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Final Summary Overview

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Assessment of Rapid, Robust, and Efficient Postmortem DNA Sampling Method
for DVI and Missing Persons Identification

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Summary of Research Project

The ability to efficiently and rapidly identify large numbers of recently deceased persons using DNA can be a significant drain on an already overburdened forensic laboratory. Depending on the scale of the event, the laboratory will likely be required to extract DNA from a variety of post mortem sample types, family or direct reference samples related to the missing, and perform matching of these results in a very short period of time. Most forensic institutions are well equipped to handle both family reference samples and direct reference samples obtained from items related to the missing individual. Post mortem samples from the missing themselves can be difficult for labs to analyze due to their lack of experience with certain types of samples, such as bone, or to efficiently deal with the arrival of a larger number of samples at once.

Current guidelines for sampling may be further complicated due to a lack of basic resources at the site of the disaster. The purpose of this research was to evaluate an easily deployable, efficient, and inexpensive method for collecting postmortem DNA samples. A goal of this study was to assess the success of DNA typing of postmortem samples collected on commercially available, chemically treated, DNA preservation cards (“FTA[®]” cards). This study was designed to benefit both persons in the field collecting samples and the labs tasked with processing the samples. The properties of FTA[®] cards make them suitable to collect samples from both recently deceased individuals and decomposed bodies and present a good alternative to other extractions methods from tissue or bones. Moreover, FTA[®] cards are widely used in forensic labs and represent an ideal media for easy shipping due to their small volume and stability at room temperature. Additional study objectives included evaluating the method’s efficacy over time (postmortem interval or PMI), its success rate in different environmental conditions through the analysis of both the quality and quantity of DNA retrieved from these

samples, and whether results can be correlated to body decomposition.

Project Subjects

Project subjects consisted of donated human cadavers from the University of Tennessee, Forensic Anthropology Center (FAC) Body Donation Program. Individuals were selected based on having a time of death within 48 hours of receiving the body. Following established FAC Intake Protocols, all subjects were documented upon arrival. Individuals were anonymized with a donation number and biological samples (blood, fingernail clippings, and hair) were obtained. All subjects were refrigerated at 38° F for 24 hours to equalize body temperatures prior to placement outdoors at the Anthropology Research Facility (ARF), to decompose naturally.

Project Design and Methods

To examine the efficacy of postmortem DNA sample collection and profiling using FTA[®] cards, we conducted replicate controlled trials using human cadavers. Initial plans were to conduct 3 trials with 4 cadavers each, totaling 12 individuals. However, the summer trial began later than anticipated and only 3 bodies meeting the study criteria were received. Initial results from the summer trial indicated a markedly high rate of body decomposition and subsequent DNA degradation, necessitating the addition of a fourth trial with 2 bodies during the following summer. The purpose was to take a shortened sampling period in an attempt to capture the window where DNA degrades beyond the limits of this protocol.

Each trial took place during a different point in the calendar year to evaluate seasonal weather patterns and temperature effects on decomposition, DNA yield and rates of degradation. Trial 1 ran between 20 February and 16 April 2015 with late winter to early spring temperatures. Trial 2, covering summer months, ran from 4 August through 05 November 2015 and was characterized by temperatures often exceeding 95 degrees Fahrenheit with levels of humidity

approaching 100%. Trial 3 was conducted between 08 January and 01 March 2016, to capture winter temperatures characterized by periodic days below freezing and occasional snow. Trial 4 ran between 30 August and 16 September 2016.

Climatological information is necessary to determine correlations between ambient temperatures, stages of decomposition and DNA degradation. All temperature data used in the analyses were converted to Accumulated Degree Days (ADD) and only temperatures above 0°C were included in calculations (Megyesi et al. 2005). Two sources were used to collect climate data. Site-specific hourly ambient temperature was collected using a temperature data logger placed adjacent to the subjects. Daily mean temperatures were collected from the University of Tennessee Agricultural Campus Weather Station located approximately 0.5 miles from the ARF.

