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A rapid, semi-quantitative test for detection of raw and cooked horse meat residues

Jongkit Masiri [a], Lora Benoit [b], Cortlandt Thienes [a], Charles Kainrath [a], Brianda Barrios-Lopez [a], Alex Agapov [a], Anatoly Dobritsa [a], Cesar Nadala [a], Shao-Lei Sung [c], Mansour Samadpour [a, b, *]

[a] Molecular Epidemiology, Inc (MEI), 15300 Bothell Way NE, Lake Forest Park, WA, 98155, USA
[b] IEH Laboratories and Consulting Group, Inc (IEH), 15300 Bothell Way NE, Lake Forest Park, WA, 98155, USA
[c] Pi Bioscientific, Inc (Pi Bio), 8315 Lake City Way NE, Seattle, WA, 98115, USA

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ABSTRACT

Intentional mislabeling and adulteration of meat products with undeclared horse meat is a concern for religious, ethnic, and health reasons and is illegal under regulations mandated and enforced by food regulatory agencies and the Federal Meat Inspection Act. Nonetheless, recent analysis of the meat industry has revealed an apparent increase in the frequency of meat adulteration including intentional horse meat contamination, necessitating a broader use of meat authentication testing. As existing methods for meat speciation are cumbersome and require specialized equipment and/or training, we developed a highly specific lateral flow immunoassay that can rapidly identify raw and cooked horse meat down to 0.01% and 1.0% contamination, respectively in xenogeneic meat sources in about 35 min with no false positive signals observed. Specificity analysis revealed no cross-reactivity with serum albumins or meat derived from chicken, turkey, pig, cow, lamb, and goat. The results of method comparison showed that the assay had similar if not better sensitivity than the commercial ELISA kit and PCR, and required considerably less time to perform than either method. The development of a highly robust and rapid test method capable of detecting trace amounts of horse meat residues should aid food control authorities in their continued efforts to monitor for horse meat adulteration.

1. Introduction

The issue of fraudulent incorporation of horse meat in foods intended for human consumption gained major public attention in 2013 following a meat adulteration scandal in Europe (O'Mahony, 2013; Premanandh, 2013) wherein ~5.0–7.5% of beef-based products in Europe were documented to contain undeclared horse meat residues at contamination levels exceeding 1.0% (Walkera, Burns, & Burns, 2013). Though consumption of horse meat is not inherently harmful, unanticipated introduction of horse meat into the food supply chain increases the risk of human exposure to veterinary drug residues such as phenylbutazone (Dodman, Blondeau, & Marini, 2010) and parasites (Murrell, 2000). Additionally, consumption of horse meat possesses certain cultural and religious concerns, such that the practice is verboten in many countries (Simoons, 1978) and it violates the Federal Meat Inspection Act in the US.

Due to increased awareness of intentional food adulteration and the need to verify labeling statements, numerous analytical techniques have been developed to perform meat authentication (Sentandreu & Sentandreu, 2014). Notably, detection platforms based on mass spectrometry, ELISA, and nucleic acid amplification have been commercialized, with detection limits ranging from 0.01–1.0% meat contamination (von Bargen, Dojahn, Waidelich, Humpf, & Brockmeyer, 2013; Köppel, Ruf, & Rentsch, 2011; Premanandh, 2013). However, these methodologies are time-consuming and require specialized equipment and training to perform. Accordingly, a rapid, simple, and equally (or improved) sensitive method for detecting horse meat residues in foods is warranted, to allow assessment of meat source identification throughout the process of procurement, processing, packing, distribution, and retail, so as to ensure product safety, as

* Corresponding author. Molecular Epidemiology, Inc (MEI), 15300 Bothell Way NE, Lake Forest Park, WA, 98155, USA.
E-mail address: msieh@iehinc.com (M. Samadpour).

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well as promote consumer confidence in the meat and poultry industry. To this end, we have developed a highly sensitive Lateral Flow Device (LFD) for horse meat detection intended for deployment outside the laboratory setting that rivals the performance of existing technologies with respect to sensitivity as well as specificity.

