OPTIMIZATION OF REDUCED VOLUME POLYMERASE CHAIN REACTIONS (RV-PCR)

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Recent studies using AmpF*l*STR® Profiler Plus® kits have shown that reducing the standard volume of the reactants (RV-PCR) increased the likelihood of obtaining useable results four-fold when limited quantity forensic DNA samples were amplified. The method of performing RV-PCR required drying DNA samples in PCR tubes to concentrate the limited quantity of DNA. Storage buffers, such as TE^{-4} and Qiagen AE (QAE), contain EDTA to protect DNA; however, EDTA inhibits DNA amplification by chelating Mg^{2+} from the reaction mixture. The lack of free Mg^{2+} caused the efficacy of the RV-PCR reaction to decrease when greater than 10 µL of DNA sample contained in TE^{-4} were dried. When greater than 20 µL of sample were dried, RC-PCR amplification was severely inhibited.

This research first investigated the effects of amplifying the dried DNA samples in two storage buffers, TE^{-4} and QAE, used at the North Louisiana Criminalistics Laboratory (NLCL). Next, this study focused on overcoming the adverse effects caused by the EDTA in these storage buffers by adding $MgCl_2$ to the AmpF*l*STR® reaction mix. And finally, PCR inhibitors such as melanin and wood were studied to determine possible effects on RV-PCR when present and dried down with the DNA extract.

Results showed that amplification of DNA contained in both TE^{-4} and QAE buffers was inhibited at all volume levels tested. The addition of $MgCl_2$ to the AmpF*l*STR® reaction mix overcame the inhibition for DNA samples dried in TE^{-4} buffer, but was unsuccessful at overcoming inhibition for DNA samples dried in QAE buffer. And finally, results showed that at the levels tested neither wood nor melanin inhibited RV-PCR.