A107 Enzyme-Based Preparation of PCR-Ready DNA From Semen and Mock Sexual Assault Samples

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After attending this presentation, attendees will have gained an understanding of the use of a neutral proteinase to extract DNA from sperm cells found in semen and mock sexual assault samples.

This presentation will impact the forensic science community by describing a novel method to extract male DNA without the use of a solid phase, which decreases analysis time and increases sample throughput.

Sexual assault samples often contain a mixture of sperm cells and female epithelial cells, typically on a cotton swab matrix. The most widespread method for the analysis of sexual assault samples, differential extraction, uses gentle lysing conditions to break open the female epithelial cells while leaving the sperm cells intact. The female DNA is removed and the sperm pellet is washed to rinse away excess female DNA. The sperm cells are lysed through the addition of a reducing agent, such as DTT, and both the male and female samples must undergo further purification to remove proteins, nucleases, and other inhibitory compounds before the DNA can be added to the polymerase chain reaction (PCR).

Typically, the samples are purified using a solid phase extraction (SPE) method, which utilizes a silica-based solid phase to reversibly bind DNA under high salt conditions while impurities are washed away. These extraction methods can have a number of sample transfer steps which may result in the loss of some DNA and reduce the probability of obtaining a full STR profile. Additionally, SPE is time-consuming, with many incubation and/or centrifugation steps and can take upwards of four hours to extract DNA from semen. [1] Finally, the chaotropic reagents used to load the DNA on the solid phase and the organic reagents used to wash away the impurities may inhibit PCR.

The issues that can hinder SPE methods can be nearly eliminated by switching to a method that does not use a solid phase. Liquid extraction has been used in the past in the form of a phenol-chloroform extraction, where proteins and lipids move to the organic phase and DNA moves to the aqueous phase; however, this method is time-consuming and hazardous. Recently, a liquid extraction procedure has been developed that uses a thermally-stable neutral protease to degrade cellular membranes, proteins, and nucleases.[2] The enzyme used (**Bacillus** sp. EA1) has optimal activity in buffers that are PCR-compatible, reducing the chance for PCR inhibition. This enzyme-based extraction method reduces the amount of sample transfer steps and, therefore, nearly eliminates any loss of sample that may occur, while yielding PCR-ready DNA after only 20 minutes. Commercially-available kits that utilize this enzyme can be applied to whole blood and saliva samples, but kits for semen sample analysis do not exist, primarily due to the incompatibility of the enzyme with most reducing agents.

The current work focuses on the development of a procedure for the liquid extraction of DNA from sperm cells. First, sperm cells are lysed during a brief incubation containing an alternative reducing agent. Then, an aliquot of the lysate is added to a solution containing buffer and enzyme and the sample is incubated for extraction of DNA in just 25 minutes. Using this method, DNA yield from sperm cells is significantly increased as compared to a traditional SPE method. Amplification results of genomic DNA from purified sperm cells show a full STR profile using this method. In addition, the use of this method towards sperm cells isolated from mock sexual assault samples will be demonstrated. Overall, this method represents an approximate three-fold reduction in average DNA extraction time, from 1.5 hours to 25 minutes as compared to conventional SPE methodologies.

References:

1. "Isolation of genomic DNA from sperm using the QIAamp DNA Mini Kit: Long Procedure" *QIAGEN, Inc.*. Accessed online at http://www.qiagen.com on 29 July 2010.
2. Moss, D, Harbison, SA, Saul, DJ. Int J Legal Med 2003;117:340- 349.
3. Lounsbury, JA, Nambiar, SM, Landers, JP. Analysis of Mock Sexual Assault Samples Using a One-Step Cell Elution and Preferential Lysis Method; Seattle, WA. American Academy of Forensic Sciences, 2010 Feb 22-27.

DNA Extraction, Differential Extraction, STR Typing

 ** Presenting Author*