DNA recovery from handwritten documents using a novel vacuum technique

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DNA recovery from handwritten documents using a novel vacuum technique

A thesis presented in partial fulfillment of the requirements for the degree of Master of Science in Forensic Science, John Jay College of Criminal Justice of the City University of New York

Patrick McLaughlin

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This thesis has been presented to and accepted by the Office of the Graduate Studies, John Jay College of Criminal Justice of the City University of New York in partial fulfillment of the requirements for the degree of Master of Science in Forensic Science.

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Also…..Yoda, Indiana Jones, Legolas, and Batman.
Abstract

Investigations of many crimes such as robberies, kidnappings, and terrorism are often associated with the recovery of a paper document which has been written by the perpetrator. Paper can provide a variety of forensic evidence such as DNA, latent fingermarks, and indented writing. The focus of this study was the collection of probative DNA profiles from the text region of a handwritten note through a vacuum suction device without altering or destroying the document. Collection of DNA evidence was carried out in two separate groups. The first group involved 11 volunteers providing a handwritten note sample with unwashed hands. The second group of DNA collection involved six volunteers providing handwritten notes before and immediately after a period of mild aerobic activity. After the collection of DNA evidence, 10 volunteers provided sebaceous and eccrine latent fingermarks placed onto separate paper documents and developed with magnetic fingerprint developer or 1,2 indanedione to observe any effects of the vacuum swabbing technique. A final step to the study was performed to observe the effects of the vacuum swab technique on indented writing which may be developed using Electrostatic Detection Apparatus (ESDA). Ten simulated robbery notes were impressed with overlay sheets and processed with the vacuum swab before being developed using the ESDA process. Approximately 80% of processed DNA samples produced potentially probative profiles. Quantities of recovered DNA in the single collection series ranged from 0.6-54.3 pg/µL. DNA quantities in the active series prior to the aerobic activity ranged from 3.1-55.8 pg/µL. DNA quantities in the active series post-activity ranged from 1.9 to 221.6 pg/µL. The vacuum swab technique did not destroy nor alter any of the observed fingermarks. Additionally, the vacuum swab technique did not interfere with any indented writing as observed through ESDA.
1. Introduction

Several types of criminal actions involve handwritten notes on paper. The investigation of crimes such as robbery, kidnapping, stalking, or a terrorist act often start with the recovery of a document which has been written by the perpetrator. The classic image of a bank robbery being committed by a perpetrator passing a note to the teller remains a common modus operandi. Domestic violence and stalking cases are often carried out over e-mail, however hand-written threatening notes left on car windshields or various personal property remain a consistent piece of physical evidence found at report scenes. Handwritten letters are often found at the scene of vandalized property or assaults in relation to hate crimes.

Handwritten notes recovered from a crime scene as physical evidence offer multiple levels of probative evidence. Forensic scientists and technicians may be able to perform evaluations based on questioned documents (QD) techniques like handwriting comparisons or indentation enhancement; fingermark analysis, as well as development of DNA profiles can be successful. The large size of the national fingerprint database and the speed with which prints can be developed and compared make latent fingermarks a valuable investigative tool (Komarinski, 2005). Therefore, the principal focus of investigators and forensic technicians is often the development of potential latent fingermarks deposited on the paper by the perpetrator(s) of the crime. There is a tendency for such fingermarks to cluster around the periphery of the paper and along margins of text, the regions where a potential suspect held the paper. Another promising avenue of associating an individual to paper evidence is the recovery of DNA for development of STR profiles. As explained below, the current DNA collection methods may interfere with the integrity of the fingermark friction ridge detail and DNA is normally scheduled after QD and fingermark processing, which reduces the DNA success rate.
(Sewell et al., 2008). There are several ways in which the workflow may be reconsidered. In the meantime, the document itself should be considered of highest evidentiary value and forensic examination processes destroying it should be avoided if possible. The ability to demonstrate an original and undamaged piece of physical evidence before a convened jury has a powerful effect for in courtroom proceedings.

**Literature Review**

There are numerous chemical and physical means by which investigators may attempt to develop latent fingermarks (Yamashita, French, 2019). Contemporary forensic response techniques call for the processing of latent prints on paper by either mechanical means such as magnetic fingerprint powder or through chemical means such as ninhydrin, 1,8-diazafluoren-9-one (DFO), or 1,2 indandione (Jelly, Patton, Lennard, Lewis, & Lim, 2009; Weisner, Springer, Sasson, & Almog, 2001). Crime Scene Technicians frequently submit paper documents to their respective crime labs for analysis and documentation rather than attempting to process the document in the crime scene location.

In forensic investigations, the main types of friction ridge marks which are likely to be encountered are classified as latent or patent. Latent friction ridge marks are those which are not readily visible to the naked eye whereas patent friction ridge marks can be seen with little or no assistance (Yamashita, French, 2019). Friction ridge marks which can be detected and individualized during a forensic investigation originate from the fingers, fingertips, or palmar region of the hands. The palmar region of the hand is divided into three sections, often termed the Liverpool Palmprint system: the interdigital, the thenar, and the hypothenar regions (Hutchins, 2019). The hypothenar region of the palm is direct contact with the substrate when writing on paper, and the region considered the ulnar border, often referred to as the “blade” of
the hand, scrapes along the surface area in question. Fingermarks may only be transferred from the donor to a substrate by physical contact. The chance impressions from the volar surfaces on a particular substrate can be detected by physical, chemical, and instrumental methods such as attenuated total internal reflectance (ATIR) and Fourier Transform infrared microscopy (FTIR) (Crane, Bartick, Perlman, & Huffman, 2007; Grant, Wilkinson, Holman, & Martin, 2005; Hytinen, Solomon, & Miller, 2017; Su, 2016; Subhani, Daniel, & Frascione, 2018; van Dam, van Beek, Aalders, van Leeuwen, & Lambrechts, 2016) Fingermarks on the surface of the paper substrate are of utmost importance to forensic investigations. Fingermark development on most surfaces is commonly achieved by latent print powders or magnetic powders (Subhani et al., 2018). Porous surfaces such as paper are known to absorb some of the biological and chemical components of fingermark residues (Lee & Joullié, 2015). As a result of this phenomenon, many laboratory technicians make use of chemical reagents to visualize potentially probative evidence. A commonly used chemical developer is 1,2 indanedione which is known to react with amino acids left behind on porous surfaces (Lee & Joullié, 2015; Yu & Wallace, 2007). The structure and function of 1,2 indanedione is similar to that of ninhydrin and will react readily to α-amino acid residues and create a pink colored visualization of the latent fingermark (Jelly et al., 2009). This visualized fingermark will fluoresce when exposed to 490-590 nm wavelengths of light, with an observed peak of photoluminescence at approximately 505 nm (Jelly et al., 2009). The process of visualizing fingermarks using 1,2 indanedione requires only a short period of time. The document is sprayed with or dipped into a 1,2 indanedione solution and then, once the document has dried, it is placed into a heat source of approximately 100 °C which is adjusted to approximately 60% humidity for a period of 15-20 minutes (Weisner et al., 2001). The speed at which the prints may be visualized makes the use of 1,2 indanedione optimal for law
enforcement agencies. Chemical reagents such as ninhydrin require 24-48 hours to develop latent fingermarks in an ideal circumstance (Yamashita, French, 2019). The overall period of time may be substantially reduced through the application of humidity, heat, or steam but this may inadvertently reduce the overall clarity of the developed fingermarks (Yamashita, French, 2019).

