Novel Collection Device for Enhanced DNA Recovery and Release from Biological Stain Samples

FINAL SUMMARY REPORT

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Summary

The Final Technical Report contains the details of the study and thus is not described in detail herein. Those interested in more explicit technical aspects should consult the Technical Report (dated 06-30-2017 and accepted by NIJ). Instead, a summary of the findings is provided to briefly capture the topic of the research.

Success of DNA typing is related to the amount of target material recovered from an evidentiary item. Amounts of DNA too small or samples that are too damaged or degraded may not allow analysts to obtain sufficient results useful in identification of the source of a sample(s). Generally, the more DNA that is recovered, the better the chance is of obtaining a typing result that will be robust and reliable. One method of collecting stain materials is by swabbing. Recovery of DNA from a number of commercially-available swabs is known to be an inefficient process. Most devices currently employed by law enforcement for crime scene sample collection (e.g. cotton swabs) are inefficient in DNA release, resulting in less than desirable recovery of DNA and possible poor analysis. A superior technology that ensnares greater amounts of biological materials from crime scenes and is engineered to release the captured DNA in a manner conducive to yield high quantities and quality (if present in a sample) will be of substantial benefit to law enforcement in developing more investigative leads, solving more crimes, and excluding individuals not associated with limited quantity samples. This type of collection device ultimately will result in significant savings for investigators and the forensic laboratory. In conjunction with optimizing sample collection and DNA release, realizing more sensitive analytical methods can aid in current difficult-to-analyze sample types, e.g., touch DNA samples, remains from mass disasters, and missing persons identifications.

Materials proficient at collecting stain samples often are not efficient at releasing DNA from them. Conversely, materials proficient at releasing DNA are not the best for recovering samples from crime scene surfaces. Collection devices in current use are designed to recover biological stain evidence with reasonable efficacy; however, the release of analytes, DNA in particular, from the collection substrate represents only a fraction of the total DNA that is available. This shortcoming reduces the potential of acquiring sufficient sample and can compromise success of obtaining a genetic profile, especially for trace level samples. Marshall et al (1) reported on a collection material composed of a synthetic polymer named Diomat™ (Diomics Corporation, San Diego, CA), which could be dissolved under certain extraction conditions. Therefore, it was hypothesized that more DNA could be collected from a substrate and be released from the swab matrix than is possible with other swabs. Results using Diomat™ showed yields of DNA from blood and saliva samples as high as 80-90%, which far exceeded the performance of current stain collection devices. The yield of DNA, especially from low quantity samples, from Diomat™ outperformed another collection device, i.e., the Copan 4N6 FLOQSwab® (Brescia, Italy), a device which is touted to maximize DNA collection and elution efficiency. These results were preliminary but very promising. Given the impressive preliminary findings, further development and validation of Diomat™ collection devices were warranted.

This project proposed to develop and validate materials and devices, fashioned from synthetic polymeric materials available at Diomics, Inc., for effective collection of biological stains and efficient release of DNA from these devices. Using controlled samples, such as defined volumes of body fluids from known donors, the efficiency of DNA recovery was determined. The pick-up and release efficacy then was compared with currently used swabs. Controlled experiments were designed to measure amounts of sample acquired and the quantity and quality of DNA obtained from them to assist in selection of best materials. Selected Diomat™-based

devices then were tested under controlled conditions and subsequently under conditions simulating crime scene collection for performance assessment.

The selection of optimum materials and devices was guided by consideration of data obtained, by manufacturing feasibility, and by broad functionality of the device(s). The properties of the selected devices were evaluated by the ability to pick up minute samples and release the majority of DNA contained within them. Other relevant properties tested included collection on porous and non-porous surfaces, integrity of the swab matrix and device, ability to retain samples for long periods under adverse conditions, and adaptability to automated analytic procedures.