All subjects were photographed at intake and during placement. Photos were taken during each sampling event and decomposition progression was recorded using the Total Body Score (TBS) method (Megyesi et al. 2005), which evaluates body segments independently. Using a disposable scalpel, a 2" incision was made through the individual's skin into muscle tissue. The sampling site was random, based on visual evaluation for the most preserved appearing tissue and may have been taken from anywhere on the body. Two sites were sampled during every sample collection event to reduce anomalous results. Disposable tweezers were used, if necessary, to maintain the opening for the foam collection swab to be inserted. The swab was placed into the incision site and pressed or "rubbed" into the muscle tissue until saturated. The swab was then rubbed on an Indicating FTA[®] card until the color change indicating transfer was observed. A Sample Collection Form was used to document the collection date, time, and sample numbers along with the incision temperature, the specific locales on the body where the sample was collected, and additional observations if any. FTA[®] cards air dried on site within minutes

and stored in labeled manila coin envelopes in neutral room-temperature conditions at the FAC until the end of each trial. All samples from a single trial were photographed, packaged together and shipped with a chain of custody form and inventory, to the International Commission on Missing Persons (ICMP) DNA laboratory for testing.

The first sample was collected at the time of placement, recorded as day 0. Subsequent samples were collected every 5 days through 45 days post-placement. Frequent sampling was an approach designed to allow for close monitoring of the DNA yield's quality and quantity, to help determine when degradation began and quantify how rapidly it progressed during different environmental conditions. High heat and humidity during Trial 2 caused rapid decomposition and subsequently a rapid accumulation of degree-days. This necessitated more frequent sampling to ensure time-of-failure was not missed during the wider, 5-day interval used during cooler temperatures. Therefore, during the first summer trial, samples and data were collected from the two donors placed in early August every other day for the first twenty days before reverting to the standard five-day interval for days 21 through 45. The third donor, placed in September, was sampled every five days for the entirety of the 45 days. The frequency of sampling during Trial 4 was further increased to try and narrow the window where DNA degradation occurred. For the first 3 days following placement sampling occurred roughly every 8 hours; samples were collected during the next 2-3 days every 12 hours, followed by 24-hour intervals through day 9.

During all trials an alternate, direct-application collection was utilized concurrently to determine if failure to recover DNA profiles was a result of intrinsic full degradation in the sample, or if it was related to lower efficiency of the primary sampling method involving FTA[®] cards. This alternate media comprised a secondary collection using a CEP[®] Swab (Fitzco, Spring Park, USA), which is a multilayered paper tipped ejectable swab that is formulated from their

FP705 paper. Following the sampling with a foam-tipped swab, a CEP[®] swab was inserted and rubbed against the muscle tissue at the opposite end of the same incision, to collect cellular material. Instead of transferring the sample to an FTA[®] card, the tip of the CEP[®] swab was ejected from the stem into a 1.5ml tube. Unlike FTA[®] cards that dry quickly, the CEP[®] swabs were transferred to the laboratory and placed into a fume hood with the cap open to thoroughly dry. Once dry, the cap was closed and the tubes were organized, documented, and shipped to the lab in the same manner as, but separate from, the FTA[®] cards.

Data Analysis

The DNA immobilized on FTA[®] cards was analyzed using the following methods. A 1.2mm punch cut from the card was submitted to a series of washes with FTA[®] Purification Reagent and Tris-EDTA aimed to remove all cell debris and PCR inhibitors, leaving clean DNA immobilized in the cellulose fibers. This well-established purification protocol is fast, straightforward and is the method originally intended for the use of these cards. This protocol is easily transferable on robotic platforms and can also be combined with automated PCR setup in order to increase the throughput and to minimize the possibility for human errors. The purified 1.2mm punches were added directly into the PCR plates for the DNA amplification using the PowerPlex 21 system (Promega). A validated protocol using one 3.0 mm punch and the QIAGEN QIAamp[®] Micro kit was successfully used to extract DNA from postmortem samples deposited on FTA[®] cards. The DNA extracts obtained from the 3mm punches were quantified prior to amplification, which is helpful to monitor the evolution of the DNA quality and quantity over time and depending on environmental conditions as well as to assess if inhibitors have been co-purified during the extraction process. Moreover, access to a DNA extract allows performing multiple amplifications using the same markers to ensure the reliability of the results but also

