Combined Extraction of Protein and DNA from Touch Evidence on Ammunition Cartridge Cases

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Abstract

DNA analysis of touch evidence on cartridge cases has proven difficult despite numerous attempts to improve collection and extraction methods for this important type of evidence. Numerous factors influence the variability and often paucity of touch evidence such as shedder status, pressure and duration of contact, surface type, and even collection and storage. In addition to these factors, retrieval of biological information from cartridge cases is even more challenging since firing abrades the surface of the case, removing material, and metal in the case can react with DNA, resulting in oxidation and cleavage of the DNA backbone. Protein is also present in biological touch evidence. Protein is more robust chemically than DNA, and it also contains genetic and contextual information that could be useful to investigators. Extraction and analysis of both DNA and proteins from touch evidence can provide the maximum amount of information needed for probative results, especially when DNA is limited. To fully exploit all available biological information on a cartridge case, all types of information need to be transferred from a surface into a technical workflow. Additional methods need to be developed to maximize retrieval of material and to do so in a compatible manner. This study evaluated the efficiency of transferring artificial DNA and protein from a glass microscope slide surface into the workflow for seven different collection and transfer methods: the standard wet-dry cotton swab was compared with alternative methods, a 'Copan microFLOQ® direct' swab, with and without lysing agent, and with and without extraction buffer, a cell scraper, and adhesive silicone gel-film sheets. Based on the results obtained, it was determined the 'Copan microFLOQ® direct' swab was the overall most efficient and consistent with an average transfer of 58 ± 22% efficiency for DNA and 55 ± 32% for protein compared to 27 ± 24% and 2 ± 27% when using cotton swabs respectively. The evaluation was taken a step further to measure transfer of DNA and protein from real fingermarks on a glass microscope slide to the workflow. It was

determined that the cotton swab and the 'Copan microFLOQ® direct' swab collected an average of 11.8 ± 20 ng and 15.7 ± 18 ng of total DNA, respectively, and 1200 ± 1200 relative densitometry units (RDU) and 2300 ± 1600 RDU of total relative protein density, respectively. This level of relative variation is high but consistent with previous studies for fingermark deposition. When normalized for individuals the 'Copan microFLOQ® direct' increased performance by 1.7-fold (p=0.23) for DNA and 1.9-fold (p=0.02) for protein. Finally, real-life scenarios were mimicked where the cotton swab and 'Copan microFLOQ® direct' were used to transfer DNA and protein from unfired and fired cartridge cases into the technical workflow. The cotton swab and the 'Copan microFLOQ® direct' swab collected an average of 1.1 ± 1.5 ng and 2.8 ± 2.4 ng of total DNA, respectively, and 660 ± 380 RDU and 1200 ± 1500 RDU of total relative protein density, respectively for unfired cases. When fired there was a significant reduction in the amount of DNA material, but the same trend was observed. The cotton swab and the 'Copan microFLOQ® direct' swab collected an average of 0.3 ± 0.5 ng and 0.5 ± 0.6 ng of total DNA, respectively, and 400 ± 340 RDU and 530 ± 450 RDU of total relative protein density. When normalized for individuals the 'Copan microFLOQ® direct' increased yields by 3.2-fold (p=0.03) for DNA and 2.0-fold (p=0.06) for protein when unfired and 3.7-fold (p=0.09) for DNA and 1.6-fold (p=0.24) for protein when fired. The firing process was harsher on DNA than on protein. Protein was 2.2-fold (p = 0.07) more persistent than the DNA on cartridge cases after firing. We conclude that transfer of residual biological material from fingermarks is improved when using 'Copan microFLOQ® direct' swabs compared to the standard wet-dry cotton swab method, and that the improvement applies to both idealized surfaces such as microscope slides, and challenging surfaces such as unfired and fired cartridge cases. We also observe that protein is more stable during the firing process and potentially can provide

additional identifying and contextual information for investigators for this difficult type of evidence.

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1. Introduction

Fingermarks, or touch evidence, on fired (expended) cartridge cases are highly probative, since information about who loaded a fired gun at a crime scene is a major investigational lead. However, information from the biological material in those fingermarks may prove difficult to obtain. The amount of material transferred may be too low and inconsistently applied [1]. Therefore, analysis of protein can also be beneficial since it provides an additional source of genetic and contextual information. Additionally, current collection methods are typically inefficient and tend to leave material on the surface or on the swab. The result is a further reduced level of probative biological material not entering into the workflow. The aim of this study is to validate a collection technique that can transfer as much of the biological material as possible into the workflow, into microcentrifuge tubes, and to do so for both DNA and protein material. This would allow probative information on a fired cartridge case to be maximized, especially considering the low and variable initial levels of both components in fingermarks. In this way the information contained in the chemically more stable protein can also be included in the analysis. Furthermore, to the degree possible this study takes a quantitative approach for both DNA and protein to explore how much of the biological material is being transferred into the workflow. By focusing on the transfer of both DNA and protein, the total amount of context and genetic containing information is maximized, increasing the potential for developing probative and investigative leads from fingermarks.

2. Background

2.1 Main Skin Components

Touch evidence results from the transfer of skin cells to a surface they come into contact with. This follows Locard's exchange principle that states "every contact leaves a trace" [1]. The

skin is made up of three anatomical layers that are known as the epidermis, dermis, and hypodermis. The epidermis is the outer layer which prevents water loss via evaporation, and it is the protective barrier for underlying tissue. The epidermis is made up of numerous cell layers where the innermost one is the stratum germinativum also known as the basal cell layer. Following that layer is the stratum spinous layer (prickle cell layer) which is held together by desmosomes. The next layer that is closer to the surface is the stratum granulosum (granular layer) and the outermost layer is the stratum corneum also known as the cornified or callus layer [2, 3]. The outer surface is comprised of terminally differentiated, or cornified, squamous cells. These are a type of keratinocyte, corneocytes, where the cytoskeletal structural elements in the cell are concentrated, radically changing the physical properties of the cell. The cells become more robust, enucleated, flattened, and attached to neighboring cells in layers of two-dimensional sheets. Cornification is similar to apoptosis, except that many cellular structures remain intact. The most superficial cells become more exposed to activated endogenous proteases that break down the attachments to surrounding cells, resulting in flaking or release of the squamous cells into the environment [3]. This process is called desquamation which allows for skin renewal. An overview of how this works is the cells migrate to the surface of the epidermis from the basal layer, which is the innermost layer of the epidermis just before the dermis, in about 30 days. These become the squamous cells on the surface of the epidermis which will evenly shed allowing for more cells to take their place continuing the desquamation process [4]. The outer layer however is complex, and keratinocytes compose about 90-95% of the epidermis cells, along with melanocytes (pigment producing cells), which are found between cells of the stratum germinativum. Langerhans cells (dendritic cells), which are found in the stratum spinosum, and Merkel cells, which are found in the stratum germinativum, are also types

of cells that are present in the epidermis [5]. The next layer of skin, the dermis, is the layer of connective tissue that supports the epidermis. The dermis is responsible for blood supply, sensory reception, and temperature regulation. It also contains eccrine (sweat) glands, apocrine glands, and sebaceous glands. The eccrine glands are used in temperature regulation, secrete sweat, and excrete metabolic waste. These also release cells and biological material such as salts, lipids, proteins and sugars onto the surface, coating the skin. The last main skin layer, the hypodermis, is loose connective tissue and is under the dermis. This layer contains adipose cells that hold fats that serve as an energy source [5].

2.2 Friction Ridges

In forensic casework, touch evidence is often in the form of fingerprints due to the transfer of skin cells to a surface they encounter. Fingerprints are based on the pattern of friction ridges. Ridges and furrows are formed on the fiction ridge skin in the dermis layer by the primary and secondary ridges. Primary and secondary ridges interlock with the dermis and strengthens the friction ridge skin. The sweat glands extend from the primary ridges to anchor in the dermis or hypodermis layers. The friction ridge creates friction between the volar surface and the contact surface which allows for grip. Friction skin ridges are found on planar and palmer surfaces of both hands and feet. These ridges are flexible and deform under pressure. The volar pads will take a certain shape that results in the primary fingerprint pattern such as a loop, whorl, or arch. Minutiae, friction ridge characteristics or details, will also form within the friction ridge pattern. The friction ridge skin contains the ducts of sweat glands that provide moisture for grip and sweat secretions that transfer to the surface, which can also accumulate biological material [5].

2.3 Components of Fingermarks

Biological material in a fingermark contains potentially identifying information that can be very useful when it comes to forensic casework [6]. It is important to note that a fingerprint is a 2D-pattern while fingermarks refer to deposited biological material and are often not useful for generating a 2D pattern. The primary components of fingermarks include sebaceous fluid, eccrine perspiration, extracellular cell-free DNA, shed skin cells and other cell types [6]. Other biological material includes substances from epidermis, secretory glands in dermis, metabolites, and traces of medications and drugs. Eccrine perspiration is composed mostly of water, organic and inorganic compounds. Some of the organic compounds include amino acids, proteins, glucose, lipids, and vitamins. Some of the inorganic compounds include chloride, sodium, potassium, iron, zinc, calcium, tin, and mercury ions. Sebaceous secretion is composed of organic compounds like lipids, fatty acids, glycerides long chain fatty acid esters, squalene, and sterols. Biological material may be transferred from elsewhere on the body such as saliva or sebaceous material. The fingermark also includes external transferred exogenous material; this includes biological material from other people including blood, or environmental material including dirt and grease, make-up, food discharges, moisturizers, sun block and hair care products. All this biological and environmental material can be useful in developing investigational leads and can tell a lot about the individuals' behavior, habits, and diets, especially when it comes to fingermarks on cartridge cases at a crime scene [7]. Some even claim to identify sex based off hormone dependent changes in fingermarks [8].

2.4 Biological Sources in Fingermarks

The two main sources of fingermark biological material that can help identify an individual are DNA and proteins. The DNA component of fingermarks includes epithelial cells, cell-free DNA (extracellular DNA) and intracellular DNA [9]. DNA from the skin is a

combination of degraded nuclei from shed corneocytes and cell-free DNA together, along with sweat and sebum [10]. Burrill *et al*. notes in touch deposits the most common cellular material is corneocytes and since they have degraded nuclei this causes their amount of DNA to be uncertain when it comes to deposits [11].

Corneocytes are fully differentiated keratinocytes that are found in the outer layer of the epidermis. These cells lose nuclei and organelles during the process of cornification. The protein in fingermarks is robust and comprised of keratin fibers and a tough envelope that is formed from cross-linked proteins at the cell periphery replacing the cell membrane [12]. The cross-linking falls into two parts: disulfide bonds from cysteine residues in keratins and keratin-associated proteins, and iso-peptide bonds formed through transglutaminase reactions initiated during the final stages of differentiation. Corneocytes, however, have DNA levels that are highly variable, manifest as a lack of correlation between cell count and DNA quantity [11]. Cell free DNA is typically found in sweat, saliva, semen, urine, and touched items. It has been determined that cell-free DNA contains an average concentration of 11.5 ng/mL in touched items yet can vary dramatically from individual to individual [13]. Wickenheiser *et al*. demonstrated that each undifferentiated skin cell, or corneocyte, contains approximately five picograms of nuclear DNA [14]. The protein component of a fingermark includes proteins from endogenous sources such as structural keratins, house-keeping proteins, and corneocyte specific proteins as well as peptides [9, 15].

2.5 Genetic content in DNA and Protein

Burrill *et al*. indicate that corneocytes contain not only proteins, but also residual DNA that can be collected through subsequent processing [11]. The majority of recoverable DNA from touch evidence is cell free DNA [16]. DNA is particularly rich in many forms of variation,

ranging single nucleotide polymorphisms from indels, which include STRs, to single nucleotide SNPs, transposable elements and structural variants [17]. While DNA levels are often low, they are often sufficient in fingermarks to detect STR alleles and to develop STR profiles using low copy DNA techniques [18]. This can provide valuable STR profiles for DNA typing in a forensic laboratory [11]. Other types of DNA-based information are also available, such as epigenetic patterns, RNA transcriptomics and SNP alleles. [19, 20, 21].

The skin is also rich in keratin and complex proteins that, like all protein populations, contain single amino acid polymorphisms (SAPs), the result of non-synonymous SNPs [22]. Peptides that contain SAPs are genetically variant (GVPs) and detection of these peptides using proteomic mass spectrometry allows inference of the corresponding SNP allele genotype [22, 23]. While this type of genetic information is restricted to non-synonymous SNPs alleles, this is a second fundamental means to obtain DNA sequence information from a biological sample. Information contained in protein has some fundamental differences to DNA: it is chemically more stable, protein is many of orders more abundant than DNA with a median number of 300 thousand copies per expressed protein per cell, and the peptides analyzed are considerably smaller than DNA amplicons, resulting in a greater tolerance for environmental degradation [24, 25, 26].

2.6 General Influences on Biological Material Deposition

Deposit of biological material from touch evidence is highly variable, and numerous factors influences the amount deposited on a surface [27]. These factors include sex, shedder status, personal grooming and behavior, pressure and duration of contact, substrate type, level of sweat production, and even body regions [22, 27]. According to Oleiwi *et al*., fingermarks deposit more DNA compared to palmar surfaces. This is important to note because it can

influence whether to swab for DNA first or develop a fingerprint first at a crime scene [27]. Stanciu *et al.* indicate holding time of objects and the pressure applied play a significant part in the amount of biological material deposited on an object. Data suggest the length of contact is not a significant factor, despite it being commonly thought that handling an object or encountering a surface longer deposits more touch DNA. Contact duration does not seem to influence the amount of biological material deposited [9]. As the deposit pressure increases, the surface area of a fingermark increases as well. Thus, low pressure deposition can vary and not be as consistent, while high pressure fingermarks are more consistent [28]. With that said, Delhaye *et al*. note that the friction ridge skin is more elastic at low deposit pressure and is more rigid at high deposit pressure. At that high pressure the skin folds towards the inner part of the finger and creates stacks allowing for more deposit of touch DNA [29]. A significant force is needed to compress the spring in the magazine to load the ammunition, and that force causes skin cells to transfer onto that ammunition. The ammunition that is loaded last into the magazine ultimately becomes more suitable for recovery of biological material due to the increased resistance of the spring, and the increased force applied to compress it. The increase in force means more pressure applied across a greater surface area, and thus more biological material deposited onto the ammunition cartridge cases [30].

Another significant factor that affects the deposit of biological material onto an object or a surface is shedder status, one's ability to deposit biological material upon contact of a surface or an object. The deposit of biological material depends on an individual, where some individuals may be 'good' shedders or 'bad' shedders. In general, 'good' shedders will deposit more biological material, like DNA or proteins, compared to 'bad' shedders who may deposit low to undetectable levels of biological material even if conducting the same tasks [31, 32, 33].