Overall the DNA yield from Diomat™ material was comparable to that reported by the Marshall et al study. DNA release and recovery from most of the Diomat™ materials did meet or exceed those observed with other commercially-available collection devices (e.g., cotton swabs, Copan 4N6 FLOQSwabs®). There were a few instances early on in which some lots of material did not yield high quantities of DNA; communication of these observations to Diomics resulted in materials with consistently high yield of DNA from stains. However, some lots of Diomat™ material remained relatively stable over time and across all storage scenarios and others varied quite substantially in performance (i.e., in terms of structural integrity and hydrophilicity). Data from our experiments suggested that the Diomat™ polymer provided to us had some level of instability and/or sensitivity to environmental factors (e.g., humidity, temperature, shipment or storage conditions, or time-since-production) that resulted in decreased and varied performance both after manufacturing and over time. In addition, there could be manufacturing issues contributing to variation in swab material which was supported by the difference in performance with the original material reported by Marshall et al (1).

In a number of lots the structural integrity of the swab material was delicate and fragile, falling apart and flaking quite easily during the swabbing process. When the swabs were rubbed on rougher surfaces (e.g., textured clothing, wood), appreciable flaking and disintegration of polymer material occurred. Therefore, testing was limited to smooth surfaces. Furthermore, while some film and card materials performed well, others fell apart during lifting of fingerprints or dissolved upon application of blood solutions. A potential solution to this instability could be the Diomat™ material to be synthesized in the form of long fibers that can be tightly wound on the end of an applicator (or stick). This approach should eliminate the structural integrity problems encountered with the material.

The hydrophobic nature of some of the swab heads/materials resulted in decreased absorption or non-absorption of hydrated stains, which is an important consideration for crime scene use in which complete as possible retrieval of a stain is paramount. The results were varied with some batches being very hydrophobic and others having some swabs being hydrophobic and others being quite hydrophilic (absorptive).

Although the Diomat™ material has shown high recovery (or yield of DNA), these other performance issues regarding hydrophobicity and lack of structural integrity have yet to be overcome. These features were not consistent and stable in a manner that would allow UNTCHI to fulfill the aims of this project. Therefore, the initial goals of developing and validating a Diomat™ material constructed swab or other collection devices could not be met. While the DNA yield is exceedingly high and promising, it is imperative that Diomics improve the other aspects that also are necessary before considering further assessment of this swab material and its performance. There is some promise as Diomics is reassessing its synthesis of the polymer approximating the conditions used to develop materials similar in nature to that used in the study

by Marshall et al (1). Once the desired features are achieved, it would be worthwhile to revisit the Diomat™ material as the yield of DNA was higher than any other currently-available swab.

Account of the Activities

The primary goal of this research was to: (1) assess performance of the Diomat™ polymer in X-Swab™ format. Due to the difficulties in obtaining consistent quality with thw Diomat™ material the next two goals, which were initially ancillary, were assessed: (2) investigate the performance of 4N6 FLOQSwab® system, with and without the Nucleic Acid Optimizer basket (NAO® Basket); and (3) compare the performance of these two substrates to the industry standard cotton swab. Both the Diomat™ X-swab™ and the 4N6 FLOQSwab® were hypothesized to enable superior recovery of DNA from stains, particularly those with low-level amounts of genetic material. In addition, the recovered DNA must be amenable to standard DNA typing procedures.

Accomplishments

Although the aims of this project could not be met as initially intended, the project did allow comparison with other swabs and some success was obtained with either improved collection and recovery or a more sensitive system with less consumption of evidence. Nylon flocked swabs, such as 4N6 FLOQSwabs® (Copan), were designed to maximize DNA collection and elution efficiency. In contrast to traditional fiber swabs, 4N6 FLOQSwabs® consist of short nylon fibers arranged in perpendicular fashion at the tip of an applicator shaft. Because these flocked swabs have no internal absorbent core to disperse and entrap the specimen, the sample remains close to the swab head surface, which facilitates analyte release and elution. Studies have shown that 4N6 FLOQSwabs® outperform traditional fiber swabs in terms of DNA recovery (2-4).

