B83 The Optimization and Validation of a Novel Direct-Lysis Differential Extraction Procedure

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Learning Overview: The goal of this presentation is to describe an innovative method for DNA extraction from sexual assault samples.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by providing a faster method for processing backlogged sexual assault cases.

Forensic analysis of DNA from sexual assault kits is a laborious process. Samples may be a mixture of sperm and male or female epithelial cells (E-cells). Since its introduction by Peter Gill in 1985, differential extraction has remained the essential pre-PCR extraction procedure adopted by most forensic laboratories.

The differential extraction procedure relies on the differences in the protein packaging of DNA in these two types of cells. The E-cells are first lysed by the addition of SDS and Proteinase K. These reagents alone are not able to lyse the sperm cells. The mixture is centrifuged leaving E-cell DNA in the supernatant and sperm cells in the pellet. After several wash steps to remove residual E-cell DNA, the sperm fraction is then subjected to lysis using SDS, proteinase K, and dithiothreitol (DTT). DTT reduces the disulfide bonds present in the sperm nucleus, thereby releasing sperm cell DNA.

The Gill method of differential extraction, while proven to be highly effective in providing two separate fractions for a simplified interpretation of DNA profiles, requires an average of approximately six hours of an analyst's concentration. To mitigate carryover from the female fraction, the sperm cell fraction is usually subjected to multiple wash steps. Furthermore, the resulting fractions must be subjected to additional pre-PCR DNA purification procedures to remove PCR inhibitors such as SDS and Proteinase K. These steps inevitably result in some DNA loss, particularly when few sperm were initially present in the sample.

Progress has been made in developing methods that allow for PCR-ready cell lysates that do not require additional purification steps (referred to as direct-lysis methods). However, thus far, none have been proven to be viable options for use in sexual assault samples. The authors' laboratory has developed a novel differential extraction procedure that is time-efficient, less laborious, and utilizes a direct-lysis procedure requiring no further pre-PCR purification for most samples.

The procedure uses two commercially available enzymatic products (ZyGEM and AcroSolv, from ZyGEM NZ Ltd) along with a nuclease (Benzonase®) to effectively lyse cells and produce PCR-ready E-cell and sperm cell fractions suitable for downstream nucleic acid amplification. The procedure uses the different optimal activity temperatures of the enzymes to perform most of the process in a DNA extraction lab thermal cycler, requiring only a single centrifugation for the usual separation of the epithelial cell fraction from the intact sperm. No subsequent washing steps for the sperm cell fraction are required.

In typical mixtures the novel direct-lysis differential extraction procedure recovers close to 100% of the sperm DNA from freshly prepared mock sexual assault samples of varied mixture ratios. Using samples with E-cell DNA: Sperm DNA ratios of 1:1, 5:1, 20:1, and 50:1 placed on cotton swatches aged for 4 weeks, 77%-100% of sperm DNA was recovered. DNA profiles generated from the sperm fractions were almost entirely from the male contributor, as indicated by 85%-100% mean peak height contribution.

Minimal, if any, female contribution with increased epithelial cell concentration was observed. The mean percentage of female peak height carryover was 8.4% for freshly-prepared samples and 14.6% for cotton swatches at a ratio of 50:1 E-cell DNA to sperm DNA. The procedure appears to be robust in producing DNA profiles from small amounts of sperm. Data from samples where the enzymatic procedures was either scaled up or scaled down will be presented.

The novel procedure can be completed in less than two hours using an extraction lab thermal cycler and requires no additional wash steps or pre-PCR purification procedures. Additionally, the procedure reduces the number of steps and sample manipulations, decreases carry-over between fractions, and increases sperm DNA recovery. It has the potential to be a rapid, robust procedure that can be easily implemented in any forensic laboratory. The presentation will describe the procedure and show additional data from progress in the procedure validation.

Differential Extraction, Sexual Assault, Sperm DNA Recovery

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