APPLICATION OF PRESSURE CYCLING TECHNOLOGY (PCT) REDUCES IMPACT OF PCR INHIBITORS

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A common problem in the analysis of forensic DNA evidence is the presence of contaminants or inhibitors. These contaminants co-purify with the DNA and inhibit downstream PCR. Typically, these challenged samples exhibit allele imbalance, allele dropout and sequence specific inhibition, leading to interpretational challenges. Contaminants may also present samples as low copy number samples, even when the DNA quantity is sufficient for PCR. Reducing the effects of inhibitors may increase the effective amplification yield of challenged low copy samples. Extreme high pressure may alter the conformation of some inhibitors and render them less effective at reducing the yield of PCR products. In an attempt to enhance the amplicon yield of inhibited DNA samples, pressure cycling technology (PCT) was applied to DNA exposed to various concentrations of hematin (0, 1.25, 2.5, 5, and 7 µM) and humic acid (0, 1.25, 2.5, 5, and 7 ng/µl). To assess the effect of high pressure on the inhibitors and subsequently, the PCR process, DNA quantity was measured by qPCR and the STR typing results were evaluated. The internal PCR control of a qPCR assay was shifted to lower Ct values in a number of pressured samples compared with non-pressured samples. In addition, these same pressured samples often yielded increased STR allele peak heights. Bone samples subjected to pressure did not show any affect with pressure with the qPCR assay; but there were improvements in STR amplicon yield. These observations support that an inhibitor may be present in a sample that has minimal effects on qPCR and yet may present inhibition during STR amplification. The results support that PCT can reduce inhibitory effects and thus enhances yield of amplified products of both hematin and humic acid inhibited samples. Based on the results obtained in this study, this method can improve the ability to type challenged or inhibited DNA samples. ⌘