Yet while some individuals demonstrate consistently high or low levels of deposition, others show variation [34]. Numerous factors can affect an individual's shedder status such as age, personal habits, and sex. Fonneløp *et al.* note that males have increased DNA shedding compared to females, which may be an important investigational lead when it comes to a crime scene [35]. Age may be a factor in shedder status, especially since a child typically is a 'good' shedder compared to the elderly. This may provide important information about the handler and who deposited that touch DNA [36]. According to Ostojic *et al*., it is important to note that there is not a strong correlation between dense deposition of skin flakes and obtaining a high-quality DNA profile [37]. Determining an individual's shedder status can be very useful to an investigation for examination of evidence [35]. One consequence of individuals potentially having consistent levels of DNA released from their fingers is the expectation of high variation in DNA yields, confirmed in the literature. This may be reduced at the data analysis level by conducting comparisons for each treatment for each individual instead of pooling data and using absolute combined values [34, 35, 36].

Personal habits and behaviors may also play a role. Washing or not washing hands is a significant factor that alters the deposit of biological material on a surface or object. Washing hands can remove biological material, especially DNA, resulting in little to no extracellular or cell pellet associated DNA. However, some biological material may still be present even with washed hands and can provide other biological information that may be useful to an investigation. It is apparent that the longer an individual goes without washing their hands the more biological material they will deposit on other objects by touching them [9, 31].

Secondary transfer is important to note since it can influence the deposited biological material on a surface or object. Secondary transfer is defined as the transfer of DNA from one

person to another and then to an object or surface, or alternatively the transfer from one person to an object and then to another person [31, 38]. Lowe *et al.* suggest that shedder status can influence secondary transfers. The secondary transfer will presumably occur with the 'good' shedders cell deposits [31]. Fonneløp *et al.* also indicates that a 'good' shedder will deposit way more DNA than a 'bad' shedder, thus causing difficulty with detection of that 'bad' shedder since the 'good' shedder will interfere with the signal of a 'bad' shedder's deposit [35]. This increases the difficulty of profile interpretation [38]. The type of surface and moisture content may be an important variable for secondary DNA transfer as well, since epithelial cells, a component of a fingermark, adhere better to porous surfaces than non-porous surfaces [39]. With forensic casework, it is very important to note the type of surface the touch evidence is applied on and the moisture at a crime scene, as well as shedder status to fully grasp the factor of secondary transfer [39].

2.7 Surface Variability

The substrate surface, environmental conditions, friction of contact and moistness of the biological sample play a factor in the presence and transfer of touch DNA. Areas that are most frequently handled or are constantly contacted will have a high amount of DNA deposited on it. Areas that are frequently cleaned will have a reduced level of DNA on them [33]. The deposition of biological material on substrates is not dependent on handling time, but dependent on individual and substrate type. Daly *et al.* notes that epithelial cells more readily adhere to porous substrates than non-porous substrates, and the best yield for DNA transfer and recovery was wood over fabric and glass. The type of substrate that biological material is deposited on can indicate whether a significant amount of DNA is likely to be available for a full profile [38]. Hedman *et al.* demonstrate that traditional cotton swab techniques perform just as well as

alternative swabs on smooth/non-absorbing surfaces. They also indicate that using a large foam swab on absorbing surfaces, like wood, can increase the DNA yield compared to using other swab types. It is important to note the type of surface for a sample so that the right type of swab can be used to optimize DNA yields [40]. Sterling *et al.* indicates reduction of biological material on ammunition surfaces such as aluminum, steel, nickel, and brass [41]. Extraction of touch evidence on aluminum cartridge cases have produced better DNA yields [42]. Yet on the other hand, brass cartridge cases have been shown to yield less DNA [10, 41, 43]. Overall, DNA will have a higher affinity for porous surfaces rather than non-porous surfaces that make up metal ammunition [44].

2.8 Metal Effects

Cartridge cases are essential components of modern ammunition. Specifically, a cartridge case is a component of a cartridge, which is a unit of ammunition that generally consists of the cartridge case, the primer, the powder, and the projectile. Cartridge cases are made up of certain metals depending on what type of cartridge case is used. These metals contain metal ions that can solubilize and ionically interact with the phosphate groups of the DNA backbone. The metal ions are highly reactive and catalytic since they contain vacant higher-level orbitals that allow easy swapping between high energy states. The resulting redox reactions result in removal and alternately addition of electrons, resulting in breakage and hydrolysis of covalent bonds. The metal ions; like Zn^{2+}, Co^{2+}, Cu^{2+}, and Ni^{2+}; are incorporated in the DNA forming M-DNA since they replace the imino protons of DNA base pairs in an environment above pH 8. When the DNA collected from the touch evidence of the cartridge cases is examined, the capillary electrophoresis produced broad peaks with low fluorescent detection due to the metal ions. These metal ions significantly affect the resulting DNA profile, and the profile is difficult to interpret.

However, studies have shown using a pH 7 buffer during extraction of the DNA can prevent the formation of M-DNA and allow for sharp and intense peaks for an interpretable DNA profile [45]. Brass cartridge cases are the most commonly used ammunition cartridge case, the major component being copper. Copper contains free divalent ions that can generate radical species, especially in the presence of hydrogen peroxide. This is generated through Habor-Weiss-like reactions. The reactive oxygen species, like hydroxyl radical, cause oxidative damage to DNA [43, 46]. Numerous studies have indicated that the addition of copper-binding tripeptides can help protect DNA from copper-mediated degradation. This purification method and other purification methods, such as solid phase extraction, need to be done to reduce copper ion contamination from the presence of DNA [43, 46, 47].

2.9 Fired vs. Unfired Effects

Environmental effects are also a factor on the amount of biological material transferred and recovered from cartridge cases. During the process of firing a firearm there is an intense, if transient, amount of heat applied to the cartridge case. Smokeless powders burn at temperatures between 1,760-1,870ºC [48]. The heat can lower the amount of biological material, especially the more labile DNA [30]. The temperature on cartridge cases can range from 62ºC to 98ºC [49]. Thanakiatkrai *et al*. note that recoverable DNA is reduced by approximately 30.8% during the firing process due to not only heat but pressure and friction generated in the processes [50]. The firing process can remove DNA as it encounters the chamber since the metal cartridge case expands after it is discharged, causing contact and shearing of biological material. Deflagration release of gases also shears the cartridge surface of biological material. In addition, DNA may be removed from the case through the extraction from the firearm chamber, and the ejection from the breech [48]. The firing process may even leave behind metallic species from gunshot residue

in the DNA. This may cause DNA degradation and PCR inhibition since the metal may have an effect on instrumentation during DNA processing [42]. The heat causes damage to the DNA when the cartridge case is fired, as well as the overall firing process. The combination with the oxidative effects from the brass cartridge case results in minimal recovery of DNA. Even unfired brass cartridge cases will cause an oxidative effect on DNA [41, 51, 52].

2.10 Collection and Storage Effects

Collection and storage of touch evidence, especially in the long-term, can cause DNA to damage and degrade. Traditionally collected swabs are air-dried; however, if they are not air-dried properly and placed into a cardboard or paper bag, then bacteria and mold may grow degrading the touch evidence. This is due to oxidation and hydrolysis breaking down the DNA causing less of the template to amplify. Contamination from collection and storage needs to be prevented and proper techniques need to be followed to prevent any additional damage to the touch evidence [43, 52]. Bille *et al.* introduces an alternative collection/storage method of cartridge cases to help minimize the degradation of DNA. The point of this collection is to minimize any possible contamination that may result in loss of DNA and to minimize handling of the cartridge cases. Devices like this can be incorporated into forensic workflow to help maximize the available DNA while minimizing the damaging effects [43]. Outdoor conditions such as rain can have a negative effect on recovery of touch evidence on cartridge cases, since water may promote degradative chemistry. It is noted by Subhani *et al.* that fired cartridge cases left outside, over a seven-day period, showed a decrease in DNA yield and a decrease in the number of alleles. This may be due to the solubilization of copper ions from the brass cartridge case. This is important to note because it indicates that environmental conditions play a significant role in the decrease of DNA [53].

2.11 Collection Methods of Touch Evidence

Numerous methods have been applied to collection, recovery, and processing of touch evidence from cartridge cases. Some collection techniques that are commonly used include cotton swabs and adhesive tape. The use of cotton swabs is considered the traditional method and current best practice, where the double swab technique is used, one dry swab and one wet swab [10, 41, 43, 54, 55]. This wet and dry method is typically used on nonporous items [55]. The swabs traditionally need to be air dried before the collected touch evidence is packaged, otherwise bacteria and mold may grow on the swabs [52]. This collection method typically yields relatively lower DNA quantities, especially compared to other alternative methods as depicted in Table 1 [10, 51]. However, studies have shown that adjusting extraction techniques such as the use of resuspension of cotton swabs can increase DNA recovery [54]. The use of adhesive tape such as Voigtlaender Neschen Foil S23 or Scotch Tape, especially on larger surface areas, can yield greater DNA recovery compared to the cotton swabs. Studies have shown that the use of adhesive tape produces considerably better PCR STR results compared to wet swabbing. For example, in one study, 8 of 10 amplifiable samples had 3 full profiles and 3 good partial profiles compared to only 6 amplifiable sample that had 1 full profile and 4 good partials profiles [41].

Since traditional collection methods have had difficulty with recovery of touch evidence, other types of methods have been developed to increase DNA yields and recovery. These collection methods include SimpleSwab, SwabSaver, nylonFLOQ swab, and the microFLOQ® direct swab [10, 52, 56]. The SimpleSwab is designed to focus on the overall swab shape to enhance collection. Studies have indicated that it is seen to release significantly more DNA than other swabs. DeWeese *et al.* demonstrated the SwabSaver collected a high average of touch

deposits compared to others when swabbing the trigger/trigger guard on a firearm [10]. The SwabSaver has been shown to preserve touch DNA for up to two months since it removes oxygen and moisture from the environment and prevents breaks in the DNA molecule [52]. The nylonFLOQ's design, lacking an internal absorbent core, entraps cellular materials. Now the microFLOQ® direct swab takes this a step further, where it has a small swab head with nylon fibers that are treated with a lysing agent. This allows for the sample to be concentrated, conduct direct amplification, and DNA profiling from the sample collection. With this small swab head there is less sample consumption compared to traditional swabbing methods, and low quantities of DNA can more easily be typed. It is seen that there is no sample loss during the workflow which is very important when dealing with touch evidence [56].

In addition to swab type used for collection, studies have demonstrated other methods and techniques to improve recovery of touch evidence from cartridge cases. Billie *et al.* developed a technique called the rinse and swab, where the cartridge cases were rinsed numerous times with certain buffers and then swabbed followed by another rinse and then a re-swab. This study demonstrated that the rinse and swab method with BTmix (BSA and GGH) increased the total DNA recovered and average peak height per locus. The study indicated DNA recovery that ranged from 200 pg to 14 ng of total DNA [43]. Other studies have also demonstrated that the use of buffers, like trypsin, or detergent in swabbing solutions have increased DNA recovery from touch evidence [23, 51, 57]. The detergent causes elements of a fingermark to become suspended in the aqueous solution, thus enhancing cellular recovery [57]. Sodium dodecyl sulfate (SDS) is another type of detergent that has been demonstrated to be highly effective. SDS is an anionic detergent that denatures secondary and non-disulfide linked tertiary structures by interfering with hydrogen-bonding. This denaturation allows for enhanced release and recovery

of DNA from a sample [58]. Studies have indicated that lysis buffer, like 1% n-lauroylsarcosine, Tris-HCL 10 mM, EDTA 0.1 mM, NaCl 50 mM and 0.01% ProteaseMax Surfactant in 50mM NH_4HCO_3, can also improve DNA concentrations, especially compared to water [51, 59]. It is noted that having an effective lysis buffer in a swabbing solution can help increase DNA yields even if the sample is stored at high temperatures for 24 hours [59]. Furthermore, Subhani *et al*. investigated a direct lysis method on both spent 9 mm cartridge cases and live ammunition. It was noted that this method recovered more DNA and improved STR profile recovery when compared to the traditional double swabbing method. The direct lysis method contained a median DNA concentration of 3 pg/µL while the double swabbing method contained a median yield of 0.2 pg/µL. This is a 15-fold increase in recovery of DNA with the use of the direct lysis method over the double swabbing method [53]. Subhani *et al.* also indicated that even though the DNA recovered from fired cartridge cases was less compared to unfired cartridge cases, both conditions were able to get similar number of alleles per STR profile. This is significant to note because the more alleles recovered the better the profile interpretation will be [53]. In general, these studies provide alternative efficient methods for recovery of touch evidence on cartridge cases that can be useful for the forensic laboratory. Table 1 depicts a summary of numerous studies that have used the double swab method as well as other alternate methods for the collection of DNA from touch evidence on cartridge cases and glass microscope slides [23, 37, 41, 43, 51, 52, 53, 57, 60].

Literature Article	Surface Used	Hands Prepared	Technique Used	Average DNA Yields (ng)
Kranes et al. [23]	Microscope slides	Washed hands with soap and made sebaceous rich for 15 secs	Wet swabbed with either 0.01% SDS or 0.01% Protease Max buffer.	0 to 0.745
Ostojic et al. [37]	Microscope slides	No washed hands for 2 hours; left as is.	Wet swabbed with water or 5% Triton X-100 ; 20 µL final volume.	HighSens: 0.64 Zygem: 0.236 One-tube: 0.198
Sterling et al. [41]	Cartridge Cases	Washed hands with soap and made sebaceous rich for 15 secs.	Adhesive tape: Pritt Sello Double Sided Tape, Scotch Magic, Transparent, Permanent Double Sided and Removable Poster Tape, Voigtlaender Neschen Foil S23, Scenesafe FAST, and SmartSolve Hydrographic Tape.	Unfired: 0.02 Fired: 0.03 Wet Swab: 1.34
Bille et al. [43]	Cartridge Cases	Handled cartridge cases as is for 10-15 seconds then loaded into magazine.	Double swab with different solutions: water, 2mg/mL BSA in water, 62.5 mg/mL GGH in water, BTmix	Double swab: 0.2 to 14
Sterling et al. [51]	Cartridge Cases	Washed hands with soap and made sebaceous rich for 15 secs.	Adhesive tape: Neschen Foil S23 Wet-Dry Cotton Swab	Tape: 1.36 ± 1.87 Swab: 1.34 ± 3.04
Tasker et al. [52]	Cartridge Cases	Rubbed unwashed hands together for 10 seconds then loaded ammunition magazine.	4N6 FLOQSwabs®, Simple Swab2, Double swab technique with 2% SDS	0 to 0.7*
Subhani et al. [53]	Cartridge cases	No hand washing; handled cartridge cases as is for 10 seconds.	Direct lysis method	1.25**
Thomasma et al. [57]	Microscope slides	Hands as is. Collected over 6 day period.	Double swab method; final volume 20 µL	0 to 17.6***
Harush-Brosh et al. [60]	Plaster wall surface with two layers of acrylic matt whitewash water-based paint	Washed hands and made sebaceous rich	Scraped using a sterile scalpel	1.57

*Estimate range based on the use of QIAamp® DNA Investigator kit (QIAGEN, Valencia, CA) following the "Isolation of Total DNA from Surface and Buccal Swabs" protocol. Estimated yield was calculated assuming 20 µL was used for final extraction volume.
** Estimate range based on the use of Investigator STAR Lyse&Prep Kit (Qiagen) protocol. Estimated yield was calculated assuming 50 µL was used for final extraction volume.
***Estimate range based on the methods provided in article. Estimated yield was calculated assuming 20 µL was used for final extraction volume.

