B103 Obtaining Typable DNA From Bloodstains That Serologically Test Negative

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The goal of this presentation is to make the forensic community aware that typable DNA may be extracted from bloodstained material even when negative presumptive and/or confirmatory test results are obtained.

This presentation will impact the forensic community and/or humanity by changing collection and screening protocols and/or interpretation guidelines for biological evidence testing to assure that valuable forensic evidence is not overlooked.

The goal of this presentation is to make the forensic community aware that typable DNA may be extracted from bloodstained material even when negative presumptive and/or confirmatory test results are obtained. This research project was undertaken to determine if typable loci could be obtained from washed and treated bloodstains and how these results compare to serological results, specifically with the ABAcard HemaTrace confirmatory test.

Human blood was diluted down to a concentration of 1:500 (undiluted, 1:20, 1:100, 1:250, 1:500), applied to a cotton t-shirt, and then subjected to treatments of machine washing in water, Tide, or bleach. Each sample was then tested using the confirmatory ABAcard test. DNA was extracted by traditional organic methods (phenol/chloroform) followed by quantification of human DNA by the Quantiblot method. Multiplex STR amplification was performed using the ABI AmpF*l*STR® Profiler Plus PCR kit. The resulting PCR products were separated and detected by capillary electrophoresis on the ABI 3100*Avant* Genetic Analyzer and data analysis performed using ABI GeneScan and Genotyper software.

The untreated whole bloodstains and undiluted Tide-treated bloodstains were the only samples to produce a positive reading for hemoglobin with the ABAcard test. All other treated *and* untreated diluted bloodstains gave a negative ABAcard result after washing. However, previously published reports suggest that the ABAcard tests are quite sensitive and possibly able to test positive for whole blood or bloodstains that have been diluted down to 1:1,000,000. Thus, it is noted that traditional washing procedures (regardless of treatment) can significantly alter the hemoglobin molecule such that negative results may be shown even when stain extracts actually contain human blood.

Results from the DNA profiles indicated that typable DNA was present in many of these treated and diluted bloodstain samples that initially tested negative using the ABAcard confirmatory test. While negative ABAcard results were obtained for all but two stains (undiluted, untreated and undiluted Tide-treated bloodstains), complete or partial STR profiles were obtained from all untreated stains (all dilutions) and several of the treated stains (bleach or Tide). In washed, untreated whole bloodstains, complete profiles were easily generated. In addition, partial profiles (>50% of STR loci successful) were obtained from untreated bloodstain samples that had been diluted down to 1:500. Interestingly, complete and/or partial profiles were also generated from the Tide-treated samples that were either undiluted or diluted down to 1:20. Furthermore, the undiluted bloodstains that were treated with bleach yielded a near complete profile, generating successful results at an average of 89% of all STR loci tested despite the fact that the confirmatory blood serology test on those bleach-treated samples had yielded negative results. This was a significant and unexpected finding because bleach is thought to severely denature most biological molecules, including DNA. Many additional trends were noted with the data generated from these experiments. As one may anticipate, the severity of DNA degradation increases with harsher treatment and along with that, the heterozygote balance decreases, the peak intensity diminishes, and the larger alleles are more prone to allelic dropout.

The data produced from these experiments show that confirmatory serology tests are not always reliable predictors of successful STR amplification. Because confirmatory tests such as the ABAcard may often be used in the field and the laboratory to determine what biological evidence to collect and submit for DNA analysis, a negative reading could (in some labs) prevent DNA analysis and thus result in lost forensic evidence. This research demonstrates the value of performing multiple serological screening tests prior to DNA analysis, as well as cautiously interpreting negative results, as typable DNA may be present at trace levels even when the serological protein marker is present at levels below the limits of detection for that test. Further research could investigate amplification of these treated samples using methods designed to detect low copy number DNA since these samples have limited quantities of DNA that vary in quality, similar to what is commonly seen in forensic case samples.

DNA, STR, ABAcard HemaTrace

 ** Presenting Author*