The Nucleic Acid Optimizer basket (NAO® Basket) (Copan) is an alternative to the traditional spin basket and reduces both labor time and sample manipulation during DNA extraction. Incubation and lysis of the sample occurs completely within the chamber of the NAO® Basket. After incubation, the NAO® Basket (designed with a collapsible grid bottom) can be used for a one-step collection of sample eluate from the swab head. With traditional spin baskets, the swab head is transferred manually to the basket and then subjected to centrifugation to recover remaining liquid (and DNA) trapped within the fibers of the swab. This manual transfer step increases the risk of cross-contamination and can result in potential loss of DNA.

In all cases, DNA recovery from the 4N6 FLOQSwab® outperformed the cotton swab with traditional spin baskets. In addition, there was added value in coupling 4N6 FLOQSwabs® with the NAO® Basket insert because it allowed for one-step processing of swab heads. Besides reducing labor and risk of sample contamination, the results suggested that processing swabs with the NAO® Basket increased DNA recovery by as much as 51.61% (compared to the traditional spin basket). The results of our study support that the most critical step in forensic DNA analyses – collection and recovery of typeable DNA − can be augmented with an efficient workflow by using the 4N6 FLOQSwab® system.

One of the newest designs of 4N6 FLOQSwabs® is the microFLOQ® Direct swab (co-developed by the French Gendarmerie Forensic Research Institute, IRCGNTM and Copan). The fibers of microFLOQ® Direct swabs are arranged in the same manner as 4N6 FLOQSwabs® but are treated with a lysing agent for direct amplification, eliminating the need for DNA extraction and quantification. The diameter of the swab head is approximately 1mm. Use of this swab can enable DNA profiling from sample collection to final result in less than two hours. Additionally,

due to the small dimension of the microFLOQ® swab head, only a minimal portion of a stain is collected, and thus there is far less sample consumption than traditional swabbing methods. Efforts that reduce sample consumption allow for more evidence to be retained for re-testing, if desired.

The efficacy of direct amplification of DNA from dilute bloodstains, saliva stains, and touch samples was evaluated using microFLOQ® Direct swabs and the GlobalFiler™ Express system. Comparisons were made to traditional methods to assess the sensitivity and robustness of this alternate workflow. Controlled studies with 1:19 and 1:99 dilutions of bloodstains and saliva stains consistently yielded higher STR peak heights than standard methods with 1ng input DNA from the same samples. Touch samples from common items yielded single source and mixed profiles that were consistent with primary users of the objects.

Samples collected with microFLOQ® swabs and direct amplification of DNA with GlobalFiler™ Express (Thermo Fisher Scientific) appear to allow for low quantities of DNA to be typed in a facile and expeditious manner. It was hypothesized that the swab design and presentation of the sample on the surface of the swab head would create an environment such that the sample essentially would be concentrated and accessible for amplification. Thus, smaller amounts of sample could be used for testing compared with traditional extraction-based DNA typing protocols. The results support that subsampling small portions of stains yields higher signal compared to full consumption of the same stains using the standard casework approach and 1ng input DNA.

The results of the microFLOQ® swabs study potentially may have important implications for analysis of low quantity and/or degraded samples that plague forensic casework. The purification step in the traditional workflow increases chances of sample contamination and results in loss of DNA. With direct amplification, no sample loss occurs and more template is available during amplification. Therefore, a better means for obtaining more complete profiles could be possible with direct amplification compared with traditional methods. Moreover, direct amplification of a sample on a microFLOQ® swab consumes a very small portion of the stain, preserving valuable evidence for re-analysis or additional testing.

