Sensitive Monoclonal Antibody-based Sandwich ELISA for the Detection of Porcine Skeletal Muscle in Meat and Feed Products

LIHUA LIU, FUR-CHI CHEN, JODEE L DORSEY, AND YUN-HWA PEGGY HSIEH

ABSTRACT: A monoclonal antibody–based sandwich enzyme-linked immunosorbent assay (ELISA) was developed for the sensitive detection of porcine skeletal muscle in raw and heat-processed meat and feed products. Heat treatment of meat samples up to 132 °C for 2 h did not affect the assay performance. The assay uses a pair of monoclonal antibodies (MAbs 8F10 and 5H9) specific to skeletal muscle troponin I (TnI). MAb 8F10, reacting to mammalian TnI, is the capture antibody and the biotin-conjugated MAb 5H9, specific to porcine TnI, the detection antibody. The sandwich ELISA is able to detect 0.05% (w/w) of laboratory-adulterated pork in chicken, 0.1% (w/w) pork in beef mixtures, 0.05% (w/w) pork meal in soy-based feed, and 1% commercial meat and bone meal (MBM), containing an unknown amount of pork, in soy-based feed. This new assay provides a rapid and reliable means to detect the contamination of meat and feed products with trace amounts of porcine muscle tissue to ensure product quality and safety.

Keywords: ELISA, pork, monoclonal antibody, meat and bone meal, feed

Introduction

An effective method for detecting pork in food or feedstuffs is essential to avoid fraudulent or unintentional adulteration. Many people restrict pork from their diet due to allergies (Asero and others 1997), for religious or humane reasons, or because of other health and food safety concerns because undeclared pork could also contaminate other meats with porcine-borne pathogens. In addition, rendered animal by-products have been recognized as the major route for transmission of the fatal Transmissible Spongiform Encephalopathies from sheep to cattle and the spread of Bovine Spongiform Encephalopathy in cattle, so animal proteins, including pork, have been banned in European countries to feed livestock animals since 2000 (European Commission 2001). As a result, food regulations and feed control both require accurate labeling of the ingredients in products. Effective analytical methods for detecting pork adulteration in agricultural products are crucial for law enforcement, animal health, and consumer protection.

Several methods such as electrophoresis (Kim and Shelef 1986), liquid chromatography (Saeed and others 1989), near-infrared spectroscopy (Ding and Xu 2000), immunoassay (Martin and others 1988; Ayob and others 1989; Sawaya and others 1990; Morales and others 1994; Chen and Hsieh 2000), and polymerase chain reaction (PCR) amplification of specific DNA fragments (Meyer and others 1994; Montiel-Sosa and others 2000, Calvo and others 2001, 2002; Lahiff and others 2001) have been developed to identify pork in meat mixtures, both heat-processed and unprocessed. However, each method suffers from some limitations, including cross-reactions, poor sensitivity, complicated data interpretations, invalidity in severely heat-processed samples, laborious sample preparation, and so on. Although the PCR approach is sensitive and specific, DNA is not tissue-specific and the techniques are thus not able to distinguish between prohibited muscle protein from allowed proteins, such as milk and blood of the same species, when the assay is used for feed control (Moncilovic and Rasooly 2000).

A porcine-specific monoclonal antibody, MAb 5H9, that specifically recognizes a porcine thermal-stable muscle protein, troponin I, has previously been reported (Chen and Hsieh 1998). MAb 5H9 was found to be capable of differentiating pork from other common meat species (beef, horse, lamb, deer, chicken, turkey, and duck) in both raw and cooked products. An indirect enzyme-linked immunosorbent assay (ELISA) using MAb 5H9 was subsequently developed to detect specifically porcine skeletal muscle but not cardiac muscle, smooth muscle, blood and non-muscle organs, and no cross-reactivity was observed with common food proteins tested. However, indirect ELISA is not suitable for routine testing of many samples because of the inconvenient procedures involved in coating unknown sample extracts onto the microplates. The sandwich ELISA method is more sensitive and easier to use than the indirect ELISA and is thus the ELISA system most frequently used in commercial kits. In this study, we paired MAb 5H9 with a mammalian TnI-specific MAb 8F10, to develop a user-friendly and sensitive sandwich ELISA capable of detecting low levels of porcine content in both heat-treated and untreated products.