In addition to the successful development and analysis of fingermarks, law enforcement and prosecutor’s offices greatly rely on short tandem repeat (STR) profiles generated by “contact traces” or “touch” DNA from touched evidence such as the fingermarks discovered on various substrates (van Oorschot & Jones, 1997), (Kanable, 2005). “Touch DNA” is the term given to DNA evidence deposited on a substrate by physical contact with the donor’s exposed skin. In the past all touch DNA protocols were often considered low copy number DNA testing. But as Butler and others stated, there are several definitions of what DNA amount can be classified as low copy number (LCN) and it has been shown that even touch DNA donors can leave sufficient quantity to be suitable for a conventional level of analysis (Butler, 2015; Gill & Buckleton, 2009). Since the presence of body fluids and the mechanism for the DNA deposit on a surface are often undetermined, the term “trace DNA” may be more appropriate. “Trace DNA” is a term used to describe the recovery of small quantities of DNA from a stain of unknown body fluid origin (Burrill, Daniel, & Frascione, 2019).

An important avenue of detection and extraction of trace DNA focuses on fingermarks. Since the first description of DNA recovery from fingermarks in 1997 (van Oorschot & Jones, 1997), multiple studies have been performed to enhance the methods of DNA extraction from a fingermark or touched substrate (Balogh, Burger, Bender, Schneider, & Alt, 2003; Plaza et al., 2016). The main concern is how these processes for DNA collection affect fingermark integrity
and vice versa, how latent print development may reduce DNA yields (Raymond, Roux, Du Pasquier, Sutton, & Lennard, 2004). Many of these methods, however, require the visualization of the fingermark for DNA processing to proceed (Bhoelai, de Jong, de Puit, & Sijen, 2011) with DNA recovery. Sewell et al. (2008) developed latent fingermarks on office paper by using ninhydrin but stated that overall quantity of DNA was reduced and required the paper to be destroyed in the process. Similar findings were reported by Raymond et al. (2004), who reported that none of their donor profiles could be developed after the use of ninhydrin. In a study performed by dipping paper into DFO and ninhydrin, Bholelai et al. (2011) observed that this reagent method not only had a deleterious effect on DNA recovery, but the dipping action seemed to introduce a potential source of contamination. Fieldhouse et al. (2016) reported that methods of DNA collection applied to developed fingermarks did cause deleterious effects of friction ridge details on a variety of substrates but specifically for paper they were able to collect DNA using adhesive tapes without compromising the friction ridge detail. The methods of dry swabbing or wet-dry swabbing which are undertaken as common DNA extraction techniques (Parsons, Sharfe, & Vintiner, 2016) have proven to provide useable DNA samples but create the issue of smudging valuable prints if the swabbing is performed over the surface area of the substrate, in this case handwritten documents, without knowing where latent fingermarks have been deposited. In cases where fingermarks have been visualized before the recovery of DNA, the chance of friction ridge loss can be overcome by photographic documentation of the fingermark prior to DNA recovery techniques being applied.

Studies have indicated that DNA recovery from rough or porous surfaces is superior to that of non-porous or smooth surfaces (Daly, Murphy, & McDermott, 2012). Often the exact opposite is encountered when processing a substrate for the development of fingermarks.
Smooth, non-porous surfaces provide an investigator with better conditions for development and recovery, where rough, porous surfaces may present a great challenge for probative result (Yamashita, French, 2019). Different types of paper provide a middle ground for the collection of both DNA and latent fingermarks. Paper is a porous surface that enables DNA to be retained and extracted while also providing physical conditions where numerous biological and chemical reagents can produce probative fingermark evidence. The extraction of DNA from paper cuttings can be inhibited by several physical properties of the paper itself. Studies into paper substrates have revealed that whitening agents used as optical brighteners, such as bleach or diaminostilbene polysulfonic acid, in the paper milling process may interfere with the DNA extraction process (Raymond et al., 2004; Sewell et al., 2008) and inhibit the successful development of STR profiles. In addition to conditioners within paper, Almog et al. (2018) suggested that DNA may form hydrogen bonds with the cellulose of the paper, further complicating extraction methods. Logistical issues with the potential destruction of probative evidence have led to a need to be selective in both method and order in recovery from paper documents. In her paper, Ferraro indicated that there was a need to first focus on the nature of the substrate before considering which manner in which evidence was to be recovered (Ferraro, 2012). Fieldhouse et al. (2016) demonstrated that various DNA collection techniques produce very different results based on the nature of the substrate. The study performed by Plaza et al. (2016) also stated that DNA collection on various substrates could be performed in a non-destructive manner by being selective on choice of recovery method based on the suspected nature of biological material and an understanding of the substrate characteristics (Plaza et al., 2016).
With fingermarks being the main forensic focus, there has been relatively little attention paid to the inner regions of the paper where the handwriting has been deposited. Logically, the author of a document would slide their hand and lower forearm across the paper surface and possibly smudge latent prints during the writing process, so the main target for print development is the margin areas. In their study, Parsons et al. (2016) noted that participants contacted the margin regions of the paper up to 65% of the time while writing. In the analysis of DNA transfer by different parts of the hand (McColl, Harvey, & van Oorschot, 2017) reported that the hypothenar region deposited the greatest quantity of DNA on glass substrates (0.100 ng), however it should be noted that the subjects in that study were asked to press their hand onto a glass plate. As a result, the reported quantity may not have included DNA from the ulnar border. Many previous studies of the overall volar surfaces of the hand involved placing hands onto glass surfaces and applying pressure for a pre-designated time-period (Daly et al., 2012; McColl et al., 2017; Oleiwi, Morris, Schmerer, & Sutton, 2015). These studies did not include more dynamic actions like the ones encountered during the writing process and the scraping motion of a hand along a porous substrate. The ulnar border is in the transition region between the volar surface and the dorsal surfaces of the hands. This region of skin may contain a different population, in quantity and quality, of pores which will meet the surface of the paper during the writing process. This region of the body may also possess a higher quantity of DNA than the volar palm surface. Another region of the body which often contacts with the surface of the paper while handwriting a document is the distal forearm. The scraping of the skin of the forearm along the paper surface may provide an increased opportunity for DNA bearing material to be transferred onto the paper substrate beyond what the fingers and hand might provide alone.
Contact trace DNA collection involves targeting of skin cells which sloughed from the epidermis through desquamation (Aditya, Sharma, Bhattacharyya, & Chaudhuri, 2011; Ferraro, 2012; van Oorschot & Jones, 1997). The biological purpose of the epidermal layer of skin is to provide a tightly sealed barrier for the body which protects against viral infections and pathogens, prevents dehydration of the body, and provides a mechanism for wound healing (Fuchs, 2008; Rittié, Sachs, Orringer, Voorhees, & Fisher, 2013). Skin cells journey through a series of layers, or strata, as they progress from a rapidly dividing basal layer to a highly cornified layer of the stratum corneum during the keratinization process (Eckhart, Lippens, Tschachler, & Declercq, 2013). The process of programmed cell death, similar to apoptosis, is referred to as cornification (Eckhart et al., 2013). The cell population of the innermost basal layer of keratinocytes are nucleated and divide rapidly. As new layers of basal keratinocytes are created the previous base layer is pressed upwards into the spinous layer linked together by small proteins called desmosomes (Eckhart et al., 2013). Once the keratinocytes have differentiated into the spinous layer, their gene expression is altered to produce caspase-14 which is often associated with cellular apoptosis (Eckhart et al., 2013). While in the spinous layer, cells can no longer divide and are compressed. As the cells pass from the spinous layer into the granular layer they are further compressed and affected by transcription factors which cause the destruction of organelles including the nuclear envelopes and very few nucleated cells survive beyond the upper granular layer into the cornified layer, also called the horny layer (Eckhart et al., 2013). Extracellular enzymes or external abrasion then cleave the linkage between the desmosomes and cause the corneocytes to be shed in the desquamation process (Eckhart et al., 2013; Iizuka, 1995). These shed corneocytes with their degraded nuclei are the target of skin cell DNA recovery methods. These corneocytes comprise the vast majority of the epidermis,
accounting for approximately 95% of the epidermis (Maceo, 2019). The rate at which these cells travel from the basal layer through the outermost horny layer varies from person to person (Alvarez, Villanueva, Budowle, Lorente, Entrala, Lorente, 1998; Iizuka, 1995). It is estimated that a skin cell spends approximately a month in the horny layer before being shed through desquamation (Wickenheiser, 2002). The horny layer is comprised of approximately 65% glycerides and fatty acids, 20% cholesterol, 15% sterol esters (Girod, Ramotowski, & Weyermann, 2012).

