## A38 Girls Not Allowed: Erase the Mixture

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After attending this presentation, attendees will learn how to separate male and female DNA fractions in sexual assault cases without going through the cumbersome differential extraction procedure.

This presentation will impact the forensic science community by discussing a procedure that can be used for identifying the semen donor and separate the DNA of the male donor from the epithelial cells of the victim.

Forensic science laboratories process numerous cases involving sexual assaults. When the victim goes to a health care facility, a sexual assault kit is used to collect body fluids from various orifices. The swabs used to collect fluids from vaginal, rectal, and oral cavities often contain a mixture of the victim's epithelial cells and seminal fluid from the suspect. If the victim does not report the crime within a few hours, the number of epithelial cells from the victim can overwhelm the number of sperm cells present in the sample collected from the victim. Normally, a time-consuming and cumbersome differential extraction procedure is used in an attempt to separate the sperm DNA from the epithelial DNA. The resulting sperm DNA profile may yield a mixture of sperm and epithelial cell DNA even after repeated differential extraction wash steps.

The Erase Sperm Isolation Kit (Paternity Testing Corporation) can degrade DNA from epithelial cells, while leaving the spermatozoa intact. The DNA from the epithelial cells is selectively degraded by nuclease capable of digesting the DNA that is in solution. The sperm cell membrane protects the DNA inside the sperm heads and the nuclease has little effect in the protected DNA. This process makes it possible to obtain a single source sperm cell autosomal DNA profile from samples that also contain overwhelming amounts of epithelial cell DNA. Therefore, it is possible to obtain a single source male DNA profile from a mixture of seminal and vaginal fluid using the reagents in the kit.

The goal of this study was to determine if the Erase Sperm Isolation Kit could separate sperm cell and epithelial cell DNA from post-coital swabs, and from samples containing mixtures of body fluids. The objective was to generate single source male and female DNA profiles from various mixtures containing female body fluids and seminal fluid containing spermatozoa.

Swabs containing a mixture of seminal fluid and vaginal epithelial cells were collected at regular intervals and mock swabs containing a mixture of various female body fluids and seminal fluid were created. Portions of the post-coital swabs and the body fluid mixtures were digested in sterile tubes using the Erase Sperm Isolation Kit method. After digestion, the sample was centrifuged to pellet intact sperm cells. The supernatant was transferred from the original tube into a new tube. DNA was extracted from the supernatant containing the non-sperm fraction. Next, the nuclease was added to the original tube containing the sperm fraction in order to degrade the DNA free in solution. After the appropriate incubation period, the nuclease was inactivated and the sperm fraction was then lysed and purified. DNA was purified from both the non-sperm and sperm fractions using two methods: the Qiagen DNA Investigator Kit and organic extraction. Samples which contained mixtures of saliva, blood, and seminal fluid were subjected to the same procedure. Prior to lysing the sperm fraction, the pellet obtained from the sperm fraction was examined for the presence of spermatozoa using Kernechtrot Picroindigocarmine staining method and microscopy.

DNA was quantified using the Quantifiler® Human DNA Quantification Kit and amplified using commercially available PCR Amplification kits. The amplified products were injected on the AB 3130xl Genetic Analyzer, followed by analysis with SoftGenetics GeneMarker® HID software.

The results of the study showed that complete, single source male and female autosomal STR DNA profiles could be generated from swabs containing mixtures of seminal and female body fluids using the Erase Sperm Isolation kit. The method is cost effective and eliminates labor intensive and lengthy procedures used in standard differential extraction.

**DNA, Differential, Extraction**

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