discussion

Both the Brai*no*stic™ and Ridascreen® kits seem to work as described by the manufacturers. Overall, results obtained from the Brai*no*stic™ kit were consistent all throughout the runs. The blots obtained per run showed bands when the NSE protein was positively detected. To avoid excessive illustrations, results of only choice grade ground shoulder clod, contaminated experimentally with SC (stored refrigerated) are presented in Fig. 1. The graph illustrates the concentrations (0, 0.1, 0.2, 0.4, 0.8, and 1.6%) of SC in meat. A summary of the results indicates that the NSE protein was detected at $\geqslant$0.4% SC in meat, and no bands were detectable at $\leqslant$0.2% SC in meat regardless of whether SC used was refrigerated or frozen (Table 1). Additional tests, at the 0.25% SC level that is the reported limit of detection of CNST, were positive via this technique.

Similar trends were observed for frozen choice meat, and for select and utility samples, regardless of prior freezing (Table 1). Quality grade of meat did not influence

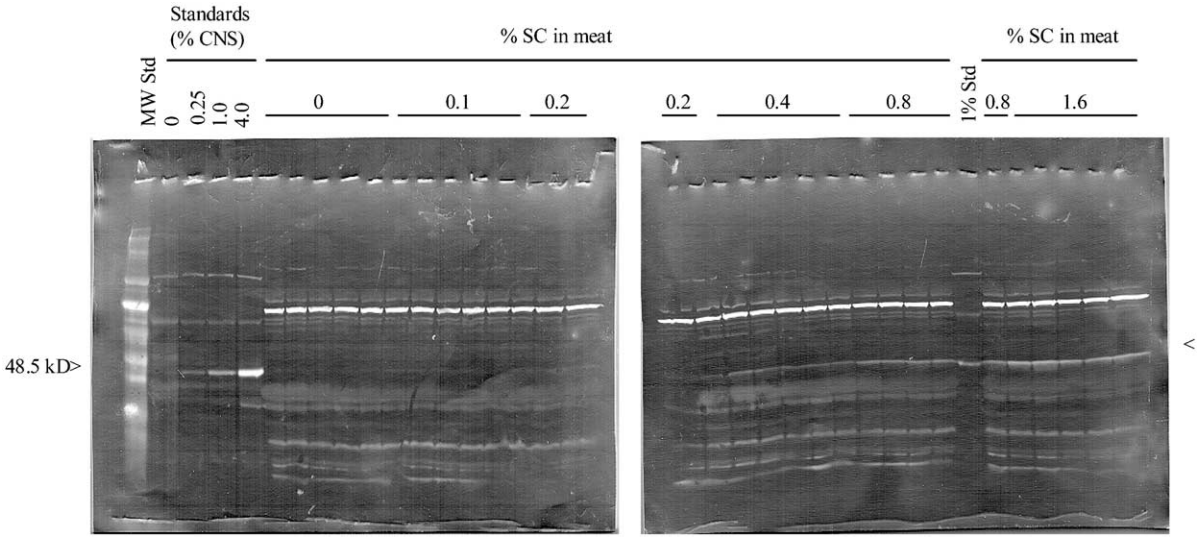

Fig. 1. Fresh choice grade ground beef shoulder clod, illustrating western blot detection of spinal cord (SC).

Table 1
A summary of ScheBo Brai*no*stic™ detection of various percentages of spinal cord added to three grades of fresh and frozen beef

Grade	Preparation	Spinal cord added (%)					
		0	0.1	0.2	0.4	0.8	1.6
Choice	Fresh	0/5[a]	0	0	5	5	5
	Frozen	0	0	0	5	5	5
Select	Fresh	0	0	0	5	5	5
	Frozen	0	0	0	5	5	5
Utility	Fresh	0	0	0	5	5	5
	Frozen	0	0	0	5	5	5

[a] Lanes positive/lanes run; denominator is always 5.

the sensitivity of this test. Thus, the inherent fat level in the lean does not seem to interfere with this assay. Lücker et al. (1999) reported that the inter-muscular or added fat in meat and the cholesterol level in some products, such as brain, contribute to variations in test sensitivity.

