Targeted Non-Destructive Evidence Detection and Collection

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1.0 Abstract

The ability to successfully detect, collect, and process individual biological samples from various evidence substrates without causing integral surface damage continually proves to be a difficult challenge in the field of forensics. The damage inflicted to evidence items during sample collection may inhibit additional evaluations of the object. Traditional methods of recovering DNA from forensic samples typically rely on chemical sprays, wet/dry cotton tip swabbing, and material cuttings. While these traditional techniques are effective collection methods for biological samples, they typically leave evidence items in an altered and damaged state. Superior and more efficient non-destructive collection methods are needed to allow the forensic community to have a confident non-destructive approach to sampling. Forensic DNA analysts need the ability to detect and collect biological materials from an item without damaging the structural integrity of evidence items and/or interfering with any subsequent examinations.

Bode was awarded Grant# 2010-DN-BX-K191 from the National Institute of Justice in 2010 to study the use of non-destructive methodologies for the targeted collection of biological materials on common forensic evidentiary substrates. The Electrostatic Detection Apparatus (ESDA®), alternative swab matrices (non-cotton swabs), adhesive evidence lifters, and the Thermal Fingerprint Developer (TFD) were each evaluated as effective non-destructive evidence processing tools. Each of the four non-destructive collection methodologies were systemically evaluated for their ability to yield DNA STR profiles from a variety of biological samples deposited on various forensically relevant substrates.

Each of these innovative DNA collection methodologies displayed positive results, and they have the capabilities for real-world forensic implementation. The results of the methodologies evaluated allowed for the non-destructive identification of biological samples on paper documents (TFD and ESDA) and also demonstrated increased success in obtaining DNA profiles from a variety of substrates and biological fluids when compared to destructive and current non-destructive methods of sample collection (i.e. dry cotton swabbing).

Having the ability to non-destructively detect and collect biological samples would greatly benefit the forensic community by enabling other disciplines the opportunity to perform more thorough forensic investigations of evidentiary items. The additional information gained from items processed in this manner could convict or exonerate individuals associated with questioned documents, entry point surfaces, various clothing items, and other handled evidentiary materials. Evidence processed in a non-destructive manner would also remain available for future evaluations which could prove pivotal to the outcome of a cold case investigation and/or criminal retrial. All of the tools and techniques suggested are relatively inexpensive or are already available in crime labs and could easily be incorporated into standard laboratory operating procedures.

2.0 Table of Contents

3.0 Executive Summary

The ability to successfully detect, collect, and process individual biological samples from various evidence substrates without causing integral surface damage continually proves to be a difficult challenge in the field of forensics. The damages inflicted to evidence items during sample collection may inhibit and/or prevent additional evaluations of the object. Traditional methods of recovering DNA from forensic samples typically rely on chemical sprays, wet/dry cotton tip swabbing, and/or material cuttings. Chemical sprays, such as ninhydrin, are also utilized frequently by forensic investigations to locate amines left behind by sloughed off cellular debris. The wet/dry double swab technique is a highly employed collection method for the sampling of biological deposits. Material cuttings provide DNA analysts with small clippings of the original sample which can elicit ample amounts of DNA through the extraction process. While these traditional techniques are effective liberators of biological samples, they do typically leave evidence items in an altered and/or damaged state. Use of the wet/dry double swab method generally causes tearing and blotting on sampled paper documents. Material cuttings also prove to be very destructive as they involve physically removing segments of evidence which could otherwise be analyzed by other forensic disciplines. These sampling techniques cause destruction to sampled items and therefore do not prove to be universally applicable in all forensic investigations.

Superior and more efficient non-destructive collection methods are needed to allow the forensic community to have a confident non-destructive approach to sampling. Forensic DNA analysts need the ability to detect and collect biological materials from an item without damaging the structural integrity of evidence items and/or interfering with any subsequent examinations. Having the ability to non-destructively detect and collect biological samples would greatly benefit the forensic community by enabling other disciplines the opportunity to perform more thorough forensic investigations of evidentiary items. The additional information gained from items processed in this manner could convict or exonerate individuals associated with questioned documents, entry point surfaces, various clothing items, and other handled evidence materials.

Bode Technology performed a thorough evaluation of several novel non-destructive DNA collection tools. The Electrostatic Detection Apparatus (ESDA®), alternative swab matrices, adhesive evidence lifters, and the Thermal Fingerprint Developer (TFD) were each evaluated as effective non-destructive evidence processing tools. Each of these innovative DNA collection methodologies have shown positive results in preliminary experiments and have the potential for real-world forensic implementation.

Each of the four non-destructive collection methodologies were systemically evaluated for their ability to generate high quality DNA STR profiles from a variety of biological samples deposited on various forensically relevant substrates. Initial testing evaluated each technique's ability to generate DNA profiles from buccal cells that were purified and spotted onto a variety of forensic substrates in various volumes. This was expanded on in subsequent testing of fingerprints and various volumes of semen, blood, and saliva deposited on additional substrates. Aged sample testing was also performed to determine the non-destructive techniques' abilities to obtain DNA profiles from samples stored at room temperature (RT) for one month and six months. Finally,

additional testing was performed that expanded upon the initial studies in order to strengthen previously analyzed data sets. All biological samples were processed with standard DNA processing methodologies utilizing the QIAGEN EZ1® DNA Investigator Kit in conjunction with the EZ1 Advanced for all DNA extractions, the AmpFℓSTR® Identifiler® Plus PCR amplification kit from Life Technologies for all STR amplifications and the 3130xL genetic analyzer for capillary electrophoresis. The ability of the non-destructive methodologies to successfully obtain DNA profiles was measured as the percent profile achieved of the applicable STR profile.

Results of the study demonstrated that the evaluated non-destructive techniques have great potential for the forensic community.

- The ESDA samples generated high partial or full DNA profiles, especially from fingerprints on a variety of paper substrates. By sampling the Mylar sheet that comes into contact with the paper document of interest, the non-destructive ESDA collection technique consistently outperformed the destructive methodology of cutting an equivalent sample from the same paper substrate.
- The overall evaluation of the adhesive evidence lifters demonstrated positive results, with several lifters displaying the potential to non-destructively obtain DNA sample from a variety of substrates. In particular, the BVDA Gellifter® worked exceptionally well on all substrates when lifting blood, semen, saliva, and fingerprints. However, utilizing the evidence lifters on paper substrates commonly resulted in ripping of the substrate.
- The evaluation of the alternative swab matrices proved to be exceptionally fruitful, as a number of the matrices performed at the same level or better than standard cotton swabs when collecting a sample non-destructively. The VWR Foam swab and the Puritan Hydraflock swab obtained the highest quality DNA profiles across the most substrates and biological samples. The results of this study led to the utilization of the Hydraflock swab during the additional ESDA testing in an effort to optimize the ESDA technique.
- The TFD evaluation demonstrated the ability to non-destructively visualize fingerprints on paper thus, limiting the area of DNA collection to detected prints only as compared to random swabbing of a large document. This greatly reduced the sample area that was subsequently dry swabbed, increasing the chance of producing high partial or full DNA profiles.

The newly developed techniques could allow DNA analysts access to evidence items prior to the performance of any other forensic type examination. It is possible that vital DNA evidence is lost when fingerprint, trace evidence (fibers, hairs, etc.), and/or chemical examinations are performed in advance of biological inspections. Allowing DNA analysts access to unprocessed evidence could increase the likelihood of collecting sufficient biological material to produce a high quality DNA profile, particularly in regards to touch type evidence items. If the proposed non-destructive DNA collection techniques are used before other examinations are performed, it would allow for non-DNA analysts to receive the processed items in a seemingly untouched state. For example, the use of an ESDA instrument to collect biological deposits from a handwritten document would allow a DNA analyst to collect biological material during the initial processing of the item, and it would not interfere with any subsequent fingerprint development and/or handwriting analysis. The implementation of these proposed techniques

would afford forensic scientists the ability gain more information from sensitive evidence type items by allowing multiple full-scale examinations to be performed.

4.0 Technical Report

4.1 Introduction

4.1.1 Statement of the Problem

The ability to successfully detect, collect, and process individual biological samples from various evidence substrates without causing integral surface damage continually proves to be a difficult challenge in the field of forensics. The damages inflicted to evidence items during sample collection may inhibit and/or prevent additional evaluations of the object. Traditional methods of recovering DNA from forensic samples typically rely on chemical sprays, wet/dry cotton tip swabbing, and/or material cuttings [4, 8, 19, 21, 22, 24, 28]. Chemical sprays, such as ninhydrin, are also utilized frequently by forensic investigations to locate amines left behind by sloughed off cellular debris [2, 11].The wet/dry double swab technique is a highly employed collection method used for the sampling of biological deposits. Material cuttings provide DNA analysts with small clippings of the original sample which can elicit ample amounts of DNA through the extraction process [24]. While these traditional techniques are effective liberators of biological samples, they do typically leave evidence items in an altered and/or damaged state. The wet/dry double swab method generally causes tearing and blotting on sampled paper documents (Figure 1). Material cuttings also prove to be very destructive as they involve physically removing segments of evidence which could otherwise be analyzed by other forensic disciplines (Figure 1). These sampling techniques cause destruction to sampled items and therefore do not prove to be universally applicable in all forensic investigations.

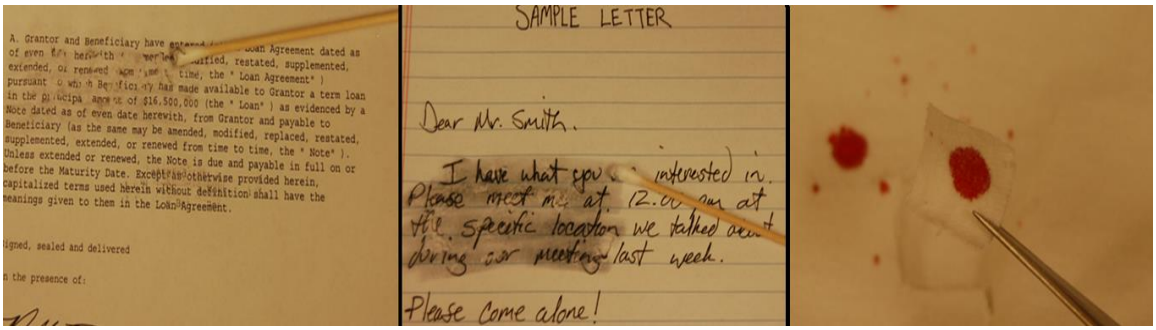

Figure 1: Example of a printed (a) and hand-written document (b) after the double swab collection method and a blood stained t-shirt (c) after a material cutting for DNA evidence.

