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DNA from Archived Fingerprints**

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## Introduction

DNA analysis was introduced to the criminal justice system in 1986, and since then, there have been several improvements in forensic technology and commercial products allowing for short tandem repeat (STR) multiplexing from minute levels of template DNA, such as 100pg or lower (1-4). Due to such sensitivity levels, there has been a greater demand for analysis of touch DNA (mixture of corneocytes and sweat gland secretions) where low levels of DNA are often encountered (1,4-8). Several challenges have been discovered with touch DNA evidence samples. For example, there is no defined relationship between the amount of DNA that is deposited and the length of time associated with the contact (2,4,9,10). Low success rates have been reported due to numerous accounts of partial/null profiles (4,11), and the resulting electropherograms often reveal mixture profiles (11) with the potential of having no probative value to the criminal case depending on the complexity of the mixture and circumstances of the criminal activity.

Historically, latent fingerprints were located, photographed, and frequently visually enhanced via physical/chemical treatment (6,12-14). Beyond this, it was common for fingerprints to be collected with adhesive tape then affixed to and stored on paper backing cards for archiving (archived latent fingerprints) (8). However, Van Hoofstat et al. and several other researchers, showed that a DNA profile could be generated from fingerprints, and, as a result, collection and storage methods for fingerprints have changed over time (6,13,14). When DNA analysis is the only desired examination, fingerprints will often be swabbed directly (*in situ*) in place of the archiving method described above (1-3,15-17). Nonetheless, archived latent fingerprints continue to exist as evidence from many older cases; these specific samples could therefore be a valuable source of DNA evidence even after numerous years of storage. In some cases, archived latent fingerprints may, in fact, be the only physical or biological evidence that is available. However, due to the minimal success rates reported and scarce research on DNA typing from archived latent fingerprints collected and stored in this manner, cases are prolonged and archived latent fingerprints are often overlooked or dismissed as viable sources of DNA (11).

Given the low success rate for STR typing from touch evidence samples and the unique challenges associated with archived latent fingerprints, it is important to consider alternative methods that can be incorporated into the traditional DNA workflow while simultaneously identifying the most optimal parameters for traditional steps of the workflow. Several previous studies have attempted to examine sampling technique and swab diluents (2,3,7,15,18-21). Beyond sampling, DNA extraction, purification and concentration methods can impact the quality and quantity of DNA yields. Forensic laboratories often utilize organic purification methods or commercially available kits for initial cell lysis and DNA purification. Nevertheless, neither an optimal collection technique, nor DNA extraction method, has been identified specifically for archived latent fingerprints. Moreover, low template DNA samples (such as these) typically require a concentration step or post-amplification purification (22). Thus, both initial template DNA concentration and post-amplification purification for removal of salts and primers should be tested as part of the DNA analysis workflow for archived latent fingerprints.

In addition to a lack of information regarding the DNA testing of the archived latent fingerprints themselves, there seems to be little research on how much DNA is actually left behind on a surface *after* a fingerprint is lifted for archiving. If sufficient touch DNA is left behind such that an STR profile could be developed, this could signal a need for investigators to change the way latent fingerprints are collected, as it may prove useful to also collect a surface

swab (post tape-lifting) for DNA testing. If the DNA is viable, this could prevent the need to destroy the fingerprint ridge patterns in order to conduct DNA analysis. Thus, it is important to determine empirically if it is possible to obtain detectable DNA and STR profiles from non-porous surface swabs taken *after* latent fingerprints had been tape-lifted.

Ultimately, there is limited knowledge regarding appropriate DNA analysis procedures for processing archived latent fingerprints or surface swabs after lifting, and there are very few reports on STR profiling success rates from these unique samples (2,4-6,11,13,15,16,18,20,21). Thus, this comparative research study focused on identifying the best combination of DNA sampling, DNA extraction and DNA concentration methods that could be used along with traditional STR amplification and detection methods for archived latent fingerprints. Furthermore, additional studies sought to evaluate supplemental analytical steps to determine if they could further improve STR typing success of archived latent fingerprints. Lastly, we sought to determine the best crime scene swabbing procedures that would maximize the ability to capture the residual touch DNA left behind after lifting of a latent fingerprint.

## Goals & Objectives

The overall objective of this project was to evaluate analytical procedures for the processing of archived, paper-backed, latent fingerprint lifts. As a result of this work, we wanted to be able to make recommendations to both the crime scene, criminal justice, and forensic DNA community on best practices for latent print collections based on substrate (when DNA may be pursued), on processing archived prints in the laboratory, and on what to expect from these types of samples based on time since collection and the collection methods used. We hope that the results of this work will fill the previous void in the literature on DNA processing of latent fingerprints. Specifically, our goals were:

- 1) Generate tape-lifts of treated and untreated latent fingerprints (and reference buccal swabs) from a minimum of 15 volunteers on both a non-porous and porous substrate, using both standard modern-day handling and historical methods. Collections would include swabs of all non-porous surfaces to be collected *after* lifting of the latent prints.
- 2) Determine the best methods for upstream laboratory processing for latent fingerprints that have been tape-lifted and backed on paper backing cards, including an evaluation of DNA sampling techniques, swab diluents, and extraction/purification methods.
- 3) Used best practices established empirically in above-stated goals to assess source attribution of major and minor profiles generated from the samples to study the potential to detect exterior surface contamination from handlers, to study the effect of multiple-use brushes on resulting DNA profiles, to determine the effects of archival time on DNA success, and to test various “low-template” methods to improve success rates.

## Materials and Methods

### *Sample Collection & Fingerprint Visualization*

A total of 15 volunteers provided latent fingerprints and reference buccal swabs for this study. All samples were collected in accordance with Virginia Commonwealth University (VCU) Institutional Review Board (IRB) regulations and personal protective equipment (lab

coats and gloves) were worn by the collector throughout the entire research study. Additional, detailed methods describing sample collection can be found in Solomon et al. and Hytenin et al. (50, 51). All samples used in this study were individually packaged and stored at room temperature for approximately four weeks or for a period of two years ("Aged" samples). A subset of samples (from either 10 or 5 volunteers) was used for initial experiments designed to optimize methods for processing (Goal 2); samples from all volunteers (15) were processed for subsequent studies (Goal 3). Fingerprint "sandwiches" are referred to throughout as archived latent fingerprints (each sample consisting of only one fingerprint from a single volunteer) and are used for all experiments described herein. After storage, all experimental samples were disassembled, sampled for DNA, and processed as described below.