typing the samples with additional STR loci or other genetic markers located on the Y chromosome or on the mitochondrial DNA. If a set of FTA[®] cards did not yield results, CEP[®] swabs were tested to evaluate the baseline DNA collected from a direct application in order to determine whether the swab to FTA[®] transfer or the postmortem interval was responsible for the reduced genetic yield. DNA extraction from the CEP[®] swab used manual larger scale protocols designed to recover the full amount of DNA that was collected.

For DNA quantification, Quantifiler Human DNA quantification kit (Applied Biosystems) was used as it allows the detection of PCR inhibitors while providing concentrations values that are sufficiently accurate to determine the optimal DNA input for the subsequent amplifications. The PowerPlex 21 system was used for DNA typing all samples. This kit amplifies simultaneously 20 different autosomal STRs (D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D21S11, CSF1PO, FGA, TH01, TPOX, vWA, Penta D, Penta E, D1S1656, D2S1338, D6S1043, D12S391, D19S433) plus Amelogenin. The PowerPlex 21 system is part of the latest generation multiplex STR kits developed to handle challenging forensic samples. The kit has been validated at the ICMP for purified FTA[®] samples and postmortem DNA extracts and falls under ICMP's scope of accreditation. PP21 has been used successfully by the ICMP to type >1000 reference samples and hundreds of bone samples.

Findings

- There does not appear to be a consistent relation between ADD and DNA decomposition.

Instead, it seems that Total Body Score (TBS) better correlates with DNA in the samples. A TBS below 13 consistently yielded a full profile (e.g., Trial 1: Day 30; Trial 3: Day 45), and full profiles were achieved through a TBS of 17 during Trial 4.

- FTA cards and CEP swabs are comparable as collection media. However, while there is a great

amount of DNA extracted from the CEP swab, a larger portion of the material is required to extract versus FTA. Moreover, FTA cards dry immediately and are self-contained. CEP swabs are ejected and therefore require an additional container (microtube), which must remain open until the swab is completely dry. Additionally, a significant drawback for sampling with CEP swabs was their tendency to ‘self-eject’ in the incision, necessitating their recovery.

- It is likely that decomposition rates were increased due to multiple sampling events; opening the bodies to the elements likely had an effect on decomposition. When comparing the results from the two summer trials – the first produced no results after day 2; the second continued to produce full profiles at day 10 (when sampling ceased), clearly indicating that another mechanism is influencing the results. The difference is likely the sampling approach. During the first trial, samples were collected from random locations throughout the body, creating openings on all limbs and the torso. Conversely, sampling during the second summer trial began at the superior aspect of the body and progressively continued inferiorly towards the feet. This approach confined consecutive openings to one end of the body while leaving the other end relatively intact. This approach may more closely mimic natural decomposition rates otherwise accelerated by multiple sampling events.

Implications for Criminal Justice Policy and Practice in the United States

Implications from this research to criminal justice policy and practice in the United States include advancing a simple, cost-effective and efficient method for collecting and preserving a large number of postmortem DNA samples for human identification. Collecting and DNA profiling postmortem samples on FTA[®] cards can streamline the way forensic practitioners approach disaster victim identification (DVI). The tested FTA[®]-swab DNA collection method has numerous advantages over current standards. First, it does not require preservation methods,

such as refrigeration, freezing, dry ice or chemical input to stave off further genetic degradation – resources that are often inaccessible following a mass disaster. The established stability of DNA immobilized on FTA[®] cards obviates the need for shipping at cold temperatures. The FTA[®] card chemistry inactivates pathogens, reducing considerations of biohazard posed by either soft tissue or bone samples; this further reduces complicated regulations regarding shipping human remains. Additionally, the FTA[®] card method does away with the odor of putrefaction that is encountered with large numbers of soft tissue samples, reducing the need for handling in laboratory hoods or other air handling systems. Conversely, successful profiling from soft tissue and bone samples is complicated by the need for refrigerator or freezer storage to impede continued decomposition of the sample, with sample retesting requiring detrimental freeze-thaw cycles. The FTA[®] collection method avoids all of these difficulties. Second, this sampling format is easier for the person taking the collection and less invasive to the victim's remains than excising a bone sample. Finally, FTA[®] cards are significantly easier, faster, and less expensive to profile than bone samples.