Table 1: Summary of DNA yields collected from touch evidence on cartridge cases and glass microscope slides from relevant literature articles.

This study is focused on comparing alternate collection methods for touch evidence to the traditional double swabbing method, wet-dry cotton swab. The alternate collection methods include 'Copan microFLOQ® direct' which contains a lysing agent. This lysing agent is on the small nylon fiber head of the swab. The lysing agent on the nylon fibers has a positive charge,

thus causing an attraction to negatively charged DNA [61]. In addition, the microFLOQ® without lysing agent is the same design, but it lacks the lysing agent in the nylon fibers. Another alternate collection method in this study is the Gel-Pak silicone gel film. The Gel-Pak silicone gel-film is an adhesive gel that is typically used for fingerprint lifting. It contains a polyethylene coversheet, gel material, and a polyester substrate [62]. The cell scraper used in this study, typically used to harvest cells or cell lysates, is also a control [63]. This study focuses on comparing these different collection methods to the traditional collection method known as the double swab method, wet-dry cotton swab, to validate an alternative way of maximizing biological material from touch evidence.

2.12 Processing Effects

Certain extraction methods may also have an effect on the DNA yield and overall profile quality. For instance, Danielson *et al.* indicated that a combination of organic extraction (phenol:chloroform:IAA) with a soak and sonication recovery method has proved to be effective. DNA yields for this combination include 430-930 picograms and >95% interpretable profiles for unfired cartridge cases with interpretable profiles from fired cartridge cases [42]. Furthermore, the use of different types of extraction kits throughout numerous studies; like Danielson *et al.,* Bille *et al.,* Montpetit *et al.,* Thanakiatkrai *et al., and* Tasker *et al.;* have been used to increase the amount of DNA recovered from cartridge cases [42, 43, 48, 50, 52]. For example, Danielson *et al*. demonstrated that organic extraction contained an estimated range of 0.43 ng to 0.93 ng, while the Prepfiler Forensic Extraction kit and QIAmp DNA Investigator Kit both had estimated ranges of 0 ng to 0.12 ng and 0 ng to 0.05 ng [42]. Different extraction kits include QIAmp DNA Mini kit, QIAmp DNA Investigator Kit, QIAmp DNA Micro kit, Qiagen EZ, Promega IQ kit, Qiagen EZ1 Investigator kit, and the Prepfiler Forensic Extraction kit. The different types of

technologies, along with modifications of these kits, have helped increase the amount of DNA recovered from cartridge cases and be incorporated into forensic laboratory workflow [42, 43, 48, 50, 52]. Processing of touch DNA is affected by the laboratory methods. It has been indicated that touch DNA samples usually need about 250 cells (1.45 ng) for a sufficient amount of DNA for amplification after extraction [18]. With the use of direct amplification there is no need for a DNA extraction step that can minimize loss. By eliminating the need to increase PCR cycle number or concentrate the amplified products, the procedure is easily adapted into working practices [64]. Currently, forensic laboratories use direct PCR only on reference samples due to their high quality of DNA and lack of inhibition [18]. Overall, direct PCR can provide a quicker and more efficient processing technique for forensic laboratories, especially with low-level DNA samples. The use of these techniques has proven to increase the recovery of touch evidence and are crucial to incorporate into forensic laboratory workflow.

2.13 Protein Preservation

Since touch evidence is mainly in the form of fingermarks, DNA is not the only form of biological material found in transferred biological material. Fingermarks also contain proteins that can provide genetic information about the touch evidence. Girod *et al*. notes that the overall protein content is roughly 384 µg per fingermark trace [4]. This is in contrast to DNA that can be less than 300 picogram when transferred to a surface and is highly variable [57]. Proteins offer better overall preservation and are preserved in remains quite well [65]. Proteins are chemically more robust than DNA and are preserved longer [26]. They are also more abundant and environmentally abundant than DNA [66]. An example of how robust proteins are is demonstrated by the Chu *et al.* study, where the hair proteome had minimal degradation after an explosion. A successful proteome profile was obtained even though the hair contained

morphological damage. The study was able to recover keratin associated protein as markers, and genetically variant peptides were identified even with the explosive conditions. Overall, the proteome of the hair still had a similar discriminating power to undamaged hair [67]. It is important to note the robustness of these proteins because similar proteins are found in touch evidence. Furthermore, Schulte *et al.* validate protein robustness compared to DNA. This study indicates that analysis of DNA from touch evidence contains challenges due to the trace nature of the DNA. It also highlights that DNA degrades over time in the touch samples, thus limiting the usefulness of the DNA. The study also demonstrated that proteins from touch samples do not contain these limitations since they are more robust with measurable quantities collected across numerous donors [68].

2.14 Use of Proteins for Human Identification

As described above, proteins are very useful for identification since they are used to detect genetically variant peptides (GVPs) in proteomic genotyping [26]. GVPs contain single amino acid polymorphisms (SAPs) that can be identified. These are the result of nsSNP alleles in an individual's genome [66]. It has been demonstrated that an average of 30 GVPs using fingermark proteins can be obtained [22]. In addition, GVPs can be obtained from as little as 60 corneocytes, providing an estimate of 62.5 nanograms of protein, and fingermarks contain an estimated range of about 5 to 50 corneocytes per print. [51]. The SAPs can be inferred through proteomic based identification of GVPs and discriminating profiles can result [69]. Borja et al. demonstrated random match probabilities for genotype frequency based on SNP alleles with a median probability of 1 in 2.4×10^6. Borja et al. also note that these resulting proteomically-inferred genotypes are compatible with STR based RMP. This is due to the closest STR locus being 2.2 Mb from the nearest GVP-inferred SNP locus [22]. The random match probabilities

therefore are compatible with those used in DNA-typing [22]. The analysis of GVPs can produce additional information in forensic cases especially in those with limiting touch evidence on cartridge cases [68]. Table 2 is a summary of numerous studies that have demonstrated protein collection from touch evidence on different surfaces [15, 23, 41, 68].

Literature Article	Surface Used	Hands Prepared	Technique Used	Average Protein Yields (µg)
Oonk et al. [15]	Microscope slides	As is. No sebaceous rich. Firmly pressed down for 5 seconds.	Wet Swab with 50 µl extraction fluid containing 50% acetonitrile in water (v/v).	0.2 to 51.0
Kranes et al. [23]	Microscope slides	Washed hands with soap and made sebaceous rich for 15 secs	Wet swabbed with either 0.01% SDS or 0.01% Protease Max buffer.	1.03
Sterling et al. [41]	Cartridge Cases	Washed hands with soap and made sebaceous rich for 15 secs.	Adhesive tape: Pritt Sello Double Sided Tape, Scotch Magic, Transparent, Permanent Double Sided and Removable Poster Tape, Voigtlaender Neschen Foil S23, Scenesafe FAST, and SmartSolve Hydrographic Tape.	Unfired: 1.7 Fired: 0.90
Schulte et al. [68]	Microscope slides	Washed hands and made sebaceous rich	100 µL of 0.1 % (w/v) RapiGest in 50 mM ammonium bicarbonate. Then scraped with cell lifter.	15 to 40

Table 2: Summary of protein yields collected from touch evidence on a variant of surfaces from relevant literature articles.

2.15 Real World Applications

In forensics, touch evidence on cartridge cases contains probative information where the collection of touch DNA may link a potential suspect to a firearm at a crime scene. However, touch DNA can be problematic and have a lack of success at developing suitable DNA profiles from cartridge cases. Numerous factors such as environment, surfaces, and deposition contribute to this low success rate, as described above. Numerous studies have been done with different methods, techniques, types of swabs, and protocols to increase the success rate of recovering DNA from touch evidence on cartridge cases [43]. The success rate has still been proven low and difficult. Thus, incorporating a combined extraction of both DNA and proteins can help provide

more information about the touch evidence when DNA alone is not enough. Proteins can be used for identification and are more robust than DNA [66]. It provides addition information on numerous factors, not just DNA, but metabolites, traces of medications and drugs, dirt and grease, make-up, food discharges, moisturizers, and hair care products [7]. The combined extraction of DNA and proteins from touch evidence on cartridge cases can be incorporated into the workflow and help improve touch evidence collection for a crime lab.

Collection and extraction of touch evidence on cartridge cases has proven difficult despite numerous attempts at improvement and optimization. Major issues that arise from this include the variability of biological material in the fingermarks themselves, the surface the evidence is on, and the loss of biological material when collected. Furthermore, the firing process itself proves very problematic where DNA is broken down and degraded due to the oxidization from the copper chemistry in the brass cartridge case. However, DNA is not the only biological material in fingermarks; protein is also present and is chemically more robust overall. Protein can provide additional information that can be probative especially when the information is just as compatible as DNA.

Transferring material is currently a limiting factor to maximizing biological material collected. This study focuses on maximizing the amount of biological material that can be made available to the investigator to maximize biological information for probative results. The goal of this study is twofold: to test and develop a collection technique to improve the collection of both DNA and protein from touch evidence on cartridge cases for additional information, and to apply the optimal method to a range of increasingly difficult contexts, from use of characterized and quantified artificial fingermarks to real fingermarks, and an optimal surface, pristine microscope slides, to unfired and then fired cartridge cases. Therefore, there will be two outcomes: an

optimization and demonstration of a method of transferring biological material from a two-dimensional surface into a technical workflow (i.e., microcentrifuge tubes), and to learn more about the character and outcomes of biological material in touch evidence on brass surfaces before and after exposure to the explosive physics and chemistries associated with deflagration and firing. Furthermore, the use of fully characterized artificial prints allows us to directly compare transferred and starting material to obtain quantitative yields for each method. As described above these transfer methods include for this study: wet-dry cotton swab, microFLOQ® direct (Copan), microFLOQ® direct without the lysing agent, Gel-Pak silicone gel-film (PF-40-X4, Gel-Pak, Hayward, CA), and a cell scraper.

3. Materials and Methods

3.1 Extraction Buffer Preparation

The extraction buffer was 0.1X solid phase extraction (SPE) filtered phosphate buffered saline (PBS) 0.1% sodium dodecanoate. 10X SPE PBS was prepared first by adding 80 grams of NaCl was to a one-liter glass bottle, 2 grams of KCl, 2 grams of KH_2PO_4, then 21.2 grams of $Na_2HPO_4 \bullet 2H_2O$, and then double distilled water (DDW) was added to the one-liter mark. This was mixed well until everything was dissolved. 50 mL of the 10X PBS was then filtered through a solid phase extraction (SPE) filter syringe to remove organic material. Next, 2% sodium dodecanoate was made by adding 200 mg of sodium dodecanoate to a clean 15 mL falcon tube. Then SPE DDW was added up to the 10 mL line on the tube. The falcon tube was placed into a 50^{o}C oven for about 10 minutes until all the sodium dodecanoate was dissolved. A clean 15 mL falcon tube was obtained where 9.4 mL of SPE DDW was added, then 100 µL of 10X SPE PBS was added, and then 500 µL of 2% sodium dodecanoate was added. This was vortexed until evenly mixed.

3.2 hTERT Plasmid DNA for Standard Preparation

Purified human telomerase reverse transcriptase (hTERT) plasmid DNA was generously provided by Ashleigh Matzoll to create standards for qPCR. The hTERT plasmid, pCDNA-3xHA-hTERT, from Addgene was a gifted to Ashleigh Matzoll from Steven Artandi (Addgene plasmid # 51637 ; http://n2t.net/addgene:51637 ; RRID:Addgene_51637) and is depicted in Figure 1. This plasmid contains 9027 base pairs [70]. The human telomerase is the target gene that is used in the commercial Quantifiler™ Human DNA Quantification Kits [71].

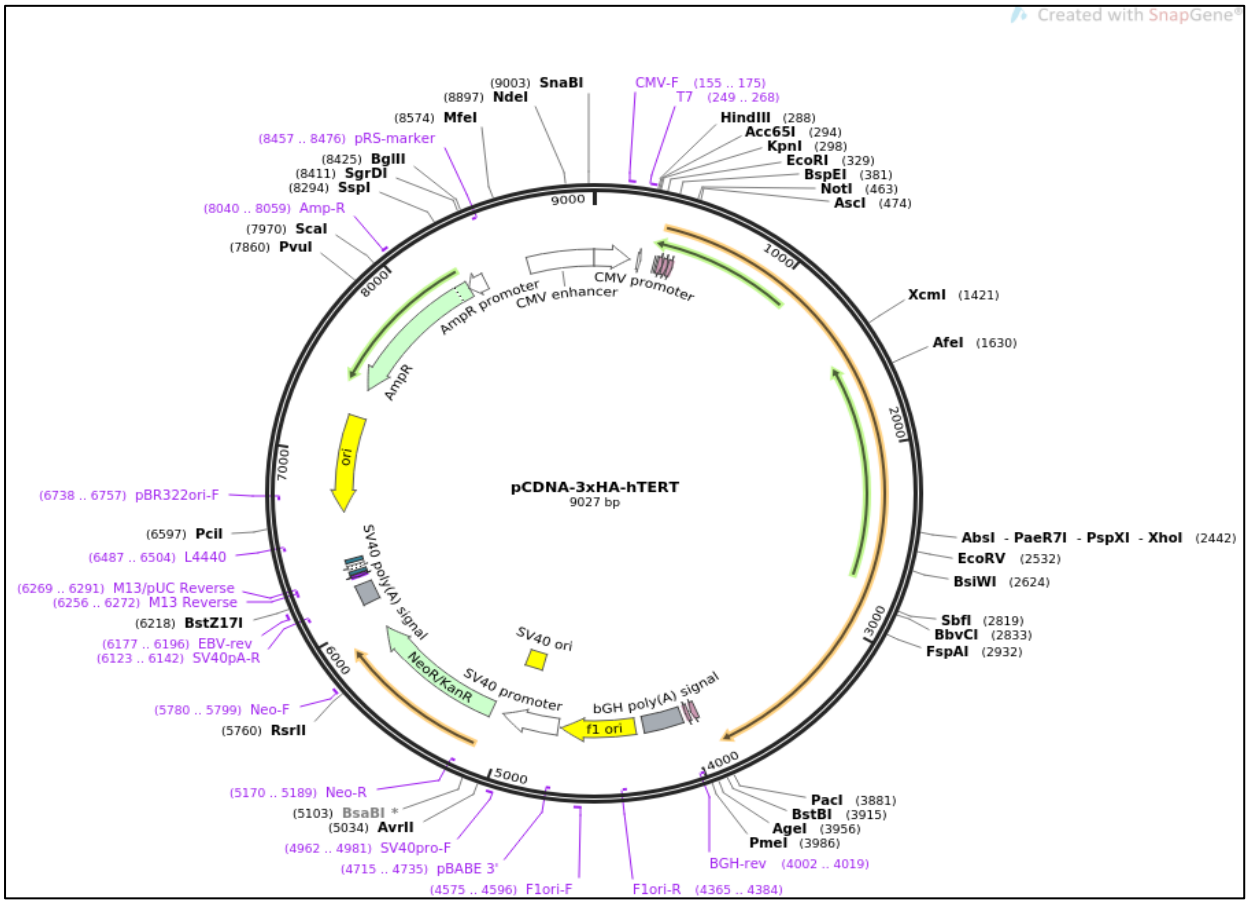

Figure 1. hTERT plasmid from Addgene.