Materials and Methods

All chemicals used in this study were analytical grade. Glycerol, thimerosal, sodium chloride, sodium phosphate, citric acid, ethylenediaminetetraacetic acid (EDTA), biotinamidocaproate N-hydroxysuccinimide (NHS-CA-biotin), 2,2′-azino-di-3-ethyl-benothiazoline-6-sulfonic acid (ABTS), horseradish peroxidase (HRP) conjugated goat anti-mouse IgG, streptoavidin-HRP conjugate, and 30% hydrogen peroxide were purchased from Sigma Chemical Co. (St. Louis, Mo., U.S.A.). Tween-20, gelatin, bovine serum albumin (BSA), egg albumin, and polyvinylchloride microtiter plates

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(Costar, Cambridge, Mass., U.S.A.) were purchased from Fisher Scientific (Pittsburgh, Pa., U.S.A.). SoyBest containing high bypass soybean protein without animal proteins was obtained from Grain States Soya Inc. (West Point, Nebr., U.S.A.).

Monoclonal antibodies

Production of porcine TnI-specific MAb 5H9 against crude porcine thermal-stable muscle protein has been described previously (Chen and others 1998). MAb 8F10 was produced against purified equine troponin I. The procedures for the mouse monoclonal antibody production and the purification of skeletal muscle TnI from animals of different species are followed according to Chen and others (2002). The specificity of MAb 8F10 is characterized using indirect ELISA (Figure 1a and 1b) using indirect ELISA. MAb 8F10 has a broad specificity of binding skeletal muscle TnI from all mammalian species tested (cattle, pig, deer, sheep, horse, and rabbit) but not other animal and food proteins (poultry, catfish, stomach, heart, intestine, soy isolate, nonfat dry milk, and gelatin). Like MAb 5H9, MAb recognizes the TnI from skeletal muscle including tongue of the particular species. They are selected because of their compatible binding properties and excellent heat-stable epitopes (data not shown). Both MAbs 5H9 and MAb 8F10 belong to the subclass IgG1 and were purified from mouse ascites fluid using a Protein A affinity column on an Econo low-pressure chromatography system (Bio-Rad, Hercules, Calif.) according to the manufacturer's instructions. The biotin conjugated MAb 5H9 was prepared using NHS-CA-biotin following a standard protocol (Harlow and Lane 1988). Concentrations of purified IgG and biotin conjugate in the final preparation were determined by UV spectrophotometer (SmartSpec 3000, Bio-Rad) at 280 nm.

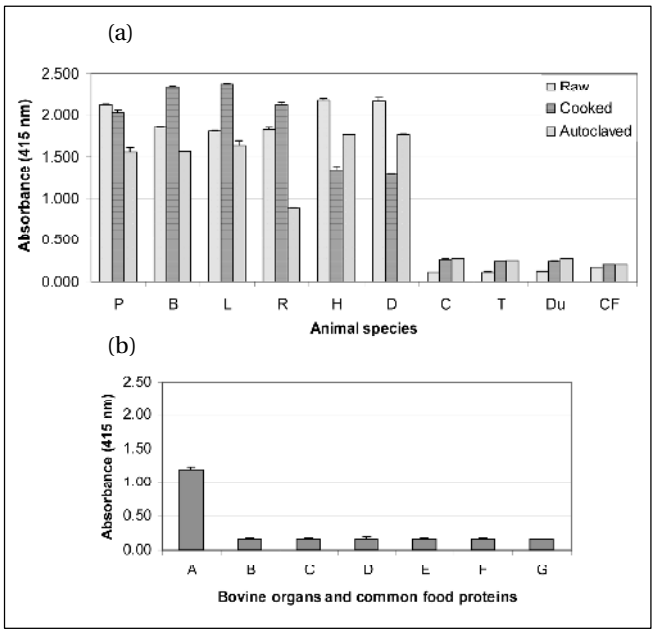

Figure 1—(a) Species specificity of monoclonal antibodies (MAb) 8F10 to raw, cooked and autoclaved meats and fish from different species as determined by indirect enzyme-linked immunosorbent assay (ELISA). B = beef; C = chicken; CF = catfish; D = deer; Du = duck; H = horse; L = lamb; P = pork; R = rabbit; T = turkey. Standard deviation bars are shown (*n* = 3). (b) Cross-reactivity of MAb 8F10 with protein extracts from autoclaved bovine organs and tissues and common food proteins, as determined by indirect ELISA. A = tongue; B = stomach; C = heart; D = intestine; E = soy isolate; F = non-fat dry milk; G = gelatin. Standard deviation bars are shown (*n* = 3).