Because of its ease of use, cost savings, and stability, the FTA[®]-swab DNA collection method could become the accepted standard for postmortem DNA sampling, preservation, and testing following a mass fatality incident or in any medico-legal setting given that the remains are not in a state of advanced decomposition. Certainly, rural jurisdictions lacking expertise and resources would benefit greatly from this comparatively cost effective and reliable method. However, even large jurisdictions with the infrastructure to adequately collect and analyze DNA according to current standards could realize significant savings. Therefore, the application of this DNA collection method in a medico-legal context can provide the criminal justice system and forensic science practitioners a tool to improve efficiencies in time and cost, as well as improve

disaster victim identification efforts.

Until now, samples taken for these purposes often include soft tissue, which continuously degrades without perseverative measures, or bone, which is costly and labor-intensive to test. A dual approach to samples could be taken to ensure the positive outcome of all cases in disasters, as the level of DNA degradation may not be known by visually assessing a case. The FTA[®] - swab collection, combined with collection of a bone sample, would allow the laboratory to expedite the analysis of FTA[®] which could, in certain instances, resolve DNA typing of the majority of cases. The remaining minority of problematic cases could then be prioritized for extraction from bone samples, thus significantly reducing the time and work load of the lab. This two pronged approach would be both cost and time effective, while minimizing the re-allocation of lab resources for infrequently encountered samples such as bone samples. As a result, the number of unidentified remains following a mass disaster, the time invested in establishing a positive identification and consequently, the financial burdens of a large-scale identification project would all decrease.

Uniform sampling, room temperature shipping and long-term viability of samples stored at room temperature, coupled with easy DNA profiling allows for efficient and rapid collection and profiling of postmortem samples in DVI events. Moreover, since this technology is widely used in laboratories across the United States, adaptation of current lab protocols to deal with postmortem samples collected on FTA[®] cards will require little validation efforts of current laboratory procedures.

A successful outcome of this research may be the significant impact it has on the US medical-legal system in the times of crisis that are occasioned by “mass disasters”. Mass fatalities may be of any scale with regard to the number of individuals and/or body parts that

need to be identified. Depending on the location/jurisdiction where an event occurs, local capacity may easily be overwhelmed with regard to the many legal and social obligations of the state to identify individuals and certify their deaths. This can be true of even small sized events in many jurisdictions (e.g., rural), and for truly large-scale events for any jurisdiction regardless of their advanced standing capacities. While seemingly a simple step, the tested sampling method can greatly enhance US capabilities at two stages that are often highly stressed in DVI response: collection of biological samples, and efficient processing in the DNA laboratory. Mass fatality events impose a chaotic environment, disrupting the normal flow of work and imposing a large additional burden to the official responsibilities of emergency responders, medical personnel and DNA laboratories. The method we developed is highly amenable to local preparedness plans. The process of sampling human remains is comparatively simple and can be performed by minimally trained personnel, as opposed requiring a forensic pathologist or anthropologist. Importantly, the materials needed are simple and easily put together as ready “go-kits” that can be on hand as part of preparedness resources: a package of sterile foam swabs, FTA[®] cards (optimally, bar-coded and integrated into a preexisting data and sample management plan), gloves and sterile disposable scalpels. The complexity of the response effort is further reduced as the need for sample preservation methods such as refrigeration, freezing, dry ice or liquid chemical are not necessary with the FTA[®] card collection method. At the level of the DNA laboratory, DNA profiling can be achieved through steps very similar to, or easily adapted from, the normal working operations of standard forensic laboratories, which could ultimately minimize the re-allocation of lab resources for an extended amount of time on infrequently encountered samples, enabling the lab to return regular casework in a very timely manner.