A New Spin on DNA Extraction: Validating GenSpin™ with SpermX™ Differential Extraction for Sexual Assault Evidence Processing

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Introduction

Differential DNA Extraction is a critical method in forensic DNA analysis, particularly for processing sexual assault evidence. The standard differential extraction process enables the separation of sperm cells from epithelial cells by exploiting the difference in cell membrane stability, ultimately allowing for the generation of discrete DNA profiles from mixed samples. Traditionally, differential extraction methods are manual, time-consuming, labor intensive, analyst- dependent, and subject to DNA loss during multiple transfer steps. These limitations have led to the development and evaluation of alternative extraction methods.

This study presents the internal validation of the GenSpin™ with SpermX™ Manual Differential Extraction Protocol, a novel method developed by a collaboration between InnoGenomics Technologies and Gentueri. The GenSpin™ with SpermX™ device utilizes a nanofiber membrane designed to selectively isolate sperm cells while allowing epithelial cell lysate to pass through, enabling a streamlined manual workflow with the potential for improved sperm recovery and cleaner separation of male and female DNA fractions.

Methods and Materials

Samples used in this validation were prepared by InnoGenomics and include a range of biological mixtures and sample types. These include controlled neat semen, saliva/semen, and semen/semen mixtures at varying ratios with varying known sample donors, and mock case samples. Additionally, proficiency test samples from Collaborative Testing Services (CTS), internal training samples N06-034 and N06-037, and post-coital vaginal swabs from anonymous donors were also used. Mixed DNA samples were applied to a variety of fabric substrates including cotton, denim, and polyester to evaluate the methods' performance across common evidence materials. UV degraded samples, that were degraded by exposure to UV light for 16 hours, were evaluated to mimic conditions that may be found in forensic casework. Samples were processed using the GenSpin™ with SpermX™ Manual Differential Extraction Protocol and compared to the laboratory's currently validated differential extraction method (EZ1). Each sample was quantified using 7500 Real-Time PCR System and PowerQuant® System and amplified using the PowerPlex® Fusion 6C System. Capillary Electrophoresis was performed with the Applied Biosystems® 3500 XL Genetic Analyzer using a 24-second injection time and a 1.2 kV injection voltage,

The following studies were assessed for the validation process:

Sensitivity Study

Precision and Accuracy Study

Mixture Study

Mock Case Samples

Contamination Study

Results

Sensitivity

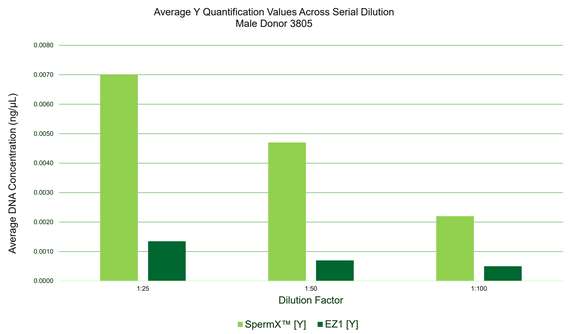

Figure 1: *Comparison of average quantification values (ng/µL) of the Y- target across a serial dilution, focused on the dilution of 1:25, 1:50, and 1:100 of semen from Donor 3805 for GenSpin™ with SpermX™ and EZ1 Extraction Methods using ABI 7500 with PowerQuant. Average was obtained from 2 replicates for each dilution ratio. All data was generated from the sperm fraction.*

	Autosomal			Y-Target		
	GenSpin™ with SpermX™	EZ1	Fold Increase	GenSpin™ with SpermX™	EZ1	Fold Increase
Neat	0.2472	0.0645	3.83	0.3220	0.0660	4.88
1:10	0.0229	0.0016	14.28	0.0281	0.0018	16.03
1:25	0.0110	0.0007	15.64	0.0104	0.0004	29.57
1:50	0.0028	0.0004	6.88	0.0045	0.0000	
1:100	0.0026	0.0002	12.75	0.0025	0.0000	

Table 1*: Comparison of fold increase in average quantification values (ng/µL) of the autosomal and Y targets across a serial dilution of semen from Donor 3805 for GenSpin™ with SpermX™ and EZ1 Extraction Methods using ABI 7500 with PowerQuant. All data was generated from the sperm fraction.*