Superior and more efficient non-destructive collection methods are needed to allow the forensic community to have a confident non-destructive approach to sampling. Forensic DNA analysts need the ability to detect and collect biological materials from an item without damaging the structural integrity of evidence items and/or interfering with any subsequent examinations. . Having the ability to non-destructively detect and collect biological samples would greatly benefit the forensic community by enabling other disciplines the opportunity to perform more

thorough forensic investigations of evidentiary items. The additional information gained from items processed in this manner could convict or exonerate individuals associated with questioned documents, entry point surfaces, various clothing items, and other touch type evidence materials.

4.1.2 Literature Citations and Review

Bode Technology proposed to perform thorough evaluations of several novel non-destructive DNA collection tools. The Electrostatic Detection Apparatus (ESDA®), alternative swab matrices, adhesive evidence lifters, and the Thermal Fingerprint Developer (TFD) were each evaluated as effective non-destructive evidence processing tools. Each of these innovative DNA collection methodologies have shown positive results in preliminary experiments and may be implemented for real-world forensic use.

ESDA

The ESDA is a well-known tool for forensic document examinations (Figure 2). The device applies an electrostatic charge over a thin polymer, similar to plastic wrap, which is securely held in place over an evidentiary document by gentle vacuum suction [26]. The polymer adheres to the form of the original document and highlights discrepancies in the document surface, such as writing impressions and latent fingerprints [7, 18, 20, 26]. These markings become visible to the naked eye when a charged toner is applied to the polymer sheet [26]. The polymer sheet can then be analyzed for handwriting styles, replica text, or fingerprint markings. Due to the close proximity of the polymer sheet to the original document during the ESDA process, electrostatic detection techniques may be used for collecting DNA evidence from documents which need to be preserved for further analysis.

Figure 2: Collection of indented writing in a sample document using an ESDA [7].

In a previous study, Bode Technology demonstrated the ability to transfer DNA from an original document to a polymer sheet using an ESDA. A letter, which had been handled by an individual of interest, was given to Bode by a government agency for analysis. The letter was sampled using non-destructive techniques; including dry swabbing of the document itself, Post-it® note adhesive collection, and wet/dry swabbing of the polymer sheet after electrostatic detection (Figure 3). The samples taken from the polymer sheet generated the highest quality DNA analysis results of all the techniques performed, and they produced three profiles matching to the reference profile provided.

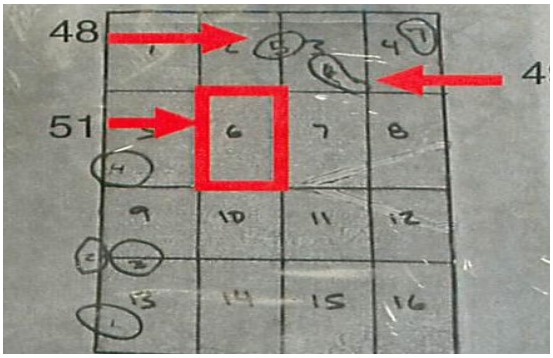

Figure 3: ESDA polymer sheet highlighting areas which produced favorable DNA results. Sampling locations were identified by toner marks showcasing touched areas.

Electrostatic detection was developed for the recovery of indented impressions created when writing occurs on a sheet of paper resting upon other pages. The study performed by Bode Technology highlights an innovative way to use the ESDA, which is already a common piece of equipment in forensic laboratories. The collection method is non-destructive and further testing was performed to determine if it could prove to be a valuable technique for acquiring DNA evidence from various substrates.

Alternative Swab Materials

Current methods of recovering DNA from forensic samples typically rely on wet swabbing techniques with a cotton swab (Figure 4). It is recognized by the forensic community that the double swab technique which, uses a dry swab preceded by a wet swab, is an effective collection method [19]. While proven efficient, the wet swab and the double swab techniques can be destructive to donor surfaces and therefore should not be used when the integrity of the substrate must be preserved. Dry cotton swabs can be employed in these situations but a decrease in overall profile quality and generation may be observed [19, 28]. It was proposed that various unconventional dry *alternative swab* matrices could be used to collect and release DNA more efficiently during the sampling and extraction process when compared to dry cotton swabs.

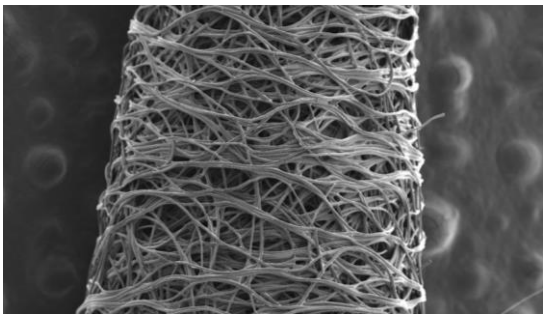

Figure 4: Electron Microscope photograph of traditional cotton fiber swab [9].

One alternative collection matrix proposed was the Nylon Flock Swab (Figure 5a). Nylon flocked swabs have demonstrated improved DNA collection due to an outwardly splayed fiber arrangement which may allow for a more efficient sample collection and subsequent sample release during elution [3, 12]. Traditional cotton fiber swabs release between eighteen to thirty percent of collected sample whereas nylon flocked swabs free cellular materials at eighty percent

efficiency [12]. This increased efficiency rate could potentially bring about a significant increase in the recovery of trace type biological deposits.

Foam swabs (Figure 5b), another proposed alternative swab type, have shown promising results in the recovery of low copy number DNA collection. In a published study investigating the swabbing of trace DNA evidence with multiple collection matrices, foam popules proved to be superior in generating complete profiles [15]. These encouraging results, in addition to an in-house evaluation of foam swabs, provide supplementary evidence supporting the potential benefits and the need for further testing of foam type matrices.

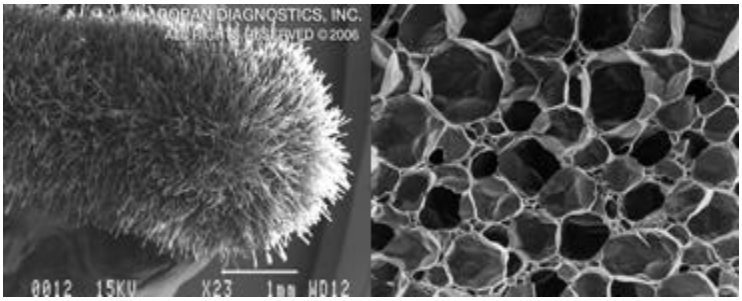

Figure 5: Electron microscope photograph of a nylon flocked swab (a) and foam swab material (b) [9].

Additional alternative swab matrices to be evaluated were microfiber and polyester swabs. Microfiber swabs are commonly used for delicate electronics cleaning and possess physical properties that potentially can be an effective non-destructive DNA collection matrix. Microfiber fabric (Figure 6) is a manufactured polymer comprised of star shaped polyamide and polyester fiber strands with a density of less than one denier (<1g/9,000m) [17]. The characteristic star-shaped fibers which comprise microfiber fabrics possess great potential for wide use in the forensic industry as a collection material. These polymers have superior absorbing qualities and are able to attract oils, water, and particles with higher efficiency than cotton fibers because polyester is lyophilic (affinity to oils) and polyamide is hydrophilic (affinity to water) [6] (Figure 6). Oils and particles can be easily released using a light detergent solution, which is standard for most DNA extraction methods used in the forensic field.

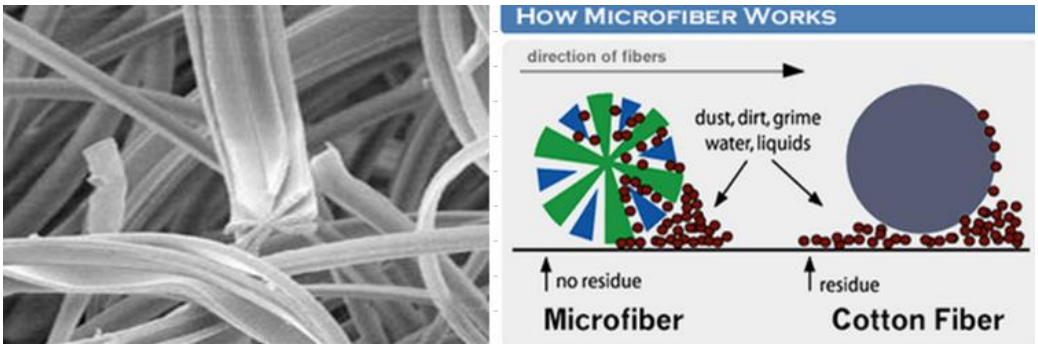

Figure 6: Electron microscope photograph of microfiber (a) and an illustration comparing the collecting efficiency of microfiber and cotton strands (b).

Bode Technology had previously performed preliminary experiments with several alternative swab collection matrices and several distinct microfibers for the purposes of evaluating non-destructive evidence collection. These non-traditional swab materials and microfibers were tested as a dry method on a glass substrate to provide possible non-destructive alternatives to cotton swabs. It was concluded from these preliminary examination that alternative swab materials and microfibers provided the encouraging results, great potential for widespread use in the forensic community, and need to be further evaluated to establish benefits and limitations.

Adhesive Evidence Lifters

Adhesive evidence lifters present a novel non-destructive collection alternative that has efficiently collected evidence samples while leaving a substrate surface relatively unmarked [13, 14]. The first proposed adhesive to be evaluated was water-soluble tape. Water-soluble tape is made of a poly-vinyl alcohol backing with a synthetic adhesive which leaves no residue after removal [1]. The tape completely dissolves during the extraction process and therefore provides an efficient collection method that allows for 100% of the obtained sample to be analyzed without loss attributable to collector retention [16]. This tape presents a novel non-destructive collection alternative that can be used on multiple substrates. Due to the portable, non-destructive nature of water-soluble tape, a forensic sample can be collected on site or in the laboratory without damage to the donor material.

In a study performed at the Institute of Forensic Medicine at the University of Oslo, Norway, soluble tape strongly indicated superior trace DNA collection from fabric substrates. Results continually showed higher DNA quantifications and stronger profiles for water-soluble tape when compared to the results generated with traditional swabs [14]. In another published study, water-soluble tape also demonstrated successful results when used to collect minimally invasive control samples from contact with various skin regions of the human body [16]. It was proposed that this tape be further tested on more common forensic-type samples.

The second adhesive to be proposed for this study was gelatin lifters. Gelatin lifters are made of a non-destructive, low-adhesive gelatin material which is capable of collecting forensic samples without disturbing the donor surface [5]. The collection method has historically provided crime scene investigators with an invaluable tool for lifting latent fingerprints and shoe prints. According to BVDA, the manufacturer of Gellifters®, gelatin lifters not only pick up shoe-marks and fingerprints but are also proficient at lifting blood, micro-trace material, and chemical residues from a wide variety of porous and non-porous materials (Figure 7). Additional studies provide supporting evidence that gelatin lifters are successful at lifting trace residues which can be further analyzed by chemical analysis [15, 23]. The capability of lifting residual evidentiary traces provides an innovative non-destructive technique for collecting biological evidence which can be further used for DNA analysis. With BVDA Gellifters already widely in use, the forensic community could greatly benefit from novel research examining these lifters as an effective collector of biological evidence.