Table 1—Industrial animal byproducts[a]

Nr 1	Meat meal A
Nr 2	Meat Meal B
Nr 3	Pork MBM
Nr 4	Dairy Blend B
Nr 5	Poultry Meal A
Nr 6	Poultry Meal B
Nr 7	Sheep MBM
Nr 8	Feather Meal

[a]MBM = meat and bone meal.

Meat, dried meat meal, and industrial meat-bone-meal (MBM) samples

Meats from 8 animal species were used to formulate laboratory samples. Fresh pork ham, beef round, lamb leg, whole chicken, turkey, and frozen rabbit meat were purchased from local supermarkets. Horse meat was obtained from the college of Veterinary Medicine, Auburn Univ. (Auburn, Ala., U.S.A.). Deer meat was supplied by the Fats and Proteins Research Foundation (Bloomington, Ill., U.S.A.). Lean muscle samples were prepared by trimming off the connective tissue and visible fat, grinding twice, and then mixing thoroughly. Meats from different species were processed separately. The grinder and utensils were cleaned carefully between samples to prevent cross-contamination.

Laboratory-prepared dry meat meals from various species were prepared according to Chen and others (2002). Industrial animal by-products including poultry meal, feather meal, dairy meal, and meat-bone-meal (MBM) samples were collected from various commercial sources (the experimenters agreed not to disclose the sources) and are listed in Table 1.

Preparation of meat protein extracts

Ten grams of each kind of ground meat was mixed (1:5 wt/vol) with 50 mL of 0.01 *M* sodium phosphate buffer containing 0.5 *M* NaCl (PB-NaCl) for raw meat sample extraction. The mixtures were then blended in a stomacher (Lab-Blender 400, Tekmar Co., Cincinnati, Ohio, U.S.A.) for 1 min. Cooked meat samples were prepared by heating 20 g of ground meat in a beaker covered with aluminum foil in a boiling water bath for 30 min. After cooling to room temperature, 40 mL of PB-NaCl (1:2 wt/vol) was added to each cooked sample. The cooked mixtures were then homogenized with a homogenizer (T 25 Basic S 1, IKA Works Inc., Wilmington, N.C., U.S.A.) at 11000 rpm/min for 2 min. Another set of meat samples was prepared in the same fashion as the cooked samples but autoclaved in a NAPCO 8000-DSE Benchtop antoclave (Jouan, Inc., Winchester, Va., U.S.A.) at 121 °C/1.2 bar for 30 min. After standing for 2 h, all the raw, cooked, and autoclaved sample homogenates were centrifuged (3220 × *g*) at 4 °C for 30 min. The supernatants were filtered through filter paper (Whatman 4, 125-mm dia, Fisher Scientific, Fair Lawn, N.J., U.S.A.) to obtain protein extracts. The protein concentration of each filtrate was determined using a Protein Assay Kit II (Bio-Rad) following the manufacture's instructions with BSA as the standard. All meat protein extracts were aliquoted into 1-mL portions in small tubes and stored at –20 °C until used.

Preparation of laboratory-adulterated samples

To study the sensitivity of the assay, fresh ground pork was mixed in ground chicken or ground beef samples at 7 adulteration levels: 0%, 0.05%, 0.1%, 0.5%, 1%, 2%, and 4% (w/w). The chicken

sample containing no pork (0%) was included as an unadulterated negative control. Each of these samples was then thoroughly mixed and subdivided into 3 portions with which to prepare raw, cooked (100 °C/30 min), and autoclaved (121 °C/30 min) samples for subsequent extraction according to the procedures described previously. Clear extracts were used without further dilution for ELISA assays.