Fingerprint secretions as well as DNA derive from different glands that can be found on every surface of the body. There are estimates that state as many as 100 sweat (eccrine) glands and 10 oil (sebaceous) glands can be found per square centimeter of skin surface (Wickenheiser, 2002). Differing anatomical sections of the body provide various amounts of DNA transfer to the volar surfaces of the hand, based partly on the type and concentration of glands found at that location (Oleiwi et al., 2015). Eccrine glands are found all over the body, including the volar surfaces (Girod et al., 2012; Zoppis et al., 2014). The number of eccrine glands found throughout the surface of the body greatly outnumbers the number of the sebaceous glands, with the highest concentration of eccrine glands found on the volar surfaces of the body (Rittié et al., 2013). The primary purpose of these glands is to assist in homeostasis, by cooling the body. As a response to parasympathetic signals, the myoepithelial cells which surround eccrine glands contract and bring the contents of eccrine sweat to the surface of the body. The principal component of eccrine sweat is water (~99%) but it is also known to contain several organic and inorganic compounds such as sodium salts, glucose, pyruvate, lactic acids, amino acids, dermcidin, and others (Girod et al., 2012; Jorizzo; Bolognoa; Schaffer, 2012).
The population of sebaceous glands are found throughout the dermis except for the surface of the hands and feet (Zoppis et al., 2014). These glands secrete a mixture of various cellular components, such as fatty acids and oils, which is referred to as sebum. Sebaceous glands tend to be found clustered around hair glands (Zoppis et al., 2014). Much like the continuing process of keratinization, secreted sebum can be brought to the surface of the epidermis through mechanical means. Sebaceous gland complexes which surround a hair follicle are composed of the sac-like sebaceous gland, the hair follicle, and an arrector pili muscle, referred to as a pilosebaceous unit (Rittié et al., 2013). Recent models have suggested that the movement of the hair follicle in conjunction with the contraction of the arrector pili muscles cause the sebaceous gland to contract and drive the sebum onto the surface of the skin (Song, Hu, Kim, & Koh, 2007). Sebum can be transported from the hair follicle to the volar surfaces by simple contact. As an individual touches other parts of their skin they may transfer the DNA containing sebum onto volar surfaces, which is detected as part of touch DNA. Studies have suggested that observed adults touch their own face as often as 23 times per hour (Kwok, Gralton, & McLaw, 2015). This action of touching regions of the body may provide the ideal mechanism for transfer of latent fingermark material as well as DNA from the donor to a substrate. The deposited fingermark material is thought to be a composition of sloughed skin cells, secretions from bodily glands, and external contaminants such as dirt or cosmetics (Girod et al., 2012). Quinones et al. (2012) showed that the deposited DNA consists of a combination of cellular DNA which could be pelleted by centrifugation and cell free DNA can be found in the supernatant.

Questioned document examination routinely involves the visualizing of indented writing. This is accomplished by using an Electrostatic Detection Apparatus (ESDA), which was first
used in forensic casework in 1977 (Barr, Pearse, & Welch, 1996) and since then has been an investigative tool for forensic document examiners that can provide crucial probative evidence. Invisible indented writing is created if a sheet of paper is laid atop of the questioned document and the indented writing is created either through the direct impression of writing on the overlay sheet or by pressure from contact between the two sheets (Yaraskavitch, Graydon, Tanaka, & Ng, 2008). The ESDA device operates by placing a document on a porous document platen. A thin mylar foil (~9 µm) is placed over the document and a voltage of approximately of 8kV from a hand-held corona wand is passed over the foil. The corona wand applies a negative charge to the mylar and a cascade of positively charged glass beads (0.5mm in diameter) coated with negatively charged toner is poured over the document surface where the negatively charged toner particles are drawn into the impressions on the document (Freeman, 2003). The settling of the toner particles in the indentations writing allows for these markings on the questioned document to be visualized and provide potential sources of investigative leads (Freeman, 2003). Realizing that the mylar foil comes into contact with the document and may have lifted off adhering cellular material, Plaza et al. (2015) swabbed the foil for DNA after the ESDA process. This allowed for non-destructive DNA collection of a large area but was only tested for fingerprints and had a lower success rate than a dry swabbing method (Plaza et al., 2015).

This study evaluated a different method for DNA recovery from a large area of paper. A vacuum device plugged with a moistened swab was used to collect shed corneocytes and possible sebaceous and eccrine residue deposited while the writer dragged their hand across the surface while writing. Collection was limited to areas which contain handwritten text and did not extend into the margins, where the majority of fingermarks from the paper would be expected. None of the donors were asked to wash their hands prior to writing a brief standardized
text. This created a realistic scenario but meant at least some of the DNA results were likely to be DNA mixtures. Aside from the testing for DNA typing success across a range of donors and after physical activity, the vacuum method was tested for its effects on fingermark integrity and indentation detectability.

2. Materials and Methods

Permission for the study was granted by the university integrated institutional review board under file # 2017-0306.

2.1 Sample Collection

White copy paper (Staples® Multipurpose letter sized (8.5”x11”, 20 lb., 94 brightness)) was made “DNA free” by UV irradiating individual sheets in an Air Science® Box (Airscience, Fort Meyers, FL) for a period of one hour. Samples of handwriting on irradiated copy paper were obtained from volunteers who transcribed a provided sample letter, ensuring the same number of words and characters were used by all participants. A group of 11 volunteers performed the letter writing process with no special preparations i.e., unwashed hands. In addition, an “active series case study” was performed with a separate set of 6 volunteers providing a written copy of the same letter prior to, and immediately after a Hatha style yoga class lasting approximately one hour and fifteen minutes. This scenario design was to simulate an excited state, such as increased perspiration, which many individuals exhibit when engaged in the stressful activities associated with criminal events. The first writing sample was used as a “baseline” of the volunteer, and the second sample as a “real world” simulation. Each volunteer
provided an exemplar DNA sample to allow for proper reference with resulting DNA profiles. Two sheets of plain paper were irradiated and processed without any handwriting samples to serve as blanks for this study.

To carry out DNA collection, a 9” Carolina® pipette (Carolina, Burlington, NC) was used to prepare a suction device. All pipettes were irradiated by UV light from Air Science® UV Box (Airscience, Fort Meyers, FL) for a period of one hour prior to use to reduce the risk of internal and external contamination. Using sterilized shears, the long spout of the pipette was trimmed down approximately 9cm. A sterile Puritan® brand cotton swab (Puritan, Anahein, CA) was inserted into the wide opening of the pipette. The swab shaft was also trimmed to allow the cotton head of the swab to align with the natural pinch point of the glass pipette. A length of one-quarter inch diameter vacuum tubing was attached to the trimmed end of the pipette and a bench top vacuum system was activated to provide suction throughout the length of the pipette. It was believed that the swab head at the natural pinch point would provide enough blockage to prevent DNA from passing through the swab head, while simultaneously allowing enough suction to remove biological material from the surface of the substrate. Swab heads were moistened with 20 µL of 0.1% Triton X (Millipore Sigma, St. Louis, MO) to assist in the collection of DNA. The overall time of vacuum swabbing for each sample was recorded.