Following tape-lifting of each set of prints, the glass surface area of a single latent fingerprint was swabbed using one of two different methods – the single swab (n=45) or double swab (n=45) technique. Additional, detailed methods for this study can be found in Hytenin et al. (51).

Reference DNA was extracted with the QIAamp® DNA Blood Mini Kit (QIAGEN®, Valencia, CA) on the QIAcube® (QIAGEN®) following manufacturer's protocol (23). After extraction, all reference samples were processed as described below.

#### *Biological Sampling of Archived Latent Fingerprints*

Three DNA sampling techniques - direct cuttings, single, and double swab techniques (24) - were compared against three visualization treatments - untreated, magnetic powder-treated and black powder-treated. Each data group included analysis of a single archived latent fingerprint from ten volunteers (n=10 for each). Each fingerprint sample was outlined on the exterior portion of the adhesive, the adhesive was pulled away from the paper substrate, the fingerprint was outlined on the interior portion of the paper, then both the adhesive and paper portions of the single fingerprint were sampled using one of three techniques. For direct cuttings, ~3mm x 3mm pieces were obtained using sterile forceps and laboratory scissors (Fig. 1). Details for sampling methods can be found in Solomon et al. and Hytenin et al. (50, 51).

#### *Evaluation of Swab Diluents*

Five magnetic powder-treated samples from each of ten volunteers were sampled using the double swab technique. One set from each volunteer was sampled using each of the following five swab diluents - sterile, deionized water, Buffer ATL (lysis buffer) and proteinase K mixture (QIAamp® DNA Investigator Kit reagents), 2% sodium dodecyl sulfate (SDS) (Bio-Rad Laboratories Incorporation, Hercules, CA), 1X Triton X-100 (Thermo Fisher Scientific Incorporation, Carlsbad, CA), and 91% isopropanol (Fisher Scientific, Pittsburg, PA). Additional, detailed methods for this study can be found in Solomon et al. and Hytenin et al. (50, 51).

#### *DNA Extraction Methods*

Four individual archived latent fingerprints treated with each of the three visualization treatments from 10 volunteers were obtained. A set from each visualization treatment was processed using each of the following DNA extraction methods - phenol-chloroform organic extraction, QIAamp® DNA Investigator Kit (QIAGEN®), Invisorb® Spin Forensic Kit (STRATEC Molecular, Berlin, Germany) method, and prepGEM™ Tissue Kit (ZyGEM®, Hamilton, New Zealand) (25-27). All DNA extracts were stored at 4°C until quantification and concentration. Additional, detailed methods for this study can be found in Solomon et al. and Hytenin et al. (50, 51).



### *DNA Concentration Techniques*

Quantified DNA extracts from four individual archived latent fingerprints visualized with magnetic powder from 10 volunteers were obtained. A set from each visualization treatment was concentrated using each of the following methods - vacuum centrifugation, Microcon<sup>®</sup> Y-100 DNA Fast Flow Centrifugal Filters with Regenerated Cellulose Membrane, Centri-Sep<sup>™</sup> Spin Columns (Thermo Fisher Scientific) and the NucleoSpin<sup>®</sup> DNA Clean-up XS Kit (MACHEREY-NAGEL GmbH & Company, Bethlehem, PA) (28-30). All DNA extracts were concentrated to 0.2ng/μL if at least 1ng (total) was available; if 1ng of template DNA was not available, the entire template DNA extract was concentrated to 5-8μL and this entire volume was used in the STR amplification (described below). All concentrated DNA extracts were stored at -20°C until STR amplification took place. Additional, detailed methods for this study can be found in Solomon et al. and Hytenin et al. (50, 51).

### *DNA Analysis Methods*

All reference DNA and archived latent fingerprint DNA samples were quantified with the Investigator<sup>®</sup> Quantiplex Kit (QIAGEN<sup>®</sup>) using the ABI PRISM<sup>®</sup> 7500 Sequence Detection System (Life Technologies<sup>™</sup>, Carlsbad, CA) and SDS software v1.4.0 (Life Technologies<sup>™</sup>) following manufacturer's protocol, with one modification (half reaction volumes were used) (31). Samples that exceeded 0.2ng/μL were diluted to 0.2ng/μL and samples below 0.2ng/μL were concentrated as described above. The AmpFISTR<sup>®</sup> Identifiler<sup>®</sup> Plus PCR Amplification Kit (Life Technologies<sup>™</sup>) was performed with the GeneAmp<sup>®</sup> 9600 PCR System (PerkinElmer Incorporation, Waltham, MA) with analysis using capillary electrophoresis (CE) methods on an ABI PRISM<sup>®</sup> 3130 Genetic Analyzer (Life Technologies<sup>™</sup>) with data analysis using GeneMapper<sup>®</sup> ID software v4.1 (Life Technologies<sup>™</sup>) (32). Additional, detailed methods for this study and resulting data analysis can be found in Solomon et al. and Hytenin et al. (50, 51).

### *Evaluation of Post-Amplification Purification Methods*

After an initial CE analysis, remaining amplicons from fresh untreated and magnetic powder-treated fingerprints sampled via direct cutting from each volunteer underwent post-amplification purification followed by reanalysis on the CE. Five untreated and five magnetic powder-treated samples were subjected to the MinElute<sup>®</sup> PCR Purification Kit (QIAGEN<sup>®</sup>, Valencia, CA) while the remaining samples (five of each) were re-purified and concentrated with Microcon<sup>®</sup>-30 Centrifugal Filters (Ultracel<sup>®</sup> YM-30) with Regenerated Cellulose Membrane (Fisher Scientific) (33, 34). Additional, detailed methods for this study can be found in Solomon et al. (50).

Post-lift surface swabs from ten sets of magnetic powder-treated fingerprints and ten sets of untreated fingerprints collected with the double swab technique were selected for an evaluation of post-amplification purification study based on overall DNA yields. Additional, detailed methods for this study can be found in Hytenin et al. (51).