According to Ashleigh Matzoll's thesis procedure the pCDNA-3xHA-hTERT is purified from a liquid bacterial culture that is inoculated according to the Addgene protocol [72]. To begin, two milliliters of liquid broth (LB) are added to a falcon tube followed by 200 µg of

ampicillin. Using a sterile pipette tip, a single colony from the LB agar plate containing the plasmid is added to the falcon tube. The falcon tube with the culture is loosely covered with aluminum foil and incubated at 37°C overnight. After visual confirmation that a colony has grown, the plasmid DNA is purified using a QIAamp® DNA Mini Kit. Once purified, the 260/280 ratio of the DNA is then measured. It is determined the total DNA copy number per microliter is 2.95×10^{10}. A serial dilution is made from 10^9 to 10^0 copies per microliter to be used as a standard [73]. From this purified hTERT plasmid DNA provided by Ashleigh Matzoll, the $1x10^9$ copy number/µL of purified hTERT plasmid DNA was used to remake and purify the hTERT plasmid DNA.

Transformation of competent cells was done following Thermo Scientific DH5α Competent Cells user guide with slight adjustments [74]. Briefly, 2 µL of $1x10^9$ copy number/µL of purified hTERT plasmid DNA was added directly into the competent cells tube. A liquid bacterial culture was prepared in LB medium (1.5 grams of Tryptone, 0.75 grams of yeast extract, 1.5 grams of NaCl, 15 µL of 10N NaOH, and 150 mL of double distilled water). Before use, the medium was autoclaved for 30 minutes. Once the liquid LB media reached room temperature, 2 mL was added to each falcon tube. Then 4 µL of 50 mg/mL ampicillin was added to each tube resulting in 200 µg of ampicillin per tube. Using a sterile pipette tip, a single colony from the LB plate was collected, and the pipette was dropped into its designated tube with liquid LB medium. A total of five colonies were selected to make five liquid LB media tubes. All tubes were swirled to mix and loosely covered with a cap that was not airtight. All tubes were incubated at 37°C for 12-18 hours in a shaking incubator at 300 rpm. After incubation the tubes were checked for growth which was indicated by a cloudy haze in the media [72]. The plasmid DNA was then purified following the Promega Wizard® Plus Minipreps DNA Purification

System with vacuum manifold quick protocol. These resources were generously provided by Dr. Robert Rice's laboratory [75].

After purification five replicated of the plasmid DNA were isolated then quantified. They were quantified by diluting them 20-fold with nuclease free water then the concentration was measured using a UV spectrophotometer with the wavelengths of 260/280. The results indicated a ratio range of 1.76 to 1.91 with an average of 1.84 for all five replicated of the purified plasmid DNA. After calculations, the concentrations ranged from 54 µg/mL to 114 µg/mL with an average of 81 µg/mL of plasmid DNA. All five replicated were pooled together and converted from µg/mL to plasmid DNA copy number/µL via calculations. Therefore, the final concentration was determined to be 8.23×10^{10} plasmid DNA copy number/µL. Serial dilutions were then done to make standards that ranged from 1×10^{9} to 1×10^{0} copy number/µL.

3.3 Quantification of Human DNA using Real-Time qPCR

All samples were quantified for DNA concentration using Applied Biosystems 7500/7500 Fast Real-Time qPCR following the protocol for TaqMan™ Fast Universal PCR Master Mix with TaqMan Gene Expression Assay that targets hTERT (Hs05045220_g1) (Figure 2) [76]. The software used was Applied

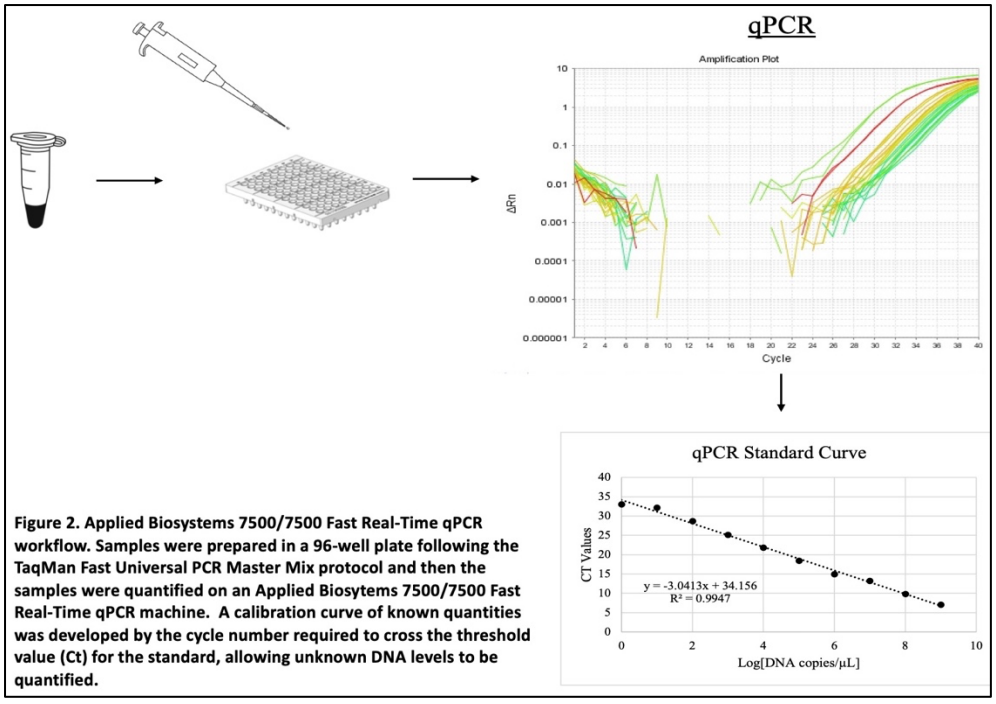

Figure 2. Applied Biosystems 7500/7500 Fast Real-Time qPCR workflow. Samples were prepared in a 96-well plate following the TaqMan Fast Universal PCR Master Mix protocol and then the samples were quantified on an Applied Biosytems 7500/7500 Fast Real-Time qPCR machine. A calibration curve of known quantities was developed by the cycle number required to cross the threshold value (Ct) for the standard, allowing unknown DNA levels to be quantified.

Biosystems Software for 7500 and 7500 Fast Real-Time PCR systems v2.3. The cycling included holding stage 95ºC for 20 seconds, cycling stage at 95ºC for 3 seconds then at 60ºC for 30 seconds for 40 cycles. The hTERT plasmid DNA, described above, was used for standards that ranged from $1x10^9$ to $1x10^0$ copy number/µL, the equivalent of $9.9x10^6$ fg/µL to 0.01 fg/µL of plasmid DNA. The cycle number required to cross the threshold value (Ct) for the standard was used to create a calibration curve of known quantities for each run, to allow unknown DNA levels to be quantified.

3.4 Quantification of Protein using SDS-PAGE Gel Electrophoresis

All samples were quantified for relative protein density using BioRad 4-15% Mini-PROTEAN® TGX™ SDS-PAGE gel electrophoresis in Mini-PROTEAN® Tetra Cell [77]. Samples were diluted by 1.4-fold by addition of a premade 4X SDS-PAGE loading buffer, heating at 95ºC for 10 min, vortexing and pulse centrifugation. An addition of 25 µL for each gel contained 2 x 10 µL pre-stained protein ladder standards. Samples were ran at 90 V for about 1 hour until the leading band reached the bottom of the gel. The resulting SDS-PAGE gels were rinsed with double distilled water three times, 75 mL of staining solution added to each gel, and they were placed on a shaker to gently mix and stain overnight [78]. The proteins in the gel were stained using a colloidal Coomassie G-250 stain protocol, made according to Neuhoff *et al* [79]. After an overnight stain, the gels were then rinsed three times and then 100 mL of 2% methanol was added to each gel to remove background stain after gently mixing for 1 hour and repeated two times. After sitting in the refrigerator overnight, the gels were scanned using an Epson scanner set at 24 color bit and 350 dpi. The gels were analyzed using ImageJ version 2.0.0-rc-43/1.50e to determine relative protein density. To analyze each lane, each lane was selected by putting it into its own individual rectangle panel. Once all lanes of interest were in their own

individual panel, as indicated in Figure 3, the lanes were then selected to be analyzed. This allowed each lane to have their own plot, where the densitometry peaks of interest were visualized. To control for background noise, editing was done by drawing lines to isolate all densitometry peaks of interest. Each peak of interest in all lanes had verticle lines drawn at the highest point of the baseline and the lowest point on the baseline to isolate a discret space coresponding to protein dependent desitometry signal. Once this was done for all definitive peaks of interest in all lanes, the area was calculated on ImageJ to determine the relative protein density expressed in relative densitometry units. The quantification of proteins is depicted in Figure 3.

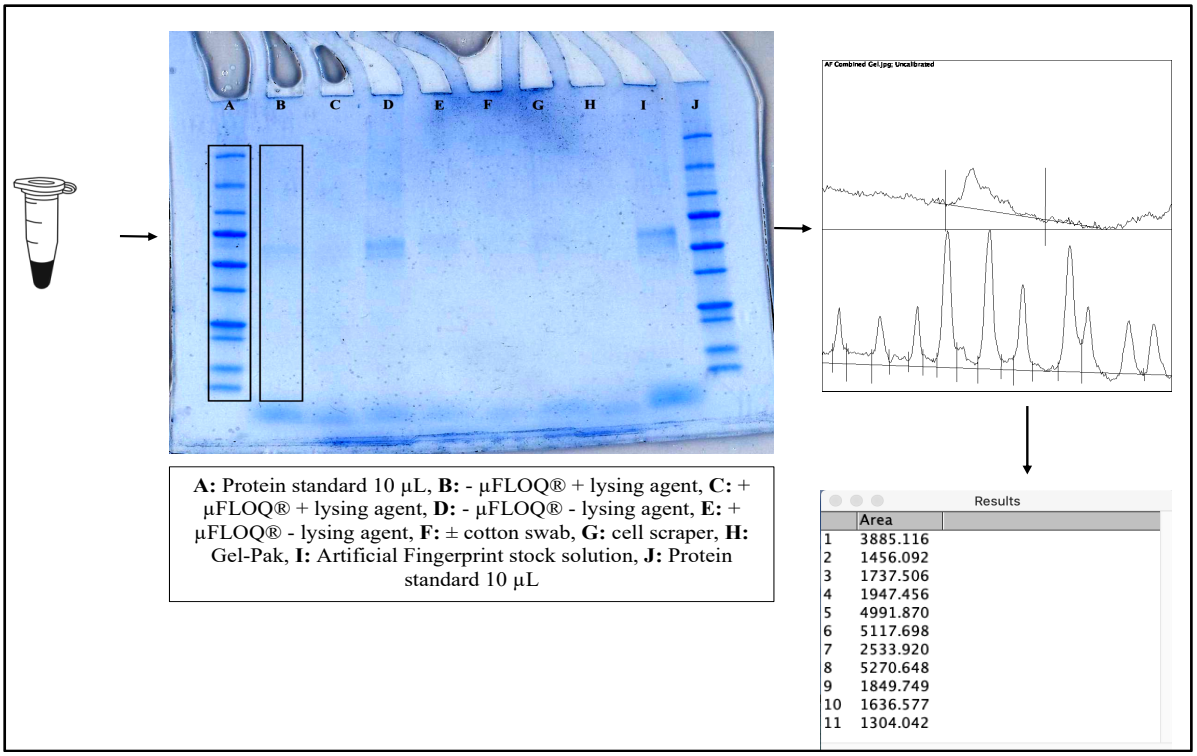

Figure 3. BioRad SDS-PAGE Gel Electrophoresis workflow. Samples were loaded into the SDS-PAGE gel where a voltage was applied to allow the samples to run towards the bottom of the gel. The gel was stained by colloidal coomassie G-250 and then scanned on an Epson scanner. To quantify the gel ImageJ was used to identify the peaks of interest and control for background noise via editing with lines. Once edited the peak was quantified to determine the relative densitometry units.

3.5 Artificial Fingerprint Stock Solution

Artificial fingerprints were made following the LeSassier *et al.* protocol with modifications [6]. Briefly, epidermal material (ESM) was collected from three individuals' fingertips and palms by gently rubbing a clean Ped Egg exfoliating grate (As Seen on TV PedEgg Professional, China), purchased at Walmart, across the fingertips and palms of three individuals' hands for about 60 seconds. The hands were facing downwards, and the grate was facing upwards to collect the skin particles into the collection chamber. ESM was transferred to a clean weigh paper by removing the grate and using an eyebrow brush to gently brush the skin particles from the grate collection chamber. The ESM was then poured into a clean Eppendorf tube. Following this, 2 mg of ESM was weighed out and added to a clean Eppendorf tube where it was resuspended with 80 µL of stabilized artificial eccrine perspiration (Pickering Labs, P/N 1700–0024) resulting in a concentration of 25 µg/µL of ESM [6, 80].

The artificial fingerprint stock solution was made by obtaining a clean Eppendorf tube where 60 µL of artificial eccrine perspiration-sebum emulsion (Pickering Lab, P/N 1700–0547) was added, then 45 µL of stabilized artificial eccrine perspiration, 30 µL of the 10,000 copies/µL concentration plasmid DNA, and then 15 µL of the 25 µg/µL of ESM. The artificial fingerprint stock solution was mixed by pipetting up and down until evenly mixed. A blank artificial fingerprint stock solution was made in the absence of the protein and DNA. This was made by adding 85 µL of artificial eccrine perspiration-sebum emulsion and 65 µL of stabilized artificial eccrine perspiration. The blank artificial fingerprint stock solution was mixed by pipetting up and down until evenly mixed [80].

Pre-cleaned microscope slides were obtained and 5 µL aliquot of the artificial fingerprint stock solution, or 'blank' artificial fingerprint samples, was dispensed and evenly spread onto a

discrete section of a microscope slide. A 5 µL aliquot of the blank artificial fingerprint stock solution was also dispensed onto microscope slides. For each 5 µL aliquot of the artificial fingerprint samples there were 10,000 DNA copies of plasmid DNA (99 fg) and 12.5 µg of ESM. All samples/blanks were left in a fume hood or covered overnight on the bench with aluminum foil tents to dry overnight. The experimental design is depicted in Figure 4 [6].