The vacuum swabbing was performed in a methodical pattern across the paper surface over the areas where the handwriting samples are visible but avoiding “margins” created by the writer where no handwriting was visible. During the vacuum swabbing process, a set of joined tweezers (see figure 1) was used to physically raise the documents off the work bench. An alternative mechanism to raise the paper was developed later in the study. Wooden blocks with a metal surface and magnetic clamps provided suspension for different sized paper fragments (see
Upon completion of the vacuum swabbing, the swab was removed from the Carolina® pipette; swab heads were cut off the stick and transferred into 1.5mL reaction tubes and either processed for DNA extraction or temporarily stored at +4°C.

Figure 1. Disassembled Vacuum Swab device and joined tweezers.

Figure 2. Benchtop view of magnetic clamp devices developed during project.
2.2 DNA Extraction and testing

DNA extraction process closely followed the protocols published by Forsberg et al. (Forsberg, Jansson, Ansell, & Hedman, 2016). Cotton swabs were cut into individual 1.5 mL reagent tubes and mixed with 100 µL of 5% Chelex solution (Biorad, Hercules, CA), 1 µL of Proteinase K (Promega, Madison, WI) (final concentration 0.1µg/µL), and 2 µL of 10% Tween 20 (Millipore Sigma, St. Louis, MO) (concentration 0.2%). Samples were incubated at room temperature for a period of 30 minutes with brief vortex approximately every 10 minutes. Upon conclusion, the samples were placed in an Eppendorf® Thermomixer C (Eppendorf, Hapauge, NY) and processed for 45 minutes at a temperature of 56°C and 1400 rpm. Incubation continued by placing the samples into a Fisher Scientific® IsoTemp heat block (Fisher Scientific, Hampton,
set to 99°C for 10 minutes, followed by being placed into an ice block for 10 minutes. Substrates were then transferred into irradiated Dolphin tubes with spin basket inserts (Midscientific, St. Louis, MO) and centrifuged for 5 minutes at 1500rcf. Afterwards the swab substrates were discarded, and the resulting flow through plus the original lysate were added to Microcon® DNA Fast Flow (MW 100) filters units (Millipore Sigma, St Louis, MO) for concentration. Microcon® filters placed in collection tubes were centrifuged at 500 rcf for 30 minutes. DNA recovery was facilitated by adding 20µL of 0.1x Tris EDTA (TE) Buffer (Fisher Scientific, Hampton, NH) and centrifuging inverted Microcon® Fast Flow filters at 1000 rcf for 3 minutes. After recording the volumes all DNA extracts were stored at +4°C. One reagent blank was processed with each extraction.

Quantitation of samples was performed using Thermo Fisher® Quantifiler Trio THP PCR kits (Thermo Fisher Scientific, Waltham, MA), a human specific real time DNA PCR assay as per manufacturer recommended guidelines (Applied Biosystems, 2017) on the Applied Biosystems® 7500 Real-Time PCR instrument (Thermo Fisher Scientific, Waltham, MA). Samples were compared to serially diluted standards and checked for internal positive performance, potential negative control contamination, and sample DNA quantitation levels. PCR amplification of polymorphic short tandem repeat (STR) loci was performed on the Applied Biosystems® Gene Amp PCR System 9700 (Thermo Fisher Scientific, Waltham, MA), set for 29 cycles using fluorescent primer and master mix supplied in the Applied Biosystems® Identifiler Plus kits (Thermo Fisher Scientific, Waltham, MA). Target DNA input was 500pg, or the maximum amount possible based on the DNA concentration in 10µL of DNA extract. PCR cycles were preset as an initial incubation of 11 minutes at 95°C, followed by 29 cycles of
denaturing for 20 seconds at 94°C, annealing for 3 minutes at 59°C, and extension for 10 minutes at 60°C. Resulting amplified samples were stored at +4°C.

Capillary electrophoresis analysis was performed using the Applied Biosystems® Genetic Analyzer 3500 which used eight 36cm capillaries. Samples for electrophoresis were composed of 1.2 µL of PCR product combined with 11 µL of a formamide: LIZ 600 internal standard size mixture (all Thermo Fisher Scientific, Waltham, MA). All injections included allelic ladder, 9947A positive control, and negative controls. Injections were set for 15 seconds at 1.2 kV. Capillary electrophoresis data were examined via Gene Mapper® ID-X Software to analyze STR profiles for both paper specimen and reference samples. Resulting profiles were compared to their known reference and evaluated as follows (colors indicate heatmap depiction:

- Only donor alleles present or both donor alleles as major component (green)
- One or both donor alleles present as part of a non-deducible mixture (yellow)
- Alleles present but not from donor (red)
- No alleles detected (blue)

Each sample was also given a mixture status designation as described in table 1.
Table 1. Mixture status criteria for STR profiles.

<table>
<thead>
<tr>
<th>Profile Designation</th>
<th>Criteria for Designation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single Source</td>
<td>Profiles containing no more than 2 alleles present at any loci.</td>
</tr>
<tr>
<td>Mixture</td>
<td>Profiles containing no more than 4 alleles present at least one locus.</td>
</tr>
<tr>
<td>Complex Mixture</td>
<td>Profiles containing 5 or more alleles at least one locus.</td>
</tr>
</tbody>
</table>

In addition, profiles were classified reflecting their investigative value in a case based on criteria listed in table 2.

Table 2. Putative probative value for STR profiles.

<table>
<thead>
<tr>
<th>Letter Code</th>
<th>Profile Determination “Category”</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>Full Profile</td>
<td>Single source detected or all loci could be resolved with the reference as the major contributor</td>
</tr>
<tr>
<td>DB</td>
<td>Database Profile</td>
<td>Reference could be determined as the major contributor at 8 or more loci of the sample</td>
</tr>
<tr>
<td>C</td>
<td>Comparison Mixture</td>
<td>Reference could be detected at all loci but a true major contributor could not be determined for that sample</td>
</tr>
<tr>
<td>NS</td>
<td>Not Suitable for Comparison</td>
<td>1 or less loci had DNA detected</td>
</tr>
</tbody>
</table>

Likelihood ratios for the observed mixture donor combinations were calculated by using LR Studio Mix® software (Gill & Haned, 2013). DNA quantification data for the different sets of male and female volunteers were tested for variability using a non-parametric Mann Whitney test. Comparison of all three sample populations was performed with the Kruskal-Wallis rank sum test using R-Studio®.
2.3 Latent Print Testing

To establish possible effects of the vacuum swabbing technique upon latent fingerprints, controlled fingerprints were developed and photographed prior to and after processing with the vacuum swab to observe any deleterious effect of the study’s technique. Ten volunteers (four females, six males) provided two sets of fingerprints on two separate sheets of the same brand of copy paper as used in the DNA extraction study (Staples, Framingham, MA). The first set of prints was made sebaceous by rubbing fingers on the surface of face and neck. The participant then placed each hand, palmar surface down, on separate papers for a period of five seconds. Volunteers were advised to use a moderate level of pressure, consistent with that of picking the sheet of paper up.

