## A13 Evaluation of Novel DNA Extraction Methods and Modifications to Existing Methods

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After attending this presentation, attendees will learn about new methods for extracting DNA from biological samples containing cellular material.

This presentation will impact the forensic science community by providing systematic data that can be used by the analyst in selecting a method for extracting DNA from crime scene or reference samples especially when considering time and cost.

DNA extraction is a method of purifying DNA from other cell contents including proteins, enzymes, and membranes. DNA extraction is employed routinely by crime labs to recover DNA from biological samples containing cellular material. While a number of methods for extracting DNA from crime scene evidence are available to the practioner, research results are presented in which new methods have been evaluated and older methods have been modified to reduce the time needed to recover the DNA. The extracted DNA is used for modern DNA typing analyses including autosomal, Y-chromosome and mitochondrial DNA typing using STRs, SNPs, and sequence comparison. The DNA extraction methods used in crime laboratories including Chelex® 100, Phenol-Chloroform-Isoamyl Alcohol, dialysis, and commercial kits (e.g., Promega's DNA IQ™ and Qiagen's QIAamp™) vary widely in terms of cost and extraction time. The 5% Chelex® method is the cheapest method but it is time-consuming due to a recommended six to eight hour, 56 ºC incubation step performed prior to vortex mixing, boiling, and centrifugation steps. The Chelex® beads chelate metal ions, including magnesium, known to be essential for DNase nuclease activity. Inactivating nuclease enzymes that digest DNA reduces DNA degradation in the extraction process. In this study, modifications to the Chelex® method were evaluated including reducing the reagent concentration and incubation time in order to decrease the overall cost and time involved in using this method for casework and other samples. In addition, extractions with Silicycle®, EDTA, and citrate were performed to determine if these methods would also yield amplifiable DNA. Like Chelex®, Silicycle®, EDTA, and citrate reagents also chelate metal ions. The extraction efficiency with consideration to DNA yield, cost and time was tabulated for all of the methods.

In this study, the Chelex® method was evaluated for DNA recovery and amplifiability using reduced incubation times (thirty to ninety minutes) and overnight without Proteinase K. The results showed that any incubation time over sixty minutes and the procedure from which Proteinase K was omitted produced results similar to those produced using the standard method. Omitting the Proteinase K that digests proteins significantly reduces the cost of employing this method. In addition, the concentration of Chelex® was varied from 1% to 5% solutions. Reduced Chelex® concentrations yielded amplifiable DNA at a reduced cost. In addition, extractions with similar concentrations of Silicycle®, EDTA, and citrate were evaluated for use in DNA extraction using varying incubation times for comparison to the Chelex® method. The Silicycle® method was observed to extract successfully amplifiable DNA as evaluated by real time-PCR using TPOX primers validated for DNA quantitation and post-PCR agarose gel electrophoresis. EDTA and citrate are very inexpensive chelating compounds that could be useful to the forensic community as DNA extraction agents.

**DNA Extraction, Real-Time PCR, Chelex**

 ** Presenting Author*