VALIDATION OF THE SPERMX™ METHOD FOR DIFFERENTIAL EXTRACTION OF SPERM AND EPITHELIAL CELL DNA FROM SEXUAL ASSAULT KITS (SAK)

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A high throughput, fully hands-off, automated SAK processing method is an unmet need for crime labs worldwide. Such an automated SAK processing method will help reduce the SAK backlog, processing errors, forensic analyst workload, and labor costs. We report here the development and validation of a novel sperm DNA recovery device (SpermX), which effectively separates sperm cell DNA from epithelial cell DNA in sexual assault evidence. Differential extraction is needed to isolate sperm DNA from victim's epithelial cell DNA. The SpermX method provides an efficient, high throughput, automated, low-cost solution to obtain "clean" sperm fraction DNA profiles from SAKs. Validation studies fulfilling the *Quality Assurance Standards for Forensic DNA Testing Laboratories,* highlight SpermX's ability to separate sperm cell DNA in the presence of high numbers of female cells in evidence samples.

The performance of SpermX was evaluated using several mock forensic case-type male-female mixture samples. The input amounts used were based on the sperm and epithelial mixtures observed in 100 actual SAK swabs previously processed. Mock case-type mixture swabs were created by mixing known amounts of sperm cells with known amounts of female epithelial cells, utilizing both buccal cells and vaginal cells. Female DNA profile carryover is dependent upon female to male DNA ratio and overall amount of female epithelial DNA present in the sample.

The validation results showed that SpermX produced full male STR profiles with samples composed of up to 10,000 ng of female epithelial cell DNA in the presence of 7-90 ng of sperm DNA. With female to male DNA ratios ranging from 10:1 to 521:1, SpermX produced sperm fractions with the male donor as the major contributor. Samples with higher ratios (up to 1044:1) were still able to generate full male STR DNA profiles with the female as the major contributor. When over 12,000 ng of vaginal epithelial DNA and 17 ng of sperm DNA were present, female DNA carryover resulted in dilution of male DNA when 1 ng total DNA was amplified for STR (Identifiler Plus) analysis, resulting in the recovery of a partial male STR profile.

Sperm DNA recovery was evaluated by serially diluting approx. 2.72 million sperm cells in neat semen. This approx. 30,000-fold dilution resulted in samples with approximately 90 sperm cells per swab.

SpermX recovered 165 pg of male DNA from these swabs, which was sufficient to produce a full STR DNA profile of the sperm donor. The remaining validation results of the SpermX differential digest method for case-type samples, sperm recoveries from various substrates, and degraded sperm cells will be presented.

In conclusion, SpermX method provides a reproducible, highly reliable, and fully hands-free automated approach with male DNA recovery and male-female DNA separation for high throughput processing of sexual assault kits.

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