### *Evaluation of Aged Archived Latent Fingerprints*

All samples collected and aged (from all 15 volunteers) were processed after 2 years (±2 weeks) using the optimized methods and workflow described herein for comparison to aged samples processed using a more traditional forensic DNA workflow. The optimized workflow includes best practices as observed during the initial studies using fresh samples (DNA sampling using cuttings, DNA extraction using the QIAamp<sup>®</sup> DNA Investigator Kit (QIAGEN<sup>®</sup>), and post-quantitation concentration (as needed) using Centri-Sep<sup>™</sup> Spin Columns). The traditional workflow consisted of DNA sampling via the double swab method (with 2% SDS as the diluent),

DNA extraction using the QIAamp® DNA Investigator Kit (QIAGEN®), and post-quantitation concentration (as needed) using Microcon® Y-100 DNA Fast Flow Centrifugal Filters as described above. At the time of this report writing, approximately one-third of the samples collected had reached the 2 year mark for processing. That limited data is reported herein.

## Results and Discussion

### *Evaluation of Biological Sampling Techniques*

Overall, the average DNA yield from individual archived latent fingerprints for each sampling method tested was 0.45ng, 0.12ng and 0.17ng for cuttings, single swabbing and double swabbing, respectively. Cutting each side of the disassembled latent fingerprint sandwich resulted in higher DNA yields; only 3% of the samples tested using this method failed to produce detectable, quantifiable DNA versus 13% and 17% from the single and double swab methods, respectively (data not shown). When the effect of visualization treatment was considered, results for DNA recovery were not significantly impacted (Table 1,  $p = 0.645$ ). As expected, similar observations were noted upon analysis of STR amplification results. Archived latent fingerprints that were sampled with the cutting method were also more likely to produce at least a partial STR profile, with 4-100% of expected alleles successfully detected and one full STR profile observed (Table 1). Again, this observation held true regardless of visualization treatment. Generally, archived latent fingerprints that resulted in higher DNA yields produced more detected STR alleles (data not shown).

Interestingly, DNA yield and STR allele detection were highest from direct cuttings of magnetic powder-treated archived latent fingerprints. It is likely that the binding and natural adsorbent properties of the carbon in the powder, coupled with the soft magna brush that is formed, assist in keeping the integrity of the fingerprint pattern while also reducing the chances of inadvertently brushing touch DNA away (36-39). Furthermore, both swabbing techniques use an indirect DNA sampling method, where the cellular material is being transferred from the storage material to a secondary substrate (the cotton swab(s)) before moving to cell lysis and DNA extraction – this process may not sufficiently trap all of the available biological material from the evidentiary sample. In contrast, the direct cutting method allows for the direct placement of the biological material (attached to the original substrate) directly into the microcentrifuge tube for initial cell suspension and lysis. While the differences in success based on sampling method noted herein were not significant, the retrieval of biological material from archived latent fingerprints through disassembling and cutting both sides of the fingerprint “sandwich” for DNA extraction often improved both DNA yields and the detection of STR allele peaks.

Unfortunately, all DNA sampling techniques performed were destructive to the archived latent fingerprint sample, but other methods which are non-destructible, such as Electrostatic Detection Apparatus (ESDA®) may be useful if latent print pattern analysis is needed. Plaza et al., for example, implemented ESDA® collection on fingerprints deposited on various paper substrates and afterwards utilized DNA sampling techniques similar to those described herein; the pairing of ESDA® and dry swabbing outperformed ESDA® with wet/dry swabbing as well as direct cuttings in regards to DNA recovery and STR allele detection (35). Reported DNA yields were similar to those noted in the current study. However, ESDA® touch DNA collection was not tested using adhesive substrates or archived latent fingerprints. Despite this, the work of

Plaza et al. shows potential for a non-destructive DNA analysis procedure that could avoid the consumption of the fingerprint pattern (35).

#### *Evaluation of Swab Diluents*

Several studies have suggested that the swab diluent used for touch DNA swabbings could have a direct impact on the quality of the resulting STR profile [2,3,7,15,18]. Consequently, water, detergents, a set of commercial reagents, and isopropanol were used as diluents to pre-wet swabs for sampling archived latent fingerprints. Although more of the 1X Triton X-100 swabs and 91% isopropanol swabs tested (9/10 for each) provided detectable, quantifiable DNA yields versus the other three solutions (data not shown), the Buffer ATL (lysis buffer) and proteinase K mixture from the QIAamp® DNA Investigator Kit provided more DNA when DNA was detected in a sample (average 0.308ng) (Table 2,  $p = 0.387$ ). Likewise, on downstream STR amplification, the Buffer ATL/proteinase K swabs were more likely to produce at least a partial STR profile with 4-25% of expected alleles successfully detected (Table 2). While our findings concur with several other studies reporting that 2% SDS performs just as well, if not better, than water as a swab diluent (18,21), our studies further suggest that the use of cell lysis reagents outperform them both. Lysis buffer solutions typically include protein degrading enzymes that may prove to be beneficial for increasing the amount of available DNA. The opportunity to lyse human cells beginning with the point of collection may allow for the release of more DNA during the extraction procedure, consequently increasing DNA yields and STR amplification success. However, despite the improvements noted when a lysis buffer solution is used to pre-wet swabs used for sampling of archived latent fingerprints, the use of direct cuttings still outperformed the swabbing technique in both DNA recovery and STR profile quality (data not shown). Thus, DNA sampling via direct cutting is recommended specifically for archived latent fingerprints. However, if the workflow of an individual laboratory is optimized for swab samples, the use of a protein degrading enzyme coupled with a lysis buffer may provide better results than other diluents.