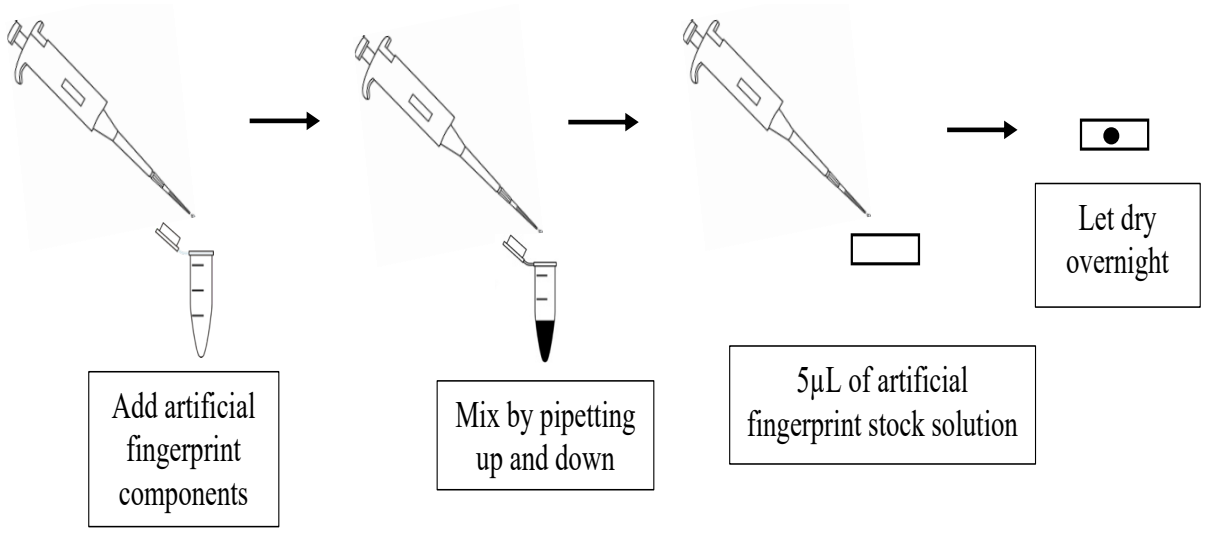

Figure 4. Creation of artificial fingerprint stock solution workflow.

3.6 Fingermark Transfer Methods

For the wet-dry cotton swab transfer method two cotton swabs were used for each sample where the first one had 20 µL of the extraction buffer added to it. The samples were first thoroughly rubbed by the wet cotton swab and then followed by the dry cotton swab. Both were left to dry for about 15 minutes. Following QIAGEN Investigator Lyse & Spin Basket Handbook with slight modifications, once dried both cotton swab tips were cut off using sterilized scissors and placed into a pre-labeled Eppendorf tube with a spin basket. The extraction buffer (350 µL) was added to each sample and all samples were placed into the hot block for 30 minutes at 56ºC. Afterwards they were centrifuged for 5 minutes at 14000 rpm and then the basket was removed [81].

Now for the dry microFLOQ® with lysing agent (Copan microFLOQ® direct) transfer method, designated samples were thoroughly rubbed for 5 minutes to transfer biological material [61]. Once thoroughly rubbed, the tip of the microFLOQ® was broken off into a microcentrifuge (Eppendorf) tube containing 100 µL 0.1X SPE-filtered PBS and 0.1% sodium dodecanoate extraction buffer. The samples were vortexed (5 s), centrifuged for 2 minutes at 8000 rpm, and placed in a hot block for 30 minutes at 56ºC. The samples were then removed and centrifuged for 5 minutes at 10000 rpm.

For the wet microFLOQ® with lysing agent transfer method the designated samples were treated identically with the exception that the microFLOQ was first dipped into an aliquot of the extraction buffer prior to rubbing and transfer. Likewise, for the dry microFLOQ® without lysing agent and wet microFLOQ® without lysing agent transfer methods the designated samples were treated identically except that a customized microFLOQ® direct swab without lysing agent was used with no pre-wetting of the swab, and with pre-wetting of the swab.

For the cell scraper transfer method, 100 µL of the extraction buffer was gradually added to the designated samples while the solution drained into its designated 50 mL falcon tube. The samples were scraped with their designated cell scraper into the 50 mL falcon tube. This was repeated with the extraction buffer that was already in the 50 mL falcon tube three more times. The cell scraper was washed with the extraction buffer numerous times to fully extract the DNA and protein from the cell scraper. The 50 mL falcon tube was then centrifuged for 2 minutes at 2000 rpm to get all the extraction buffer to the bottom of the tube. All of the extraction buffer in the 50 mL falcon tube was collected and placed into a clean pre-labeled Eppendorf tube. The Eppendorf tube was placed in the hot block for 30 minutes at 56ºC, then centrifuged for 5 minutes at 10000 rpm.

Finally, for the Gel-Pak silicone gel-film sheets (PF-40-X4, Gel-Pak, Hayward, CA), appropriate size squares were cut out of the Gel-Pak silicone gel-film sheets with pre-cleaned scissors. The covers of the gel-film sheet squares were carefully peeled off and the adhesive gel-surface were placed on the designated samples. The back of the gel-film sheet squares were thoroughly rubbed to make sure they fully stuck to the samples, and they were left to sit for about 10 minutes while thoroughly rubbing the gel-film sheet squares occasionally. The gel-film sheet squares were carefully peeled off using pre-cleaned forceps and placed into clean pre-labeled Eppendorf tubes. The extraction buffer (100 µL) was added to each tube where the gel-film sheet squares were constantly washed with that added extraction buffer numerous times. The Eppendorf tubes were then vortexed for 5 seconds and centrifuged for 2 minutes at 8000 rpm. They were then placed in the hot block for 30 minutes at 56ºC then centrifuged for 5 minutes at 10000 rpm. The Eppendorf tubes were repeatedly centrifuged at 10000 rpm until extraction buffer was fully separated from the gel-film sheet squares. All of the extraction buffer was taken from the bottom of the Eppendorf tubes and placed in clean pre-labeled Eppendorf tubes. The Eppendorf tubes that contained the gel-film sheets were centrifuged again, and the rest of the extraction buffer was added to the clean pre-labeled Eppendorf tubes. All samples were stored in an -80ºC freezer until they were quantified. These exact collection and extraction techniques, described above, are demonstrated in Figure 5.

4. Results

4.1 Artificial Fingerprints Experiments

The first set of experiments involved the collection, extraction, and quantification of artificial fingerprints from microscope slides to determine the percent efficiency of the seven different transfer methods, described above (Figure 5). To reduce variables an ideal surface was

used and the starting quantities for DNA and protein were known. These sets of experiments were designed to determine how efficient our collection techniques are when used on a fully characterized artificial fingerprint and an ideal surface. The artificial fingerprints experiments each contained a pair of artificial fingerprints along with a blank per transfer method. All artificial fingerprints experiments were replicated exactly the same for six triplicate samples per transfer method including three blank replicates for each method. After all samples and blanks were collected and extracted, they were quantified for DNA and protein as described above.

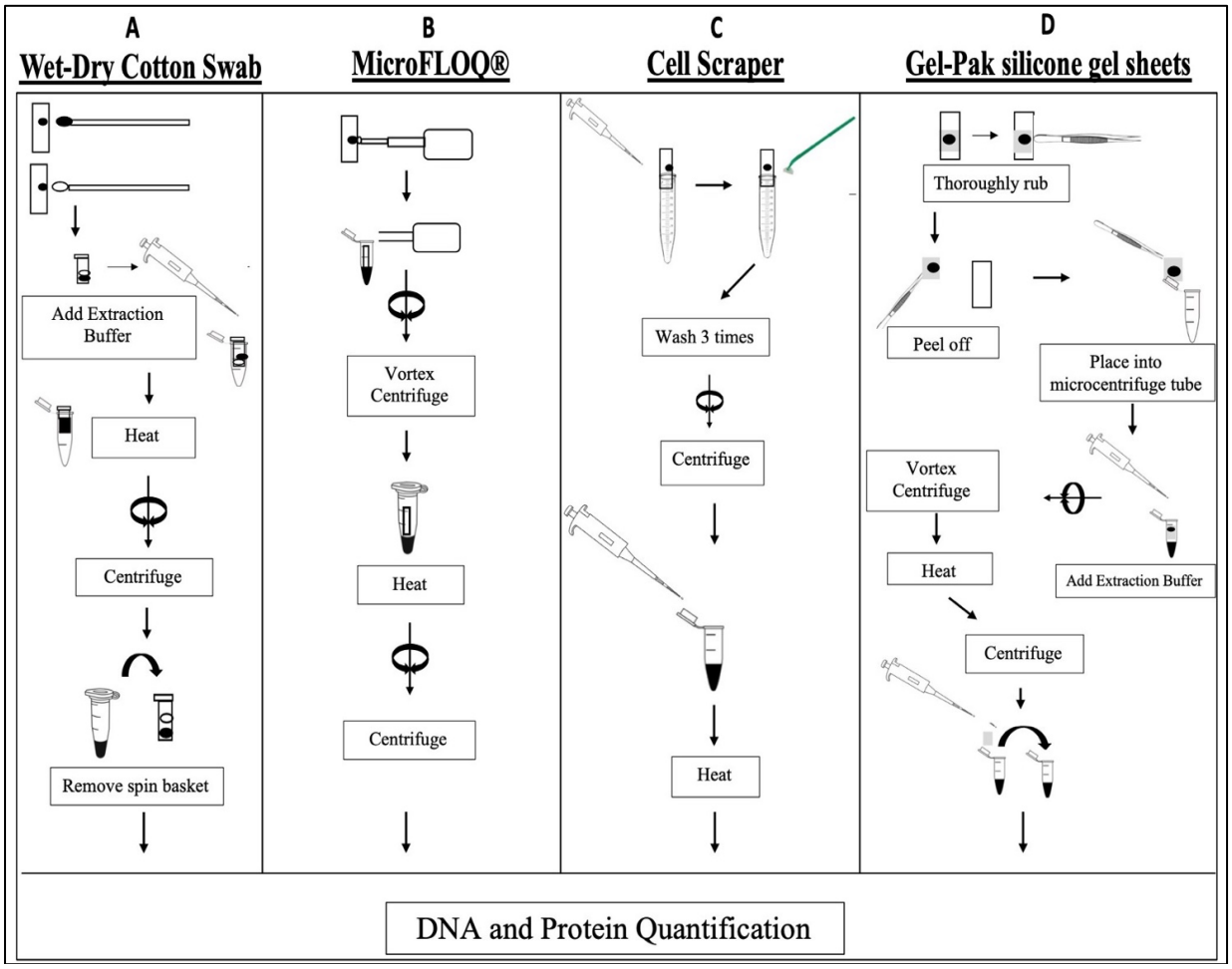

Figure 5. Description of workflow for the collection techniques. (A) The wet-dry cotton swab workflow is depicted. (B) The workflow of the dry microFLOQ® with lysing agent, wet microFLOQ® with lysing agent, dry microFLOQ® without lysing agent, and the wet microFLOQ® without lysing agent is demonstrated. (C) The workflow of the cell scraper is demonstrated. (D) The Gel-Pak silicone gel-film (PF-40-X4) workflow is depicted.

4.1.1 Artificial Fingerprints Experiments Results

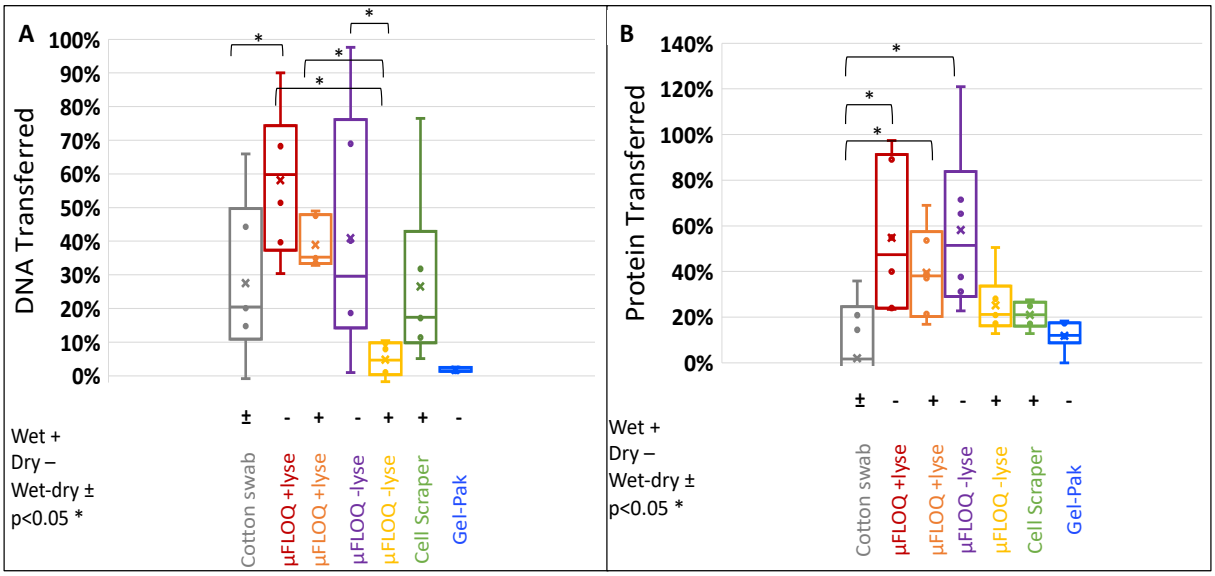

Figure 6. Biological material transferred into workflow for artificial fingerprint experiments. Cotton swab is wet-dry cotton swab, - µFLOQ +lyse is dry microFLOQ with lysing agent, + µFLOQ +lyse is wet microFLOQ with lysing agent, - µFLOQ -lyse is dry microFLOQ without lysing agent, + µFLOQ – lyse is wet microFLOQ without lysing agent, and Gel-Pak is silicone gel-film (PF-40-X4). The whisker plot graphs show median (middle horizontal line), mean (x), lower quartile (lower horizontal box line), upper quartile (upper horizontal box line), the minimum (lowest line outside box), the maximum (highest line outside box), and full range of both DNA and protein percent efficiencies for each collection technique (n = 6 each). (A) DNA transferred into workflow for all collection techniques expressed in whisker plots. (B) Protein transferred into workflow for all collection techniques expressed in whisker plots.