With these features it may be worthwhile to consider an alternate workflow in which subsampling is performed first on all stains, and if the results are acceptable, no additional testing is performed. This approach would preserve precious sample for additional forensic analyses. If the results are limited or inconclusive, then the entire stain can be collected and extracted using traditional methods. One criticism of the direct amplification approach is that there is no quantification step, which is a current SWGDAM recommendation. However, one should keep in mind that quantification of extracted DNA is to ensure primarily that a sample is not over consumed (although other benefits occur with current quantitation methodologies). With the microFLOQ® direct amplification approach, the amount of sample used is so minimal that consumption is not a real concern. Essentially the entire sample is available if subsequent collection/extraction is deemed necessary. Additionally, since the surface area of a microFLOQ® swab is so small, it may be a better way to collect samples in areas that are difficult to access, such as seams of mechanical or electronic devices and cracks in flooring. Future studies should 1) evaluate the benefit of this alternate workflow in terms of time, cost, and sample consumption; 2) further validate this direct amplification method for collection of samples deposited on porous surfaces, such as textiles, and 3) determine if other direct amplification kits may be more refractory to the effects of inhibition.

References

1. Marshall PL, Stoljarova M, Larue BL, King JL, Budowle B. Evaluation of a novel material, Diomics X-Swab™, for collection of DNA. *Forensic Sci. Int. Genet*. 12 (2014) 192-198.

2. Brownlow RJ, Dagnall KE, Ames CE. A comparison of DNA collection and retrieval from two swab types (cotton and nylon flocked swab) when processed using three QIAGEN extraction methods. *J. Forensic Sci*. 57 (2012) 713-717.

3. Dadhania A, Nelson M, Caves G, Santiago R, Podini D. Evaluation of Copan 4N6 FLOQSwabs® used for crime scene evidence collection. *Forensic Sci. Int. Genetics Supp. Series* 4 (2013) e336-e337.

4. Verdon TJ, Mitchell RJ, van Oorschot RAH. Swabs as DNA collection devices for sampling different biological materials from different substrates. *J. Forensic Sci.* 59 (2014) 1080-1089.

Products Produced

In addition to the research results obtained, numerous presentations and a peer-review published paper have been produced that document the work.

1. Presentations at National and International Meetings that were supported by this work:

a. 4N6 FLOQSwabs® and NAO® Baskets: Innovative tools for recovering DNA from crime scene evidence. 27th International Symposium on Human Identification (ISHI), Minneapolis, Minnesota *(October 2016)*
b. Improved DNA recovery with the Copan FLOQSwab® system. 27th International Symposium on Human Identification (ISHI), Minneapolis, Minnesota *(October 2016)*
c. Increasing DNA recovery with nylon flock swabs and one-step spin baskets. American Academy of Forensic Sciences (AAFS) 69th Annual Scientific Meeting, New Orleans, Louisiana *(February 2017)*
d. Micro sample swabbing for reduced sample consumption, increased sensitivity of detection, and enhanced intelligence for processing biological evidence. 27th Congress of the International Society for Forensic Genetics (ISFG), Seoul, South Korea *(August 2017)*
e. Front-end DNA analysis enhancements: Collection, recovery, sample integrity, and improved workflow. 21st Triennial Meeting of the International Association of Forensic Sciences (IAFS), Toronto, Canada *(August 2017)*
f. Enhanced front end sample collection and extraction can improve DNA typing results and laboratory workflow. 9th Asian Forensic Sciences Network Annual Meeting and Symposium, Singapore (August 2017)
g. An alternate workflow for DNA analysis featuring increased sensitivity of detection and reduced consumption of evidence: Casework and legal implications. 28th International Symposium on Human Identification (ISHI), Seattle, Washington *(October 2017)*
h. Increasing DNA typing success with improved front-end processing and alternate workflow strategies. American Academy of Forensic Sciences (AAFS) 70th Annual Scientific Meeting. Seattle, Washington *(February 2018)*

2. Publications

a. Ambers A, Wiley R, Novroski N, and Budowle B. Direct PCR amplification of DNA from human bloodstains, saliva, and touch samples collected with microFLOQ® swabs. Forensic Science International Genetics 32 (2018) 80-87.

Invention Report:

There were no inventions or patents related to this research.

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Impact:

a. What is the impact of the project on the criminal justice system?