#### *Evaluation of DNA Extraction Methods*

Selecting a DNA extraction technique suitable for archived latent fingerprints is important given the unique nature of this particular substrate, which incorporates both a paper substrate and an adhesive substrate in a single sample. Thus, direct cuttings were taken from individual archived latent fingerprints (treated and untreated) and processed using one of four DNA extraction methods. This process was repeated three times with replicate sets processed using the other three DNA extraction methods. Overall, average DNA yields ranged from 0.45ng (QIAamp® DNA Investigator Kit) to 0.68ng (organic) ( $p = 0.91$ , data not shown). The use of the QIAamp® DNA Investigator Kit more often produced detectable, quantifiable DNA (29/30 samples tested) versus the organic method, prepGEM™ Tissue Kit, and the Invisorb® Spin Forensic Kit (28/30, 26/30, and 21/30, respectively) (data not shown). However, when the effect of visualization treatment was considered, there was no clear, single DNA extraction method that significantly improved DNA recovery (Table 3,  $p = 0.336$ ). Interestingly, inhibition was only detected in sample sets processed using the ZyGEM prepGEM™ method, regardless of visualization treatment (1/10, 3/10, and 4/10 samples untreated, magnetic powder-treated, and black powder-treated, respectively) (data not shown). Le Roux et al. reported the same observation noting inhibition in FTA paper samples whose DNA was purified with the same prepGEM™ method (40). Thus, given the many challenging attributes associated with touch DNA and archived latent fingerprints specifically, it is recommended that a post-extraction

purification/concentration step be considered if samples are processed using enzyme-based liberation assays or that these assays be avoided altogether when processing these sample types.

Samples processed using the QIAamp<sup>®</sup> DNA Investigator Kit also provided the most valuable STR profile data versus the other methods tested, regardless of visualization treatment (Table 3). This included one magnetic powder-treated sample in the group that produced a complete STR profile that was fully concordant with the associated reference (Fig. 2). It is important to note, however, that all STR data obtained in this study showed a traditional “ski-slope” effect (regardless of extraction method) with the shorter STR amplicons amplifying more efficiently than the larger amplicons. Absent any clear signs of amplification inhibition in all sample groups (other than the ZyGEM prepGEM<sup>™</sup> method), one could most likely attribute this amplification pattern to probably DNA degradation. Steadman et al. reported similar observations of partial STR profiles from archived latent fingerprints showing the same “ski-slope” pattern after one week room temperature storage (11). Taken together, these data indicate that a silica-column based method may be the best approach when processing archived latent fingerprints, specifically the QIAamp<sup>®</sup> DNA Investigator Kit. In addition to the performance detailed above, the QIAamp<sup>®</sup> DNA Investigator Kit protocol includes fewer tube-to-tube transfer steps than many methods (less opportunity for contamination), offers a safer alternative to traditional organic chemicals, is easily automatable, and is already widely used within the forensic community (41,42).

#### *Evaluation of DNA Concentration Techniques*

The aforementioned studies exclusively utilized Microcon<sup>®</sup> Y-100 filters for concentration of template DNA prior to STR amplification. However, to determine the most effective method for post-extraction concentration of low yield DNA samples (as obtained herein), the DNA retrieved from one set of archived latent fingerprints from different individuals (n=10) were quantified then concentrated using only vacuum centrifugation, while two additional replicate sets of these samples were processed identically, but with either the Centri-Sep<sup>™</sup> Spin Columns or the NucleoSpin<sup>®</sup> DNA Clean-up XS Kit for concentration. At least partial STR profiles were obtained from 9/10 Centri-Sep<sup>™</sup> samples tested (with 7-100% of expected alleles observed), 2/10 samples tested using the Microcon<sup>®</sup> Y-100 method (with 4-100% of expected alleles observed) (Table 4). One complete DNA profile was obtained from the Microcon<sup>®</sup> Y-100 sample set while two complete profiles were obtained from the Centri-Sep<sup>™</sup> samples (Fig. 3). Unfortunately, none of the 10 samples concentrated using the NucleoSpin<sup>®</sup> method produced any detectable STR alleles and only one sample exhibited a single allele from samples tested using vacuum centrifugation only for concentration (Table 4). The total absence of STR alleles in profiles obtained after using the NucleoSpin<sup>®</sup> method is likely due to the concluding step in the NucleoSpin<sup>®</sup> manufacturer’s protocol, which includes a 90°C incubation period (29,30). In the scientific literature, DNA degradation has been noted when high incubation temperatures are used; as such, several studies recommend omitting heat incubation periods if feasible (43,44).

The quality of the resulting STR electropherograms from archived latent fingerprint samples whose DNA was concentrated using the Microcon<sup>®</sup> Y-100 and Centri-Sep<sup>™</sup> methods was comparable. Both sample sets averaged STR allele peak heights greater than 900rfu (Fig. 4,  $p = 0.293$ ). As reported in the aforementioned studies, the ski-slope effect was consistently present in the electropherogram data. However, the profiles from the Centri-Sep<sup>™</sup> samples displayed an overall improved interlocus peak balance than those from the Microcon<sup>®</sup> Y-100 samples ( $p = 0.388$ , data not shown). Lastly, both the Microcon<sup>®</sup> Y-100 and Centri-Sep<sup>™</sup>



samples produced acceptable intralocus heterozygous peak balance ( $\geq 0.80$ ) in loci that displayed both expected allele peaks (Fig. 5,  $p = 0.231$ ). These data clearly indicate that the method used to concentrate low yield DNA samples prior to STR amplification can significantly affect the quality of the resulting STR profile. Work described herein suggests that the combined use of the QIAamp® DNA Investigator Kit followed by concentration using the Centri-Sep™ gel columns for processing of archived latent fingerprints will more likely lead to an STR profile sufficient for a human identification ( $\geq 8$  loci) or for exclusionary purposes (45).

#### *Evaluation of Post-Amplification Purification Methods*

In order to determine if post-amplification purification would improve STR profiles obtained from archived latent fingerprint samples, remaining STR amplicons from untreated and magnetic powder-treated samples were subjected to two different post-amplification purification methods. In initial testing without post-amplification purification, STR alleles were detected in four of the 20 samples tested from these sample groups (3 from the MinElute® samples and 1 from the Microcon®-30 samples). Adding a post-amplification clean-up step using MinElute® columns resulted in an additional two samples with partial STR profiles (Table 5) and an improvement in STR allele peak signal intensity (data not shown). However, this only amounted to a net gain of 6 additional STR alleles detected, which included 2 unexpected drop-in alleles. While no additional samples displayed partial STR profiles when post-amplification clean-up was added using the Microcon®-30 method, three additional STR allele peaks were detected from a single untreated fingerprint sample when using this method (Table 5). Overall, these results concurred with previous studies reporting higher signal intensity after performing post-amplification purification, but with an increase in STR artifacts (drop-in alleles), which further convolute the interpretation of the STR profile (22,46). This finding, in addition to the inherent contamination risk associated with crime scene processing of latent fingerprints, indicates that it may be best to avoid the addition of post-amplification steps in the DNA workflow (such as post-amplification purification) when processing DNA from archived latent fingerprints.

