B129 The Use of Direct Polymerase Chain Reaction (PCR) on Semen and Spermatozoa and the Development of a Differential Isolation Protocol

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After attending this presentation, attendees will recognize the potential of direct PCR use in cases of alleged sexual assault to provide a higher level of sensitivity. Attendees will also learn a novel differential isolation protocol for use with direct PCR to obtain both female and male profiles.

This presentation will impact the forensic science community by demonstrating a more rapid method to determine the DNA profiles within sexual assault samples that also provides greater sensitivity. A differential isolation protocol for use with direct PCR will be presented to separate the male and female fractions of a simulated sexual assault mixture.

Sexual assault samples can be some of the most common samples encountered in forensic analysis. These samples can require a significant time investment due in part to differential extraction processes. This presentation reports on the first recorded use of direct PCR to successfully amplify semen for STR analysis. Amplification without prior DNA extraction, known as direct PCR, has gained increased interest in forensic science due to the reduced time to DNA profile and increased sensitivity of the technique. Benefits of direct PCR include reduced time and expense compared to standard DNA extraction prior to amplification. In this study, the potential for direct amplification of spermatozoa and seminal fluid is investigated in order to determine the donor. Stains containing seminal fluid (pure and mixtures) were subjected to the technique of direct PCR and a differential isolation method was investigated prior to direct amplification.

Neat seminal fluid, dilutions ranging from 1:5 to 1:160, and German DNA Profiling (GEDNAP) samples were successfully amplified using a direct method. All samples amplified full profiles to the 1:160 dilution in which approximately 50% of a full profile were obtained. All GEDNAP stains produced low-level but complete and reproducible profiles containing the same alleles as when analyzed using a traditional differential extraction technique. GEDNAP samples represent simulated case samples that are used for proficiency testing relating to presumptive testing and Short Tandem Repeat (STR) analysis. A mild differential isolation technique to enrich spermatozoa was developed and successfully implemented to separate and directly amplify a mixture of semen and female epithelial cells. Profiles resulting from the differentially isolated samples show high levels of amplification with good balance across all loci. Global balance ranged from 0.78 to 0.87 for liquid mixtures and 0.78 to 0.9 for dried mixtures. Aliquots of samples subjected to the differential isolation protocol were stained with hematoxylin and eosin for sperm scoring. To verify that the spermatozoa were being lysed during the direct PCR, samples stained after PCR showed a complete lack of intact spermatozoa. While the differential isolation protocol adds a

small amount of time to the direct PCR process, it resulted in strongly enhanced male profiles. Even in samples with an excess of female epithelial cells, direct PCR can produce male profiles with surprisingly strong peak heights if combined with the differential isolation protocol. Direct PCR can offer increased sensitivity with reduced time and cost for sexual assault samples compared with traditional differential extraction methods.

Direct PCR, Differential Isolation, Spermatozoa