The same ten volunteers were asked to repeat the process for the collection of eccrine prints. The participants wore latex examination gloves for a period of 30 minutes prior to sample donation while carrying out non-strenuous activities. The volunteers then followed the same protocol as the sebaceous latent fingerprint deposit. Each hand was placed, palmar surface down, onto two separate sheets of paper for a period of five seconds.

All latent fingerprint samples were maintained at room temperature for a period of approximately 24 hours prior to development and analysis. Samples were developed as follows: one set of prints from each target print category (eccrine and sebaceous) was developed using Sirchie® magnetic latent print processing powder (Sirchie, Youngsville, NC) and the other set was developed using 1,2 indandione reagent also purchased from Sirchie (Weisner et al., 2001). Latent prints developed with 1,2 indandione were sprayed with a working solution of the reagent and then allowed to incubate for a period of 20 minutes within a Bio-Rad® heat box at 70°C (Weisner et al., 2001). Sheets treated with 1,2 indanedione were visualized with 505nm
wavelength of light from a Crimescope® CS-16 Alternate Light Source (Spex Forensics, Piscataway, NJ). All developed prints were photographed with a Nikon® D-7100 affixed with a 60mm Nikkor f/2.8D macro lens and an orange Tiffen® filter. Camera was set at f-stop 11 with an ISO of 100 and a 6 second shutter release delay. Photographs were taken with camera set as level to surface of photo stand surface. Prints for analysis were photographed at a 1:1 scale, unless the physical size of the print prevented inclusion of scale marker and print identifying labels. In such cases, these prints were photographed at 1:4 ratio.

2.4 Questioned Document Analysis

To determine if the vacuum swabbing method interfered with basic questioned document (QD) ESDA examination, 10 robbery notes were generated with “known” indentations. To simulate torn paper evidence, five sheets of Staples® Multipurpose white copy paper were torn in half. A sheet of paper was laid over each of the torn sheets and simulated “investigative lead” information like phone numbers, random names, and locations were written on the top sheet using a ball point pen and moderate pressure. The overlay sheets were removed, and the robbery note text was written on the bottom sheets. This process was repeated for all ten robbery notes. All notes were written by the same author and using the same Zebra® Brand F-402 Ballpoint pen (Zebra Pen, Edison, NJ) to ensure consistency. An additional robbery note was generated to evaluate the effect of not elevating the paper while vacuuming. The additional note was generated with the same paper brand and pen as all the other tested notes.

The robbery notes were photographed and then processed with the vacuum swab method. The additional robbery note, referred to as “Note X”, was processed with the vacuum swab method however it was left flat on the benchtop during the swabbing process. All other tested robbery notes were elevated off the benchtop using magnetic clamps (see figure 2 and 3).
Following the vacuum swab process, each note was placed into a Foster and Freeman® ESDA\textsuperscript{2} (ESDA Mark II) humidity chamber for the period of one-half hour. Chamber humidity was set to 65%, as per manufacturers recommendations to maintain a minimum of 60% humidity. The humidified documents were placed onto the Foster and Freeman® ESDA\textsuperscript{2} (ESDA Mark II) instrument. Each note was covered in ESDA processing film and indented writing which carried over from the top overlay “investigative lead” paper was developed using Foster and Freeman ESDA2 Cascade Developer (Batch A14/02). The subsequent visualized ESDA film was covered in Foster and Freeman® fixing sheets to preserve the integrity of the ESDA film (all instruments and supplies Foster and Freeman, Sterling, VA).

All robbery note indented writing visualization evidence was scanned and recorded with careful observation for any lines or gouging of the paper which may have been caused by the passing of the vacuum swab pipette.
3. Results

3.1 Method Development

The vacuum swabbing technique was optimized in three steps. Leaving the paper on the bench, the vacuum swab tended to snag on imperfections on the paper or the bench surface, making the process sporadic and more difficult to control. By raising the paper using the fused tweezers shown in Figure 1, it was easier for the user to manipulate the paper to vacuum connection, get sufficient suction, and perform smooth and steady movements. In addition, carrying out the vacuum swab process on the bench surface would “sandwich” the document between the hard bench surface and the hard surface of the pipette and potentially create indentations that would likely smudge prints. Holding the paper up with tweezers in one hand while vacuuming required a lot of practice. A more stable mechanism to lift the paper off the surface could have been a type of elevated frame, but discussion with members of the New York City Police Department Police Laboratory Questioned Documents Unit revealed that many of the evidence intake notes are torn sheets of paper in different sizes. This information led to the development of the elevated magnetic clamps where the clamps could be oriented in any desired configuration around sheets of paper of any size (see Figure 2).

The mean overall time to vacuum swab letter size sample documents was 27 minutes. The “active” series mean time of processing was recorded to be 25.3 minutes. The “passive” series mean time of processing was recorded as 28.8 minutes. Variations in time of processing were noticed in some instances where vacuum flow through the tubing seemed to decrease slightly. In addition, the application and operator skill level with the vacuum swab apparatus improved over the course of study, resulting in faster overall processing periods. The magnetic clamp design provided an easier to handle, more stable platform to physically raise the document
off the bench top and reduced the overall document processing time to approximately 8.5 minutes.

3.2 DNA Results

DNA quantitation revealed similar average DNA concentrations for the three sets of samples, with the exception of the letters “post activity.” This last series had a similar median, but the average was higher due to a single outlier with a 4x higher concentration than any other sample.

Figure 4. Quantities of recovered DNA for three different sets of written letters; single collection set n=11, pre- and post-activity n=6 each.

Whisker plots shown in Figure 4 depict interquartile range (boxes), median values (line), and averages (X) for the three sample sets. Quantities of recovered DNA in the single collection unwashed hands series ranged from 0.6-54.3 pg/µL with an average detected quantity of 14.73pg/µL (SD ±14.44). Quantities of recovered DNA in the active series prior to the aerobic
activity ranged from 3.1-55.8 pg/µL with an average detected quantity of 14.92 pg/µL (SD ±18.65). Quantities of DNA in the active series ranged from 1.9 to 221.6 pg/µL with an average detected quantity of 44.07 pg/µL (SD ±79.69). A Kruskal Wallis One Way Analysis of Variance test was performed using RStudio™ v 1.2.1335 to evaluate the single collection, pre-, and post-activity groups (R-Team, 2018). A chi-squared value of 0.32312 with 2 degrees of freedom, and a p value of 0.8508 indicated that none of the mean differences between were statistically significant. The wide range of DNA concentration reflects the donor to donor variability typical for touch DNA. Individual donor propensity to deposit DNA into surfaces had previously been determined as “shedder status” and based primarily holding an inanimate object for a preset period of time (Lowe, Murray, Whitaker, Tully, & Gill, 2002). Lowe et al. (2002) concluded that there was no clear distinction in shedding status based on gender. More recent studies, such as those carried out by Quinones et al. (2012), included more complex chemical composition such as sweat components and cell free DNA. Their findings suggested that the definitions of shedder status may be open to interpretation and more difficult to predict based on one-time collection and testing. The variation of daily activities of individual donors prior to donation can also provide a variation in the amount of DNA collected throughout relatively short time periods (Goray, Fowler, Szkuta, & Van Oorschot, 2016).

A statistical comparison of the DNA quantities collected from male and female donors for this study was generated. The difference between male and female volunteer DNA deposits was not significant. The DNA collected from seven male volunteers throughout the study was an average of 55.56 pg/µL (SD ±72.59). The DNA collected from 16 female volunteers throughout the study was calculated to be an average of 9.69 pg/µL (SD ±7.12). The Mann-Whitney U value was 38 for male-female comparison. The critical value of U at p< 0.05 was
calculated at 26. Therefore, the result is not significant at p<0.05. A z-score value was determined to be -1.16927, the p-value was determined to be 0.242. This value is also not significant at p<0.05. Variations within the amount of deposited DNA from male and female donors had been documented in several studies (Lacerenza et al., 2016). The study performed by Lacerenza et al. (2016), demonstrated through a variety of tests including mRNA testing of recovered transfer samples to determine cellular origin, that male donors tended to produce a higher quantity of DNA, although the cause of this difference was not clear.