Although repeatable results were obtained with this procedure and they were easy to interpret, running the test was time-consuming and labor-intensive, as the protocol included a large number of steps. It took ~11 h to run the test, excluding sample preparation time. This test allows for a procedural break, whereby analysis may be split over 2 days instead of being run continuously on a single day. At the time the experiments were run, the estimated associated cost per test was ~30 US dollars (preparation and labor cost excluded).

When samples were tested using the Ridascreen® ELISA kit, the results almost always fell out of the semi-quantifiable range specified by the manufacturer's information (i.e. except for control or 0% SC) (Figs. 2–4). Therefore, diluted SC concentrations of 0.0125, 0.025 and 0.05% in meat (on a 1:1 basis) were prepared and evaluated. As a consequence of lowering the concentration tested, the optical density values obtained fell within the range of the kit's standard curve

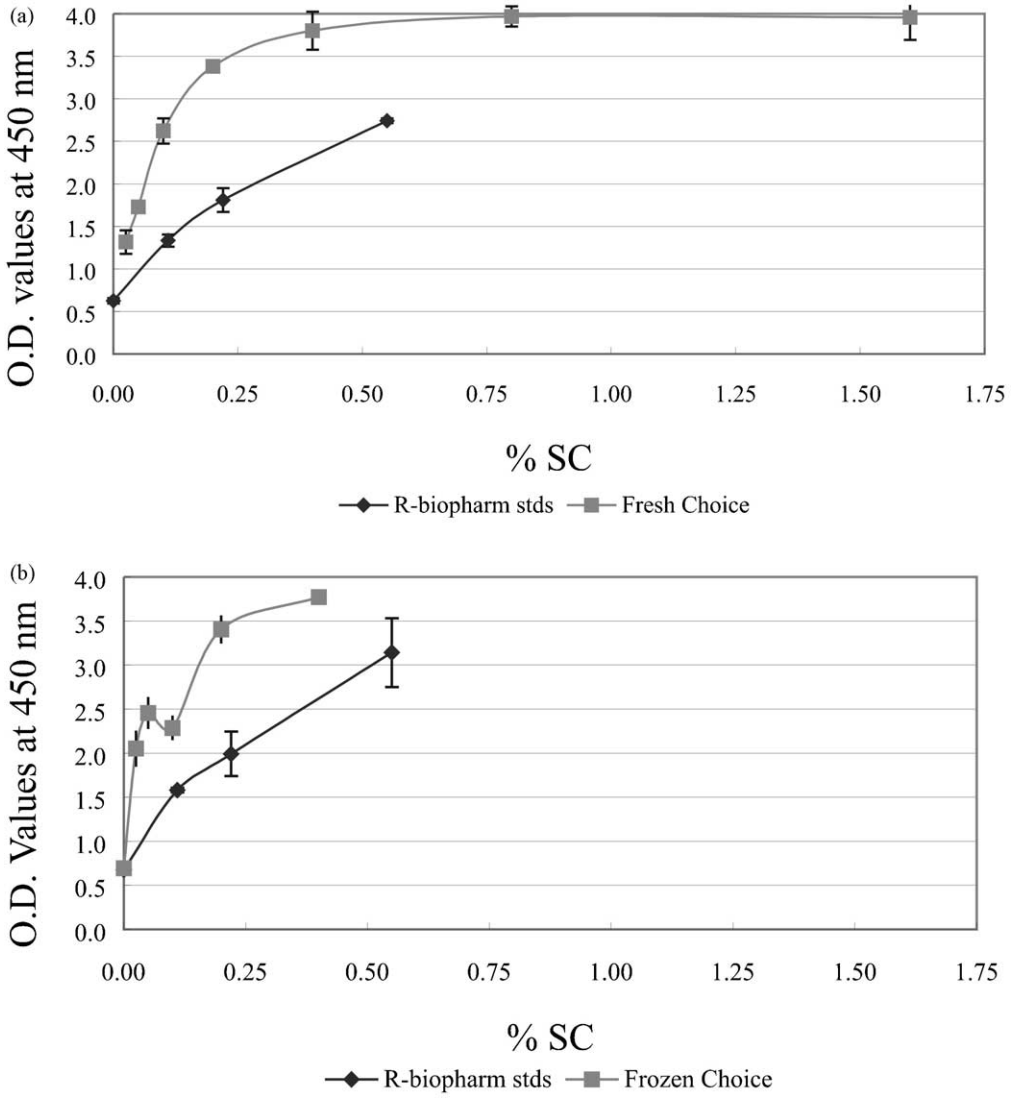

Fig. 2. Choice grade ground beef shoulder clod (a) fresh (b) frozen, illustrating ELISA detection of spinal cord (SC).