The total amount of DNA was 14,764 DNA copies and 25 ng of protein (3400 RDU) for each mark. When measuring the DNA of each individual collection technique the wet-dry cotton swab had a percent transfer efficiency of 27 ± 24 % (average percent transfer efficiency ± standard deviation) with an average of 4101 ± 3541 total DNA copies (average total DNA copies± standard deviation) collected. the dry microFLOQ® with lysing agent contained a percent transfer efficiency of 58 ± 22% with an average of 8663 ± 3262 total DNA copies collected. The wet microFLOQ® with lysing agent contained a percent transfer efficiency of 39 ± 7.4% with an average of 5794 ± 1093 total DNA copies collected, dry microFLOQ® without

lysing agent had a percent transfer efficiency of 41 ± 37% with an average of 6091 ± 5403 total DNA copies, and the wet microFLOQ® without lysing agent had a percent transfer efficiency of 4.8 ± 5%, an average of 711 ± 772 total DNA copies. The cell-scraper had a percent transfer efficiency of 27 ± 26% with 3961 ± 3876 total DNA copies collected, and the Gel-Pak silicone gel-film sheets (PF-40-X4) had a percent transfer efficiency of 1.8 ± 0.6% and an average of 263 ± 86 total DNA copies collected (Figure 9A).

Comparing the dry microFLOQ® with lysing agent to the wet microFLOQ® with lysing agent the dry microFLOQ® with lysing agent, the dry microFLOQ® with lysing agent increased the transfer of DNA from the surface into the workflow by 1.5-fold ($p=0.07$), thus increasing performance. The dry microFLOQ® without lysing agent had an increased performance of 8.6-fold ($p=0.04$) compared to the wet microFLOQ® without lysing agent. The microFLOQs® with lysing agent performed overall better with the collection of DNA compared the microFLOQs® without lysing agent. The dry microFLOQ® with lysing agent increased performance by 2-fold ($p=0.04$) compared to the traditional method, the wet-dry cotton swab. Compared to the wet-dry cotton swab, the wet microFLOQ® with lysing agent increased performance by 1.4-fold ($p=0.29$) and the dry microFLOQ® without lysing agent increased performance by 1.5-fold ($p=0.47$). The DNA in transferred, collected material that was measured across all collection techniques contained a range of -256 to 14,540 DNA copies with a total spike of 14,764 DNA copies per sample. The percent efficiency had a range of -1.7% to 98% across all collection techniques. It is to be noted that negative values were potentially due to a background contribution from environmental dust in some samples due to overnight placement in the chemical fume hood. Both the cell scraper and Gel-Pak contained the lowest collection amounts and percent efficiencies, thus underperforming. From the above data we observe that both forms

of the ‘Copan microFLOQ® direct’ swab when used without pre-wetting was most efficient at transfer of both DNA and protein. The presence or absence of pre-treatment with lysis buffer did not appear to affect transfer efficiencies, although the commercial product containing the pre-treated swab was more consistent in that it had a slightly lower coefficient of variation.

Similar results were obtained when measuring protein transfer. For each individual collection technique, the wet-dry cotton swab had a percent transfer efficiency of 2 ± 27% (average percent transfer efficiency ± standard deviation) with an average of 180 ± 870 RDU (average relative densitometry units ± standard deviation) collected, the dry microFLOQ® with lysing agent contained a percent transfer efficiency of 55 ± 32% with an average of 1800 ± 980 RDU collected. The wet microFLOQ® with lysing agent had a percent transfer efficiency of 40 ± 19% and an average of 1300 ± 600 RDU collected, dry microFLOQ® without lysing agent had a percent transfer efficiency of 58 ± 36% with an average of 1900 ± 1000 RDU, and wet microFLOQ® without lysing agent percent transfer efficiency was 25 ± 13% with an average of 940 ± 740 RDU. The cell-scraper’s percent transfer efficiency was 21 ± 6% with an average of 700 ± 320 RDU collected, and the Gel-Pak silicone gel-film sheets’ (PF-40-X4) percent transfer efficiency was 12 ± 6.5% with an average of 400 ± 250 RDU collected. All percent transfer efficiencies for all collection techniques are shown in Figure 9B. The dry microFLOQ® with lysing agent had a 1.4-fold ($p=0.34$) increase in performance compared to the wet microFLOQ® with lysing agent. The dry microFLOQ® without lysing agent showed an increased in performance by 2.3-fold ($p=0.06$) compared to the wet microFLOQ® without lysing agent. There was no significant increase in performance between dry microFLOQ® with lysing agent and the dry microFLOQ® without lysing agent. The wet microFLOQ® with lysing agent performance was 1.6-fold ($p=0.17$) higher than that of the wet microFLOQ® without lysing

agent. Comparing all the types of microFLOQs® to the wet-dry cotton swab, the dry microFLOQ® with lysing agent had a 28-fold ($p=0.01$) increase in performance, the wet microFLOQ® with lysing agent had a 20-fold ($p=0.02$) increase, dry microFLOQ® without lysing agent had a 30-fold ($p=0.01$) increase, and the wet microFLOQ® without lysing agent had a 13-fold ($p=0.09$) increase in performance. The total relative protein density collected had a range of -940 relative densitometry units (RDU) to 3400 RDU where the average to total spike per sample was estimated to be 3400 RDU. The percent of transfer efficiency therefore ranged from -37% to 121%. It is to be noted that negative values were due to a background contamination from some samples dried in a chemical fume hood. The cell scraper and Gel-Pak had lower collection amounts and percent efficiencies compared to all microFLOQ® techniques. However, both had higher collection amounts and percent efficiencies compared to wet-dry cotton swab. It is important to note that the high background material plausibly interfered with the wet-dry cotton swab causing it to be lower than expected. Therefore, the wet-dry cotton swab is estimated to have a total relative protein density of 1100 RDU and 25% percent transfer efficiency without background material interference. The estimated average total mass of protein collected for the wet-dry cotton swab was 6.6 ng, for the dry microFLOQ® with lysing agent it was 17 ng, 12 ng for wet microFLOQ® with lysing agent, 19 ng for dry microFLOQ® without lysing agent, 7.7 ng for wet microFLOQ® without lysing agent, 11 ng for cell scraper, and 3.7 ng for Gel-Pak. These are estimated via the protein ladders since the amount of some of the ladder proteins are known. Across all gels the average coefficient of variation for protein ladder densitometry was 13% (16% 100 kD 10 µL, 12% 50 kD 10 µL, 17% 20 kD 10 µL, 12% 100 kD 5 µL, 10% 50 kD 5 µL, and 13% 20 kD 5 µL). The assumptions made for these are the proteins are proportional to the signal, and they are the same type of proteins as the protein ladders. These

assumptions should be considered contingent. Based on the DNA and protein results, it was established that the 'Copan microFLOQ® direct' swab was overall most effective and consistent at transferring biological material for both DNA and protein. Thus, it was further evaluated by comparing it to the current best practice, the wet-dry cotton swab, on more challenging surfaces.

4.2 Fingermarks on Glass Microscope Slides Experiment

After identification of the most efficient transfer method, the next step was to apply the method to actual fingermarks. This experiment involved the collection, extraction, and quantification of real fingermarks from four individuals on the 'ideal' surface of a microscope slide. The bench working area and analytical scale was sterilized with 70% isopropyl alcohol and covered with clean paper and kimwipes. Four pre-cleaned microscope slides were obtained for each individual and labeled accordingly, resulting in sixteen microscope slides total. Each individual washed their hands for 20 seconds with water only and then let them air dry. Once hands were fully dried each individual rubbed their forehead and hands together for 15 seconds to make the finger surface both consistent and 'sebaceous rich' [23, 41, 51]. A microscope slide was placed on the sterilized scale and each individual placed their left thumb on the microscope slide where they applied pressure of 510 $\pm$ 15 grams (5 N) for 5 seconds as shown in Figure 7.

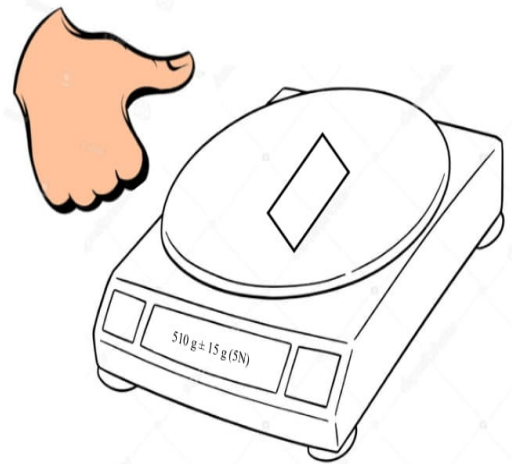

Figure 7. 'Sebaceous rich' fingermark made on microscope glass slide using 5N (510 ± 15g) of force.

The pressure applied at 5 N is the upper limit of the force needed to move a movable object [82]. This was repeated for the right thumb and one more set of both thumbs prints for

each individual resulting in 2 set of thumb prints each individual. All thumb prints were outlined and split in half on the opposite side of the microscope glass. The second half of the left and right thumb prints were collected and extracted by the wet-dry cotton swab transfer technique. as previously described, for each individual. For the first set of thumb prints the first half of the left and right thumb prints were collected and extracted by the dry microFLOQ® direct technique ('Copan microFLOQ® direct'), as previously described, for each individual. For the second set thumb prints the first half of the left and right thumb prints were collected and extracted by the wet-dry cotton swab technique, as previously described, for each individual. The second half of the left and right thumb prints were collected and extracted by the 'Copan microFLOQ® direct' technique, as previously described, for each individual. This resulted in 16 total samples for each collection and extraction technique.

All samples were quantified for DNA and relative protein density, as previously described. Both collection methods and quantification are illustrated in Figure 8.

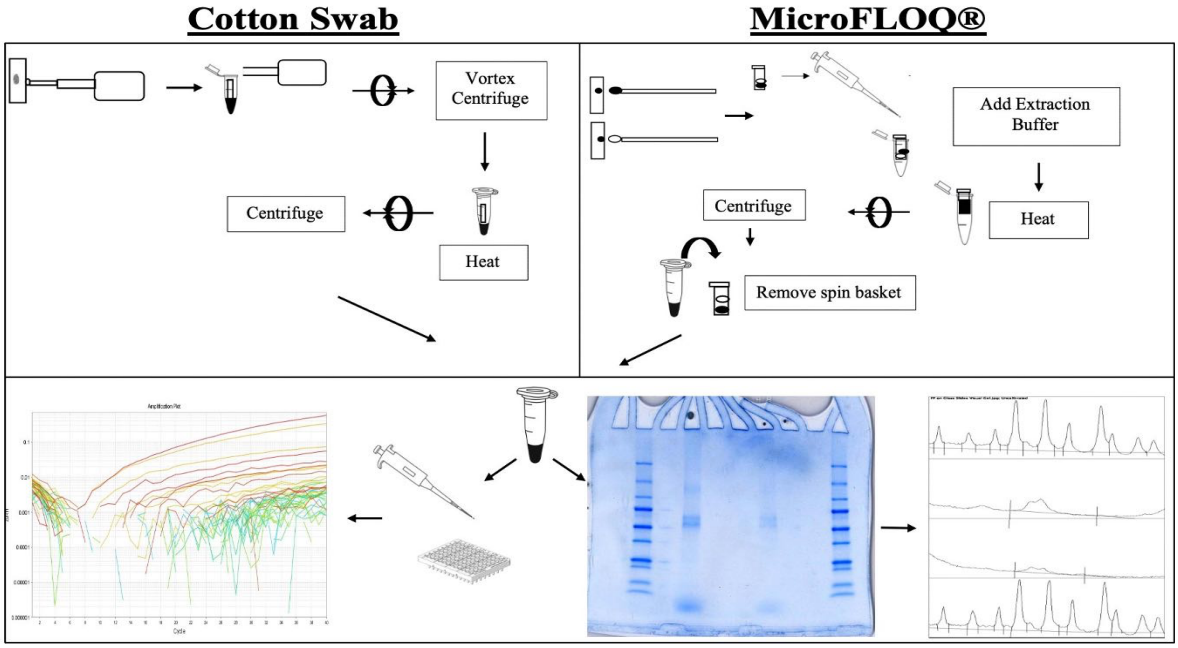

Figure 8. Workflow descriptions of cotton swab and 'Copan microFLOQ® direct' for sebaceous rich fingerprints on glass microscope slides experiment. Followed by qPCR analysis workflow and SDS-PAGE gel electrophoresis analysis workflow.

4.2.1 Fingermarks on Glass Microscope Slides Experiment Results

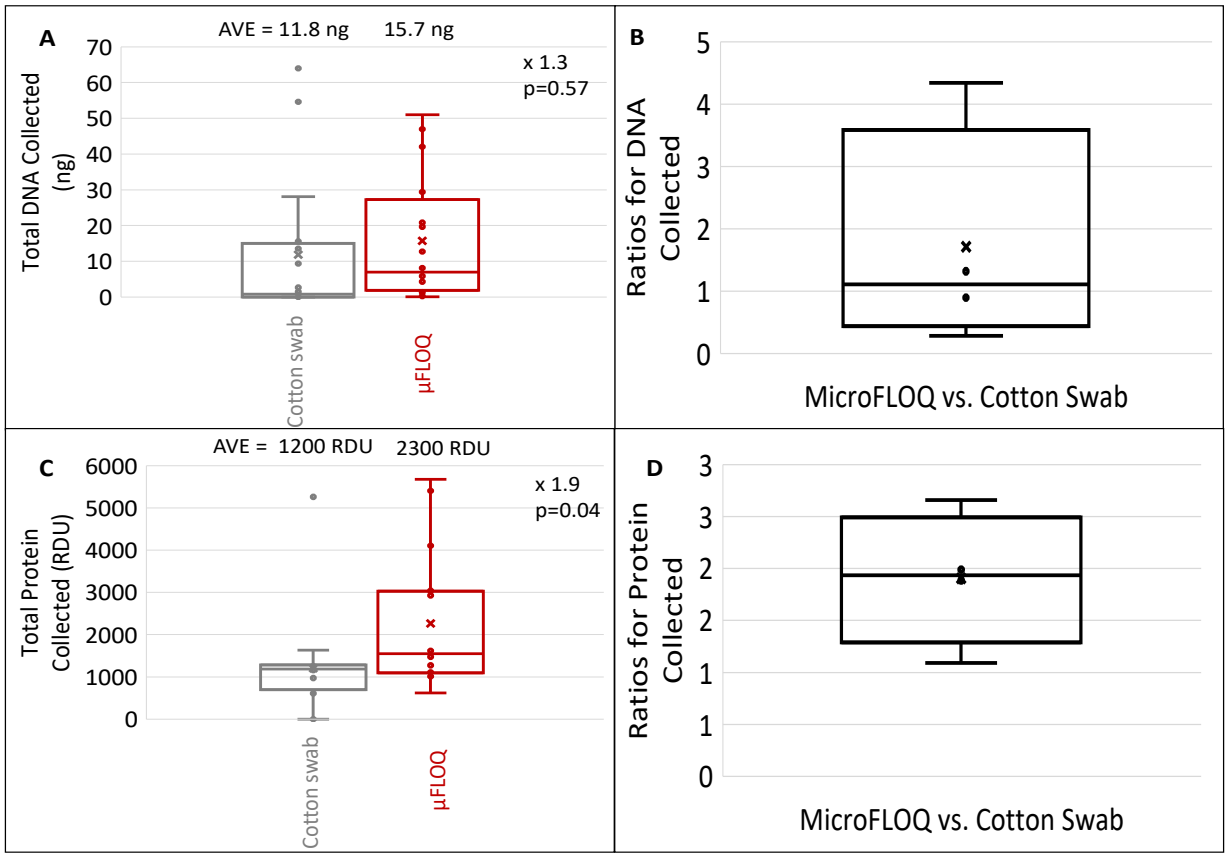

Figure 9. Biological material collected from fingermarks on glass microscope slides. The whisker plot graphs show median (middle horizontal line), mean (x), lower quartile (lower horizontal box line), upper quartile (upper horizontal box line), the minimum (lowest line outside box), the maximum (highest line outside box), and full range of both DNA and protein yields for each collection technique (n = 16 each) and ratios. (A) DNA collected in ng for both the wet-dry cotton swab (left) and 'Copan microFLOQ® direct' (right). (B) Ratios for the DNA collected on glass microscope slides when normalized for individuals comparing the transfer efficiency of 'Copan microFLOQ® direct' to the wet/dry cotton swab. Expressed as the average quotient of 'Copan microFLOQ® direct' over cotton swab for each individual. (C) Protein collected in RDU for both the wet-dry cotton swab (left) and the 'Copan microFLOQ® direct' (right). (D) Ratios for the protein collected on glass microscope slides when normalized for individuals.

