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Development and validation of a rapid test system for detection of pork meat and collagen residues

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ABSTRACT

Mislabeling, contamination, and economic adulteration of meat products with undeclared pork tissues are illegal under regulations promulgated by numerous regulatory agencies. Nonetheless, analysis of the European meat industry has revealed pervasive meat adulteration, necessitating more extensive application of meat authentication testing. As existing methods for meat speciation require specialized equipment and/or training, we developed a detection system based on a lateral flow device (LFD) assay format capable of rapidly (~35 min) identifying porcine residues derived from raw meat, cooked meat, and gelatin down to 0.01%, 1.0%, and 2.5% contamination, respectively. Specificity analysis revealed no cross-reactivity with meat derived from chicken, turkey, horse, beef, lamb, or goat. Comparison with a commercial ELISA kit and PCR method revealed similar if not improved sensitivity, with the added feature that the LFD-based system required considerably less time to perform. Accordingly, this test system should aid the food industry and food control authorities in monitoring for adulteration with pork.

1. Introduction

The mixing of undeclared meat species in meat products is illegal under various food safety regulations worldwide. In particular, the US Federal Meat Inspection Act (FMIA) and the European Parliament under Regulation (EC) No. 178/2002 strictly prohibit the act of meat adulteration (Anonymous, 2002; Anonymous, 2015b). Aside from the economic consequences that stem from gross intentional adulteration, contamination with pork can lead to increased risks associated with *Trichinella spiralis* (Matsunaga, Shibata, Yamada, & Shinmura, 1999), *Toxoplasma gondii* (Robert-Gangneux & Dardé, 2012), and *Yersinia enterocolitica* (von Bargen, Dojahn, Waidelich, Humpf, & Brockmeyer, 2013) infections. Furthermore, adulteration of meat products with undeclared pork residues poses substantial religious concerns for Jews, Muslims, and select Christian denominations (Anonymous, 2013b; Gamble, 2014; Saez, Ssanz, & Toldra, 2004).

Though data regarding mislabeled meat in the US have not been published (Anonymous, 2015a), recent analysis of the meat industry in Europe has identified inaccurate or fraudulent labeling of beef-based products contaminated to varying degrees with horse and pig meat, where pork-specific DNA was identified in 85% of beef samples tested (Regenstein, Chaudry, & Regenstein, 2003). A parallel study in South Africa analyzing processed meat products using both ELISA and PCR reported that 37% of meat tested was contaminated to varying degrees with pork residues (Cawthorn, Steinman, & Hoffman, 2013). To address this problem, numerous analytical techniques have been developed for meat authentication purposes based on detection of specific genes and proteins including the polymerase chain reaction (PCR), polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), random amplified polymorphic DNA (RAPD)-PCR, single nucleotide polymorphism analysis, enzyme-linked immunosorbent assay (ELISA), and lateral flow devices (LFD) (Aida, Che Man, Raha, & Son, 2007; Ghovvati, Nassiri, Mirhoseini, Moussavi, & Javadmanesh, 2009; Giaretta, Di Giuseppe, Lippert, Parente, & Di Maro, 2013; Kwon et al., 2007; Liu, Chen, Dorsey, & Hsieh, 2006; Martinez & Yman, 1998; Rohman, Sismindari, Erwanto, & Che Man, 2011). Collectively, commercial kits based on these approaches demonstrate detection limits in the range of 0.01–1.0% contamination. However, a major limitation for DNA- and protein-based detection methods is the quality of target analyte following food processing, including thermal treatment and acid/base mediated hydrolysis as employed in gelatin production (Köppel, Ruf, & Rentsch, 2011; Matsunaga, Chikuni et al., 1999). Speciation methods based on improved PCR has been reported to detect ~0.1% highly autoclaved pork meat contaminated into cooked mixed-meat background (Razzak, Hamid, & Ali, 2015). Of significance, using primers designed to amplify amplicons <90 base pairs in length, species-specific PCR methods have been successfully applied to speciating animal meal as well as animal lard (Natonek-Wiśniewska, Krzyścin, & Piestrzyńska-Kajtoch, 2013). Though speciation of gelatin has not been reported

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using PCR, additional methods have recently been developed based on high-performance liquid chromatography–tandem mass spectrometry (LC/MS), Fourier transform infrared (FTIR) spectroscopy, and ultra-performance liquid chromatography (UPLC), with speciation detection capabilities ranging from 0.1 to 5.0% (w/w) meat contamination (Anonymous, 2013a; Simoons, 1978). However, these methodologies are time-consuming and require highly specialized equipment. Ambient liquid extraction surface analysis mass spectrometry (LESA-MS), which requires much less preparatory and operation time, have yielded promising results for cooked pork meat contamination, at ~10% (w/w) contamination in cooked meat background (Montowska, Alexander, Tucker, & Barrett, 2014, 2015). However, a rapid, simple, and equally (or more) sensitive method for detecting pork residues and gelatin in foods is warranted to allow field-based assessment of meat source identification throughout the process of procurement, processing, packing, distribution, and retail, so as to ensure product safety, as well as promote consumer confidence in the meat and poultry industry. To this end, we have developed a lateral flow device (LFD) system intended for deployment outside the laboratory setting that rivals the performance of these existing technologies with respect to sensitivity as well as specificity.