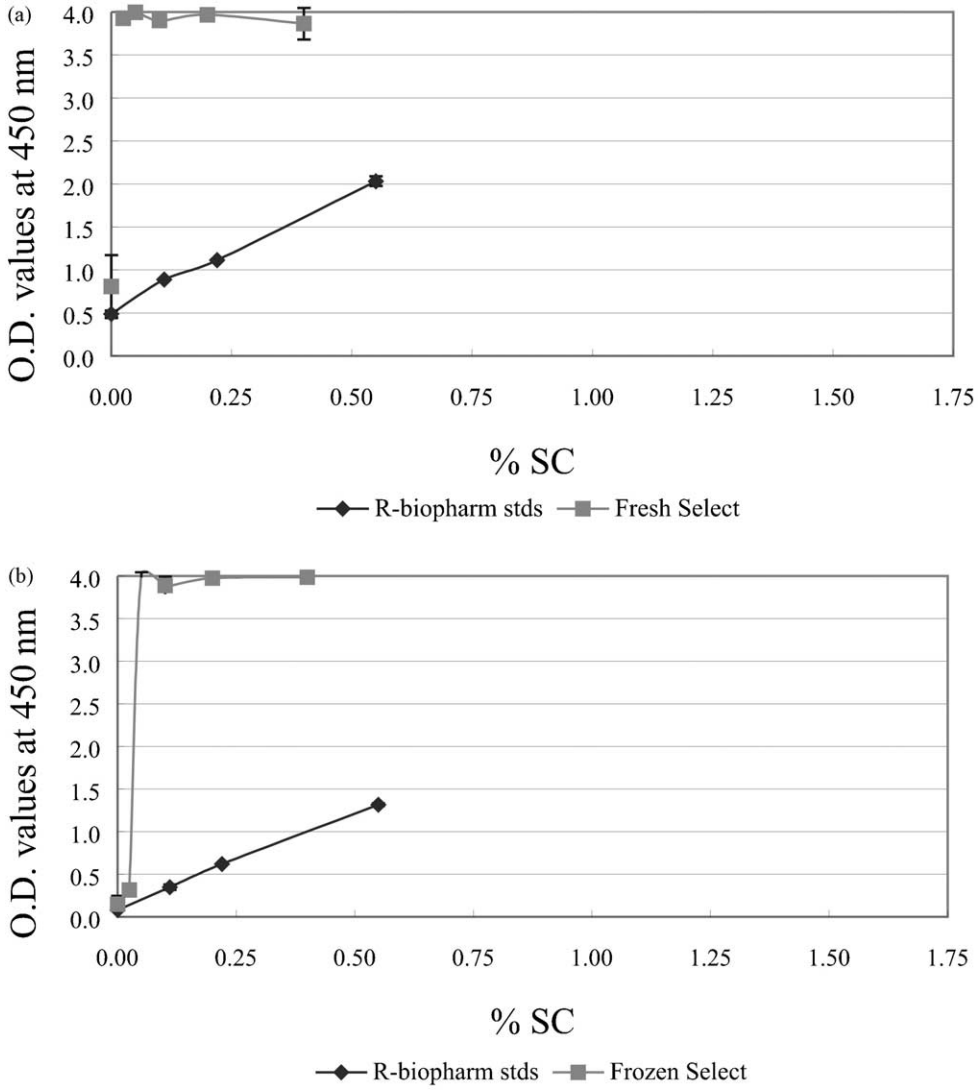

Fig. 3. Select grade ground beef shoulder clod (a) fresh (b) frozen, illustrating ELISA detection of spinal cord (SC).

as shown in Table 2. However, the determined detection limit was at 0.025% for SC, which meant that the kit was ~4.5× more sensitive to SC than what was reported by the manufacturer (i.e. 0.11%). This also indicated that the Ridascreen® kit is ~10× more sensitive to SC than the Schebo Brai*no*stic™ kit (limit of detection 0.25%). Reports indicate that the GFAP is a major part of the glial filaments in astrocytes that are restricted to CNST such as SC (Eng & Lee, 1995), and higher levels of the GFAP are found in SC, 55–220 μg/mg, relative to brain, 9–55 μg/mg (Schmidt, Hossner, Yemm, Gould, & O'Callaghan, 1999). The standards provided with the Ridascreen® assay are based on GFAP concentrations in spinal cord (2000 ng GFAP/mg) and brain (600 ng GFAP/mg) combined. Based on that information, the test used has a sensitivity of 0.03% SC versus 0.1% brain (R-biopharm representative, personal communications, 2001). The SC levels used earlier in the project had much higher concentrations of the GFAP in the samples than what the kit standards could account for, which would explain the relatively high O.D. values obtained.

Overall, quality grade had no effect on the sensitivity of the Ridascreen® ELISA, with the exception of choice samples at lower levels of SC in meat. Results with choice samples differed, at the 5% level of significance, from those with select and utility at 0.1% SC or below, regardless of storage temperature (Figs. 2–4). At 0.2% SC, results with choice samples were also significantly different from those with all other samples, with the exception of the utility-grade samples that had been stored frozen. Differences were also observed between fresh and frozen choice samples at levels of $\leqslant$0.1% SC. This suggests that factors in the system, other than intra-muscular fat, are contributing to the observed variations. Differences associated with quality grades and storage conditions diminished above 0.2% SC in meat. The semi-quantitative nature