Figure 7: Collection of a latent shoeprint using BVDA gelatin lifters.

Additional adhesive lifters to be evaluated were Mikrosil™ and Scenesafe™ FAST™ tape. Mikrosil is a casting material that has been formulated to show tremendous detail when used for casting evidentiary items such as tool marks, cartridge casings, and fingerprints [27]. Scenesafe FAST tape is an adhesive lifter that was designed by the manufacturer to maximize evidentiary integrity and for practical use out in the field at crime scenes that can be also processed for DNA analysis [25].

TFD

The TFD (Foster Freeman Ltd., UK) is a fingerprint detection device that is easily deployed in the field (Figure 8). The device passes a document of interest through a heating element that raises the temperature of the document, causing a chemical reaction between the latent fingerprint and the surface of the paper. This produces a fluorescent by-product that is visible under intense visible light such as Crime-light Blue [10]. Bode proposed to utilize the TFD's chemical free detection of fingerprints in conjunction with a non-destructive direct swabbing of the developed fingerprint to non-destructively collect DNA from paper documents.

Figure 8: Thermal Fingerprint Device

4.1.3 Statement of Hypothesis or Rationale for Research

It was the goal of this research to improve the methods of DNA sample detection and collection from various evidence types without causing integral surface damage. Current standard operational procedures typically involve processing evidence items with chemical sprays, wet/dry cotton tip swabs, and/or material cuttings. While these traditional techniques have been effective liberators of biological sample deposits, they do typically leave the evidence items in an altered and/or damaged state. The preliminary research presented above suggested that there may be multiple ways to effectively detect and collect biological materials from an item while causing minimal substrate damage. Bode Technology proposed to perform thorough evaluations of the ESDA, adhesive evidence lifters, alternative swab matrices, and TFD as effective non-destructive evidence processing tools. Each of the techniques was tested on a multitude of forensically relevant samples. The research proposed will provide novel tools for forensic scientists processing sensitive items and will allow forensic biologists earlier access to trace amounts of evidence that may have otherwise been lost during prior processing.

4.2 Methods

Bode proposed to provide the forensic field with the tools to non-destructively process evidence materials containing biological samples by meeting the following four objectives:

1. Evaluate the ability of the ESDA to effectively detect and collect various biological samples off of a wide range of substrates during determined time points in a non-destructive manner.
2. Evaluate the ability of adhesive lifters to effectively collect various biological samples off of a wide range of substrates during determined time points in a non-destructive manner.
3. Evaluate the ability of alternative swabs to effectively collect various biological samples off of a wide range of substrates during determined time points in a non-destructive manner.
4. Evaluate the ability of the TFD to effectively collect various biological samples off of a wide range of substrates in a non-destructive manner.

It is the goal of these objectives to improve the methods for non-destructive evidence detection and collection in the forensic laboratory.

General Methodology

For the tasks described below, all samples were processed using the Qiagen EZ1® DNA Investigator kit in conjunction with the EZ1 Advanced Instrument, Quantifiler® Duo DNA Quantification Kit, and AmpFℓSTR® Identifiler® Plus PCR Amplification Kit. A target template DNA concentration of 1 ng/µl of DNA was added to the amplification reaction (28 cycles, 12.5µl volume reaction) and if necessary, samples were concentrated with Vivacon 500-30K columns. Samples were run on the 3130xL Genetic Analyzer with standard injection parameters of 3kV for 10 seconds, (injection parameters ranged from 22 to 44kV/s depending on the instrument utilized per internal validation standards). Results were analyzed with an analytical threshold of 50 RFU using ABI GeneMapper® v3.2.1 software. Appropriate substrate controls,

extraction positives, reagent blanks, positive controls, and negative controls were processed for each task.

SAS JMP® statistical software and JMP Design of Experiments (DOE) software were used to create a randomized design sample setup for all tasks except for Phase IV and any additional testing performed under other phases. DOE allows for experimental setup and trend analysis of a response (percent profile recovered) based on the effects of multiple factors (substrate, biological fluids, collection method, etc.).

a) Phase I - Evaluate the ability of the **Mylar-ESDA** method to effectively detect and collect various biological samples from three substrates during determined time points in a non-destructive manner.
    A. *Task 1: Substrate Testing*. Buccal swabs were collected from three donors. Cells were eluted, purified, and re-suspended in 1X PBS. Cell equivalents of 0.5 ng, 1.0 ng, and 2.0 ng of DNA were spotted in 25 µl volumes onto glass, paper, and cotton substrates. A total of 24 buccal cell samples were dried overnight at room temperature (RT). Samples were collected using the ESDA-Lite® by placing them on a sheet of paper on the metal plate. The vacuum was started, and the Mylar film was carefully pulled over the samples. The corona wand was turned on and waved horizontally and vertically over the plate at a height of about three to five cm to initiate an electrostatic charge. A blue light on the ESDA indicated charging of the Mylar by the corona wand. Once the light began to flash, the corona wand was set aside as the electrostatic image formed. Once charged, the light turned off and the Mylar film was cut and developed by pouring cascade developer over the imaging film. The Mylar was then fixed with a transparent fixing film for easy removal of samples. The vacuum was turned off and the samples were removed. The bottom side of the Mylar was then wet/dry swabbed with a cotton swab and processed for DNA.
    B. *Task 2: Biological Sample Testing*. Fingerprints from three donors were deposited and blood, semen, and saliva from three donors were spotted in 5 µl, 25 µl, and 50 µl volumes onto glass, paper, and cotton substrates. A total of 48 samples were dried overnight at RT. Samples were collected using the ESDA-Lite in the same manner as described in Task 1.
    C. *Task 3: Aged Sample Testing*. Fingerprints from three donors were deposited and blood, semen, and saliva from three donors were spotted in 5 µl, 25 µl, and 50 µl volumes onto glass, paper, and cotton substrates. A total of 96 samples were stored at RT. Forty-eight samples were collected at a one month time point and 48 samples were collected at a six month time point using the ESDA-Lite in the same manner as described in Task 1.
    D. *Additional Testing.* Fingerprints from three donors were deposited on copy paper, resume paper (stronger bond rating and higher quality than copy paper), magazine, newspaper, currency, and cotton paper substrates. A total of 75 samples were processed using the ESDA-Lite in the same manner as described in Task 1. To compare this non-destructive collection technique with a destructive collection technique, one sample was collected from each paper substrate by directly cutting the area where the fingerprint was applied.

A larger study was performed to further investigate the use of the ESDA in collecting fingerprints on various paper substrates. Fingerprints from three donors were deposited on copy paper, resume paper, magazine, newspaper, currency, and cotton paper substrates. A total of 162 samples were dried overnight and collected in triplicate utilizing three techniques. Fingerprints were collected using the ESDA-Lite in the same manner as described in Task 1, but swabbed with the best performing swab (nylon flocked) from Phase III instead of the cotton swab. Fingerprints were also collected from the paper substrate with nylon flocked swabs via direct dry swabbing of the area where the fingerprint was applied as a direct comparison of another non-destructive collection technique. To compare these non-destructive collection techniques with a destructive collection technique, paper substrates were cut in the area where the fingerprint was applied except for currency where a wet/dry technique with nylon flocked swabs was utilized in lieu of cutting.

b) Phase II - Evaluate the ability of **adhesive lifters** to effectively collect various biological samples off three substrates during determined time points in a non-destructive manner.
   A. *Task 1: Substrate Testing*. Buccal swabs were collected from three donors. Cells were eluted, washed, and re-suspended in 1X PBS. Cells equivalent to 0.5 ng, 1.0 ng, and 2.0 ng of DNA were spotted in 25 µl volumes onto glass, paper, and cotton substrates. A total of 60 buccal cell samples were dried overnight at RT. Samples were collected with five different adhesive lifters:
      1. Scenesafe FAST tape (Scenesafe, UK)
      2. 2"x2" BVDA Instant Lifters (Evident® Crime Scene Products, USA)
      3. Mikrosil Silicone Casting Material (Evident Crime Scene Products, USA)
      4. 3M Water Soluble Wave Solder Tape (HMC Electronics, USA)
      5. BVDA Transparent Gellifters (Forensic Source, USA)
   B. *Task 2: Biological Sample Testing*. Fingerprints from three donors were deposited and blood, semen, and saliva from three donors were spotted in 5 µl, 25 µl, and 50 µl volumes onto glass, paper, and cotton substrates. A total of 204 samples were dried overnight at RT. Samples were collected with the five adhesive lifters described in Phase II Task 1.
   C. *Task 3: Aged Sample Testing*. Fingerprints from three donors were deposited and blood, semen, and saliva from three donors were spotted in 5 µl, 25 µl, and 50 µl volumes onto glass, paper, and cotton substrates. A total of 408 samples were stored at RT. Two hundred and four samples were collected at a one month time point and 204 samples were collected at a six month time point utilizing the five adhesive lifters described in Phase II Task 1.
   D. *Additional Testing*. One donor deposited fingerprints on glass, painted drywall, and cotton substrates. Blood and semen samples were diluted to a 0.04 ng/µl solution, and a total of 1 ng of each fluid was spotted onto glass, painted drywall, and cotton substrates. A total of 81 samples were dried overnight. Each fluid on each substrate was collected in triplicate for each type of lifter. Samples were collected with the three best performing lifters determined from the previous tasks: Scenesafe FAST tape, BVDA Instant Lifters, and BVDA Gellifters.

c) Phase III - Evaluate the ability of **alternative swab matrices** to effectively collect various biological samples off three substrates during determined time points in a non-destructive manner.

A. *Task 1: Substrate Testing*. Buccal swabs were collected from three donors. Cells were eluted, purified, and re-suspended in 1X PBS. Cells equivalent to 0.5ng, 1ng, and 2ng were spotted in 25 µl volumes onto glass, paper, and cotton substrates. A total of 60 buccal cell samples were dried overnight at RT. Samples were collected with five different swabs:
    1. Foamtec MiraSWAB® Microfiber Swab
    2. VWR® Foam Swab
    3. Puritan® Hydraflock® Flocked Swab
    4. Texwipe® Knitted Polyester Swab
    5. Puritan Cotton Swab (for comparison)

B. *Task 2: Biological Sample Testing.* Fingerprints from three donors were deposited and blood, semen, and saliva from three donors were spotted in 5 µl, 25 µl, and 50 µl volumes onto glass, paper, and cotton substrates. A total of 120 samples were dried overnight at RT. Samples were collected with the five swabs described in Phase III Task 1.

C. *Task 3: Aged Sample Testing*. Fingerprints from three donors were deposited and blood, semen, and saliva from three donors were spotted in 5 µl, 25 µl, and 50 µl volumes onto glass, paper, and cotton substrates. A total of 240 samples were stored at RT. One hundred and twenty samples were collected at a one month time point and 120 samples were collected at a six month time point utilizing the five swabs described in Phase III Task 1.