No DNA was detected on the sheets used as a blank control for this study. Analysis of recovered mixture profiles was based on the complexity criteria and the ability to estimate potential number of DNA donors in the sample (Schneider et al., 2009). Electropherograms of collected DNA samples provided a blend of single source profiles, database eligible mixtures, and comparison only mixtures. The German Stain Commission stated that a mixture containing more than 6 alleles at any locus would not allow a reliable number of donors to be determined (Schneider et al., 2009). No single locus presented more than 5 alleles for any of the developed profiles. Sample mixtures at each individual locus were evaluated for type A, type B, or type C (Schneider et al., 2009) and further evaluated to assess if a major donor to that sample could be determined. A type A mixture was defined as a mixture which presented as demonstrating no clear major contributor. A type B mixture was defined as a mixture with a clear major and minor component. A class C mixture was defined as a mixture with no clear major and minor contributor which also presented with possible stochastic effect commonly associated with low level DNA. A naming system to characterize the profile types was used, each was assigned a letter code which was represented on the resulting heatmap as “Category.” Figures 5, 6, and 7 show examples for different profile types.
Figure 5. Electropherogram of single source full profile.
Figure 6. Electropherogram of a complex mixture determined to be used for comparison only. Major contributor was unable to be deduced for this profile.
Figure 7. Electropherogram of a Database eligible mixture.

A total of four single source samples were observed, six samples were classified as “database mixture”, nine samples were classified as “comparison mixtures”, and four samples were classified as “not suitable for comparison.” Only one sample, P44, presented loci
(D8S1179, D16S539, and D2S1338) which contained foreign DNA without presenting any donor alleles. One pre-activity sample did not show any DNA, thus was negative and included in the not suitable for comparison count.

Table 3. Heat map for all STR profiles generated for this study.

<table>
<thead>
<tr>
<th>Sample</th>
<th>D8 S1179</th>
<th>D21 S11</th>
<th>D7 S80</th>
<th>CSF 1PO</th>
<th>D3 S1358</th>
<th>THO1</th>
<th>D13 S317</th>
<th>D16 S539</th>
<th>D2 S1338</th>
<th>D19 S433</th>
<th>VWA</th>
<th>TPOX</th>
<th>D18 S51</th>
<th>D5 S818</th>
<th>FGA</th>
<th>Category</th>
<th>Mixture status</th>
<th>LR Mix</th>
</tr>
</thead>
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<td>PMS 2</td>
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<td></td>
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<td>3.48x10^8</td>
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<td></td>
<td></td>
<td></td>
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<td>Complex</td>
<td>9.78x10^5</td>
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<td>C</td>
<td>Single</td>
<td>7.54x10^7</td>
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<td></td>
<td></td>
<td></td>
<td>DB</td>
<td>Mix</td>
<td>4.76x10^10</td>
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<td>P33A</td>
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<td></td>
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<td>2.08x10^20</td>
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<td></td>
<td></td>
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<td>UNK</td>
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<td>UNK</td>
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<td></td>
<td></td>
<td>NS</td>
<td>UNK</td>
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</tr>
</tbody>
</table>

- **Full donor alleles** or Major contributor
- **Donor Alleles Present** or Major contributor but Not Deducible
- **Alleles Present but No Donor Alleles**
- **No alleles detected**
Heat map shown in table 3 is a representation of all tested samples and allele results for all loci tested. Each cell of the heat map in an indication of the results for a sample at that indicated location.

### 3.3 LR Studio Mix Results

All mixtures suitable for comparison were compared to profiles of provided reference samples. The strength of positive association was calculated using LR Studio Mix® version 2.1.3 determining the likelihood ratio (LR) between the hypothesis that the volunteer is the source of the DNA (single source profiles) or the volunteer contributed to the mixture, versus the hypothesis that another unrelated individual is the source or contributed. Results were classified based on Butler’s defined table of predicates for likelihood ratios as follows (Butler, 2015):

<table>
<thead>
<tr>
<th>Likelihood ratio value</th>
<th>Verbal designation of Probative Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 to 10</td>
<td>Limited Support</td>
</tr>
<tr>
<td>10 to 100</td>
<td>Moderate Support</td>
</tr>
<tr>
<td>100 to 1000</td>
<td>Moderate Strong Support</td>
</tr>
<tr>
<td>1000 to 10,000</td>
<td>Strong Support</td>
</tr>
<tr>
<td>10,000 and greater</td>
<td>Very Strong Support</td>
</tr>
</tbody>
</table>

Values for chart taken from Butler (Butler, 2015).

One of the samples designated suitable for comparison had an LR< 1, thus not supporting the volunteer as a contributor to the mixture. The P44 sample was the only sample with foreign DNA and none of the donor’s reference alleles at several locations (D8S1179, D16S539, and D2S1338). The remaining likelihood ratios all exceeded the level of very strong support (see
Table 3 “LR Mix” Column). The “Full” profiles all demonstrated verbal designation of “Very Strong Support” for their likelihood ratios. The “Database Profiles” showed variation in the likelihood ratio values but all samples demonstrated a verbal designation of “Very Strong Support.” The profiles designated as “Comparison Mixtures” samples are only suitable to directly compare a suspect profile to the DNA result and thus cannot provide investigative leads via database searches. Samples falling under the Comparison Mixture were designated as “Very Strong Support” but showed the weakest overall statistical weight which is consistent with the complexity of the results. Likelihood ratios for database mixtures with a major component would have been higher if the study had used a continuous probabilistic tool. LR Studio mix is a semi-continuous modelling software (Gill & Haned, 2013). The values generated by semi-continuous models of LR consider the allele call values of the sample mixture and the alleles of the reference sample (Butler, 2015; Gill & Haned, 2013). A fully continuous model of LR determination considers not only allele calls but the peak height ratios as reported on electropherograms, mixture ratios, as well as stutter within the sample (Butler, 2015). Taking peak heights into account strengthens the statistical weight for a mixture when the major component is consistent with the volunteer.

3.4 Latent Print Results

Latent fingermark development had a high success rate for sebaceous prints and demonstrated the advantage of 1,2 indandione targeting amino acids over magnetic powder for eccrine prints. All developed prints were photographed before and after vacuum swabbing and evaluated for content and composition of friction ridges. Fingermarks were deemed to be “of value” (OV) to an investigation as follows: developed fingermarks were evaluated for clarity of
friction ridge lines to first ensure the fingermark in question was a single impression, and not an overlapping composite of multiple prints. The prints were then evaluated to observe identifiable minutiae. Prints which demonstrated at least eight unique and notable ridge characteristics were considered to be OV for the case. Having been a New York City Police Department Detective and an evidence collection technician with 14 years of field experience, these determinations were made based on this work experience. Figures 8A and 8B show examples for sebaceous print developed with magnetic powder before and after vacuum swabbing. There are no visible signs of loss of minutiae after using the vacuum.
Figure 8(A&B). Developed sebaceous latent fingermarks on paper with Sirchie® Magnetic Fingerprint powder. Figure 8A shows a print prior to application of the vacuum swab technique. Figure 8B is the same latent fingermark after vacuuming.

Table 4. Summary of all latent print results.