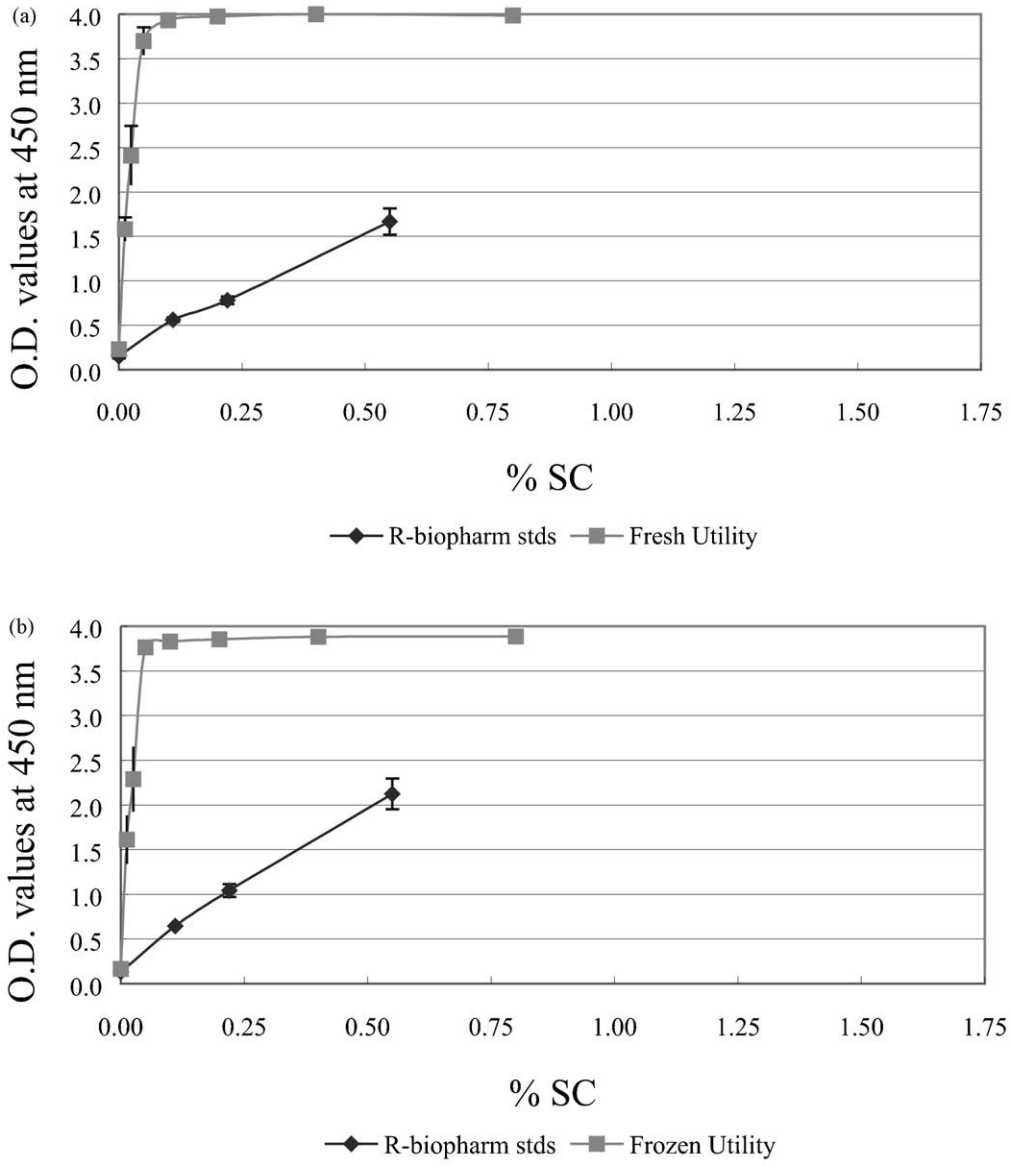

Fig. 4. Utility grade ground beef shoulder clod (a) fresh (b) frozen, illustrating ELISA detection of spinal cord (SC).

of this test may have contributed to these minor problems; the results are clear if they are simply interpreted qualitatively.

Overall, the Ridascreen® kit was attractive because of its ease of use and quick yield of results. As many as 30 samples can be tested in ~2 h (i.e., after meat samples have been mixed with SC) with an estimated associated cost of ~$7 each (excluding labor). However, care needs to be exercised when interpreting the results. If the test results are evaluated *qualitatively* (i.e. positive or contaminated when neural tissue is detected at levels well above the level of the GFAP standards provided in the kit, and negative or not contaminated when neural tissue is not detected), interpretation of results becomes straightforward and simple. This may suffice for many users. In contrast, *semi-quantitative* assessment of the test apparently applies only at optical density (OD) values within the range of those obtained with the standards provided. It is also significant that the standards of the Ridascreen® assay comprise an arbitrary mixture of SC and brain; and since GFAP level is higher in SC than brain, the level of CNST cannot be validly estimated. Based on this, and the results of our study, if a meat sample is contaminated with SC, even at a level as low as 0.1% in meat, the OD value obtained from the test will fall outside the range the standards would account for (Figs. 2–4).

In conclusion, both kits are useful in detecting SC contamination on fresh beef, thus provide objective means to detect neural tissue (specifically SC). One, of course, needs to consider nature of test, associated cost, and speed when selecting a kit to use.