Based on the results above, the cotton swab contained a range of 0 ng to 64 ng of total genomic DNA collected with an average of 11.8 ± 20 ng (average total DNA ± standard deviation) of total genomic DNA. The 'Copan microFLOQ® direct' contained a range of 0.1 ng to 51 ng of total genomic DNA collected with an average of 15.7 ± 17 ng of total DNA (Figure

9A). The ‘Copan microFLOQ® direct’ had a 1.3-fold (p=0.57) increased performance for the collection of total DNA compared to the cotton swab. When normalized for individuals, the ‘Copan microFLOQ® direct’ had a 1.7-fold (p=0.23) increased performance for the collection of total DNA (Figure 9B). The total genomic DNA collected across both collection techniques contained a range of 0 ng to 64 ng.

Evaluating the protein material collected, the cotton swab contained a range of 0 RDU to 5300 RDU of total relative protein density collected with an average of 1200 ± 1200 RDU (average relative densitometry units ± standard deviation) (Figure 9C). The ‘Copan microFLOQ® direct’ contained a range of 620 RDU to 5700 RDU of total relative protein density collected with an average of 2300 ± 1600 RDU (Figure 9C). The ‘Copan microFLOQ® direct’ had a 1.9-fold (p=0.04) increased performance for the collection of total relative protein density compared to the cotton swab. When normalized for individuals, the ‘Copan microFLOQ® direct’ had a 1.9-fold (p=0.02) increased performance for the collection of total relative density (Figure 9D). The total relative protein density collected across both collection techniques contained a range of 0 RDU to 5700 RDU. Variability was reduced when normalized for individual for both DNA and protein, since this would control for individual ‘shedder status’. Based on the results obtained for both DNA and protein, the ‘Copan microFLOQ® direct’ significantly outperformed the cotton swab. When RDU is converted into ng the estimated average total ng of protein collected for the ‘Copan microFLOQ® direct’ was 14.4 ng and 13 ng for the cotton swab.

4.3 Ammunition Cartridge Cases Scenarios

Real-life scenarios were mimicked where the cotton swab and ‘Copan microFLOQ® direct’ were used to transfer DNA and protein from unfired and fired cartridge cases into the

technical workflow. In this study, a Glock Model 17 Generation 4 pistol was used, provided, and fired by members of the UC Davis Police Department. This pistol was chambered for the 9mm Luger (Parabellum) cartridge.

4.3.1 Unfired Cartridge Cases Scenario

Four subjects made their fingertips 'sebaceous' rich by rubbing their forehead for 5 seconds before handing a cartridge case. The four participants took a 9 mm cartridge from a brand-new unopened ammunition box and loaded it into the magazine. The cartridge was removed from the magazine by the participant, and then collected into a coin paper envelope. The coin paper envelope was labeled with the scenario, participant, and cartridge number. The participants wiped off their fingers with a clean kimwipe before making their fingertips 'sebaceous' rich again to load another cartridge into the magazine. The four participants did this a total of 12 times each where the cartridge was in the same position in the magazine for each time. The cartridge was disarmed using a kinetic bullet puller and the cartridge case was put back into the coin envelope. The process resulted in a total of 48 cartridge cases where 24 cartridge cases were used for the dry-wet cotton swab collection technique and 24 for the 'Copan microFLOQ® direct' collection technique. Each collection/extraction method extracted the touch evidence on 12 cartridge cases for DNA and 12 cartridge cases for proteins. One participant wore a pristine pair of nitrile gloves and took a 9 mm cartridge from a brand-new unopened ammunition box and loaded it into the magazine. These were sampled and collected as described above and provided the negative control samples. This resulted in a total of 12 negative control cartridge cases collected where 6 were used for the wet-dry cotton swab collection technique and 6 for the 'Copan microFLOQ® direct'. Each collection/extraction method extracted the touch evidence on 3 cartridge cases for DNA and 3 cartridge cases for proteins.

4.3.2 Fired Cartridge Cases Scenario

The process was repeated as described above; however, instead of the ammunition being removed from the magazine the cartridge was fired off using the Glock 17 pistol provided and fired by members of the UC Davis Police Department, and the ejected cartridge case was collected in a coin paper envelope. The collection process as described previously was repeated as above, thus resulting in in a total of 48 cartridge cases where 24 cartridge cases were used for the cotton swab collection technique and 24 for the 'Copan microFLOQ® direct' collection technique. Each collection/extraction method extracted the touch evidence on 12 cartridge cases for DNA and 12 cartridge cases for proteins. The control of this scenario was repeated as described above, however, the ammunition was fired (expended) instead of being removed from the magazine and disarmed. Once fired, the cartridge cases were collected in a coin paper envelope as previously stated above. This resulted in a total 12 cartridge cases collected where 6 were used for the cotton swab collection technique and 6 for the 'Copan microFLOQ® direct' collection techniques. Each collection/extraction method extracted the touch evidence on 3 cartridge cases for DNA and 3 cartridge cases for proteins. The workflow for both scenarios is illustrated in Figure 10.

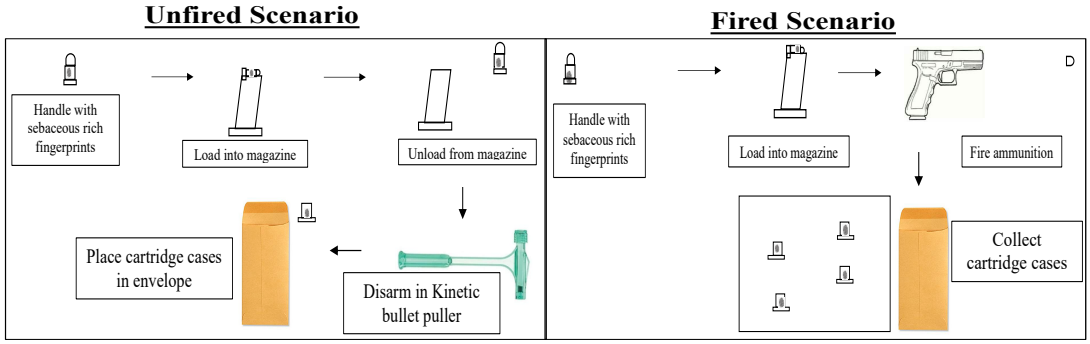

Figure 10. Unfired and fired scenarios workflow description for cartridge cases experiment.

Half of the samples from both scenarios were extracted using the wet-dry cotton swab collection technique, as previously described, and the other half using the microFLOQ® direct

collection technique, as previously described. A total of 60 samples were quantified for DNA, as previously described. A total of 60 samples were quantified for relative protein density, as previously described The workflow for the cotton swab and the 'Copan microFLOQ® direct' along with DNA and protein quantification are illustrated in Figure 11.

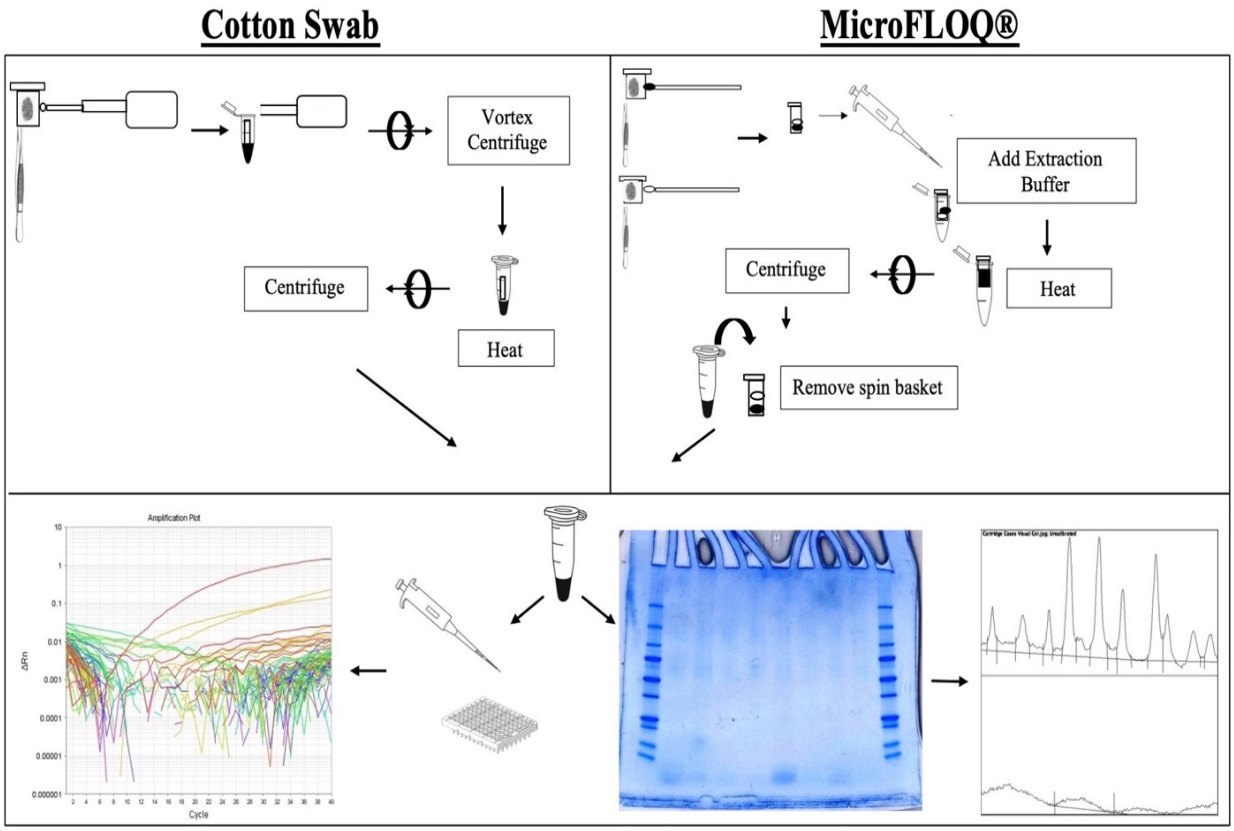

Figure 11. Workflow description of cotton swab and Copan microFLOQ® direct' for cartridge cases experiment. Followed by qPCR analysis and SDS-PAGE gel electrophoresis analysis workflow.