D. *Additional Testing.* One donor deposited latent fingerprints onto glass substrates. Blood, semen, and saliva samples were diluted to a 0.04 ng/µl solution, and a total of 1 ng of each fluid was spotted onto glass substrates. A total of 60 samples were dried overnight. Each fluid was collected in triplicate for each type of swab. Samples were collected with the swabs described in Phase III Task 1.

   Because all blood and semen samples gave full profiles, an additional set of samples further diluting these fluids was tested to determine the optimal concentration to spot for additional testing on various substrates. Blood was diluted to a 0.02 ng/µl solution and a total of 0.5 ng spotted, while semen was diluted to a 0.004 ng/µl solution, and a total of 0.1 ng spotted onto glass substrates. Samples were dried overnight and each fluid was collected in triplicate for each swab described in Phase III Task 1.

   From here, further substrate testing was performed. One donor deposited latent fingerprints on copy paper, painted drywall, and cotton substrates. Blood and semen samples were diluted to a 0.04 ng/µl solution, and a total of 1 ng of each fluid was spotted on copy paper, painted drywall, and cotton substrates. A total of 81 samples were dried overnight. Each fluid on each substrate was collected in triplicate for each type of swab. Samples were collected with the three best performing swabs determined from the previous tasks: Microfiber, VWR Foam, and Hydraflock swabs. Puritan cotton swabs

were also tested for comparison purposes, as this is the most widely used swab for collection.

d) Phase IV – Evaluate the ability to analyze DNA collected from fingerprints placed on paper using the **Thermal Fingerprint Developer (TFD)** visualized with different power settings and collected in a non-destructive manner.
   A. Buccal swabs were collected from one donor. Cells were eluted, purified, and re-suspended in 1X PBS. A cellular equivalent of 5 ng of DNA was spotted onto copy paper, resume paper, and magazine paper substrates. A total of 27 buccal cell samples were dried overnight at RT. Samples were collected in triplicate for each substrate for each TFD processing technique. Nine control samples were collected using cotton swabs via direct dry swabbing of the substrate area where the cells were deposited, without TFD visualization of the samples. Eighteen samples were processed using TFD detection with two different intensities of heat and time: 70% power at 750 mm/min and 90% power at 1000 mm/min. The paper substrate on which the samples were spotted was placed on the motor driven conveyor and passed through the TFD-2 optimized heating element. Briefly raising the temperature of the paper substrate causes a chemical reaction between the sample and the paper surface and allows the sample to be fluorescently visualized under intense visible light with appropriate filters. After detection, samples were collected via dry swabbing. 85% power at 1000 mm/min was used on select magazine paper substrates to see if it would improve collection; however, no difference in performance was shown, so 85% power data was combined with 90% power data.
   B. Fingerprints from four donors were deposited onto copy paper, resume paper, and magazine paper substrates. A total of 72 samples were collected in triplicate for each substrate for each TFD processing technique. Nine control samples were collected via direct dry swabbing of the substrate area where the fingerprints were deposited, without TFD visualization of the fingerprints. In addition, nine fingerprints on paper substrates were collected via cutting the area where the fingerprint was applied, without TFD visualization of the fingerprints. In total, 54 samples were processed using TFD detection with two different intensities of heat and time: 70% power at 750 mm/min and 90% power at 1000 mm/min in the same manner described above. After detection, samples were collected via dry swabbing. 85% power at 1000 mm/min was used on select magazine paper substrates to see if it would improve collection; however, no difference in performance was shown, so 85% power data was combined with 90% power data.

4.3 Results

4.3.1 Statement of Results

Phase I- ESDA

Task 1: Substrate Testing. No profiles were obtained for 29 out of the 33 buccal cell samples tested, and low partial profiles were obtained for the other four samples (Figure 9). These low partial profiles were obtained from cellular equivalents of 0.5 ng (approximately 75 cells) spotted on glass and cotton samples and two samples of 1 ng (approximately 150 cells) spotted on cotton. Based on these results, it can be inferred that the buccal cells did not transfer to the Mylar

film because the cells were likely bonded too tightly to the matrix when dried on the substrate. Due to the low recovery rate of DNA profiles from the samples tested above, an additional evaluation of three buccal cell samples was performed using 25 µl of a cellular equivalent of 50 ng purified DNA solution on glass, cotton, and paper substrates. A full profile (32 alleles) was obtained for 50 ng of cells on glass. No profiles were obtained from the 50 ng spotted on paper and cotton.

Task 2: Biological Sample Testing. The capability of the ESDA-Mylar film technique to produce STR profiles is demonstrated in Figure 10. This technique was able to produce useful profiles (full or high partial profiles) in 21 out of the total 48 samples tested; however, the remaining 27 samples resulted in low or no profiles. The ESDA-Mylar film technique worked markedly better on glass (65% of samples produced full or high partial profiles) than on paper (36% of samples produced full or high partial profiles) or cotton substrates (18% of samples produced full or high partial profiles). Full profiles were obtained from 23% of total samples, the majority of which were blood and semen spotted onto glass slides. Although the results from fingerprints on the three substrates appeared more varied than the other fluids, this was attributed to the use of three different donors and the nature of fingerprint deposition.

Task 3: Aged Sample Testing. Trends observed for the aged sample testing reflected those of the biological sample testing with DNA profiles obtained from glass for blood, semen, and saliva being typically higher than those profiles obtained from cotton or paper substrates (Figures 11-13). No trends were observed that indicated the ability to recover DNA profiles from biological fluids decreased over time. The aged fingerprint study indicated a decrease in percent profile recovery in the six month time samples, but the results were inconclusive due to the number of replicates tested (Figure 14).

Additional Testing. Figure 15 demonstrates the results from all three donors when comparing the average percent profiles obtained from fingerprints collected from various paper substrates. From the 75 total samples, 30 generated high partial profiles (40%) and 11 provided full profiles (15%). Donor three exhibited higher average percent profiles than the other two donors; however, amongst all three donors, a higher percent average profile was consistently associated with cotton paper. The results from the direct substrate cuttings indicated that the average percent profile collected from the cut fingerprints were similar to the ESDA-Mylar film technique. Analysis of variance (ANOVA) testing was performed to see if results from all donors could be combined. With an estimated F statistic of 9.6872 and a significance level of 95% (i.e. p=0.05), the hypothesis that the results from each of the donors were not significantly different was rejected, and therefore the results could not be combined.

Figures 16-18 demonstrate the results of the additional ESDA testing that compared the three collection techniques used to collect fingerprints from various paper substrates. Full to high partial profiles were obtained for 52 out of 54 samples (96%) utilizing the non-destructive dry swabbing technique, 40 out of 54 (74%) samples utilizing the non-destructive Mylar-ESDA technique, and 34 out of 54 (62%) using the destructive direct cutting technique. Overall, non-destructive dry swabbing outperformed the other two collection techniques for all donors on all types of paper substrates, except newspaper, with destructive direct substrate cutting working

best on newspaper. Though results of collection from currency differ slightly depending on donor, the non-destructive Mylar-ESDA technique outperformed destructive direct substrate cutting for all other types of paper. ANOVA testing was performed to see if results from all donors could be combined. With an estimated F statistic of 3.4827 and a significance level of 95% (i.e. p=0.05), the hypothesis that the results from each of the donors were not significantly different was rejected, and therefore the results could not be combined.

Phase II- Adhesive Lifters

Task 1: Substrate Testing. Full profiles were obtained for 11 out of 60 buccal cell samples with the majority recovered using Scenesafe FAST tape and BVDA Instant Lifter on both porous and non-porous substrates (Figure 19). High partial profiles (16-31 alleles) were obtained for 16 out of the 60 samples. The majority of lifters associated with high partial profiles are Scenesafe FAST tape on glass and cotton, BVDA Instant Lifter on glass and cotton, and Mikrosil on cotton. Low partial to no profiles (0-15 alleles) were obtained for the remaining 33 samples. Scenesafe FAST tape and BVDA Instant Lifter performed equally well, showing the most promising results. Gellifters performed well on glass, a non-porous substrate, but no profiles were obtained from porous-substrates. Eleven out of 12 samples lifted with water-soluble tape resulted in no profile. All tape lifters were destructive on paper; therefore, painted drywall was added as an additional porous substrate for future adhesive lifter testing.

Task 2: Biological Sample Testing. Figures 20-23 demonstrate the results obtained from blood, semen, saliva, and fingerprints collected using five different adhesive lifting techniques. Full profiles were obtained for 141 out of 204 samples tested; BVDA Instant lifters and Gellifters each obtained 35 full profiles, Mikrosil and Scenesafe tape each obtained 32 full profiles, and water-soluble tape obtained 7 full profiles. With the exception of water-soluble tape, all lifters performed equally well on cotton for all biological fluids. Scenesafe tape, BVDA Instant Lifters, and Gellifters also performed equally well on glass for all biological fluids. These three lifters obtained high partial profiles for a total of 26 samples. Water-soluble tape was eliminated from further testing due to its poor performance and handling difficulties. While Mikrosil generally performed well, the Mikrosil itself was difficult to work with in a timely manner, with the pastes often hardening before effectively being applied to the sample area. While the results seemed varied for blood, semen, and saliva on the four substrates, the lifters appeared to have worked well in lifting fingerprints on all substrates.

Task 3: Aged Sample Testing. No trends were observed that indicated the ability to recover DNA profiles from biological fluids decreased over time with the exception of saliva on painted drywall collected with the BVDA Instant Lifters (Figures 24-26). The Mikrosil lifter consistently underperformed as compared to the other adhesive lifters for saliva and semen on cotton, painted drywall, and glass substrates (Figure 25 and 26). While no trends were observed indicating that the ability to recover DNA profiles from the aged fingerprint samples decreased over time, the Mikrosil collector underperformed as compared to the other adhesive lifters (Figure 27).

Additional Testing. Figures 28-30 demonstrate the results obtained from blood, semen, and fingerprints collected using the three top performing adhesive lifters from Tasks 1 and 2: Scenesafe FAST tape, BVDA Instant Lifters, and BVDA Gellifters. Each lifter was subjected to

27 individual trials (three substrates by three fluids by three replicates). Full to high partial profiles were obtained for 19 out of 27 samples collected with the Gellifters (70%), 11 out of 27 samples collected with the BVDA Instant lifters (40%), and 13 out of 27 samples collected with the Scenesafe lifters (48%). Scenesafe and Gellifters performed equally well on glass for all biological fluids, while BVDA Instant lifters obtained useful profiles only from fingerprints on glass. Gellifters obtained high partial to full profiles from all fluids on painted drywall, while the other two lifters obtained useful profiles only from fingerprints on painted drywall. Very few samples on cotton produced useful profiles, although the Gel and Scenesafe lifters produced promising results when lifting fingerprints only. The use of BVDA Instant lifters on cotton produced very few useful profiles for any biological fluid.