#### *Evaluation of Aged Archived Latent Fingerprints using Optimized Workflow*

The first of three large sets of aged samples has reached the two year mark at the time of this writing. These aged samples have now been processed using the workflow determined to be most ideal for this unique sample type and described above. For comparison, an additional set of aged archived latent fingerprints was processed using a traditional DNA analysis workflow.

Thus far, the data show that when latent fingerprints are lifted from paper (porous) surfaces and archived, the optimized workflow allows for a significant increase in the number of expected STR alleles detected. Unfortunately, the number of latent fingerprint samples processed from glass (non-porous) surfaces led to only a few samples that produced detectable alleles, thus no conclusions can be made with the samples collected from a non-porous substrate. Most notably, however, the aged samples tested show the presence of “drop-in” alleles (unexpected alleles from unknown contributors), regardless of workflow (in 5/11 traditional samples tested and 4/10 optimized samples). Drop-in alleles were not observed in the fresh samples processed in this study (described above). However, it is worth noting that the number of drop-in alleles observed above threshold in the magnetic treated samples was significantly less than those observed in the traditional workflow. Additional sample sets, as well as the source attribution and cause of the unexpected alleles detected in these samples will be further explored in the last reporting period of this grant (June-Sept. 2017) and as a part of goal 3, as proposed.

#### *Evaluation of DNA left on Non-Porous Surfaces After Fingerprint Lifting*

During the preliminary period of the research study, it was noted that upon disassembly of an archived latent fingerprint sample, only one-half or less of the total available DNA was found on the adhesive side of the fingerprint “sandwich” implying that the adhesive tape used for lifting may not be capturing all available DNA from a specific surface (50). As such, when adhesive is used to tape-lift latent fingerprints (and thus biological material) from a non-porous surface, one must consider the possibility that up to one-half of the DNA could be left behind on the surface from which the fingerprints were lifted. For this work, both a single swabbing method and the double swabbing method were evaluated. In this study, 64% of non-porous surfaces swabbed with a single wet swab after latent fingerprint visualization and tape-lifting produced detectable, quantifiable DNA, versus 51% for those swabbed with the double swab method (Table 1,  $p = 0.278$ ). Interestingly, none of the samples in this study (regardless of treatment) showed signs of inhibition, as all IPCs were within the expected range. Although the single swab technique was more likely to produce detectable levels of DNA in this study, when DNA was detected, surfaces swabbed with the double swab method (regardless of visualization treatment) yielded more DNA, on average, than those swabbed with a single swab technique (Fig. 6,  $p=0.882$ ). Using the double swab method, surface swabs yielded 0.07 – 0.25ng of DNA on average (across all treatment groups) while the single swab method yielded only 0.05-0.18ng. This is consistent with our findings when sampling techniques were evaluated for use with disassembled archived latent fingerprints (50) as well as the findings of other research studies, such as the Pang and Cheung study reported in 2007 (2).

As previously noted in our preliminary studies, more DNA resulted from samples taken from surfaces that had been treated with magnetic fingerprint powder for visualization versus black fingerprint powder and untreated surfaces, regardless of the swabbing method (Fig. 6). This difference was significant when compared to swabs obtained from surfaces that had been treated with black carbon powder ( $p = 0.023$ ).

All post-lift surface swabs used in this study were subjected to multiplex STR amplification for the purposes of developing an STR profile for human identification, regardless of DNA yield or concentration. Unfortunately, no STR alleles were detected above the validated analytical threshold (75rfu, data not shown). Nonetheless, this study provides concrete evidence that adhesive tape-lifting from a non-porous surface does not capture all of the viable biological material that is available. Furthermore, there is often enough biological material left behind from *a single fingerprint* such that valuable levels of quantifiable, detectable DNA are present and it is not being used for investigative purposes. While this may not yield enough DNA for a standalone STR profile, it is important to remember that individual fingerprints were the subject of this study. If used in combination with other sources of DNA (such as the DNA obtained from the associated tape-lifted archived latent fingerprint), the chances of obtaining a STR profile from the primary contributor would greatly improve. Moreover, surfaces in criminal investigations may result in the visualization of several fingerprints from the perpetrator; this study suggests that DNA from even swabbing one additional fingerprint area after lifting (for a total of two) would likely provide enough quality DNA to produce at least a partial STR profile of the culprit.

#### *Post-Amplification Re-Purification of Post-lift Surface Swabs*

Unfortunately, no improvement in STR allele detection was observed when post-amplification re-purification was used. There were no STR alleles detected from any of the post-lift surface swabs whose remaining STR amplicons underwent post-amplification re-purification



with either the QIAGEN MinElute® PCR Purification Kit or the Millipore Microcon®-30kDa Centrifugal Filter Units (data not shown). As such, other post-amplification alterations (such as those mentioned previously), as well as modification of pre-amplification steps (i.e. alternate DNA concentration methods prior to STR amplification) ought to be explored in an attempt to improve both the STR typing success as well as the STR quality of DNA profiles generated from archived latent fingerprint samples *and* their post-lifted surface swabs.

## Conclusions & Future Implications

Given the limited success rates already reported with modern touch DNA evidence and the lack of literature exploring methods specific for the DNA processing of archived latent fingerprints, forensic laboratories often understandably deter investigators who wish to submit these types of samples and/or deprioritize them (47-49). Despite these concerns, the work reported herein provides some evidence that should encourage laboratories and investigators to consider pursuing DNA analysis from archived latent fingerprints, particularly if they are the only potential source of physical or biological evidence available. These studies show that viable DNA is available in some archived latent fingerprint samples as well as in the surface area left behind after swabbing, and it can be retrieved for DNA profiling.

