A122 Differential Extraction Conditions: Effects of Dehydration on DNA Mixture Quantification and Amplification

Elyse S. Cooper, BA, Robin W. Cotton, PhD, and Catherine M. Grgicak, PhD, Boston University School of Medicine, Biomedical Forensic Sciences, 72 East Concord Street, Room R806B, Boston, MA 02118*

After attending this presentation, attendees will be able to discern the effects Proteinase K concentration, SDS concentration, incubation duration, and temperature have on differential extraction efficiencies and the premature lysis of spermatozoa of dried samples.

This presentation will impact the forensic science community by aiding in the determination of appropriate differential extraction conditions that should be utilized to ensure efficient separation of the non-sperm and sperm fractions during a differential extraction.

Biological mixtures comprised of multiple individuals' tissues or bodily fluids are commonly encountered and gathered during crime scene evidence collection; however, the unraveling and distinguishing of individual contributors in mixed samples continues to be a difficult and complex facet of DNA analysis and interpretation. Determination of the total number of donors to the sample, the relative amount of DNA from each respective contributor, whether or not all data is accounted for, and the ultimate inclusion or exclusion of a known individual to the mixture are all complications that increase the intricacy of forensic DNA testing.

If the sample is comprised of sperm and epithelial cells, the DNAs can theoretically be successfully separated through differential extraction. This method relies on differences in cell membrane composition of epithelial cells and sperm heads. The first step of the differential extraction process typically involves incubation of the sample and the addition of Proteinase K and a surfactant (e.g., Sodium Dodecyl Sulfate (SDS)) to lyse epithelial cells. Because sperm heads contain a rigid network of protein disulfide bonds in their outer membrane, they are resistant to treatment with Proteinase K and surfactant and are left intact. After epithelial cell lysis, the sperm is pelleted by centrifugation and the supernatant, which contains DNA from the epithelial cells, is removed. This is referred to as the "non-sperm fraction." Following removal of the non-sperm fraction, a second cell lysis is performed to extract the DNA from the sperm cells. In addition to Proteinase K and SDS, dithiothreitol (DTT) is added to reduce the disulfide bonds in the sperm head, thereby lysing it. The release of the sperm's DNA results in the "sperm fraction." Work previously performed investigated how various differential extraction conditions affected the method's ability to yield two single source DNA extracts. It was concluded that only the presence of Proteinase K significantly affected the extraction. Additionally, the male contribution in the non-sperm fraction did not exceed 9% for any of the conditions tested.[1] This project is a progression of the aforementioned research and, in similar fashion, explores and advances upon those same parameters. In contrast to the aforementioned results, it has previously been reported that a significant portion of the sperm fraction is lost during the differential extraction process.[2] One of the differences between the two studies was whether or not the samples were dried. Since the

majority of samples received by crime laboratories are dried stains, it would be most beneficial to optimize the yield of DNA from the sperm fraction for these types of samples. Therefore, this study set out to discern the effects Proteinase K concentration, SDS concentration, incubation duration, and temperature have on the differential extraction efficiencies and the premature spermatozoa lysis of dried samples. The effect was quantified using qPCR and STR analysis and the concentrations of male and female DNA in the non-sperm and sperm- fractions were compared.

When comparing dried and wet samples, the results indicate that dried samples exhibit significantly more sperm lysis in the absence of DTT at all conditions analyzed. Preliminary results suggest the concentration (e.g., 0-300 µg/ml) of Proteinase K does not have a significant impact on the quantity of male DNA in the non-sperm fraction of dried samples. Likewise, SDS concentration had minimal impact on the male contribution in the non-sperm fraction when measured with qPCR.

Furthermore, modifications to storage conditions and rehydration steps typically associated with differential extractions were examined. If simple rehydration techniques aid in the reconstitution of destabilized cell membranes (thereby negating the premature lysis of spermatozoa during the extraction process), then such action would be advocated in the preservation and storage of biological mixture samples.

References:

1. Hennekens, Catherine, et al. Differential Extraction Conditions and the Premature Lysis of Spermatozoa: Effects on DNA Mixture Quantification and Amplification. *Proceedings of the 62nd Annual Meeting of the American Academy of Forensic Sciences,* Feb 22-27, 2010, Seattle, WA. Colorado Springs, CO: American Academy of Forensic Sciences, 2010.
2. Norris, Jessica V., et al. "Expedited, Chemically Enhanced Sperm Cell Recovery from Cotton Swabs for Rape Kit Analysis." Journal of Forensic Sciences 52 (2007): 800-5.

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 ** Presenting Author*