Dry pure pork meal was mixed with a soy-protein based feed sample (SoyBest) to assess the assay performance in a feed matrix. The dry pork meal was mixed with SoyBest feed at 7 adulteration levels: 0%, 0.01%, 0.05%, 0.1%, 0.5%, 1%, and 2%, and then extracted as described previously. Protein extracts of these pork meal adulterated samples were used to perform the porcine sandwich ELISA without further dilution.

Study of heat treatment

To evaluate the assay efficiency for meat samples heat-processed under various conditions, a series of ground lean pork samples (20 g each) were weighed into beakers, covered by aluminum foil, and processed at 1 of 7 conditions: boiled in a water bath (100 °C) for 30 min, or autoclaved at 121 °C for 30 min, 128 °C for 30 min, 132 °C for 30 min, 132 °C for 60 min, 132 °C for 90 min, or 132 °C for 120 min. After the heat treatment, samples were allowed to cool and then extracted with a 2-fold (2 mL/g) of PB-NaCl. Meat extracts of all heated samples, along with a raw sample for comparison, were prepared as previously described. The supernatants were used to perform the sandwich ELISA.

Indirect ELISA

For characterization of the MAb 8F10, polyvinylchloride microtiter plates (Costar, Cambridge, Mass., U.S.A.) were coated with 100 μL of extracts containing 2 μg of various animal or food proteins in carbonate buffer (pH 9.6) and blocked with 1% BSA in phosphate buffered saline (PBS: 0.01 *M* sodium phosphate and 0.15 *M* sodium chloride, pH 7.2).

Undiluted MAb 8F10 supernatant (100 μL) were added to the wells and incubated for 1 h at 37 °C followed by the addition and incubation of goat antimouse IgG peroxidase conjugate (Bio-Rad, 1:3000 diluted in PBS containing 0.05% of Tween 20 [PBS-T] and 1% BSA). Plates were washed 3 times with PBS-T between each step. The bound enzyme activity was revealed by the addition of the enzyme substrate (22 mg of ABTS and 15 μL of 30% hydrogen peroxide in 100 mL of 0.1 *M* phosphate-citrate buffer; pH 4.0). The enzyme reaction was stopped by adding 100 μL of 0.1 *M* citric acid and the absorbance was measured at 415 nm using a microplate reader (Bio-Rad, Model 450).

Sandwich ELISA

MAb 8F10, reacting to mammalian (including pork, beef, sheep, horse, rabbit, and deer) TnI, was selected for coating on the microtiter plate as the capture antibody and the biotin-conjugated porcine-specific MAb 5H9 was selected as the detection antibody. Each well of the microplate was coated with 100 μL of diluted MAb 8F10 (0.5 μg/mL in PBS). After incubation at 37 °C for 1 h and washing 3 times with 0.01 *M* PBS-T using a microplate washer (Bio-Rad, Model 1575), the plate was filled with 200 μL per well of blocking buffer. After a further incubation at 37 °C for 1 h and repeating the washing steps, raw sample extracts diluted with the same volume of assay buffer (1% BSA in PBS containing 0.05% of Tween 20 and 10 m*M* EDTA) or undiluted heated sample extracts were added to the wells (100 μg/well) and incubated at 37 °C for 1 h. One hundred microliters of biotin-conjugated MAb 5H9 (0.1 μg/mL in 10 m*M* PBS-T containing 1% BSA) was added to each well and the plate was incubated for 1 h at 37 °C. The plate was washed 5 times, followed by the addition of 100 μL per well of 1:3000 diluted streptavidin-peroxidase conjugate (Sigma) in antibody buffer. After further 1-h incubation at 37 °C, the plate was washed 3 times, and the color development procedures were the same as described in the indirect ELISA.

Statistical analysis

One-way ANOVA and Duncan's multiple range tests were preformed using SPSS software (12.0 for Windows, SPSS Inc., Chicago, Ill., U.S.A.) for differences among treatment groups. A student *t* test was used to determine the detection limit by comparing the differences in the ELISA readings between the baseline and the laboratory-adulterated samples. All data were obtained at least in triplicate and experiments were repeated twice. P values < 0.05 were considered statistically significant.