DNA laboratories have many choices when selecting specific analytical protocols to employ for each step of the DNA workflow. As expected, certain protocols may work best with specific sample types or substrates. Our findings suggest that when processing archived latent fingerprints for DNA analysis, disassembly of the fingerprint “sandwich” and making direct cuttings of the latent print area results in more template DNA available for downstream amplification and improved STR profiles versus commonly used swabbing methods. However, we also show that the double swabbing method can, too, be a reliable method and may work best if the initial swab is pre-wet with a diluent that includes a lysis buffer and protein degrading enzyme. Additionally, the use of a silica-based column extraction method that has specifically been optimized for use with forensic/challenged samples, such as the QIAamp® DNA Investigator Kit, followed by a gel-column concentration method, Centri-Sep™, may further increase the chances of obtaining informative STR profiles if used rather than other common methods/combinations. Conversely, our studies show that the addition of a post-amplification purification step fails to improve the STR profiles obtained from these samples and that the increased sensitivity is more likely to increase the presence of artifacts that further complicate data interpretation.

Unfortunately, DNA from surface swabs collected after visualization treatment and tape-lifting of individual latent fingerprints did not produce STR profiles, however, quantifiable high quality DNA was obtained from many samples. Thus, it should be advocated for crime scene investigators to consider adding post-lift surface swab collections to their routine procedures for fingerprint development and collection. In doing so, an additional, supplemental and valuable source of DNA could be made available to the forensic laboratory. If the surface swab is used in conjunction with other samples taken from the same source (swabs from other individual prints or from the lifted archived print itself), or if used with a modified workflow for low template DNA analysis, an otherwise low yield sample that produces a null or non-probative STR profile could be substantially improved and potentially provide a probative STR profile for human identification. This valuable source of contributor touch DNA should not be overlooked.

The studies detailed herein provide a DNA workflow tailored specifically for archived latent fingerprints that can improve STR results and increase the odds of obtaining CODIS-eligible DNA profiles. Furthermore, more than 200 latent fingerprints were collected, treated with reusable brushes, and stored for several weeks at room temperature prior to DNA processing for this work, and despite this, no unexpected or unidentified alleles were observed when using the recommended workflow. Thus, in cases where there are no other viable sources of biological material, such as older/cold cases or post-conviction cases, archived latent fingerprints should be strongly considered as a source for STR profiling.

The findings of the work described herein can be found in Solomon et al. and Hytenin et al. (50, 51). At least one additional manuscript is anticipated describing the results achieved when the developed optimized workflow is used to analyze the aged samples collected in this study.

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**Tables:**

TABLE 1 - *Effect of sampling technique and visualization treatment on STR data*

DNA Sampling Technique	Treatment (n=10)	Average DNA Yields (ng) $\pm$ SD†	# Samples with Allele Peaks Detected	# Allele Peaks Detected per Fingerprint**	Average % STR Alleles Observed**
<b>Cuttings</b>	Magnetic*	0.667 $\pm$ 1.490	2 / 10	(1/27, 26/26)	52
	Black	0.401 $\pm$ 0.637	3 / 10	(1/27, 2/26, 2/28)	6.2
	Untreated	0.255 $\pm$ 0.298	2 / 10	(4/26, 7/24)	22
<b>Single Swab</b>	Magnetic	0.100 $\pm$ 0.129	2 / 10	(8/28, 2/25)	18
	Black	0.025 $\pm$ 0.024	0 / 10	N/A	N/A
	Untreated	0.274 $\pm$ 0.338	1 / 10	(1/28)	3.6
<b>Double Swab</b>	Magnetic	0.146 $\pm$ 0.274	1 / 10	(1/28)	3.6
	Black	0.078 $\pm$ 0.130	0 / 10	N/A	N/A
	Untreated	0.276 $\pm$ 0.502	1 / 10	(1/28)	3.6

\*One complete profile \*\*Does not include Amelogenin

† p = 0.645

Table 2 - *Effect of swab diluent on DNA yields and STR data*

Swab Diluent (n=10)	Average DNA Yields (ng) $\pm$ SD†	# Samples with Allele Peaks Detected	# Allele Peaks Detected per Fingerprint**	Average % STR Alleles Observed**
<b>Water</b>	0.223 $\pm$ 0.383	1 / 10	(3/29)	10
<b>Lysis Buffer/ Proteinase K</b>	0.308 $\pm$ 0.380	3 / 10	(1/26, 7/28, 7/29)	18
<b>2% SDS</b>	0.041 $\pm$ 0.324	2 / 10	(1/28, 8/28)	16
<b>Triton X-100</b>	0.161 $\pm$ 0.239	0 / 10	N/A	N/A
<b>91% Isopropanol*</b>	0.119 $\pm$ 0.149	3 / 10	(3/28, 5/26, 12/27)	25

\*One allele drop-in \*\*Does not include Amelogenin

† p = 0.387



TABLE 3 - *Effect of DNA extraction method and treatment on STR data*

DNA Extraction Method	Treatment (n=10)	Average DNA Yields (ng) $\pm$ SD§	# Samples with Allele Peaks Detected	# Allele Peaks Detected per Fingerprint**	Average % STR Alleles Observed**
<b>Invisorb</b>	Magnetic	0.362 $\pm$ 0.318	0 / 10	N/A	N/A
	Black	0.291 $\pm$ 0.339	0 / 10	N/A	N/A
	Untreated	0.908 $\pm$ 1.092	0 / 10	N/A	N/A
<b>Investigator</b>	Magnetic†	0.667 $\pm$ 1.490	2 / 10	(1/27, 26/26)	52
	Black	0.401 $\pm$ 0.637	3 / 10	(1/27, 2/26, 2/28)	6.2
	Untreated	0.255 $\pm$ 0.298	2 / 10	(4/26, 7/24)	22
<b>Organic</b>	Magnetic	0.434 $\pm$ 0.508	2 / 10	(6/23, 6/27)	24
	Black	1.479 $\pm$ 3.071	0 / 10	N/A	N/A
	Untreated	0.173 $\pm$ 0.216	0 / 10	N/A	N/A
<b>ZyGEM*</b>	Magnetic	0.544 $\pm$ 1.190	6 / 10	(1/26, 1/27, 1/27, 1/27, 6/24, 12/28)	14
	Black‡	0.404 $\pm$ 0.492	2 / 10	(3/26, 5/29)	14
	Untreated	0.558 $\pm$ 0.967	1 / 10	(19/28)	68