<table>
<thead>
<tr>
<th></th>
<th>SEBACEOUS PRINTS</th>
<th>ECCRINE PRINTS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=50</td>
<td>n=50</td>
</tr>
<tr>
<td>POWDER 1,2 INDANEDIONE</td>
<td>44 38</td>
<td>0 42</td>
</tr>
<tr>
<td>OF VALUE (OV)</td>
<td>27 31</td>
<td>N/A 29</td>
</tr>
<tr>
<td>NOT OF VALUE</td>
<td>17 7</td>
<td>N/A 13</td>
</tr>
</tbody>
</table>
As can be seen in Table 4, of the 50 deposited fingermarks from the sebaceous set 44 were visible with magnetic powder. Of these 44 developed fingermarks, 27 were considered OV, a rate of 61.3%. The detection rate was slightly lower for 1,2 indanedione where 38 of 50 fingermarks of the sebaceous set were detected. Of these 38 developed fingermarks, 31 were considered OV, a rate of 81.5%. No fingermarks from the eccrine set were successfully developed with magnetic powder. Eccrine prints could be visualized with 1,2 indanedione. 42 of 50 fingermarks of the 1,2 indanedione eccrine set were detected. Of these 42 developed fingermarks, 29 were considered OV, a rate of 69%. A comparison of the friction ridge content both before and after the application of the vacuum swab method showed that none of the observed prints from either method demonstrated loss or deletion of visualized friction ridge quality as a result of the vacuum swab method. Figures 9A, 9B, 10A, 10B, 11A, 11B, 12A, and 12B show more examples for this, this time after 1,2 indanedione development. This indicates that using the method of vacuum swab collection prior to development of latent fingermarks may not affect the overall quality of the latent fingermarks. A rinse or dip into chemical reagents may cause the accidental loss of probative DNA from the paper surface.
Figure 9(A&B). Sebaceous latent prints on paper developed with 1,2 indanedione. Figure 9A is latent fingermark produced prior to application of the vacuum swab technique. Figure 9B is the same latent fingermark after application of vacuum swab technique. Again, there are no visible signs of loss of minutiae using the vacuum swab technique.
Figure 10(A&B). Sebaceous latent prints on paper developed with 1,2 indanedione considered not “of value”. Figure 10A is latent fingermark produced prior to application of the vacuum swab technique. Figure 10B is the same latent fingermark after application of vacuum swab technique.

The developed fingermarks in figure 10(A&B) were not to be considered “of value” in furthering an investigative case due to a low quantity of observed minutiae as well as a high degree of smudging. Despite not being of value for latent print examiner comparison purposes the fingermark does not show any signs of minutiae destruction due to the use of the vacuum swab technique.
Figure 11(A&B). Eccrine latent prints on paper developed with 1,2 indanedione. Figure 11A is latent fingermark produced prior to application of the vacuum swab technique. Figure 11B is the same latent fingermark after application of vacuum swab technique. There are no visible signs of loss of minutiae after vacuum swab technique.
Figure 12(A&B). Eccrine latent prints on paper developed with 1,2 indanedione considered not “of value”. Figure 12A is latent fingermark produced prior to application of the vacuum swab technique. Figure 12B is the same latent fingermark after application of vacuum swab technique.

The developed fingermarks in figure 12(A&B) were not to be considered “of value” in furthering an investigative case due to a low quantity of observed minutiae as well as being very faint making the existing minutiae very difficult to see without additional photo enhancements or editing. Despite not being of value for latent print examiner comparison purposes the fingermark does not show any signs of minutiae destruction due to the use of the vacuum swab technique.
3.5 Indentation Visualization Results

ESDA processing visualized not only the controlled indentations but also some latent fingerprints. All ESDA documents were reviewed for the location and legibility of the indented writing carried through from the overlay sheet, as well as for additional indentations possibly generated by the glass pipette during the vacuuming step. No indentations which could be attributed to the vacuum swab technique were observed in the set of ten robbery notes processed while suspended above the benchtop. “Note X” on the other hand, the note directly placed on the bench demonstrated a considerable amount of developed lines running along the same path as the vacuum swab (see figure 13 and 14).

Figure 13. Simulated robbery note “X” after processing with vacuum swab technique.
Figure 14. Details of impression evidence detected on robbery note “X”, which was not raised off benchtop as the other notes within this study, during ESDA processing.

Figures 15-17 show images of torn robbery notes after the vacuum swab technique and before and after ESDA processing. Indentations and latent fingermarks are clearly visible and are not negatively affected by the vacuum process.
Figure 15. Simulated robbery note on torn paper after processing with vacuum swab technique.

Figure 16. Simulated robbery note from figure 14 ESDA film after processing. No indication that vacuum swab created impressions onto paper during the application of the technique.
Four different sections of indented writing created while preparing the “robbery note” are clearly visible in the example shown in Figure 16. The vacuum swab did not create additional impressions on the paper. The indentations are sharp and readable and were not damaged by the vacuum swab technique. A total of 30 examples of indented writing within the samples were observed. None of these additional impressions demonstrated any sign of damage from the vacuum technique. For some documents, the ESDA process revealed latent fingermarks (example in figure 17A) and notation (figure 17B) evidence on the same sheet, both were apparently not damaged during the application of the vacuum swab.

![Image](A)

![Image](B)

Figure 17(A&B). Details of impression evidence detected during ESDA processing.

The above results demonstrate that DNA evidence can safely be collected prior to questioned document examination.
4. Discussion and Conclusions

Handwritten documents recovered from a crime scene can provide a great deal of probative evidence which may aid in the successful arrest and prosecution of the perpetrator. DNA, fingermark, and indentation evidence recovered from a paper document can combine to provide both information and the identity of the perpetrator. The principle focus of this study was to collect DNA from handwritten documents with sufficient quantity to produce full profiles which could be compared to available databases. The target region of the developed vacuum swabbing technique was the text region of the handwritten document where it was not expected to see latent fingermarks. The challenge was to develop a means of collecting the DNA in the manner least destructive to the paper substrate and other evidence types such as fingermarks and indentations. It has been suggested that the palmar areas of the hand do not possess significant quantities of DNA for reliable testing (Oleiwi et al., 2015). Studies using shed corneocytes on touched samples have shown that these samples do not possess enough nuclear DNA to provide full profile without considering the addition of other DNA sources such as secretions from eccrine or sebaceous glands or saliva (Stanciu, Philpott, Kwon, Bustamante, & Ehrhardt, 2015).

In this study, participants were not asked to wash their hands and wrote several sentences thus scraping their hand and lower forearm over the paper. This produces a more realistic contact trace containing a mixture of cellular components from fingers, palms, and forearm from the donor, but also extraneous DNA from other individuals. The developed vacuum swabbing method allows the forensic scientist to recover adhering material from porous copy paper surfaces of any size or shape and was successful for this sample type. Almost 80% of the samples resulted in potentially probative DNA profiles, mixed or single source, either eligible for FBI DNA database upload or direct comparison to a person of interest. Only five, or 21%, of the
profiles were negative, not suitable for comparison, or after comparison showing a likelihood ratio smaller than one, making the result more likely if another person but not the known contributor is part of the mixture. This is a high success rate for contact traces, where success rates range from 98% useful DNA profiles for worn clothing to 18% for touched bullets (Mapes, Kloosterman, & de Poot, 2015). This is also higher than the 25% of the samples that produced DNA results in the targeted DNA collection from handwritten documents performed by Parsons et al. (2016).