Phase III- Alternative Swabs

Task 1: Substrate Testing. Figure 31 demonstrates the results obtained from buccal cells collected from various substrates using four different alternative swabs and the most widely used cotton swab. A total of 60 samples were collected and full profiles were obtained for 8 buccal cell samples with the majority swabbed with Hydraflock and Microfiber swabs. High partial profiles were obtained for 13 out of the 60 samples with the majority swabbed with the knitted polyester and Microfiber swabs. Low to no profiles were obtained for 39 samples, 16 of which were samples collected from paper substrates. None of the swabs were destructive to any of the substrates; however, foam swabs themselves were easily damaged on glass. It also appeared that paper was not an optimal substrate for dry swab collection of buccal cell samples.

Task 2: Biological Sample Testing. Figures 32-35 demonstrate the results obtained from blood, semen, saliva, and fingerprints collected using five different swabbing techniques. Full profiles were obtained for 83 out of 120 samples. Out of the 83 full profiles, Hydraflock and Microfiber swabs each obtained 18 full profiles, cotton and foam swabs each obtained 16 full profiles, and knitted polyester swabs obtained 15 full profiles. All samples swabbed with the cotton and Microfiber swabs resulted in high partial to full profiles regardless of biological fluid, volume spotted, or substrate. All five swabs performed equally well on the cotton substrate regardless of biological fluid spotted. Knitted polyester did not perform as well as the other swabs on glass, and foam swabs did not perform as well as the others on paper.

Task 3: Aged Sample Testing. No trends were observed that indicated the ability to recover DNA profiles from biological fluids decreased over time (Figures 36-39). Additionally, while lower percent DNA recovery was observed for saliva as compared to the other biological fluids, no differentiation in performance was observed for the five alternative swabs across the three biological fluids or fingerprints over time.

Additional Testing. Figure 40 demonstrates the results obtained from blood, semen, saliva, and fingerprints collected using five different swabbing techniques from glass. Full profiles were obtained from all fingerprint samples, regardless of the swab used. Full profiles were obtained for all semen samples regardless of the swab used when 1 ng was spotted. When 0.1 ng semen was spotted, full profiles were obtained from all samples swabbed with cotton, Hydraflock, Foamtec Microfiber, and VWR Foam; full to high partial profiles were obtained when swabbed with knitted polyester. Full profiles were obtained for all blood samples regardless of the swab

used when 1 ng was spotted. When 0.5 ng blood was spotted, an average percent profile of 59% was obtained swabbing with Hydraflock, 58% with cotton, 54% with Foamtec Microfiber, 55% with VWR Foam, and 41% with knitted polyester.

Figures 41-43 demonstrate the results obtained from blood, semen, and fingerprints spotted onto various substrates and swabbed with the three best performing swabs; Hydraflock, Foamtec Microfiber, and VWR Foam. Each swab was subjected to 27 individual trials (three substrates by three fluids by three replicates). Standard cotton swabs were utilized as well as a comparison of standard methodology. Full to high partial profiles were obtained for 11 out of the 27 samples swabbed using Hydraflock swabs (41%), 8 out of the 27 samples swabbed using Foamtec Microfiber (30%), 11 out of the 27 samples swabbed using VWR Foam (41%), and 9 out of the 27 samples swabbed using cotton swabs (33%). Both Hydraflock and VWR Foam swabs performed equally well across all substrates when collecting fingerprints. Hydraflock outperformed all other swabs when swabbing blood from painted drywall and cotton substrates, and no useful profiles were obtained with any swabs from blood on copy paper. Cotton and VWR Foam swabs produced the best results when swabbing semen on all substrates. However, it is important to note that there appeared to be issues with the semen samples spotted for this exercise, with the results including positive controls displaying much lower values than anticipated. The semen testing was repeated with the same low level results obtained, thus the results from the semen portion of this study cannot be reliably analyzed.

Phase IV- TFD

There was no difference in results between the two intensities at which the TFD was used to detect 5 ng (approximately 750 cells) of buccal cells on a variety of paper substrates (Figure 44); however, results differed based on paper type. Full profiles were obtained from cells detected on resume paper with the TFD, as opposed to low partial profiles obtained from undetected cells on resume paper collected with cotton swabbing. Buccal cells on magazine paper gave full profiles when TFD was not performed, as opposed to low partial profiles when samples were exposed to either TFD setting. Buccal cells on copy paper showed roughly no difference in results between both TFD settings and no TFD used, with high partial to full profiles obtained for each group.

Fingerprints were spotted onto various substrates by four different donors and visualized with one of two TFD intensity settings or collected without TFD. As depicted in Figure 45, percent profiles for donor three were consistently lower than for any other donor throughout the TFD study. This may explain why donor three does not necessarily follow the same trends observed in the other donors (Figure 46). Figure 46 shows that latent prints detected on magazine paper with the TFD regardless of intensity settings gave far lower percent profiles than prints collected when TFD was not performed. However, overall trends show that detection with the TFD improved STR profiles for both resume and copy paper.

4.3.2 Tables

Non-Destructive Processing Recommendations					
Biological Sample and Order of Recommendation		**Cotton**	**Drywall**	**Glass**	**Paper**
Blood	*Primary*	Alternative Swabs	Adhesive Lifters	Adhesive Lifters or Alternative Swabs	Alternative Swabs
	Secondary	Adhesive Lifters	Alternative Swabs	ESDA	ESDA
Fingerprints	*Primary*	Adhesive Lifters	Adhesive Lifters	Adhesive Lifters	Alternative Swabs
	Secondary	Alternative Swabs	Alternative Swabs	Alternative Swabs	ESDA or TFD
Semen	*Primary*	Adhesive Lifters or Alternative Swabs	Adhesive Lifters	Adhesive Lifters or Alternative Swabs	Alternative Swabs
	Secondary	ESDA	Alternative Swabs	ESDA	ESDA
Saliva	*Primary*	Adhesive Lifters	Adhesive Lifters	Adhesive Lifters	Alternative Swabs
	Secondary	Alternative Swabs	Alternative Swabs	Alternative Swabs	None

Table 1: Summary of the non-destructive processing technique recommendations for each substrate and biological sample type based on the complete results this study.

4.3.3 Figures

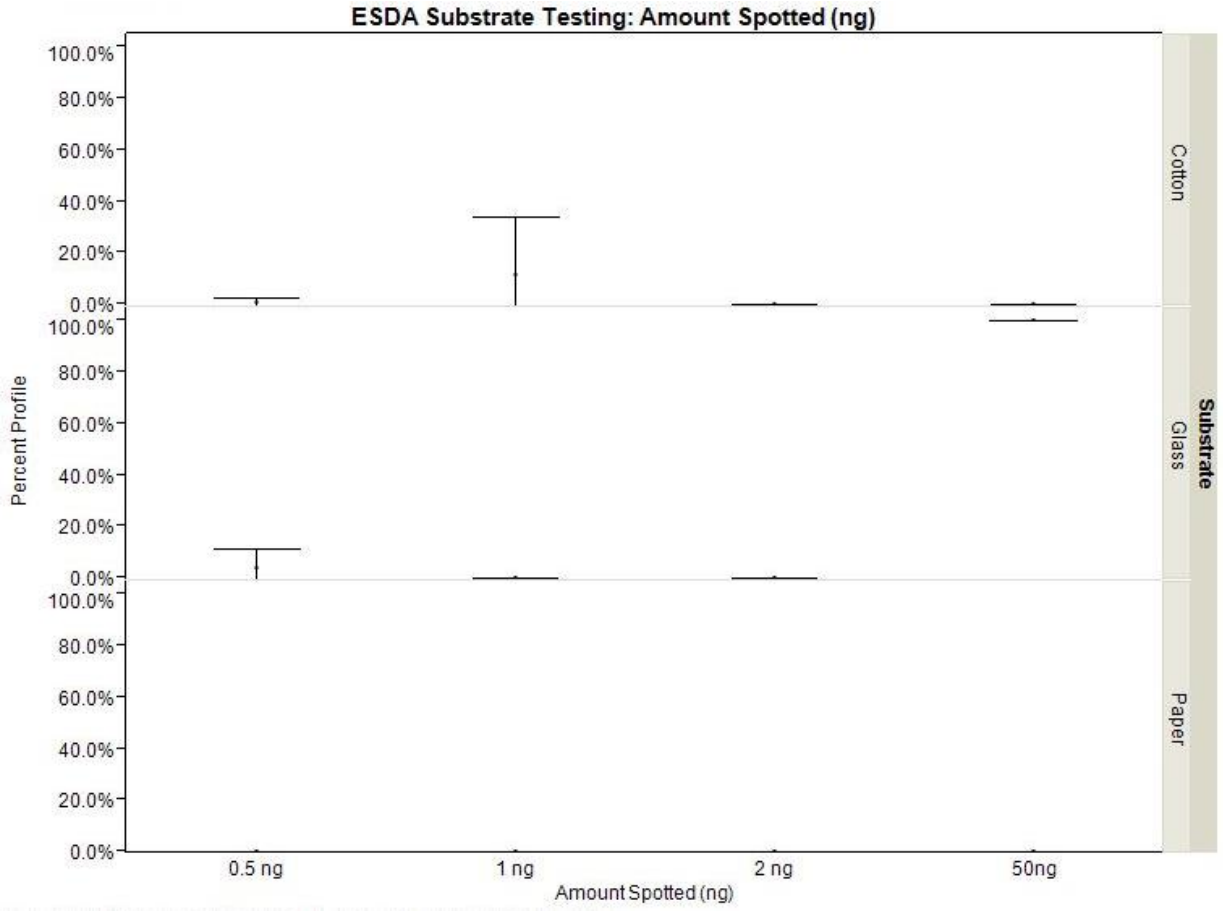

Figure 9: Summary of results obtained from 0.5 ng, 1.0 ng, 2.0 ng, and 50 ng buccal cells spotted on various substrates and collected using the ESDA-Mylar film technique.