2. Materials and methods

2.1. Reagents and assay buffers

Pig serum albumin (PSA) was purchased from Sigma-Aldrich (St. Louis, MO). Porcine thermal-stable meat protein (P-TSMP) was isolated from raw pork meat according to Liu et al. (2006). Complete Freund's Adjuvant (CFA) and Incomplete Freund's Adjuvant (IFA) were purchased from Sigma-Aldrich. To make PSA-agarose or P-TSMP-agarose columns for affinity purification of polyclonal antibodies, agarose beads (Agarose Bead Technologies, Miami, FL) were glyoxalated, periodate oxidized, and then conjugated to primary amines on the target proteins. The ensuing matrices were rinsed and packed into chromatographic columns. Sample extraction buffer and LFD running buffer were obtained from Pi Bioscientific Inc. (Seattle, WA).

2.2. Generation of polyclonal antibodies

Polyclonal antibodies (pAbs) against PSA and P-TSMP were obtained from Pi Bioscientific Inc. In brief, the pAbs were raised in goats following standard immunization protocols, purified from serum on Protein G columns and then additionally purified on affinity columns using an ÄKTA prime FPLC unit (GE Healthcare Life Sciences, Pittsburgh, PA). Ensuing IgG antibodies were assessed for purity and functionality by denaturing polyacrylamide gel electrophoresis and indirect ELISA using 10 μg/ml PSA or P-TSMP bound to polystyrene 96-well plates using horseradish peroxidase conjugated rabbit-anti-goat IgG (KPL, Gaithersberg, MD) as a detection reagent. Note that anti-PSA antibodies were used to prepare the raw pork LFD and the anti-P-TSMP antibodies were used to generate the cooked pork LFD.

2.3. Preparation of gold conjugates

Citrate-capped 40 nm gold nanoparticles were obtained from Pi Bioscientific Inc. as a gold colloid. The affinity-purified pAbs were individually diluted in borate buffer to a final concentration of 0.1 mg/ml, and then 7.5 ml was added drop-wise to 250 ml of gold nanoparticles (A530 = 1) while stirring for 30 min. To block, 2.5 ml of 10% BSA (in borate buffer) was added, and the colloid was pelleted by centrifugation at 3000 ×*g* for 1.5 h. The pellet was resuspended, its spectrum was analyzed and the suspension absorbance was adjusted to a final reading of A = 20 (at the absorption maxima) by using 1% BSA, 10% sucrose in 8 mM borate buffer as a diluent.

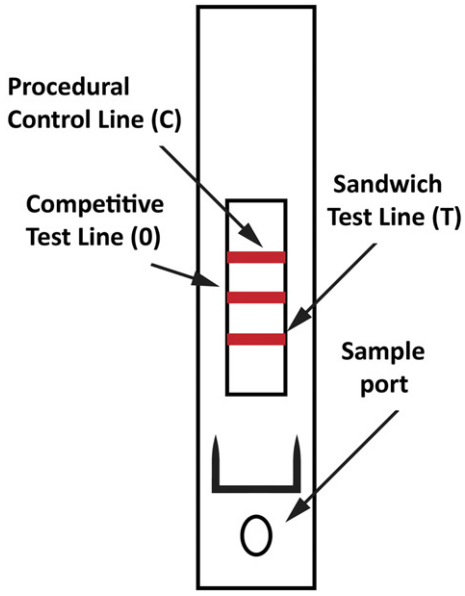

Fig. 1. Schematic diagram of lateral flow device (LFD).

2.4. Preparation of lateral flow devices

Nitrocellulose membrane (Sartorius, Goettingen, Germany) was lined with affinity-purified antibodies for either PSA or P-TSMP to prepare the sandwich format test line (T1), PSA or P-TSMP for the competitive format test line (T2), and chicken anti-goat antibodies (Pi Bioscientific Inc.) for the procedural control line (PC) using an IsoFlow ™ Reagent Dispenser (Imagene Technology, Hanover, NH). To prepare the conjugate pad, the gold conjugates were sprayed on strips of glass fiber conjugate pad material (Ahlstrom, Mt. Holly Springs, PA) using the IsoFlow Dispenser. To assemble the test strips, the nitrocellulose membrane, conjugate pad, sample pad (Ahlstrom, Mt. Holly Springs, PA), and absorbent pad (Advanced Micro Devices, India) were adhered to the laminate of the backing card (Lohmann, Precision Die Cutting, San Jose, CA) with overlapping surfaces to ensure continuous capillary transfer. The assembled cards were then cut into 5 mm wide strips using a Matrix 2360 programmable shear (Kinematic Automation, Sonora, CA), the strips were housed in plastic cassettes (Advanced Micro Devices, India), and stored with desiccant in sealed foil bags at room temperature (RT) until use. The LFD was configured such that the sample first encounters the T1 line (sandwich assay), then the T2 line (competitive assay), and lastly the PC line (consisting of chicken anti-goat IgG antibodies) (Fig. 1).

2.5. Preparation of reference materials

Serum albumins were obtained as follows: bovine serum albumin (BSA) from Calbiochem (EMD Millipore, Billerica, MA), horse serum albumin (HSA), chicken serum albumin (CSA), and goat serum albumin

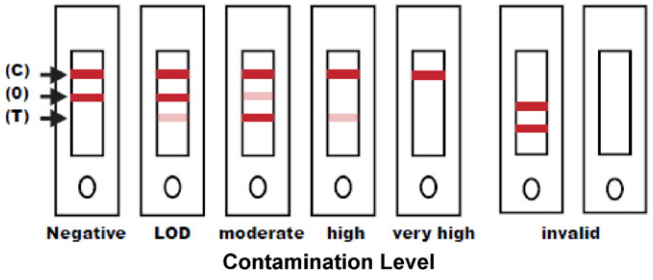

Fig. 2. Interpretation of the assay.