The results of the latent print development suggest that there is no deleterious effect to developed fingermarks by the vacuum swabbing device. The application of 1,2 indanedione to eccrine fingermarks provided a higher rate of successfully visualized fingermarks than for sebaceous prints. The eccrine developed fingermarks, under cursory observation, presented greater levels of fluorescence in monochromatic conditions. This supports the results of Lee and Joullie (2015) that sebum rich fingermarks on paper produced poor results in comparison to eccrine fingermarks. The number of developed OV prints in the study varied per person as would be expected based on individual physical traits, deposit pressure of the donor upon the paper, and donor propensity to deposit fingermarks. The overall quality of the fingermarks was not the focus of this study, the goal had been to determine if there was any destructive effect upon the fingermarks using the vacuum swabbing technique. Nevertheless, only one of the 10 donors provided samples in which no latent fingermarks were observed throughout all processing techniques. In their study, Almog et al. (2018), concluded that an increased weight downward by the user, to a maximum studied 10kg, increased both the latent fingermark quality and the DNA quantity. With pressure being an important variable for latent print studies, Fieldhouse (2011) developed a mechanical method to control the amount of force of the donor to develop
fingermarks on a variety of surfaces, including paper, in a consistent manner. For this study, pressure was not controlled and the downward hand pressure for each participant was based on their individual characteristics.

ESDA processing results strongly indicated that the swabbing technique did not interfere with indented writing nor create new indentations during the passing of the pipette body over the target document. The single “robbery note X” sample demonstrated significant levels of developed indentations along the path followed by the vacuum swabbing device. This strongly underscored the need to keep the document elevated during the swabbing process to avoid potential destruction to indentation evidence. In their study, Parsons et al. (2016) reported success in recovering DNA from handwritten notes using wet/dry swabbing and dry/dry swabbing techniques. Both of these techniques were considered to be non-destructive, however the authors reported that both methods had deleterious effects on ESDA processing with the dry/dry techniques obscuring fewer indentations on paper documents compared to the wet/dry swabbing techniques (Parsons et al., 2016). In their study using ESDA as a mechanism to recover DNA, Plaza et al. reported that the applied method of directly swabbing mylar ESDA film resulted in recovery of full and high partial profiles for 65% of their samples (Plaza et al., 2016). This result is slightly lower than the nearly 80% potentially probative rate detected in this study. The Plaza et al. (2015) study did not indicate if the direct swabbing of the mylar film interfered with indented writing or latent fingermarks detected on the paper.

With neither fingermarks nor indentations being negatively affected by this DNA collection method, this technique has the potential to reverse the fundamental workflow of many crime labs. Many current lab protocols call for the processing of the document using ESDA processing first, followed by latent fingermark development, and finally DNA processing (Plaza
et al., 2015). The vacuum swab method will allow analysts to collect DNA evidence first and then process the document using ESDA and latent fingermark development all in a non-destructive manner preserving the original paper evidence. This has the advantage of not subjecting the DNA to deleterious loss while spraying with or dipping into 1,2 indanedione or ninhydrin (Bhoelai et al., 2011). Minimal pre-collection handling also reduces the DNA contamination risk (Szkuta, Harvey, Ballantyne, & Van Oorschot, 2015).

In using a method of swabbing the entire surface area of the target, it is not necessary anymore to guess where a porous surface was touched or visualize fingermarks for targeted collection. Other non-destructive methods to recover DNA from paper require this type of processing first (Fieldhouse, Oravcova, & Walton-Williams, 2016). Increasing the area of collection raises the recovered DNA yield. An advantage of using a focused collection apparatus such as the vacuum swab is, that it allows for the entire surface area of a substrate to be processed efficiently while simultaneously granting the user the precision to omit areas if necessary that would not be available using mass collection methods. In some case examples, a surface may provide several contamination hazards for DNA but prove to be extremely valuable for print development. An example for selective vacuuming would be this case scenario: a robbery has been committed within a convenience store or gas station where a gun-wielding perpetrator presented a note to the employee. During an ensuing struggle, the perpetrator discharged his firearm multiple times. One or more of the bullets struck and penetrated a container of common motor oil on the counter, another struck the victim. The blood of the victim, as well as a quantity of motor oil spilled onto the document, which the perpetrator left behind as he fled the scene. The document can potentially contain the latent fingermarks and DNA of the perpetrator, as well as indentation evidence which may provide alternate
investigative leads. The motor oil is likely to inhibit collection of DNA or make the swabbing process too complex. The blood of the victim is a source of DNA but is less probative in this case in comparison to that of the perpetrator. The blood evidence may result in a mixture too great to detect the minor contributor, in this case, the perpetrator. The option of employing the smaller, precise collection device gives the user the surgical precision to collect vital biological evidence areas while efficiently avoiding sections which may inhibit DNA processing such as motor oil stains, known inhibitors, or debris. A method of collection such as the one described by Plaza et al. (2015) with mylar foil would require the entire surface of the document to be processed as a whole and with body fluids present may cause contamination issues. Selectively processing regions of interest on the foil by cutting them out may be an option, but with the foil being very thin technicians would have to be very careful while handling the material. The method presented here is more directional, and avoids additional swabbing transfers. The vacuum method and the elevation above the bench are critical for optimizing recovery and protecting the friction ridge detail and indentations from being distorted. Directly swabbing the paper surface with wet swab techniques would damage the paper surface (Fieldhouse et al., 2016), while dry swabs may not damage the other evidence types but will not efficiently recover DNA (Dalal 2018). The vacuum swab method also only uses parts that are easy to decontaminate between evidence items. Pipettes and swabs are disposable and can be made DNA free prior to processing; the clamps are easy to clean with bleach, water, and ethanol.
5. Future Studies

The use of copy paper provided strong results which indicate that the vacuum swab method can be quite effective to collect DNA evidence while preserving other valuable probative evidence such as fingermarks and indentations. Participants were not required to wash their hands prior to providing a sample. The resulting profiles were a reflection of real-world scenarios where it is unlikely that a perpetrator would take the time to wash their hands prior to scribing a note. This aspect of the test likely provided a blend of cellular components as well as a higher rate of mixtures than would be seen if the donor had washed prior to writing a sample. It may be of interest to conduct a separate exploration of the difference in DNA quantity and quality of donor-only material with the donor washing prior to sample writing.

The initial focus of this study was to recover DNA profiles from regions of the paper document which were not traditionally processed for latent fingermark evidence, specifically the interior region of the text. The vacuum swabbing technique avoided the margin regions of the document as not to destroy potential latent fingermarks. As the study developed it became apparent that the vacuum swab technique did not affect the quality of latent fingermark friction ridge detail. For further studies, the region of the vacuum swabbing should be expanded to include the entire surface of the paper document, including the margin regions.

Prior to casework implementation, testing needs to be expanded to cover a variety of paper grades and quality, such as newspaper, cardstock, photo, and magazine quality papers. The flexibility of copy paper may have provided a mechanism for the DNA to be collected by the vacuum swab device without damaging fingermarks or indentations. The positive results obtained from copy paper may not translate into varying thicknesses of paper which may be too rigid to flex and transfer sufficient friction onto the substrate and smudge the print. Thinner
papers may also be subject to being deformed by the pull of the vacuum and delete some indentation evidence.

In its current form, the vacuum swabbing method faced mechanical challenges which would benefit from optimization. The pipette was connected to a benchtop vacuum source by means of simple plastic tubing. Throughout the collection process, the tubing was subject to collapsing upon itself when the vacuum was activated. A stronger quality tubing may be able to prevent this collapse and the improved vacuum flow may allow for a greater yield of DNA collection.
REFERENCES


https://doi.org/10.1111/1556-4029.12779


Yaraskavitch, L., Graydon, M., Tanaka, T., & Ng, L. K. (2008). Controlled electrostatic