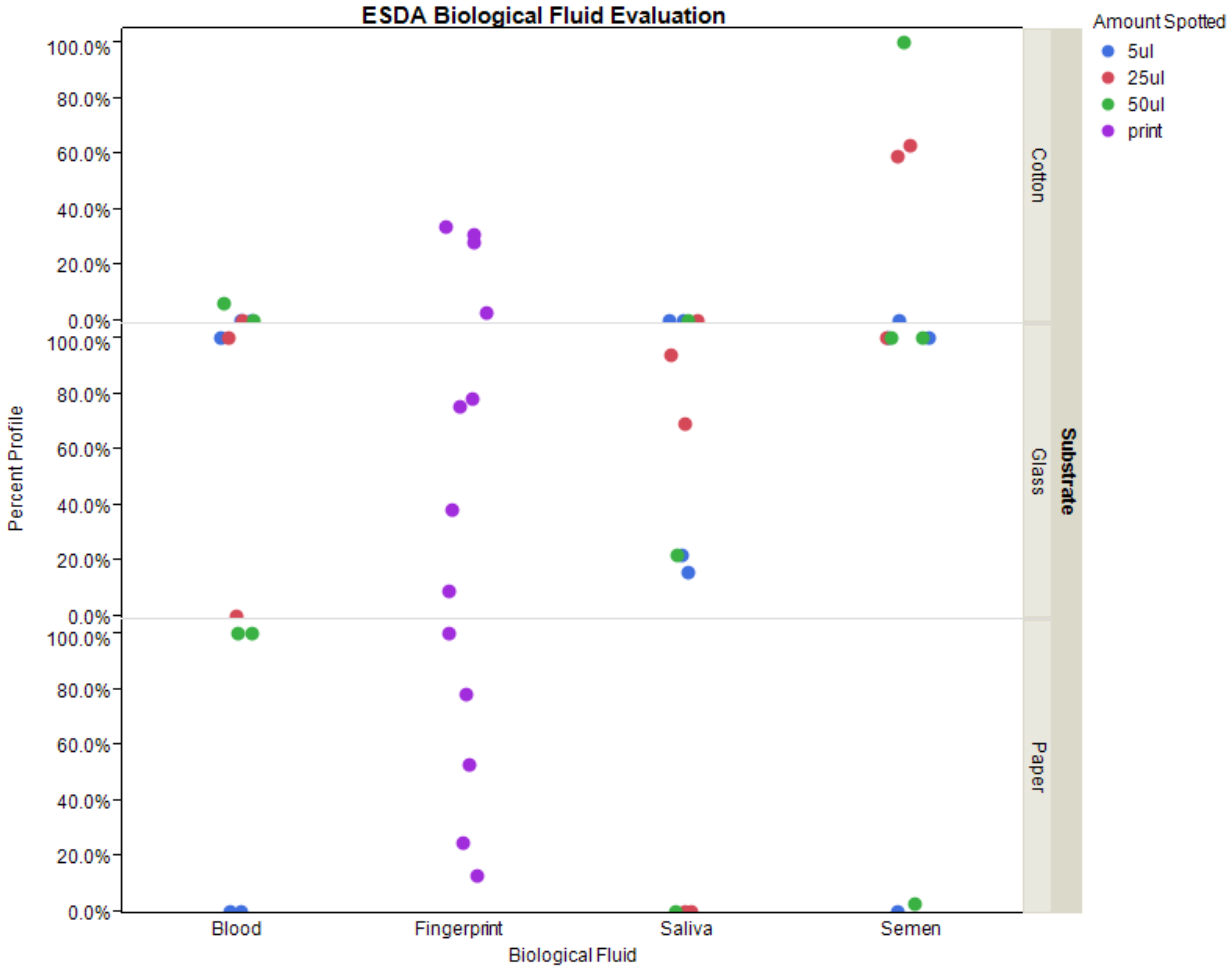

Figure 10: Summary of results obtained from blood, saliva, semen, and fingerprints spotted on various substrates and collected via the ESDA-Mylar film technique.

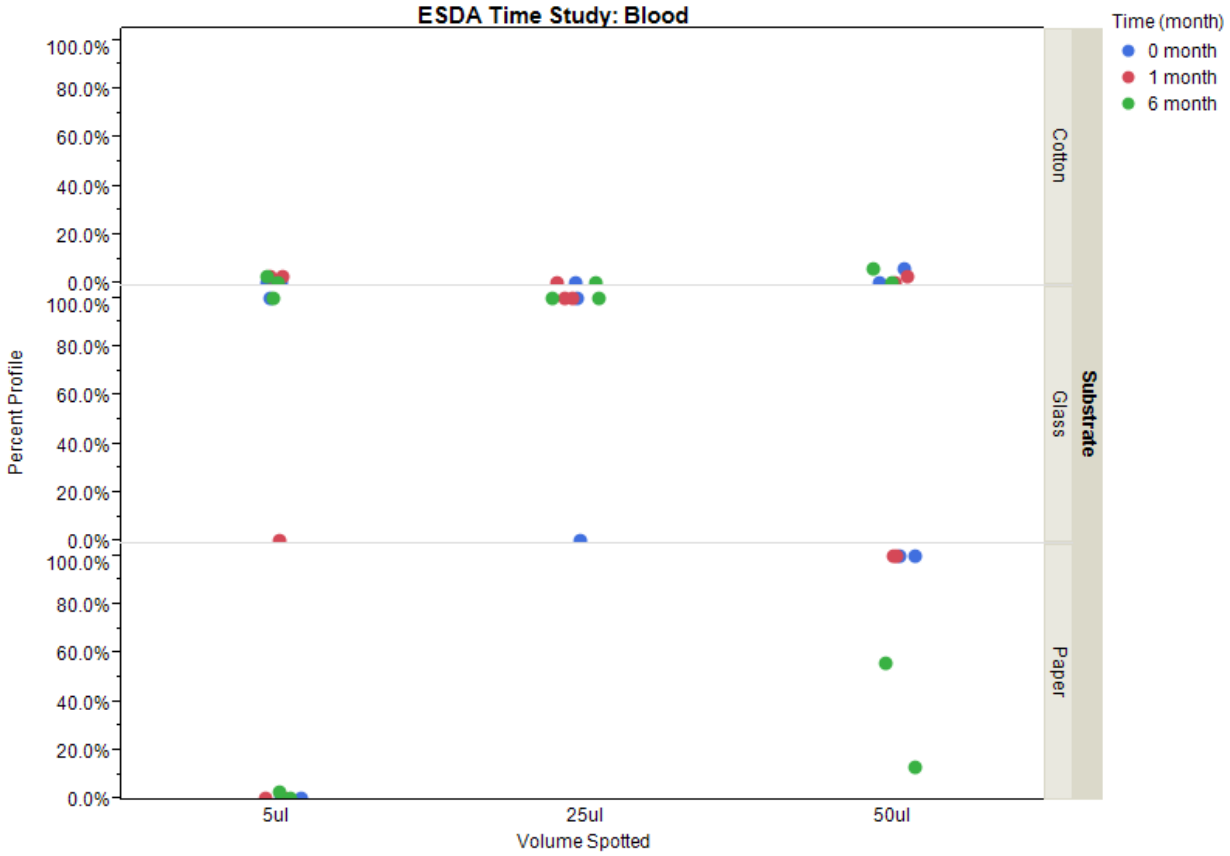

Figure 11: Summary of results obtained from blood spotted on various substrates and collected after 0, 1 month, and 6 month time points via the ESDA-Mylar film technique.

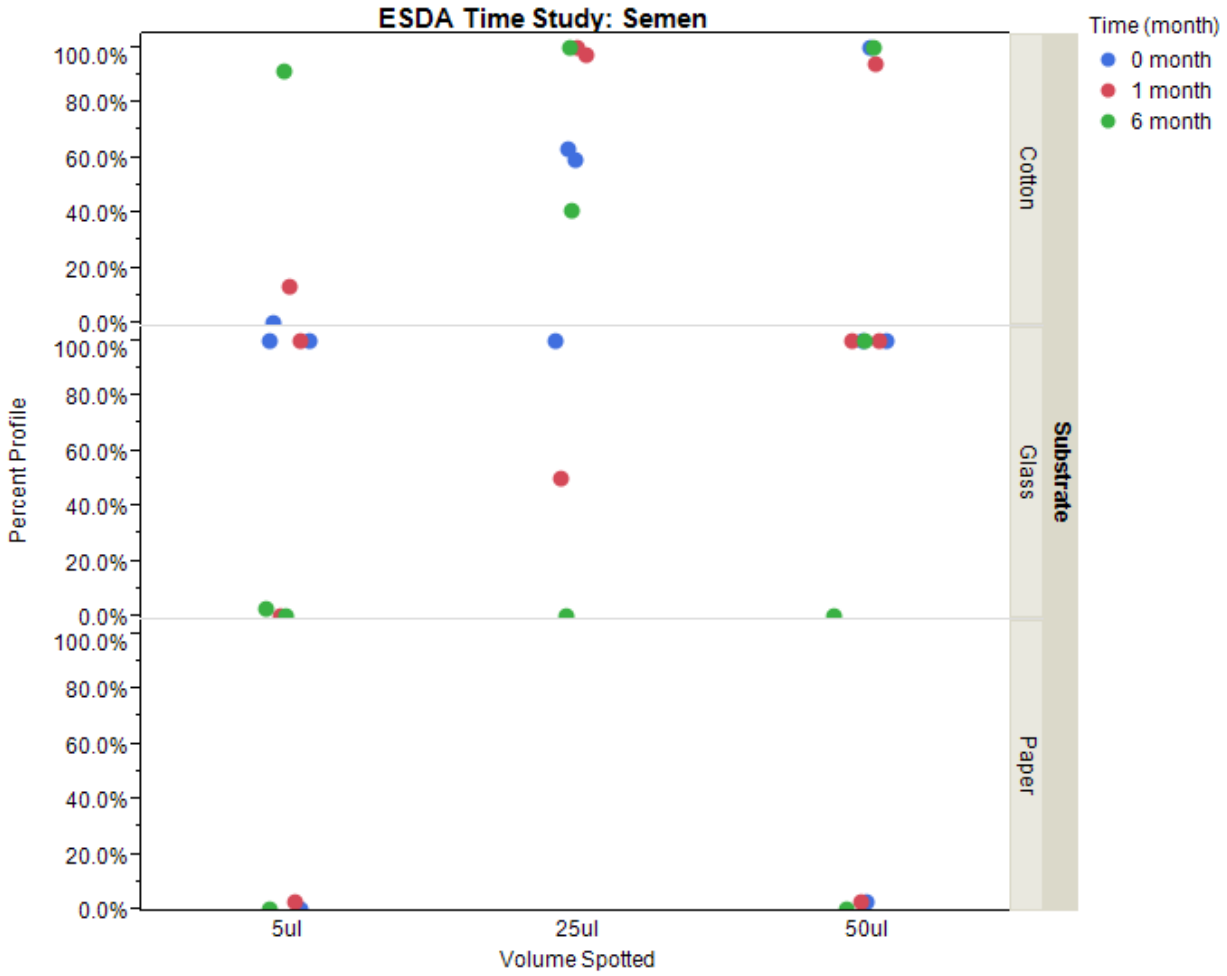

Figure 12: Summary of results obtained from semen spotted on various substrates and collected after 0, 1 month, and 6 month time points via the ESDA-Mylar film technique.