Results and Discussion

Specificity of the sandwich pork assay

The species specificity of the porcine sandwich ELISA using MAb 8F10 as the capture antibody and biotinylated porcine-specific MAb 5H9 as the detection antibody is presented in Figure 2. The porcine assay exhibited a strong positive reaction in all raw, cooked (100 °C for 30 min), and autoclaved (121 °C for 30 min) pork extracts with no cross-reaction with extracts from any of the other species tested, including bovine, equine, ovine, rabbit, deer, chicken, and turkey meat extracts. Previous studies reported MAb 5H9 to be porcine skeletal muscle specific without cross-reaction with smooth muscle, cardiac muscle, and common food proteins, such as egg

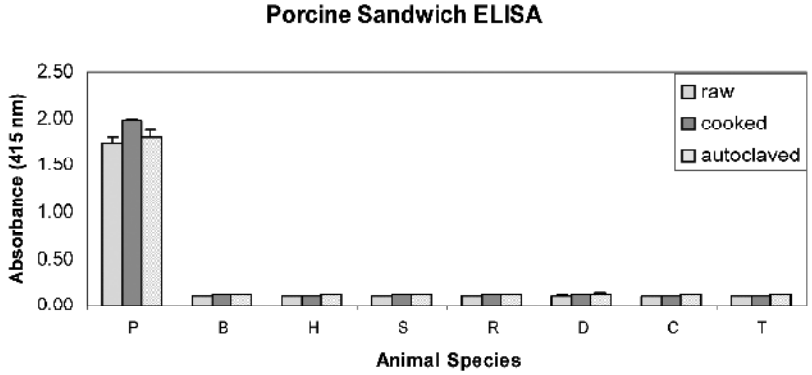

Figure 2—Species specificity of monoclonal antibodies (MAb)-based porcine sandwich enzyme-linked immunosorbent assay (ELISA). Capture antibody = MAb 8F10; detecting antibody = biotinylated 5H9. B = beef; C = chicken; D = deer; H = horse; P = pork; R = rabbit; S = sheep; T = turkey. Standard deviation bars are shown (*n* = 3).

Table 2—Absorbance (415 nm) of the porcine sandwich enzyme-linked immunosorbent assay (ELISA) for samples of pork in chicken, and pork in beef meat mixtures

Adulteration level (%)	Pork in chicken Mean (SD)			Pork in beef Mean (SD)		
	Raw	Cooked	Autoclaved	Raw	Cooked	Autoclaved
0%	0.152 (0.001)	0.109 (0.001)	0.118 (0.001)	0.299 (0.004)	0.154 (0.004)	0.163 (0.001)
0.05%	0.569 (0.025)**	0.138 (0.007)**	0.227 (0.006)**	0.32 (0.008)**	0.161 (0.002)*	0.166 (0.004)
0.1%	0.971 (0.002)	0.191 (0.01)	0.417 (0.02)	0.325 (0.013)	0.166 (0.002)**	0.168 (0.001)**
0.5%	1.952 (0.051)	0.786 (0.041)	1.479 (0.054)	0.436 (0.017)	0.186 (0.003)	0.187 (0.008)
1%	2.078 (0.032)	1.354 (0.022)	1.792 (0.042)	0.604 (0.03)	0.221 (0.008)	0.216 (0.01)
2%	2.198 (0.045)	1.761 (0.045)	1.938 (0.054)	1.009 (0.052)	0.356 (0.021)	0.315 (0.013)
4%	2.221 (0.031)	1.976 (0.033)	2.044 (0.018)	1.728 (0.098)	0.802 (0.034)	0.624 (0.032)

**$P < 0.01$, *$0.01 < P < 0.05$ ($n = 3$).

albumin, soy proteins, gelatin, milk proteins, and blood (Chen and others 1998). We confirmed this result using this MAb 5H9-based sandwich ELISA (data not shown), indicating that the porcine sandwich assay is highly specific to porcine skeletal muscle in raw, cooked, and autoclaved meat samples. This study also confirms the species specificity and thermal stability of the antigen, TnI, which can be used as an effective biomarker suitable for assaying both raw and heat-processed meat samples.

