B148 The Evaluation and Optimization of DNA Recovery and Amplification From Bullet Cartridge Cases

Heather V. Milnthorp, MSFS, Center for Forensic Science Research & Education, 2300 Stratford Avenue, Willow Grove, PA 19090; Heather E. McKiernan, MSFS, Center for Forensic Science Research & Education, 2300 Stratford Avenue, Willow Grove, PA 19090; and Phillip Danielson, PhD, University of Denver, Dept of Biology, 2101 E Wesley Avenue, Lab 223, Denver, CO 80210*

After attending this presentation, attendees will better understand the complexities and challenges associated with the collection, extraction, amplification, and analysis of touch-type DNA samples commonly encountered with ballistics evidence. Attendees will also learn techniques for improving the probability of recovering sufficient DNA to generate interpretable Short Tandem Repeat (STR) profiles.

This presentation will impact the forensic science community by providing information on the optimization of commonly employed DNA collection methods and extraction techniques in an effort to improve the ability to successfully obtain interpretable DNA profiles from both fired and unfired cartridge cases without resorting to Low-Copy Number (LCN) DNA profiling methodologies.

Cartridge cases, both fired and unfired, are commonly encountered at crime scenes. According to the Federal Bureau of Investigation (FBI) crime statistics for 2015, 71.5% of homicides, 40.8% of robberies, and 24.2% of aggravated assaults involved the use of a firearm. Despite the high frequency and latent value of cartridge case evidence, these samples are not routinely submitted for DNA analysis, as it is a commonly held belief that it is difficult or impossible to recover DNA from this type of evidence. Underlying this belief is a preponderance of published studies on generating STR profiles from cartridge cases that indicate low success rates and/or minimal profiles that are unsuitable for comparative purposes.

To optimize DNA recovery from cartridge cases, numerous collection and extraction techniques were paired and evaluated. Five collection techniques (a tape-lift method, a double-swabbing wet:dry method, a double-swabbing wet:wet method, a soaking method accompanied by sonication and lyophilization, and a soaking method accompanied by vortexing and lyophilization) were paired with each of three extraction techniques, including phenol:chloroform organic extraction, PrepFiler™ Forensic DNA Extraction Kit, and the QIAamp® DNA Investigator Kit. First, 1,800 unfired 9mm cartridge cases of various metal compositions (450 each of brass, nickel-plated, aluminum, and steel) were tested with all pairings of techniques to assess possible impact of cartridge metal-type. Next, an additional 450 fired brass 9mm rounds were tested with all pairings of methodologies to assess the effects gunshot residue can have on profile quality. Finally, 230 unfired and 230 fired brass 45 Automatic Colt® Pistol (ACP) rounds were tested to assess the impact larger surface areas would have on optimum collection methods. All samples were quantified using the Life Technologies™ Quantifiler® Trio quantification kit and subjected to post-amplification concentration using an Eppendorf™ Vacufuge® plus vacuum concentrator. Samples were then amplified with the Life Technologies™ GlobalFiler® amplification kit and analyzed using Capillary Electrophoresis (CE) or analyzed using Massively Parallel Sequencing (MPS) methods.

For unfired samples of all metal types, extraction technique had the greatest effect on DNA recovery ($\bar{x}$ *Fs*=5.775; *df*=2,8; *p*=0.0103) with organic (phenol:chloroform) extraction producing optimum results. Recovery technique optimization was dependent on caliber size/surface area of the sample. For 9mm rounds, the tape-lift collection method consistently provided higher yields of DNA than other collection methods; however, for larger caliber ammunition the tape-lift method was inferior when compared to the two soaking methods. Analysis of fired samples indicates even greater differences in the success of profile generation when using optimized methodologies resulting in full and partial genetic profiles as compared to alternative combinations of recovery and extraction methods, which generated a total loss of genetic information. Comparison of these CE data to MPS data is underway.

This study demonstrates that by optimizing the methods employed for DNA collection and extraction, it is possible to increase the likelihood of obtaining good-quality STR profiles from cartridge cases without resorting to LCN DNA profiling methodologies. The presence of Gun Shot Residue (GSR) as well as the caliber of the cartridge case may impact the choice of optimal methodology and workflow. Further studies are targeted at identifying and mitigating the impact of possible co-eluting components of GSR or reactive metallic species from the casings themselves that compromise DNA profiling success through inhibition and/or augmented DNA degradation.

Touch DNA, Cartridge Casings, DNA Extraction