\*Inhibition detected from IPC values

\*\*Does not include Amelogenin

† One complete profile

‡ One allele drop-in

§ p = 0.336

Table 4 - Effect of DNA concentration method on STR data

Concentration Method (n=10)	# Samples with Allele Peaks Detected	# Allele Peaks Detected per Fingerprint**	Average % STR Alleles Observed**
Microcon® Y-100 *	2 / 10	(1/27, 26/26)	52
Vacuum Centrifugation	1 / 10	(1/24)	4.2
Centri-Sep™†	9 / 10	(2/27, 2/29, 5/28, 11/26, 16/27, 18/28, 21/28, 24/24, 27/27)	53
NuceloSpin®	0 / 10	N/A	N/A

\*One complete profile \*\*Does not include Amelogenin

† Two complete profiles

Table 5 - Effect of post-amplification purification on STR data

Post-amp Purification Method (n=10)	Treatment	# Samples with Allele Peaks Detected		# Allele Peaks Detected per Fingerprint**		Average % STR Alleles Observed**	
		Without purification	With purification	Without purification	With purification	Without purification	With purification
QIAGEN MinElute®	Magnetic*†	2 / 10	4 / 10	(1/27, 26/26)	(2/28, 3/24, 2/27, 26/26)	52	32
	Untreated	1 / 10	1 / 10	(7/24)	(7/24)	29	29
Microcon® -30	Magnetic	0 / 10	0 / 10	N/A	N/A	N/A	N/A
	Untreated	1 / 10	1 / 10	(4/26)	(7/26)	15	27

\*One complete STR profile \*\*Does not include Amelogenin

†Allelic drop-in observed

Table 6 – Aged Archived Latent Fingerprint Analysis

Effect of DNA extraction method and treatment on STR data								
DNA Extraction Method	Original Surface	Treatment (n=5)	Average DNA Yields (ng) ±SD	# Samples with Allele Peaks Detected	# Correct Allele Peaks Detected	Average % Correct STR Alleles Observed**	# Allele Peaks Detected**	Average of Drop-In per Fingerprint**
Traditional	Paper	Magnetic <sup>^^</sup> n=8	0.366715763 ± 0.570909	6/8	74/158 (28/28, 26/26, 9/24, 3/27, 7/27, 1/26)	46.4	96/158 (28/28, 30/26, 26/24, 3/27, 8/27, 1/26)	3.7
		Untreated <sup>^</sup> n=3	0.825231483 ± 1.403224	2/3	26/53 (26/26, 0/27)	50.0	31/53 (28/26, 3/27)	2.5
Optimized	Paper	Magnetic <sup>^</sup>	0.767770338 ± 1.167301	3/5	61/80 (26/26, 16/28, 19/26)	76.7	69/80 (27/26, 16/28, 19/26)	2.7
		Untreated <sup>^</sup>	0.913284083 ± 1.347961	3/5	50/78 (1/24, 28/28, 21/26)	61.6	62/78 (2/24, 32/28, 29/26)	4.0
Traditional	Glass	Magnetic	0.02693375 ± 0.031678	5/5	10/131 (2/26, 0/24, 0/27, 2/28, 6/26)	7.6	34/131 (9/26, 1/24, 1/27, 2/28, 21/26)	4.8
		Untreated	0.032212433 ± 0.028466	2/5	2/53 (0/26, 2/27)	3.7	3/53 (1/26, 2/27)	0.5
Optimized	Glass	Magnetic	0.019228333 ± 0.02357	1/4	0/24 (0/24)	0.0	2/24 (2/24)	2.0
		Untreated	0.129032383 ± 0.107382	2/5	2/50 (1/26, 1/24)	4.0	6/50 (3/26, 3/24)	2.0

\*\* Does not include Amelogenin

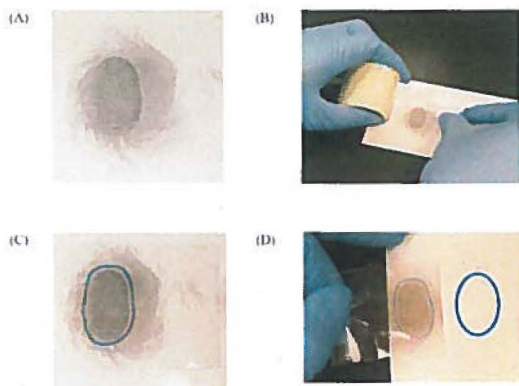
<sup>^</sup>one complete profile

<sup>^^</sup>two complete profiles

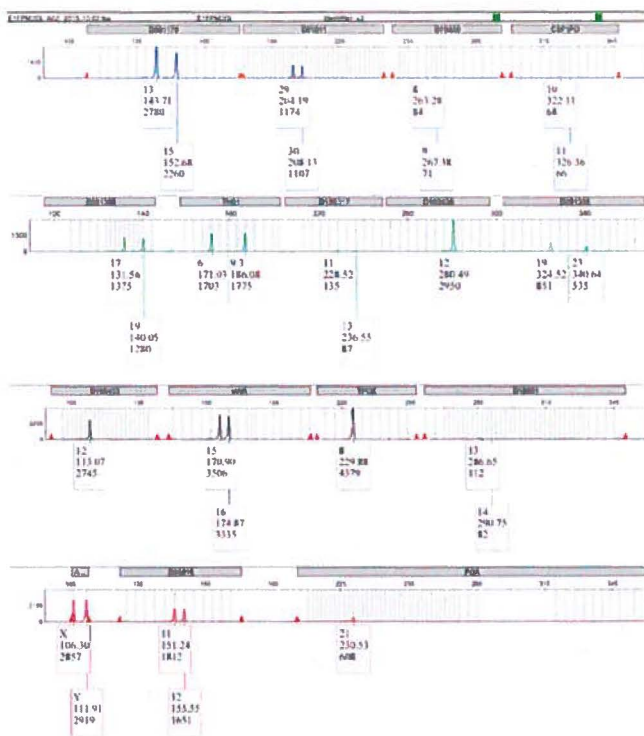
Table 7 - DNA detected from post-lift fingerprint surface swabs