Success of DNA typing is related to the quality and quantity of the DNA recovered for analysis. Sample collection is the first part of DNA analysis, and the amount of DNA recovered is critical to the outcome of a DNA typing result. As more challenging samples are being presented for DNA analyses, the limited amounts of DNA housed in such samples must be efficiently recovered. Devices that yield greater recovery of DNA will facilitate downstream DNA typing and allow for more challenged samples to be analyzed.

Although the X-Swab™ from Diomics Corporation still needs further development, the material showed promise with DNA yield. Because of this high yield, we encourage

Diomics to continue development of the Diomat material and improve the integrity and solubility of the swabs. In addition, if solubility can be attained as was observed in a study by Marshall et al (1), the swabs would be well-suited for automation.

Studies with other collection devices – such as the nylon 4N6 FLOQSwabs® (and associated NAO® Baskets) and microFLOQ® Direct Swabs do offer potential for improving DNA recovery from forensic evidence. The microFLOQ® Direct swab, a miniaturized version of the 4N6 FLOQSwab®, has a small swab head that is treated with a lysing agent which allows for direct amplification and DNA profiling from sample collection to final result in less than two hours. Additionally, the microFLOQ® system subsamples only a minute portion of a stain and preserves the vast majority of the sample for subsequent testing or re-analysis, if desired. The efficacy of direct amplification of DNA from dilute bloodstains, saliva stains, and touch samples was evaluated using these swabs. Comparisons were made to traditional methods to assess the sensitivity and robustness of this alternate workflow. Controlled studies showed consistently higher yields (based on STR typing results) than standard methods with 1ng input DNA from the same samples. Touch samples from common items yielded single source and mixed profiles that were consistent with primary users of the objects. With this novel methodology/workflow, no sample loss occurs. This approach may have important implications for analysis of low quantity and/or degraded samples that plague forensic casework. In addition, this approach reduces sample consumption so that material may be available for re-testing or additional analyses.

b. How has it contributed to crime laboratories?

The current contribution cannot be assessed, but the anticipated outcome of this effort will be development and/or validation of a collection device (i.e., microFLOQ® Direct swabs) that increases DNA typing sensitivity of detection, particularly from low-quantity or trace materials. Samples that would otherwise provide limited results could be typable. Overall, a superior collection device that fits well into laboratory workflow and consumes only small portions of stains or touch evidence will increase the types of forensic samples that can be analyzed.

The results of the microFLOQ® study potentially may have important implications for analysis of low quantity and/or degraded samples that plague forensic casework. The purification step in the traditional workflow increases chances of sample contamination and results in loss of DNA. With direct amplification, there is less manipulation of a sample and essentially no sample loss occurs. A better means for obtaining more complete profiles was possible with direct amplification compared with traditional methods. Moreover, direct amplification of a sample on a microFLOQ® swab consumed a very small portion of the stain, preserving valuable evidence for re-analysis or additional testing.

The work here is foundational and will add to the base of peer review studies necessary to support the reliability and feasibility of direct amplification in casework. A large audience of international practitioners will be exposed to the outcome of this research

due to the "open access" publication of results from the microFLOQ® direct amplification study in an international journal. It is likely that some will undertake applying the methods or similar methods to their own work.

c. What is the impact on technology transfer? N/A

Changes/Problems:

The inconsistent performance of materials developed by Diomics seriously impacted the chance of success of the original research aims to provide a novel collection device that improved DNA recovery of crime scene stains and would be amenable to automation. However, Diomics has incorporated several of our recommendations and noticeable improvements have been seen in performance and development of the product over the course of the project period. Diomics continues to attempt to overcome current manufacturing challenges and to produce an X-Swab™ that consistently is hydrophilic, has sufficient structural integrity to remain intact during stain collection, and yields higher DNA recovery than other commercially available swab formats.

Towards the end of the project a greater focus was on the microFLOQ® swab as this swab coupled with direct amplification allowed for increased sensitivity of detection with nominal consumption of evidence.

Proprietary Information:

There was no proprietary information related to this work.