4.3.3 Cartridge Cases Experiment Results

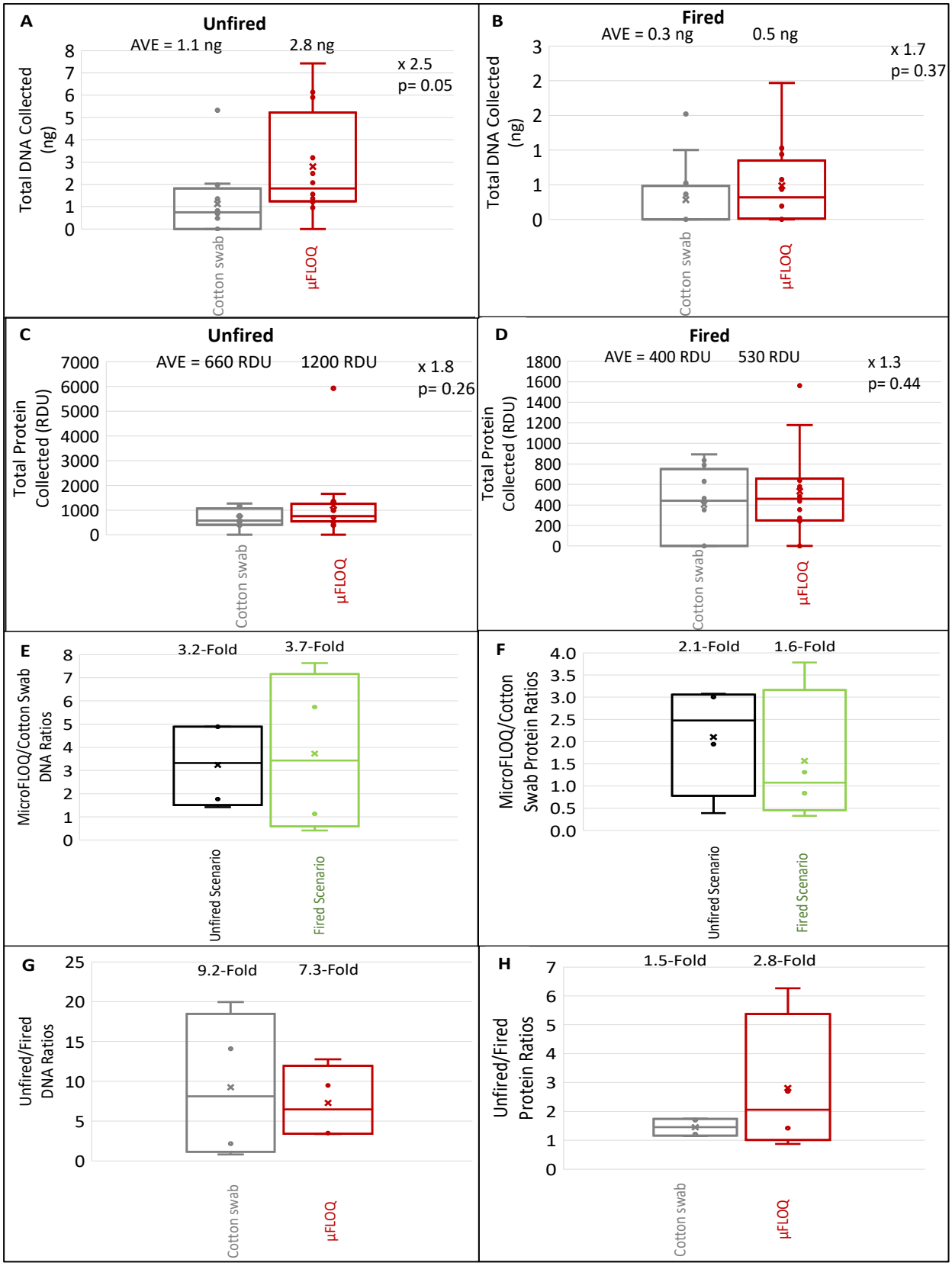

Figure 12. Biological material collected for cartridge cases experiment. The whisker plot graphs show median (middle horizontal line), mean (x), lower quartile (lower horizontal box line), upper quartile (upper horizontal box line), the minimum (lowest line outside box), the maximum (highest line outside box), and full range of both DNA and protein yields for each collection technique (n = 24 each) and ratios. (A) DNA collected in ng for the unfired scenario for both cotton swab and 'Copan microFLOQ® direct'. (B) DNA collected in ng for the fired scenario for both cotton swab and 'Copan microFLOQ® direct'. (C) Protein collected in RDU for the unfired scenario for both cotton swab and 'Copan microFLOQ® direct". (D) Protein collected in RDU for the fired scenario for both cotton swab and 'Copan microFLOQ® direct'. (E) Ratios for the DNA collected on both unfired and fired cartridge cases when normalized for individuals among the cotton swab and 'Copan microFLOQ® direct'. Expressed as 'Copan microFLOQ® direct'over cotton swab. (F) Ratios for the protein collected on unfired and fired cartridge cases when normalized for individuals among the cotton swab and 'Copan microFLOQ® direct'. Expressed as 'Copan microFLOQ® direct' over cotton swab. (G) Ratios for DNA collected for both the cotton swab and the 'Copan microFLOQ® direct' when normalized for individuals between unfired and fired cartridge cases. Expressed as unfired over fired cartridge cases. (H) Ratios for protein collected for both the cotton swab and the 'Copan microFLOQ® direct' when normalized for individuals between unfired and fired cartridge cases. Expressed as unfired over fired cartridge cases.

Based on the results above, the cotton swab contained a range of 0 ng to 5.3 ng with an average of 1.1 ± 1.5 ng (average total DNA ± standard deviation) of total DNA collected for the unfired cartridge cases. For the fired cartridge cases the cotton swab ranged from 0 ng to 1.5 ng of total DNA collected with an average of 0.3 ± 0.5 ng. The 'Copan microFLOQ® direct' contained a range of 0 ng to 7.4 ng with an average of 2.8 ± 2.4 ng of total DNA collected for the unfired cartridge. For the fired cartridge cases 'Copan microFLOQ® direct' ranged from 0 ng to 2 ng of total DNA transferred with an average of 0.5 ± 0.6 ng. The total DNA collected for the unfired cartridge cases across both collection techniques, the cotton swab and the 'Copan microFLOQ® direct', ranged from 0 ng to 7.4 ng. The fired cartridge cases had a noticeably lower amount of material where the total DNA collected ranged from 0 ng to 2 ng. All amounts of total DNA collected are depicted in Figure 12A and Figure 12B for both the cotton swab and the 'Copan microFLOQ® direct'. The DNA material lost on the cartridge cases when fired was

estimated to be 75% ± 67% when using the cotton swab and 83 ± 75% for the 'Copan microFLOQ® direct'. The remaining DNA material for the cotton swab was estimated to be 25%, while the 'Copan microFLOQ® direct' was estimated to be 17%. The 'Copan microFLOQ® direct' performed better for total DNA transferred into the workflow than the cotton swab. It had a 2.5-fold (p=0.05) increased performance for the unfired cartridge cases and a 1.7-fold (p=0.37) increased performance for the fired cartridge cases (Figure 12A and 12B). All negative control samples for both unfired and fired cartridge cases were free of DNA.

Regarding the total relative protein density collected, the cotton swab contained a ranged of 0 RDU to 1300 RDU for the unfired cartridge cases with an average of 660 ± 380 RDU (average relative densitometry units ± standard deviation). For the fired cartridge cases the cotton swab ranged from 0 RDU to 890 RDU with an average of 400 ± 340 RDU of total relative protein density collected. The 'Copan microFLOQ® direct' contained a range of 0 RDU to 5900 RDU with an average of 1200 ± 1500 RDU of total relative protein density collected for the unfired cartridge cases. For the fired cartridge cases the 'Copan microFLOQ® direct' ranged from 0 RDU to 1600 RDU with an average of 530 ± 450 RDU of total relative protein density collected. Across both techniques for the unfired cases, the cotton swab and the 'Copan microFLOQ® direct', ranged from 0 RDU to 5900 RDU. However, the fired cartridge cases had considerably less material with a total relative protein density range of 0 RDU to 1600 RDU. All amounts of total relative protein density collected are depicted in Figure 11C and Figure 11D for both the cotton swab and the 'Copan microFLOQ® direct'. When RDU is converted into ng the estimated average total ng of protein collected for the 'Copan microFLOQ® direct' is 6.6 ng when cartridge cases are unfired and -3 ng when fired. For cotton swab it is 2.9 ng for unfired and -14 ng when fired. These are estimated via the protein ladders since the amounts of some of

the ladder proteins are known. Across all gels the average coefficient of variation for protein ladder densitometry was 13% (16% 100 kD 10 µL, 12% 50 kD 10 µL, 17% 20 kD 10 µL, 12% 100 kD 5 µL, 10% 50 kD 5 µL, and 13% 20 kD 5 µL). The assumptions made for these are the proteins are proportional to the signal, and they are the same type of proteins as the protein ladders. These assumptions should be considered contingent. The relative protein lost on fired cartridge cases was estimated to be 39 ± 11% for the cotton swab and 55 ± 71% for the 'Copan microFLOQ® direct'. The remaining protein material for the cotton swab was estimated to be 61% and 55% for the "Copan microFLOQ® direct'. The 'Copan microFLOQ® direct' had a 1.8-fold (p=0.26) increased performance for the unfired cartridge cases and a 1.3-fold (p=0.44) increased performance for the fired cartridge cases when compared to the cotton swab (Figure 12C and 12D). All negative control samples for both unfired and fired cartridge cases were free of protein.

When normalized for individuals, the 'Copan microFLOQ® direct' had a 3.2-fold (p=0.03) increased performance in transferring DNA material into the workflow for the unfired cartridge cases and a 3.7-fold (p=0.09) increased performance for the fired cartridge cases. This fold increase is depicted in Figure 12E above. The DNA material lost on cartridge cases when fired was estimated to be 55% ± 57% for cotton swab and 81% ± 12% for 'Copan microFLOQ® direct' when normalized for individuals. Furthermore, when normalized for individuals the 'Copan microFLOQ® direct' had a 2.0-fold (p=0.06) increase in performance in transferring protein material into the workflow for unfired cartridge cases and a 1.6-fold (p=0.24) increased performance for fired cartridge cases (Figure 12F). The protein lost when fired was estimated to be 50 ± 35% for the cotton swab and 41% ± 43% for the 'Copan microFLOQ® direct' when normalized for individuals.

Further evaluation indicated that the amount of biological DNA transferred by the cotton swab decreased by 9.2-fold (p=0.06) upon firing, while the 'Copan microFLOQ® direct' decreased performance by 7.3-fold (p=0.02) upon firing when normalized for individuals (Figure 12G). Therefore, the overall DNA material lost upon firing was estimated to be 76 ± 11% when normalized for individuals with 24 ± 11% remaining. The protein material transferred by the cotton swab decreased by 1.5-fold (p=0.01) upon firing, while the 'Copan microFLOQ® direct' decreased performance by 2.8-fold (p=0.09) upon firing when normalized for individuals (Figure 12H). Thus, the overall protein material lost upon firing was estimated to be 35 ± 48% with 65% ± 48% remaining. With these results, protein material was 2.2-fold (p = 0.07) more persistent than the DNA material on cartridge cases after firing.

5. Discussion

The aim of this study was to determine an efficient collection/extraction technique for both DNA and protein from touch evidence that can be easily incorporated into forensic laboratory workflow. In addition, this study focuses on being quantitative and looking at how much of biological material is being transferred into the workflow by an efficient collection technique. The established technique would primarily be useful in collecting that biological material from fired cartridge cases especially when DNA is limited.

5.1 Artificial Fingerprints Experiments

Following the LeSassier *et al*. protocol with the use of artificial fingerprints to validate the seven different collection techniques, both types of dry microFLOQs® transferred DNA from the microscope slide surface to the workflow better than both types of wet microFLOQs® [6]. Therefore, dry microFLOQ® performs better than a wet microFLOQ® for DNA, regardless of lysing agent. The microFLOQs® with lysing agents performed better for DNA. All types of

microFLOQs® performed better than the current practice, the wet-dry cotton swab. The cell scraper and Gel-Pak silicone gel sheets underperformed for DNA. For protein there was no definitive difference between the microFLOQs® with lysing agent and those without the lysing agent. Both types of microFLOQs® performed better than the current practice, wet-dry cotton swab, for protein. The cell scraper and Gel-Pak silicone gel sheets also underperformed for protein. Since the Gel-Pak silicone gel sheets underperformed, a better alternative would have been to use either Voigtlaender Neschen Foil S23 or Scotch Tape as described in previous studies [41, 51]. Based on the results obtained the dry microFLOQ® with lysing agent proved to be the most consistent collection technique under these controlled conditions. It is important to note that the artificial fingerprint results indicated some high fold increases. It is plausible that this was due to the high background material that introduced noise into the findings which artificially lowered collection technique values.

With the artificial fingerprints plasmid DNA was used instead of genomic DNA, involving advantages and disadvantages. The disadvantages to using plasmid DNA is the amount of DNA used was very small, which may potentially increase non-specific binding and loss of DNA. However, an advantage to using plasmid DNA is that it is small and can come off the glass slide easier compared to genomic DNA. Genomic DNA is larger than the plasmid DNA and contains associated proteins, which could also increase non-specific binding to the glass surface and reduce yields. This study assumes that the plasmid DNA is a valid substitute for genomic DNA when it comes to the transfer of material from a two-dimensional surface into liquid in a microcentrifuge tube. We assume that what works for plasmid DNA will work for genomic DNA.

5.2 Fingermarks on Glass Microscope Slides Experiment

With real fingermarks on glass microscope slides the ‘Copan microFLOQs® direct’ had an increase in performance compared to the cotton swab for DNA. When normalized for individuals the ‘Copan microFLOQ® direct’s’ performances increased even more for DNA. The ‘Copan microFLOQ® direct’ had a significantly better performance for protein than the cotton swab. When normalized for individuals the ‘Copan microFLOQ® direct’s’ performance increased even more. Overall, the ‘Copan microFLOQ® direct’ was better at transferring DNA from the glass microscope slide surface than the cotton swab for both DNA and protein where with protein it was significantly better. Comparing to other studies, Thomasma *et al.* indicated similar DNA yields on glass microscope slides with the double swab technique (wet-dry cotton swab). This study depicted DNA yield ranges of 0 pg/µL to 881 pg/µL, which translates into an estimate range 0 ng to 17 ng of total DNA using a final volume of 20 µL after extraction [57]. However, compared to Kranes *et al*., DNA yields were higher for use of a wet cotton swab on glass microscope slides. This study indicated an estimated average range of 0 pg to 745.26 pg of total DNA yield. Numerous reasons could contribute to the difference such as the use of soap when washing hands and the use of only one cotton swab [23]. Nevertheless, similar results in other studies were obtained for the double cotton swab techniques on glass microscope slides.

5.3 Ammunition Cartridge Cases Scenarios

For the cartridge cases experiment the ‘Copan microFLOQ® direct’ performed better for DNA than the cotton swab when the cartridge cases were not fired. It also had an increase in the transfer of DNA from the fired cartridge cases to the workflow compared to the cotton swab. When normalized for individuals, the ‘Copan microFLOQ® direct’ performed significantly better for DNA on the unfired cartridge cases compared to the cotton swab. For the fired cartridge cases, the ‘Copan microFLOQ® direct’ had even better performance for DNA when

normalized for individuals. For protein the 'Copan microFLOQ® direct' had better performance for both the unfired cartridge cases and the fired cartridge cases. When normalized for individuals, the 'Copan microFLOQ® direct' increased its performance even more for both the unfired cartridge cases and the fired cartridge cases when compared to the cotton swab. The 'Copan microFLOQ® direct' did have an overall better transfer of DNA and protein from the unfired and fired cartridge cases compared to the cotton swab. However, it did lose more biological material than the cotton swab when fired. Upon firing, there was more DNA material lost overall compared to protein material. Sterling *et. al* did show a similar pattern of protein being more robust [41]. Thanakiatkrai *et al.* further supports this by indicating DNA median recovery percentage decreases by 30.8% upon firing [50]. Thus, the protein material held up better after going through the firing process. The total DNA yields for the cotton swab did reflect similar collection amounts as seen in Bille *et al.,* with theirs ranging from 0.2 ng to 14 ng [43]. Danielson *et al.* also indicates similar DNA yields after firing with ranges of 7.2 pg to 895 pg (0.007 ng to 0.895 ng) and 6.9 pg to 7,960 pg (0.0069 ng to 7.96 ng) [42].

5.4 Future Work and Important Findings

Further work can be done based on the findings described here. Such work would include doing STR profiling on the samples to see if there was a useful profile obtained especially on the fired cartridge cases samples. In addition, mass spectrometry can also be done to further understand the types of proteins that were collected and whether the different transfer methods introduced a bias for different proteins. Furthermore, different ammunition sizes and types could be used to see how that will affect the workflow and further validate it. Pre-cleaning ammunition can be done to see if touch evidence is from manufacture or from the handler. If the biological material from manufacture is extracted as well then it may lose probative value, possibly causing

a mixed profile obtained which may be less probative. Also, using fingermarks that are not made 'sebaceous' rich and instead use fingermarks that are as is to be mimic real life even more.

Important findings from these experiments include that the 'Copan microFLOQ® direct' transfer swab works better for both DNA and protein. Thus, there is a better type of swab than the traditional cotton swab method that can be incorporated into forensic laboratory workflow. When we apply this new collection technique, the 'Copan microFLOQ® direct', important things are indicated about touch evidence on cartridge cases. The first is we can quantify the amount of material lost upon firing. Second, we find out that protein is more stable than DNA. Third, the surface that biological material is on is a factor in the amount that is available to transfer into the workflow. Finally, this has implications for how we obtain genetic (identifying) evidence from fired cartridge cases. Specifically, we should aim to collect both types of information, given that we would predict that protein-based information would be more of a contributor as the sample becomes more degraded.

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