B71 Methodologies to Employ Porcine Tissue to Simulate Human Tissue: Determining the Efficacy of Radiation for Decontamination and the Subsequent Recovery of DNA From Tissue for PCR DNA Testing

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The goal of this presentation is to describe assays designed to measure DNA recovery and STR profiles from porcine tissue in order to provide a model to test decontaminated human remains.

This presentation will impact the forensic community and/or humanity by presenting studies which suggest that decontamination of human tissue by irradiation is a possible solution for the safe return of reamins to families. Moreover, these methodologies for porcine tissue provide an easily accessible model for other examinations that may require human tissue samples.

Currently, there are few viable alternatives regarding the identification and subsequently the interment of biologically or chemically contaminated human remains. Studies have demonstrated that irradiation of human tissue, specifically at 51 kGy, destroyed *Bacilus subtilis*, a surrogate for *Bacillus anthracis*, but preserved identifying DNA sequences. Chemical decontamination, however, will likely require much higher doses of radiation. Therefore, the laboratory of the OCME in conjunction with Titan Corporation performed a dose response with electron beam radiation and evidentiary items such as blood, semen, and saliva stains. Even at doses as high as 90 kGy, sufficient DNA to produce usable DNA profiles was recovered, although the absolute DNA yield was compromised. In order to better define the limits of DNA testing of irradiated samples, and to assess penetration through a large tissue sample, pork slabs, simulating human tissue, were irradiated at higher doses, and methodologies were adjusted to accommodate this porcine DNA.

Inserting dosimeters deep within a substantial piece of pork addressed the issue of radiation penetration. With the effective dose known, one could evaluate DNA testing results taken from a pork sample proximal to a dosimeter, with certainty. Moreover, the efficacy of decontaminating tissue could be studied with pork similarly “inoculated” with spore test strips for *Bacilus subtilis*.

In this experiment, six pork shoulder shanks were sliced into four one pound slabs of boneless meat. A small portion of each of twenty slabs was sliced wherein one dosimeter and one spore strip, sealed in waterproof miniature kpac pouches, were inserted. The gap was sewn together with fishing line, and wrapped in brown bench paper. Two slabs of pork thus prepared were packaged in each of ten boxes, where one slab was placed near the bottom of the box and one near the top. Sandwiched between the two slabs were an additional dosimeter and a spore strip. Surrounding these items were dry ice and newspaper.

Titan Scan, San Diego, CA, irradiated two boxes for each of the following doses: 0 kGy, 30 kGy, 60 kGy, 90 kGy, and 120 kGy. The dosimeters and the spore strips were returned to Titan Scan and Raven Laboratories, respectively, for processing. Three 25 mg samples of each of twenty slabs of pork were extracted with DNA IQ™ (Promega) according to a modified methodology developed in the laboratory on the Biomek 2000 that encompassed the use of a stronger digestion buffer and three times more resin than recommended.

DNA recovery was measured with a SYBR Green I based real time PCR assay for ALU fragment. Primers described by J Walker *et al*. (Analytical Biochemistry, 2003) produced a 134 bp amplicon. The assay was modified for the Rotorgene 3000 and a home brew master mix with the addition of 0.5 1g/1L of BSA, 8% DMSO, and 0.251L of a 1/100 dilution of SYBR Green I (Molecular Probes). The dynamic range of the assay was from 0.78 pg to 6400 pg. The control DNA for the standard curve was procured from control slabs of pork that were extracted as described above, and measured with a spectrophotometer.

Amplification of the ALU amplicon predicted successful genotyping of porcine microsatellites. Three microsatellites from the USDA database, Sw520, S0147, and TNFB, were selected due to their varying lengths, 102124 bp, 146-174 bp, and 174-213 bp, respectively. Each forward primer was labeled with 6’FAM, and collectively they were multiplexed according to the parameters described by G. Yue *et al.* (Electrophoresis, 1999) with additional KCL. PCR products were separated on an ABI Genetic Prism Analyzer using LIZ (ABI) as a size standard. DNA recovery and STR typing results were similar to previous experiments. Although, as radiation increased DNA recovery decreased, if sufficient DNA was present genotypes were generated.

Radiation, Microsatelites, Animal Model

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