It should be noted that the ideal architecture of the sandwich ELISA would be to coat the porcine-specific MAb 5H9 on a microtiter plate as the capturing antibody and use the mammalian-specific MAb 8F10 as the detecting antibody, so that only the target porcine antigen would be captured on the assay plate. Unfortunately, the sandwich ELISA initially constructed in this order became cross-reactive to several non-porcine species, such as rabbit and horse (data not shown). This change in the specificity is possibly due to the fact that the conformation of the specific epitope on MAb 5H9 for porcine TnI be altered by the immobilization of the MAb 5H9 molecule on the plate. Therefore, we reversed the order of the 2 MAbs, coating the plate with MAb 8F10 as the capture antibody and using biotinylated MAb5H9 as the detecting antibody. With this format, all mammalian antigens could be captured on the plate by MAb 8F10. Although this latter format was not our 1st choice, the results demonstrated the exclusive porcine specificity of the assay, indicating that the biotinylation of the detecting antibody, MAb 5H9 did not affect its binding characteristics with the porcine antigen and thus enabling the assay to retain its exclusive porcine-specificity.

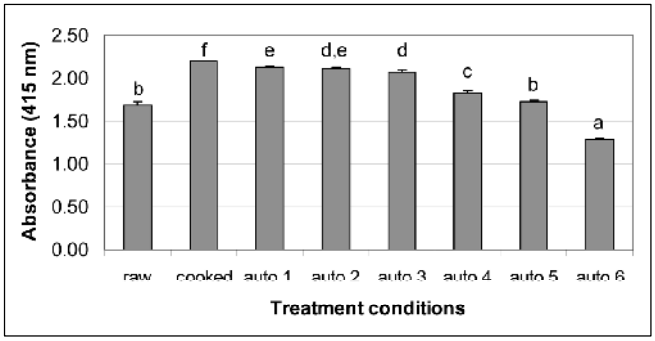

Figure 3—Changes of absorbance for the pork samples heat-processed to various conditions. Cooked = 100 °C/30 min; auto 1 = 121 °C/30 min; auto 2 = 128 °C/30 min; auto 3 = 132 °C/30 min; auto 4 = 132 °C/60 min; auto 5 = 132 °C/90 min; and auto 6 = 132 °C/120 min. Mean absorbance values with same letters are not significantly different ($P > 0.05$). Standard deviation bars are shown ($n = 3$).

Effect of heat treatment of the meat samples

To assess the effectiveness of the porcine sandwich assay in testing severely heat-processed samples, ground pork samples were treated under 7 different temperature-time conditions, described earlier. Protein extracts from these heated samples were assayed, along with a sample from an untreated pork sample. The results showed that the cooked (100 °C/30 min) sample extract had a stronger reaction compared with the raw and autoclaved samples (Figure 3). This stronger reactivity in the cooked samples than in raw samples was attributed to a concentration effect of the thermal-stable antigen in cooked extract. The majority of proteins extracted from raw meat are heat labile sarcoplasmic proteins. The ratio of the thermal-stable target myofibril muscle antigen to the total amount of the soluble proteins is lower in raw extracts than in cooked extracts (Hsieh and others 2002). Therefore, when equal volumes of raw and cooked meat extracts containing similar amounts of total proteins reacted with the capture antibodies, the cooked extract, which has a relatively high antigen content, produced a stronger reaction signal than the raw sample.

The reaction signal slightly decreased in autoclaved samples. As the temperature/time used for the autoclaving increased, the immunoreactivity gradually decreased ($P < 0.05$), indicating that prolonged heating under pressure partially and gradually destroyed some of the antigenic component. However, the absorbance reading of the ELISA in the most severely treated sample (132 °C/120 min) remained strong (absorbance: 1.3), suggesting that this assay is eminently suited to the analysis of excessively heat-treated samples.