2. Materials and methods

2.1. Reagents and assay buffers

Horse serum albumin (HSA) was purchased from Equitech-Bio (Kerrville, TX). Horse thermal-stable meat protein (H-TSMP) was isolated from raw horse meat according to Liu, Chen, Dorsey, and Hsieh (2006). Complete Freund's Adjuvant (CFA) and Incomplete Freund's Adjuvant (IFA) were purchased from Sigma-Aldrich (St. Louis, MO). Extraction buffers consisting of 0.9% saline solution or 0.9% saline solution and 1% SDS were used to extract meat proteins from raw or cooked samples, respectively. To make HSA-agarose or H-TSMP-agarose conjugated 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 HSA and H-TSMP were prepared by Pi Bioscientific Inc. In brief, the pAbs were raised in goats following standard immunization protocols, purified on Protein G columns, and then on affinity columns using an AKTA prime FPLC unit (GE Healthcare Life Sciences, Pittsburgh, PA). Anti-HSA antibody was used to prepare the Raw Horse LFD and the anti-H-TSMP antibody was used to generate the Cooked Horse LFD. Ensuing IgG antibodies were assessed for purity and functionality by denaturing polyacrylamide gel electrophoresis and indirect ELISA using 10 μg/ml HSA or H-TSMP bound to polystyrene 96-well plates using horseradish peroxidase conjugated rabbit anti-goat IgG (KPL, Gaithersberg, MD) as a detection reagent.

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. Spectral analysis was performed on the resuspended soft pellet, and the 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.

2.4. Preparation of immunochromatographic test strips, lateral flow device

Nitrocellulose membrane (Sartorius, Goettingen, Germany) was lined with affinity-purified antibodies for each of the targets to prepare the sandwich format test line (T1 or T), HSA or H-TSMP for the competitive format test line (T2 or O), and chicken anti-goat antibodies (Pi Bioscientific Inc.) for the procedural control line (C) 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, Mumbai, 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), and stored with desiccant in sealed foil bags at room temperature until use. The LFD was configured such that the sample first encounters the T1 line (sandwich assay, T), then the T2 line (competitive assay, O), and lastly the procedural control line (C) containing of chicken anti-goat IgG antibodies. Please see Fig. 1 for a schematic of the layout for the LFD assay.

2.5. Preparation of reference materials

The reference meat samples (beef, pork, and meats from sheep, goat, chicken, and turkey) used for this study were purchased from local grocery stores. Horse meat was obtained from a licensed veterinarian. Meats were finely chopped and minced in a manner to avoid cross-contamination. Reference meat samples spiked with horse meat were prepared by serial 1/10 dilutions of a mixture 6 g of horse meat and 54 g of a reference meat (lean ground beef). For detection of horse meat using Raw Horse LFD, raw meat samples were extracted by adding 5 g of meat into 10 ml of 0.9% saline solution with the following stomaching for 5 min in Whirl-Pak stomacher bags using the high speed setting. The extracts were diluted 1/10 in meat LFD running buffer. Cooked meat samples were extracted with 1% SDS, phosphate buffered saline by adding 10 g of sample into 10 ml of extraction buffer, and after stomaching, diluted 1:1 in meat LFD running buffer. To prepare cooked meat samples, reference and horse meats were incubated in extraction buffer under boiling conditions for 15 min. When Cooked Horse LFD was used, cooked samples were extracted in 0.9% saline solution using 5 g sample to 10 ml of extraction buffer, stomached, then diluted 1/10 in meat LFD running buffer.

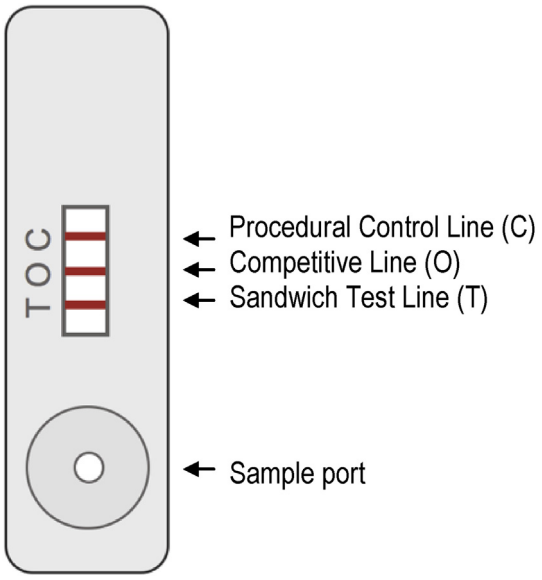

Fig. 1. Schematic diagram of horse lateral flow device (LFD).