InnoGenomics Technologies, LLC
Final Summary Overview

Agency: National Institute of Justice
Award number: 2015-DN-BX-K072

Project Title: Highly Efficient Sperm Cell Separation from Sexual Assault Samples for DNA Analysis Using Novel Polymer Filter Technology

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Award Period: 01/01/2016 to 06/30/2018

Reporting Period End Date: 06/30/2018

Purpose of the project

The aim of the proposed project is to address the problem of differential DNA extraction from sexual assault samples, which is a time-consuming, laborious process for many forensic DNA laboratories. In addition, the commonly used standard differential extraction method for separating sperm cells from epithelial cells frequently results in low quantity or quality of mixed DNA profiles that are difficult to interpret, especially if the female to male DNA ratio is very high. In some cases, this method fails to generate a conclusive profile from the male assailant, thereby hampering the criminal justice system's ability to identify and prosecute offenders.

The proposed project utilized nanotechnology derived polymer filters ("nanofiber filters") that function as a filtration medium to effectively trap sperm cells while allowing efficient flow-through of digested epithelial cell DNA. This enables development of a method that could significantly increase a forensic DNA laboratory's ability to obtain "clean" sperm fraction DNA profiles while minimizing sample manipulations, thus providing a rapid, reproducible procedure that is easy to implement in a single-tube format as well as high-throughput 48 sample automated workflows.

In alignment with the stated goals of the NIJ's Research and Development in Forensic Science for Criminal Justice Purposes Program, this project focuses on the development of novel materials and methods that can separate the various components of a sexual assault kit mixture and have a strong potential to increase the quality of results, while also substantially reducing time, manipulations and cost for forensic analyses as compared to current standard practices.

It should also be noted that this project focuses on the application of nanotechnology to the forensic sciences, an innovative area of research identified by the National Institute of Justice (NIJ) for special consideration given its contribution to an important national research initiative.

Project subjects – Not applicable

Project design and methods

Objective #1

To evaluate multiple types of nanofiber filters (pore size & thickness)

After multiple evaluations and extensive research and development of differing nanofiber materials, InnoGenomics has successfully identified a suitable nanofiber material with specific diameter size, thickness and fiber orientation that meets the specifications for optimal sperm capture and isolation from sexual assault samples. The material does not bind any male/female DNA and has appropriate chemical characteristics to withstand enzymatic treatment, digestion and wash conditions. The final selected material can separate sperm cells from female DNA with over 90% recovery efficiency from samples containing both low and high sperm concentrations.

Objective #2:

To design and produce prototype cartridges of a nanofiber extraction filter column & optimize procedure

After several prototypes, InnoGenomics has successfully created a 3D printed prototype cartridge containing the nanofiber filter, and after extensive experimentation, evaluated it to be successful. This process included the creation and testing of numerous mock sexual assault samples and collaboration with a government operated forensic DNA testing laboratory that served as a beta testing site. Based upon this research and development, InnoGenomics has now successfully developed an injection mold to create these parts with polypropylene; a plastic that is low DNA binding. The unique design provides a functional, low touch interaction in order to process the sexual assault samples with a standard table top centrifuge. The developed product

is described as a 2 mL tube, filter and basket design that accommodates swing bucket centrifuges and thus reduces the amount of manipulation steps in the sperm isolation and DNA extraction process.

Objective #3:

To validate the nanofiber extraction filter columns according to the *FBI QAS for DNA Testing Laboratories* document, in part, as detailed below:

> *8.2.1 Developmental validation studies shall include, where applicable, characterization of the genetic marker, species specificity, sensitivity studies, stability studies, reproducibility, case-type samples, population studies, mixture studies, precision and accuracy studies, and PCR-based studies. PCR-based studies include reaction conditions, assessment of differential and preferential amplification, effects of multiplexing, assessment of appropriate controls, and product detection studies. All validation studies shall be documented.*
>
> *8.2.2 Peer-reviewed publication of the underlying scientific principle(s) of a technology shall be required.*