Detection of adulterated meat samples

To determine the sensitivity of the porcine assay, 2 types of laboratory adulterated meat samples were prepared. Lean pork was artificially inoculated in chicken and in beef each at 7 low adulteration levels, 0%, 0.05%, 0.1%, 0.5%, 1%, 2%, and 4% (wt/wt). As shown in Table 2, the ELISA absorbance increased rapidly as the adulteration levels increased in the pork-in-chicken samples. The detection limit for pork in all raw, cooked, or autoclaved chicken mixtures was determined to be 0.05%, the lowest adulteration level tested. The detection limit was defined as the lowest adulteration level where the reading was significantly ($P < 0.05$) higher than the baseline (0% adulteration level). The assay in the raw chicken samples was highly sensitive, with the absorbance reaching above 0.5 for chicken sample containing as little as 0.05% of pork and a reading of almost 2 at a 0.5% adulteration level. The results suggested that the detection level of the assay is likely to be even lower than 0.05%.

On the other hand, the baseline readings for the pork-in-beef samples were slightly higher, and the increases in the absorbance readings with increasing adulteration level were smaller than for the pork-in-chicken samples, indicating that the overall assay sensitiv-

ity for pork-in-beef samples was lower. However, the ELISA absorbance readings were significantly higher than the negative control baseline readings at a 0.05% adulteration level for raw samples, and at a 0.1% adulteration level for both cooked and autoclaved meat samples (Table 2). The overall assay signals were thus apparently less sensitive in pork-in-beef samples compared with the pork-in-chicken samples but the detection limits were comparable.

The results demonstrated that the porcine assay was highly sensitive in detecting pork in non-mammalian meat, such as chicken, but was not as sensitive in detecting pork in mammalian meat, such as beef, as in poultry samples. The difference in sensitivity for pork in beef compared with pork in chicken can be explained by the structure of the sandwich assay, where MAb 8F10 is used as the capturing antibody and the porcine specific MAb 5H9 as the detecting antibody. MAb 8F10 has a broad species specificity. It can capture the muscle TnI from any mammalian species, including beef. Thus, when the sample consists of mainly beef, the majority of the binding sites on MAb 8F10 would be occupied by bovine protein (the matrix) and the pork antigen must compete with the beef antigen for binding sites. This competition dramatically decreases the chance that the pork antigen will be captured on the ELISA plate, especially when the antigen is present at very low levels. However, poultry meat protein cannot bind to MAb 8F10, only porcine TnI will be captured and thus detected by this assay, which resulted in an extremely sensitive response for detecting porcine muscle in a non-mammalian sample.

Even under these less favorable conditions, this sandwich assay can still achieve a satisfactory detection limit of as little as 0.05% to 0.1% level of pork adulteration in raw, cooked, and autoclaved beef samples, suggesting the strong affinity and high specificity of the anti-porcine antibody. For detection of the presence of low levels of pork in non-porcine mammalian meat mixtures, it is recommended that a microreader should be used to record the absorbance to differentiate between very low levels (<1%) of pork contamination, as the color development was not strong enough for an accurate visual detection. The average intra- and inter-assay coefficients of variation (CVs) were 2.9% and 8.7%, respectively, for adulterations ranging from 0% to 4% of raw, cooked, and autoclaved samples.

Detection of porcine muscle in feed samples

Laboratory-prepared pure pork meal was mixed with soy-based feed at 7 low adulteration levels: 0%, 0.01%, 0.05%, 0.1%, 0.5%, 1%, and 2% (w/w). Results showed that the porcine assay was able to differentiate as little as 0.05% (w/w) dry pork meal in soy-based feed with the average intra- and inter-assay CVs being 3.4% and 6.3%, respectively. (Table 2). The dried pork meal was prepared by autoclaving ground lean pork at 132 °C for 120 min and then dried in an oven overnight. The results indicated that the porcine assay was highly sensitive to even trace amounts of severely heated pork in soy-based feed without interference due to the plant proteins or any other added ingredients in the feed samples tested. The application of the assay in detecting trace amounts of pork meal in feed samples further demonstrates the validity of the assay in analyzing samples treated with severe conditions.