Precision and Accuracy

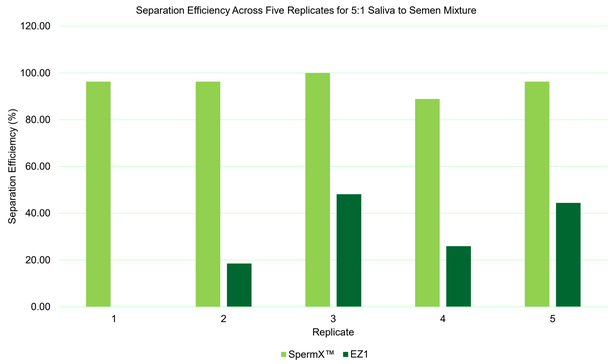

Figure 2*: Comparison of separation efficiency (%) across five replicates of a 5:1 ratio of female saliva (Donor 3809) to semen (Donor 3803) mixture using GenSpin™ with SpermX™ and EZ1 extraction methods. Separation efficiency was assessed by evaluating the presence of Donor 3809 (female) specific alleles; efficiency was calculated as the percentage of loci where no female alleles were detected. All data was generated from the sperm fraction.*

Mixture

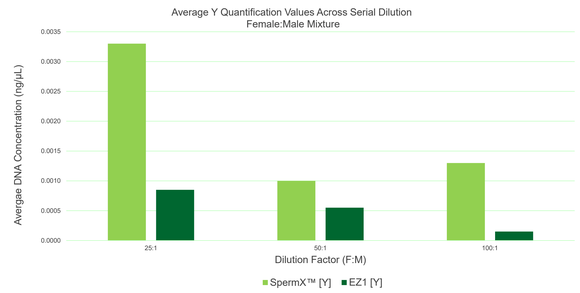

Figure 3: *Comparison of average quantification values (ng/µL) of the Y target across a serial dilution of a female saliva (Donor 3808) to male semen (Donor 3599) sample, focused on the dilution of 25:1, 50:1, and 100:1. GenSpin™ with SpermX™ and EZ1 Extraction Methods were used and quantification was done with ABI 7500 with PowerQuant. Average was obtained from 2 replicates for each mixture ratio. All data was generated from the sperm fraction.*

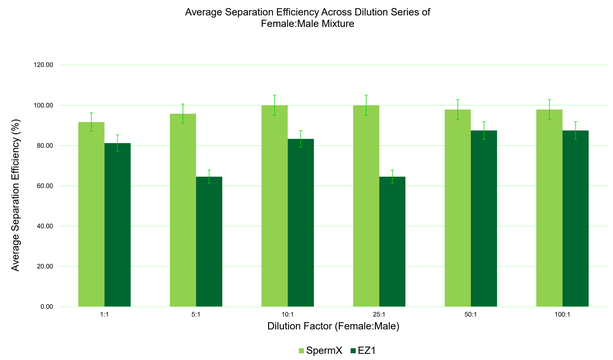

Figure 4: *Separation efficiency (%) across a dilution series of female saliva (Donor 3808) to semen (Donor 3599) using both the GenSpin™ with SpermX™ and EZ1 extraction method. Separation efficiency was assessed by evaluating the presence of Donor 3808 (female) specific alleles; efficiency was calculated as the percentage of loci where no female alleles were detected. The error bars represent the variability in the data of the 2 replicates for each mixture ratio. All data was generated from the sperm fraction.*

Mock Case

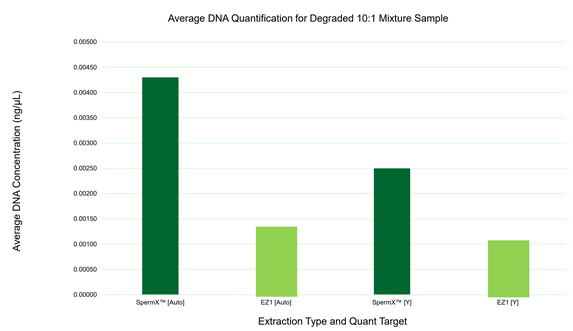

Figure 5*: Average DNA quantification results of autosomal and Y- target DNA for a degraded mixture of a 10:1 female saliva (Donor 3809) to male semen (Donor 3805) sample extracted using GenSpin™ with SpermX™ and EZ1 methods. Average was obtained from 2 replicates for each extraction method. All data was generated from the sperm fraction.*

Conclusions

Sensitivity

- DNA concentrations decreased with increasing dilution; however, the GenSpin™ with SpermX™ protocol demonstrated improved recovery at each dilution level compared to the EZ1 extraction method, including the most dilute samples (1:100).

Precision and Accuracy

- The GenSpin™ with SpermX™ method outperformed EZ1 in all replicates for mixture separation, with four of the five replicates achieving over 95% separation efficiency.

Mixtures

- The GenSpin™ with SpermX™ method consistently recovered higher DNA concentrations across all mixture ratios.
- The GenSpin™ with SpermX™ method consistently outperformed the EZ1 method across all dilution ratios for mixture separation.

Mock Case

- The GenSpin™ with SpermX™ method recovered over three times more autosomal DNA and 2.4 times more male DNA than the EZ1 method.

References

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