Sensitivity studies, reproducibility studies, mixture studies, accuracy studies and PCR-based studies were all extensively performed during the project period and after the prototype, "SpermTrap" filtration device was developed. Results of these studies are detailed in the Final Progress Report and have been presented at the 2018 Spring California Association of Criminalists Conference as well as scheduled to be presented in the 2018 AFFDA meeting in Houston. In addition, an abstract has been accepted for presentation at the International Society of Human Identification (ISHI) September 2018 meeting in Phoenix, Arizona entitled: **Novel Y-Screening and SpermTrap™ Differential Extraction: Streamlined Sexual Assault Kit Processing.**

Plans are also currently underway to complete the validation of the SpermTrap system and publish these findings in a peer-reviewed publication for dissemination to a wider audience of forensic scientists.

Objective #4:

To evaluate the ability to create a high throughput differential extraction method

InnoGenomics is collaborating with Hamilton, to make the process completely automated for sexual assault sample processing. Innogenomics has successfully re-configured the SpermTrap™ device to be compatible with the Hamilton automated liquid handling platform systems for ease of use and increased sample throughput at forensic DNA testing laboratories. InnoGenomics is actively discussing potential partnerships with organizations that currently sell automated platform systems to forensic DNA testing laboratories. The automated system can process 48 sexual assault swabs at a time resulting in 96 male and female DNA extracts, which can either be further purified by utilizing the laboratories already existing validated DNA extraction process or on the same robotic platform. The SpermTrap device is also embedded with a 2D barcode for automated sample tracking.

InnoGenomics is actively seeking partnerships with crime laboratories having the appropriate liquid handling platform, in order to validate the automated format version of the Sperm Trap test cartridges. InnoGenomics is finalizing the validation of the manual single tube format. Once completed, this validation will be published in an appropriate peer reviewed journal.

Data analysis

In order to evaluate the efficiency, sensitivity and accuracy of this novel sexual assault evidence collection and processing device, mock samples were prepared and processed utilizing the traditional, standard differential digestion protocols that are currently in use at most forensic DNA laboratories. These same samples were then processed utilizing the SpermTrap prototype and its associated protocols. Comparisons between these two methods were made. Efficiency was assessed based upon the time required to process samples with these two processes.

Sensitivity was assessed based upon serial dilutions of mock sexual assault swabs consisting of differing concentrations of male (sperm) and female (epithelial) cell samples. The SpermTrap system can successfully be used to process sperm samples that are fixed and stained on a microscope slide. The system has the ability to process and obtain results from mock sexual assault swabs containing up to 2000 fold more female DNA than the male DNA. Accuracy was assessed based upon the qPCR quantification results for the male and female DNA in each sample and subsequently generating STR DNA test results for the isolated and extracted DNA obtained for the male and female fractions, after processing.

Project findings and implications for criminal justice policy and practice in the US

The aim of this project was to address the problem of differential DNA extraction from sexual assault samples, which is a time-consuming, laborious process for many forensic laboratories. In addition, the commonly used standard method for differential extraction, often results in low quality, mixed DNA profiles that are difficult to interpret. In some cases, this method fails to enable generation of a conclusive DNA profile from the male assailant, thereby hampering the criminal justice system's ability to identify and prosecute offenders.

The developed SpermTrap system has the potential to provide a highly efficient and cost-effective alternative to standard differential extraction procedures. The SpermTrap system can enable forensic laboratories to process on an automated platform sexual assault kits (SAK) in an efficient, low cost process in order to obtain high quality sperm fraction DNA profiles from a greater percentage of sexual assault cases. This NIJ funded research has resulted in the development of a novel tool for crime laboratories in the processing of sexual assault samples and, thus enhancing the criminal justice system's ability to identify and prosecute sexual assault

offenders, and thus facilitating a reduction in the backlog of sexual assault evidence in the United States.

The SpermTrap™ device and reagents will be commercially available to any crime laboratory that processes sexual assault samples either manually or on an automated platform from InnoGenomics, by the end of 2018.