B31 Extraction and Quantification of Human DNA and the Amplification of Human STRs and Amelogenin From Fly Larvae Found on Decomposing Tissue

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After attending this presentation, attendees will learn that fly larvae found on decomposing human tissue could be an alternative means of identifying human remains.

This presentation will provide support of the forensic science community and the popular media hypothesis that human nuclear DNA can be recovered from fly larvae found on human remains.

The purpose of this experiment was to determine if human deoxyribonucleic acid (DNA) could be extracted, quantified, and amplified from fly larvae that had fed on decomposing human tissue.

Blowfly (Diptera: Calliphoidae) eggs were collected and placed on donated human skin, muscle, or liver tissue in rearing chambers. After two to twelve days, the larvae were collected in 70% ethanol, 2propanol, acetone, or by freezing. One to sixteen larvae were washed and dissected by mincing, splitting, or bisecting the larvae. The DNA was extracted using digest buffer and either phenol: chloroform: isoamyl alcohol (PCIA)or by using silica spin columns. The human DNA was quantified using a biotinylated primate specific probe. The DNA extract was then amplified using the polymerase chain reaction (PCR) for 28 cycles and primers for 13 specific Short Tandem Repeats (STRs) and the sex identification marker amelogenin. An additional seven PCR cycles were used on extracts that failed to produce any amplified human DNA. Washes from the exterior of the larvae that produced amplifiable human DNA were also analyzed. Finally, the PCR product was separated and characterized using capillary electrophoresis.

Out of the 30 DNA extracts, one sample had approximately 4.59.0ng of human DNA. The remaining 29 extracts were either void of human DNA or the amount of human DNA fell below the test detection limits. Three of the thirty extracts produced amplified products for the sex identification marker and seven to eight STRs. The STR profiles produced by these three extracts matched the STR profiles of the corresponding positive controls. Some of the extracts subjected to the seven additional PCR cycles provided limited and often inaccurate identifying information. The experiment also provided evidence, but not conclusive proof, that the human DNA originated from inside the larvae.

The following conclusions were drawn from the experiment: Human tetranucleotide STR analysis is highly sensitive and selective. Macromolecules survive larval ingestion. Three to four larvae are required for this type of analysis. Muscle and liver tissue are the best feed tissues from which to collect the larvae. Digest buffer and PCIA are more suitable than silica spin columns for recovering human DNA from fly larvae. 2-propanol is a better collection medium than 70% ethanol, acetone, or by freezing for this type of analysis. Mincing or splitting the larvae provided better results than bisecting the larvae and using the anterior portion of the larvae. The drawbacks of additional PCR cycles outweighed any additional benefits.

Larvae, DNA, STRs

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