B176 Polymerase Resistance to PCR Inhibitors in Bone

Ken D. Eilert, BS, and David R. Foran, PhD, Forensic Science Program, Michigan State University, 560 Baker Hall, East Lansing, MI 48824*

After attending this presentation, attendees will understand the utility of different DNA polymerases for amplifying difficult or inhibited DNA from skeletal material.

This presentation will impact the forensic community and/or humanity by surveying a series of commercially available DNA polymerases for their capability to facilitate PCR in the presence of elements co-extracted during phenol-chloroform extractions of recent and aged forensic bone specimens. Greater success in DNA typing from bone should result.

This presentation outlines the results of a three-part study intended to 1) use mass spectrometry to identify and quantify the major co- extracted inhibitory elements in recent versus aged/damaged bones, 2) target polymerase weakness and resistance by stressing a series of selected polymerases with increasing levels of the identified inhibitors and 3) determine the most resistant polymerase by stressing the enzymes with varying concentrations of whole bone extract.

Successful recovery of DNA from poor quality bone samples necessitates optimization of the polymerase chain reaction. This includes the ideal concentrations of thermostable polymerase, template DNA, free nucleotides, Mg2+, and buffering salts. The reaction environment must permit the appropriate interaction of polymerase with template DNA. Any substance present in the PCR reaction that interferes with this interaction is categorized as an inhibitor.

Many agents of inhibition in bone have been identified, though the exact mechanisms of inhibition remain unclear. Type I collagen has been shown to be a potent inhibitor of PCR and likely co-extracts with DNA during the extraction process. Additionally, bones left in contact with soil can accumulate humic and fulvic acids, which have also been proven inhibitory. The presence and concentration of endogenous and exogenous inhibitors varies as to the circumstantial history of the received sample.

The study attempts to utilize MALDI-TOF and ICP-MS to identify and quantify possible inhibitory elements found in the DNA extractions of both fresh and aged bone samples. Determining these elements allows for a targeted polymerase inhibitor resistance assay. Because the phenol-chloroform method of extraction is standard for bone sample amplification, the study focuses on products obtained by this technique.

The inhibitor resistance assay tests several polymerases for their amplification capability in the presence of the identified inhibitory elements. PCR reactions are designed to amplify human mtDNA stretches HVI and HVII. Each polymerase is stressed with increasing concentrations of the individual inhibitors as well as concentrations of whole bone extractions. Challenging bone sample extracts recovered by the phenol-chloroform method often require considerable dilution before inhibition is relieved. This dilution factor is used to determine efficacy of the polymerases exposed to whole bone extract.

DNA Polymerase, Skeletal DNA, PCR Inhibition

 ** Presenting Author*