Swab Method (n=45)	Detected DNA (%)
Single Swab	64
Double Swab	51
p = 0.278	

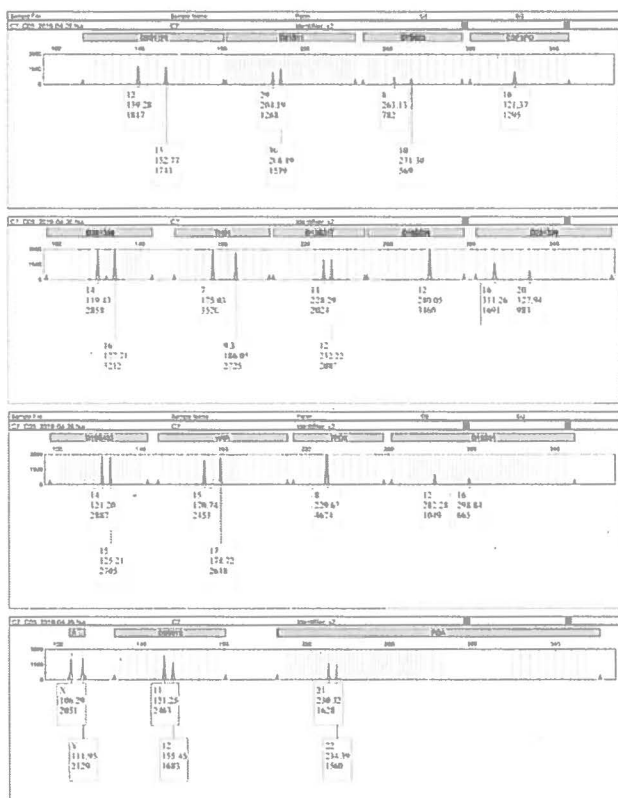
**FIG.1** Archived latent fingerprint collection and disassembly. (A) Latent fingerprint deposited on paper and visualized with magnetic fingerprint powder. (B) Adhesive placed over the fingerprint prior to room temperature storage (~4 weeks). (C) For DNA sampling the fingerprint is outlined on the exterior, adhesive is peeled back from the paper and (D) the indentation on the paper side is outlined so that the examiner can easily locate biological material on both sides of the fingerprint “sandwich.”



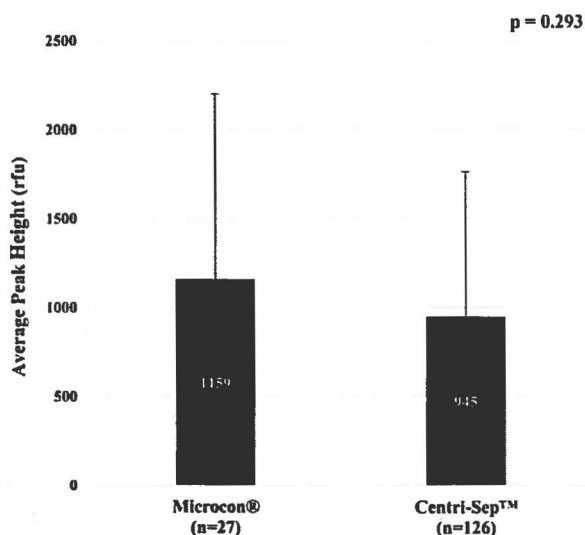
**FIG. 2.** Complete STR profile from archived latent fingerprint sample extracted with the QIAGEN QIAamp® DNA Investigator Kit. The profile is fully concordant with the reference sample profile.



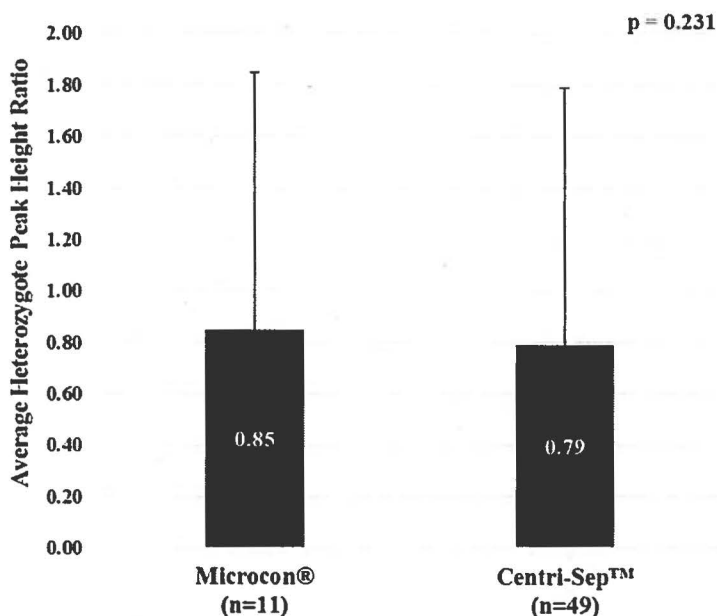
**FIG. 3** Example of complete STR profile from archived latent fingerprint sample concentrated with a Centri-Sep™ gel column. The profile is fully concordant with the reference sample profile.



**FIG. 4** Average STR allele peak heights from archived latent fingerprint samples using different DNA concentration methods. Allele peaks detected from all samples that provided at least partial profiles were averaged together for each group.



**FIG. 5** Intralocus heterozygous STR peak height ratio from archived latent fingerprint samples using different DNA concentration methods. Heterozygous loci from all samples that included loci with both expected allele peaks were averaged together for each group. To obtain the ratio for a heterozygous locus, the allele peak with the lowest peak height was divided by the allele peak with the highest peak height.



**FIG. 6** DNA recovery from surface swabs collected after latent fingerprint visualization treatment and tape-lifting using two DNA sampling methods. Regardless of the treatment, surfaces swabbed with the double swab method yielded more DNA than those sampled using the single swab method ( $p = 0.882$ ). Samples that were collected after treatment with magnetic powder yielded significantly more DNA, on average, regardless of the swabbing method used ( $p = 0.023$ ).