Table 3—Absorbance (415 nm) of the porcine sandwich enzyme-linked immunosorbent assay (ELISA) for samples of Pork Meal (PM) and nr 3 pork meat and bone meal (MBM) inoculated in SoyBest feed

Adulteration level (%)	PM in feed (mean ± S.D.)	Adulteration level (%)	MBM in feed (mean ± S.D.)
0%	0.132 ± 0.002	0%	0.112 ± 0.005
0.01%	0.134 ± 0.002	0.05%	0.113 ± 0.005
0.05%	0.156 ± 0.006**	0.10%	0.114 ± 0.003
0.10%	0.196 ± 0.001	0.50%	0.115 ± 0.003
0.50%	0.891 ± 0.033	1%	0.120 ± 0.003*
1%	1.640 ± 0.160	2%	0.131 ± 0.007**
2%	2.325 ± 0.085	4%	0.162 ± 0.007
		100%	1.650 ± 0.020

**P < 0.01, *0.01 < P < 0.05 (n = 3).

The performance of the porcine assay was also examined in 8 commercial meat by-product samples (Table 1). The actual ingredients in these industrial samples were unknown. The results showed that only 1 sample exhibited a positive reaction to pork (Figure 4). As expected, nr 3 Pork Meal gave a strong reaction with the porcine ELISA, whereas the Feather Meal (nr 8), Poultry Meal A and B (nr 5 and nr 6), Sheep Meal (nr 7), and Dairy Blend B (nr 4) had a clean negative result for pork. Two Meat Meal samples (nr 1 and nr 2) did not show a pork-positive result, indicating the meat ingredients used were from non-porcine species. Subsequently, we selected nr 3 Pork MBM for a further study of the detection limit of the assay in feed mixtures. The porcine MBM sample was mixed in SoyBest at 8 levels: 0%, 0.05%, 0.1%, 0.5%, 1%, 2%, 4%, and 100%. Although absorbance readings for samples containing up to 4% of pork adulteration were low, we were able to consistently distinguish as little as 1% of pork MBM in soy-based feed using the porcine assay (Table 2). The average intra- and inter-CV were 3.2% and 5.6%, respectively, for the adulterations ranging from 0% to 4% for MBM in feed samples. The assay not only successfully distinguished pork MBM from other animal by-products, but was also able to detect 1% of this MBM sample in soy-based feed without the need for complicated sample cleaning procedures.

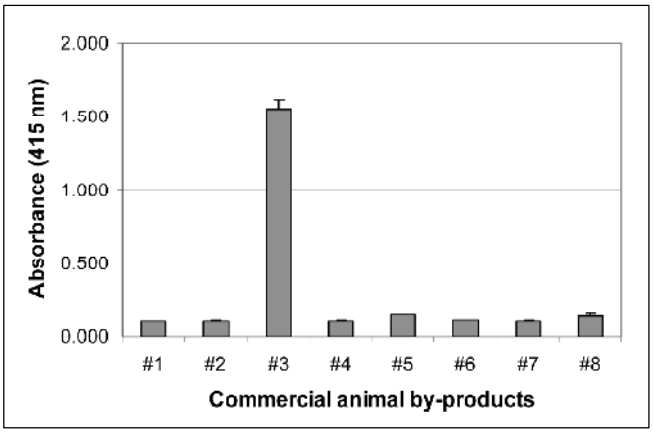

Figure 4—Detection of porcine muscle protein in commercial animal by-products using the porcine sandwich enzyme-linked immunosorbent assay (ELISA). Standard deviation bars are shown (n = 3).

Conclusions

This MAb-based immunoassay is highly sensitive, specific, and rapid. The specificity of the assay is 100% with no false-positive result being found. The only factor that could affect the sensitivity (detect positive sample as positive) of the assay was the detection limit. This is the 1st report of a MAb-sandwich ELISA that allows the simple and reliable detection of porcine muscle in a wide range of raw and heat-processed meat and feed products without the need for complicated sample preparation and with no problems due to cross-reactivity. Although the reaction signals are slightly stronger in pork spiked non-mammalian meat and feed mixtures than in mammalian mixtures, the detection limits of the assay for these 2

types of samples are comparable. The simple sample preparation, in conjunction with the high throughput capability, will enable the immunoassay to be used for the routine analysis of many samples and the development of rapid test kits, such as lateral flow or immunosticks, for field use.

Acknowledgments

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