Having such kits commercially available is definitely an advance. Analytical results can be obtained much faster

Table 2
A summary of Ridascreen® detection of various percentages of spinal cord added to three grades of fresh and frozen beef

Grade	Preparation	Standards (% central nervous system tissue)				Spinal cord added (%)								
		0	0.11	0.22	0.55	0	0.0125	0.025	0.05	0.1	0.2	0.4	0.8	1.6
Choice	Fresh	0.626[a]	1.333	1.809	2.742	Err.[c]	NTP[d]	1.316	1.729	2.622	3.383	3.801	3.970	3.957
		(0.031)[b]	(0.073)	(0.139)	(0.029)			(0.225)	(0.119)	(0.263)	(0.163)	(0.033)	(0.027)	(0.026)
	Frozen	0.677	1.579	1.992	3.141	0.963	NTP	2.051	2.455	2.287	3.405	3.772	NTP	NTP
		(0.054)	(0.025)	(0.252)	(0.392)	(0.025)		(0.205)	(0.182)	(0.139)	(0.160)	(0.068)		
Select	Fresh	0.488	0.888	1.114	2.032	0.807	NTP	3.928	3.996	3.900	3.967	3.864	NTP	NTP
		(0.040)	(0.003)	(0.009)	(0.057)	(0.365)		(0.017)	(0.021)	(0.048)	(0.054)	(0.184)		
	Frozen	0.079	0.348	0.619	1.316	0.147	NTP	0.316	4.012	3.889	3.973	3.987	NTP	NTP
		(0.005)	(0.031)	(0.004)	(0.016)	(0.101)		(0.023)	(0.030)	(0.103)	(0.019)	(0.025)		
Utility	Fresh	0.150	0.563	0.781	1.668	0.229	1.580	2.410	3.696	3.930	3.974	3.999	3.986	NTP
		(0.023)	(0.025)	(0.042)	(0.148)	(0.032)	(0.132)	(0.335)	(0.158)	(0.066)	(0.029)	(0.046)	(0.027)	
	Frozen	0.132	0.646	1.043	2.124	0.163	1.611	2.288	3.763	3.828	3.852	3.882	3.887	NTP
		(0.029)	(0.005)	(0.072)	(0.172)	(0.021)	(0.271)	(0.365)	(0.039)	(0.021)	(0.025)	(0.035)	(0.038)	

[a] Mean OD ($n = 5$).
[b] Values of standard deviation in parentheses.
[c] Experimental error.
[d] No test performed.

using these types of tests compared to histopathological examinations, which are cumbersome, costly, and tedious. Also, these analytical procedures can assist meat companies in determining effective removal of SC from beef carcasses, especially companies that need to meet USDA–FSIS regulatory requirements regarding AMR systems (USDA, 1998). Finally, our experience with the kits indicates that both have room for further improvement. Examples include possible automation of the tests to allow for faster sample analysis, and inclusion of user friendly software, compatible to other commercially available ones, for data analysis and interpretation. Improvement on the kits' ability to differentiate the neural tissue among species (e.g. bovine versus porcine) is another area for potential future work.

It should be noted that these kits, in the context of this study, were evaluated on fresh beef only; and no cooked, or other types of products were tested. Scientists (e.g. Tersteeg, Koolmees, & van Knapen, 2002) have reported that heating or certain meat manufacturing processes could cause conformational and chemical changes in the reactive sites of the antigen, whereby the antibody fails to recognize the altered antigen. Others, however, have successfully used certain antibodies (e.g. anti-NSE) in their research studies that included heated meat products (Lücker et al., 1999; Wenisch, Lucker, Eigenbrodt, Leiser, & Bülte, 1999).

Acknowledgements

We thank Carrie Hew, Tinyee Hoang, Dr. Tadassa Mariam, Katlyn Wainwright, and Jake Burns for their technical and laboratory assistance. Also, we acknowledge the help of Dr. Bruno Chomel and Dr. Rick Kasten.

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