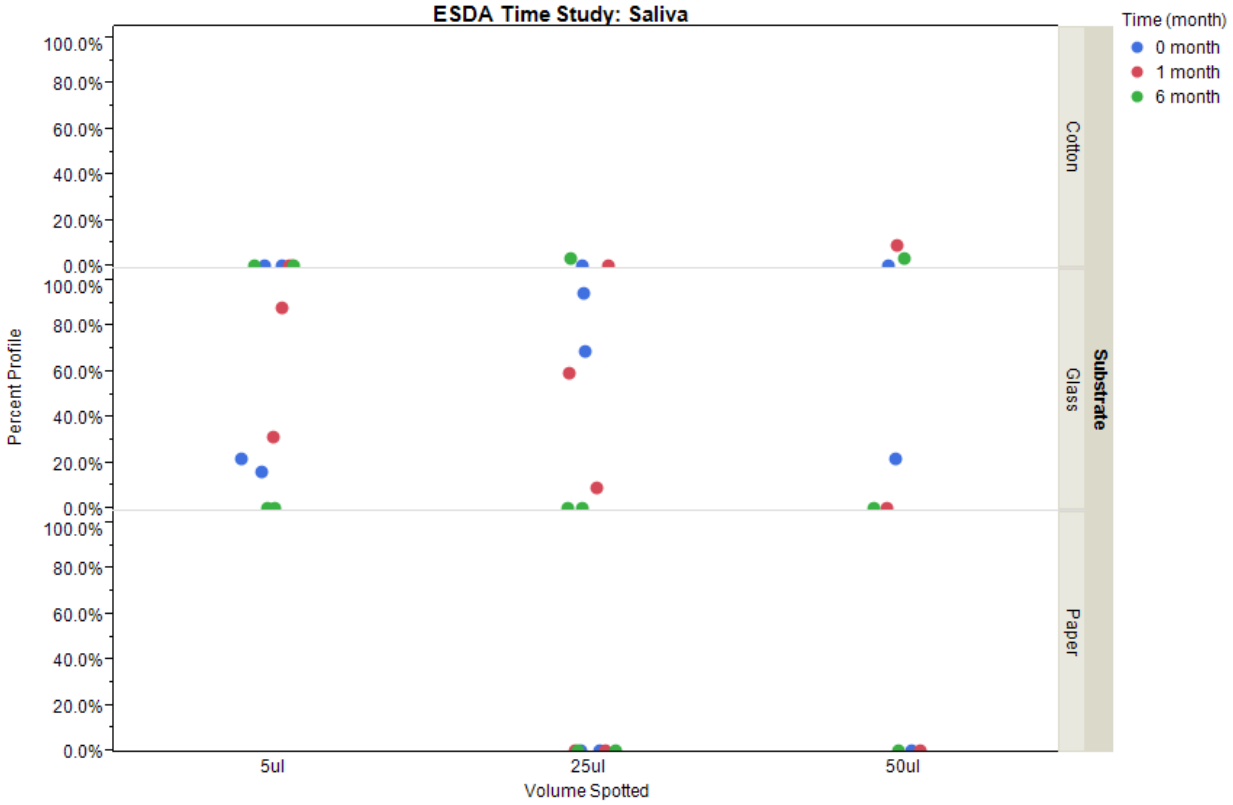

Figure 13: Summary of results obtained from saliva spotted on various substrates and collected after 0, 1 month, and 6 month time points via the ESDA-Mylar film technique.

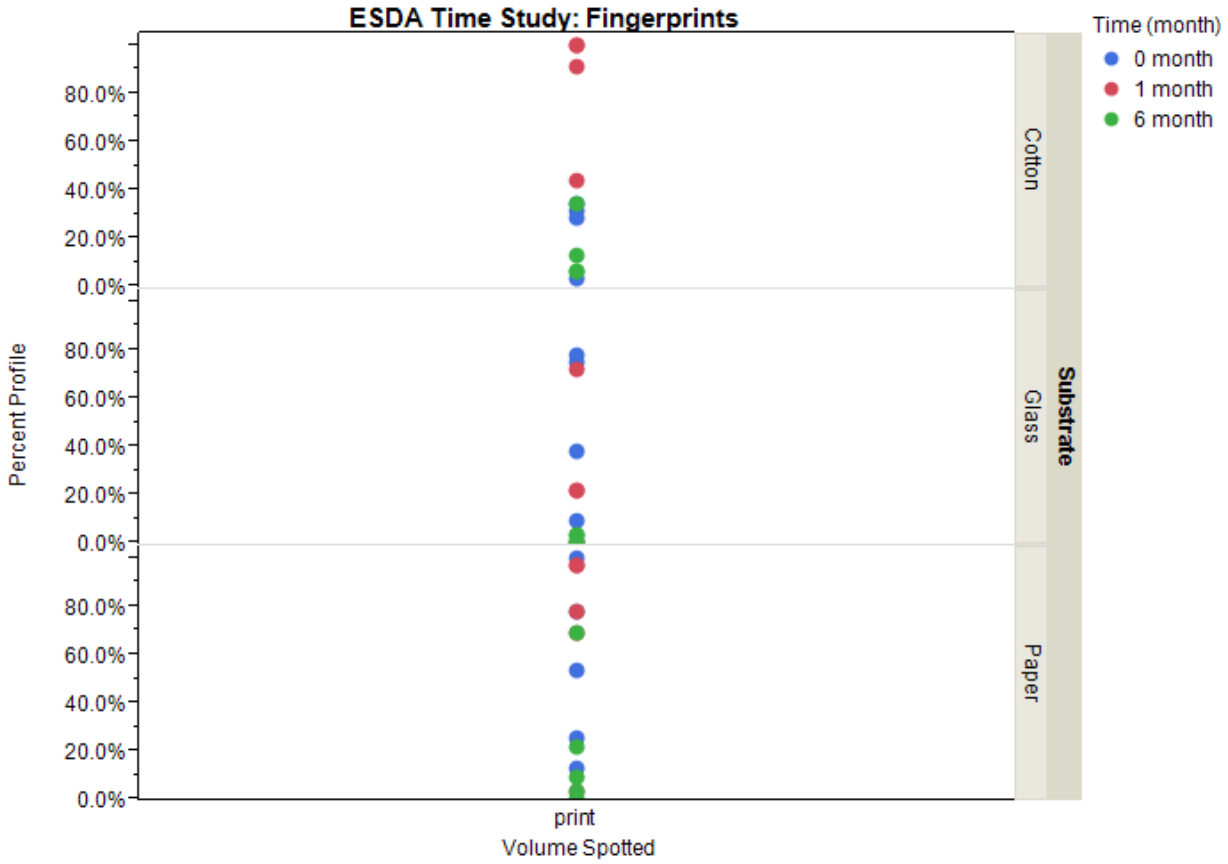

Figure 14: Summary of results obtained from fingerprints spotted on various substrates and collected after 0, 1 month, and 6 month time points via the ESDA-Mylar film technique.

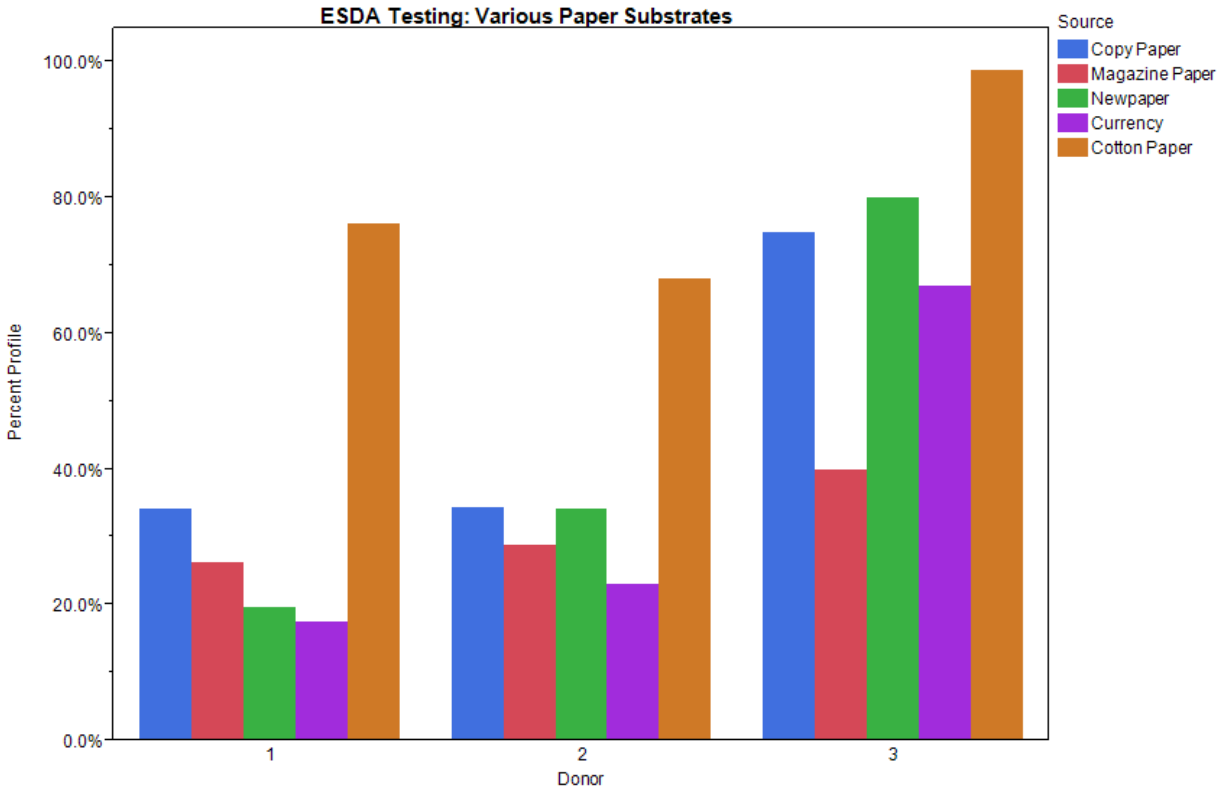

Figure 15: Summary of results obtained from fingerprints of Donors 1, 2, and 3 spotted on various paper substrates collected via the ESDA-Mylar film technique.

