



### **B114 Dyed Denim and DNA Extraction: A Comparative Study of Extraction Methods and Their Ability to Yield Inhibitor Free DNA Samples From Dyed Substrates**

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The goal of this presentation is to identify specific dye formulations that lead to inhibition of downstream DNA analysis steps when working with commonly used DNA extraction methods. In addition, alternative approaches are presented which can reverse and prevent observed inhibition.

This presentation will impact the forensic community by providing insight into the well established challenges of dealing with denim substrates as forensic evidence.

Denim is frequently encountered as evidence in the forensic community. The dyes present in denim substrates often present challenges as some will co-extract with DNA and interfere with subsequent analysis steps by inhibiting DNA amplification. This research evaluates three extraction methods on ten denim dye formulations. Phenol chloroform organic, Chelex-100 and Promega DNA IQ, were evaluated for their ability to minimize downstream inhibition. Two organically extracted dye formulations inhibited the polymerase chain reaction (PCR), while, Promega DNA IQ and Chelex- 100 were most successful at isolating DNA free of inhibitors. Having identified inhibiting dyes, various approaches to treating the observed inhibition were employed: dilution of extract, addition of bovine serum albumin, addition of AmpliTaq Gold, post extract purification using Promega DNA IQ, and NaOH clean up method. All of the above approaches except the NaOH treatment were able to reverse the inhibition. Finally, preparations of inhibiting dye concentrate were made and added directly to amplification reactions in varying amounts to demonstrate a range of inhibition. Bovine serum albumin, AmpliTaq Gold and the two in combination were then added in varying amounts to evaluate their efficacy in reversing inhibition.

As a DNA analyst, there are many options available for isolating DNA from a variety of substrates. Based on the results observed here, the phenol-chloroform organic method is least successful with denim samples while the DNA IQ method is the optimal approach of the three examined here. Furthermore, when inhibition due to denim dyes is observed, the analyst has several approaches available: dilution of the extract, a clean up step using the DNA IQ kit, or the addition of more Taq Polymerase to the amplification reaction. All of these methods have been shown to reverse inhibition due to the dyes in the denim substrates.

#### **DNA, Dyed Denim, PCR Inhibition**