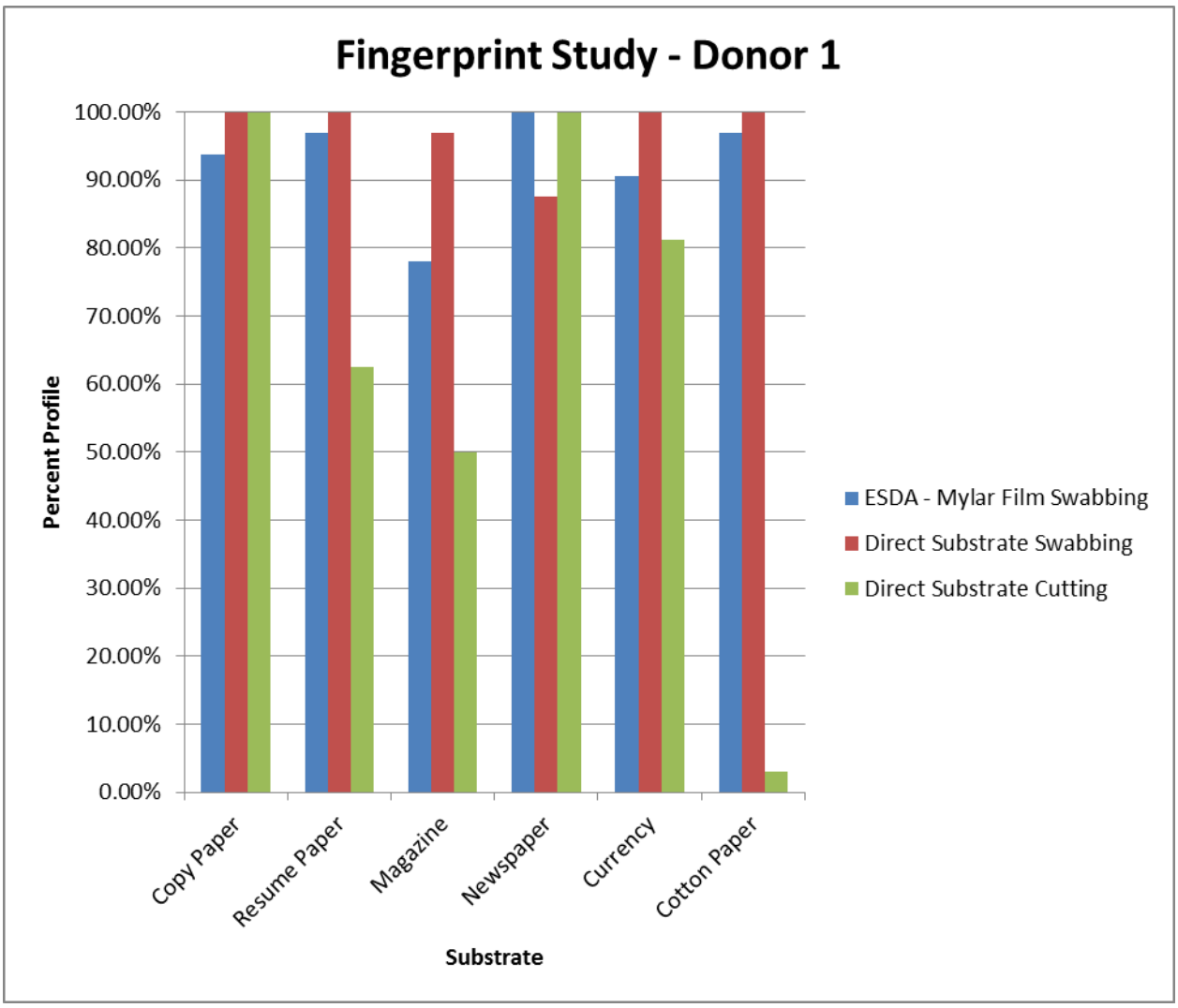

Figure 16: Summary of results obtained from fingerprints of Donor 1 spotted on various paper substrates collected via the ESDA-Mylar film technique, direct substrate dry swabbing, and direct substrate cutting.

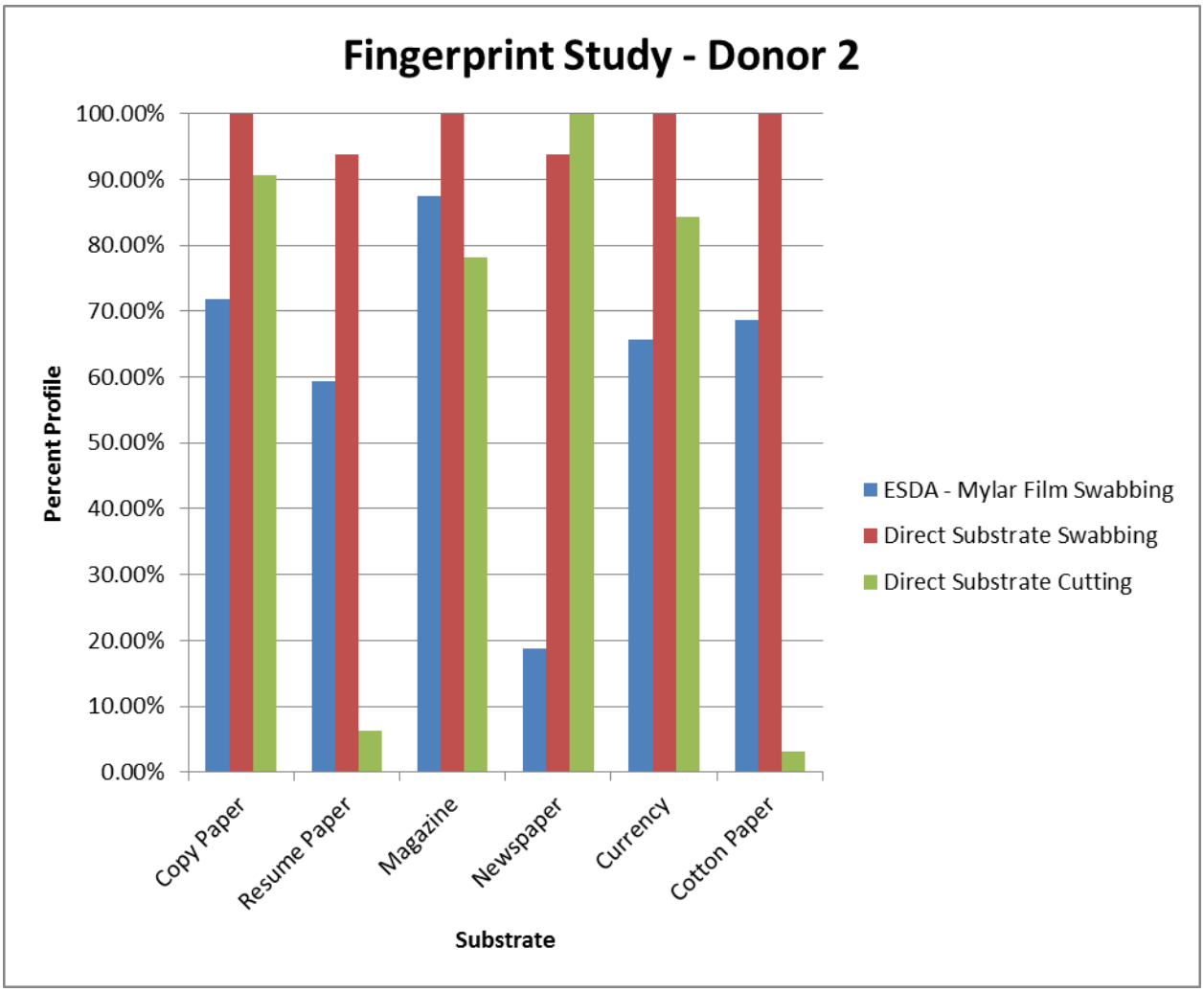

Figure 17: Summary of results obtained from fingerprints of Donor 2 spotted on various paper substrates collected via the ESDA-Mylar film technique, direct substrate dry swabbing, and direct substrate cutting.

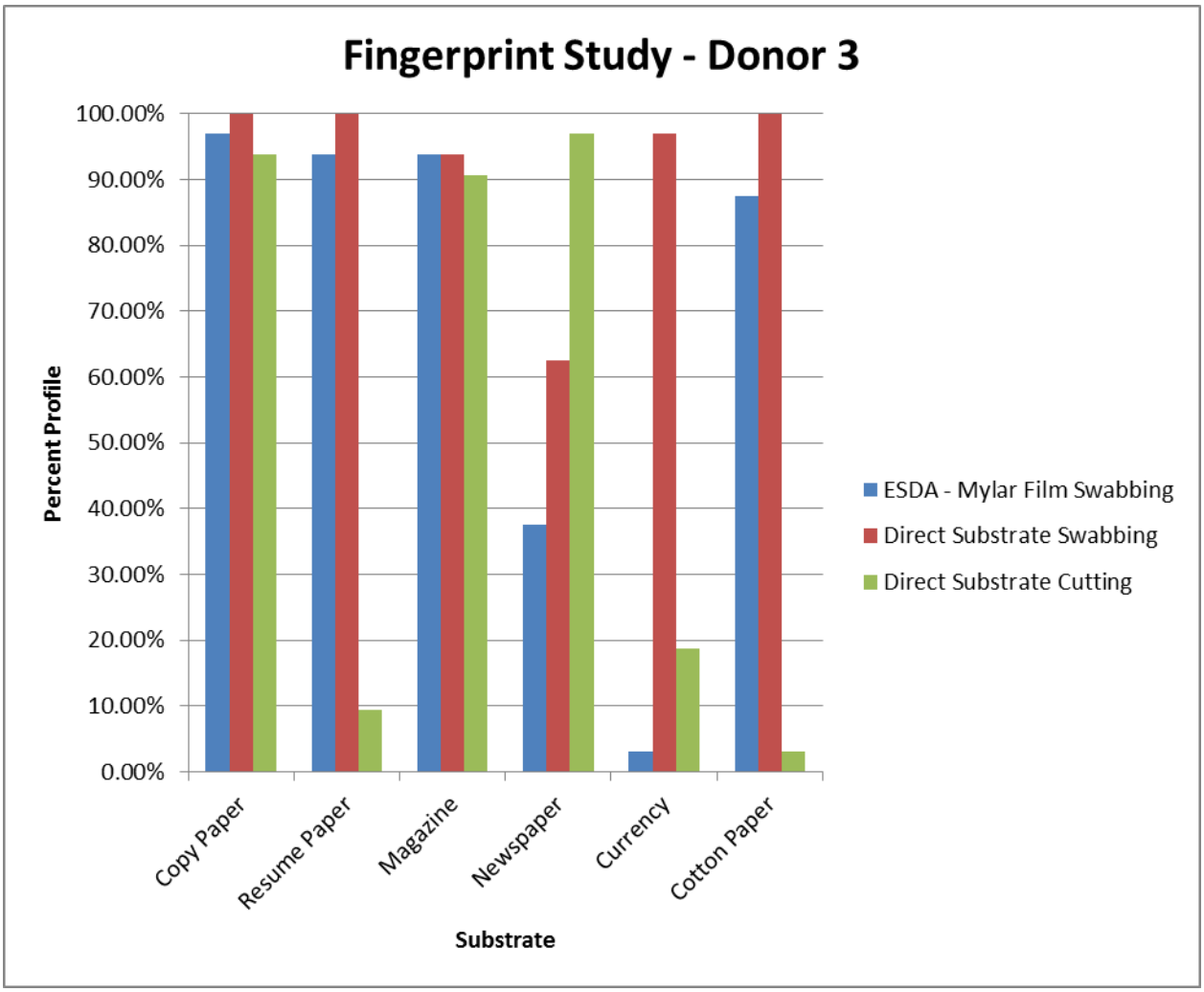

Figure 18: Summary of results obtained from fingerprints of Donor 3 spotted on various paper substrates collected via the ESDA-Mylar film technique, direct substrate dry swabbing, and direct substrate cutting.

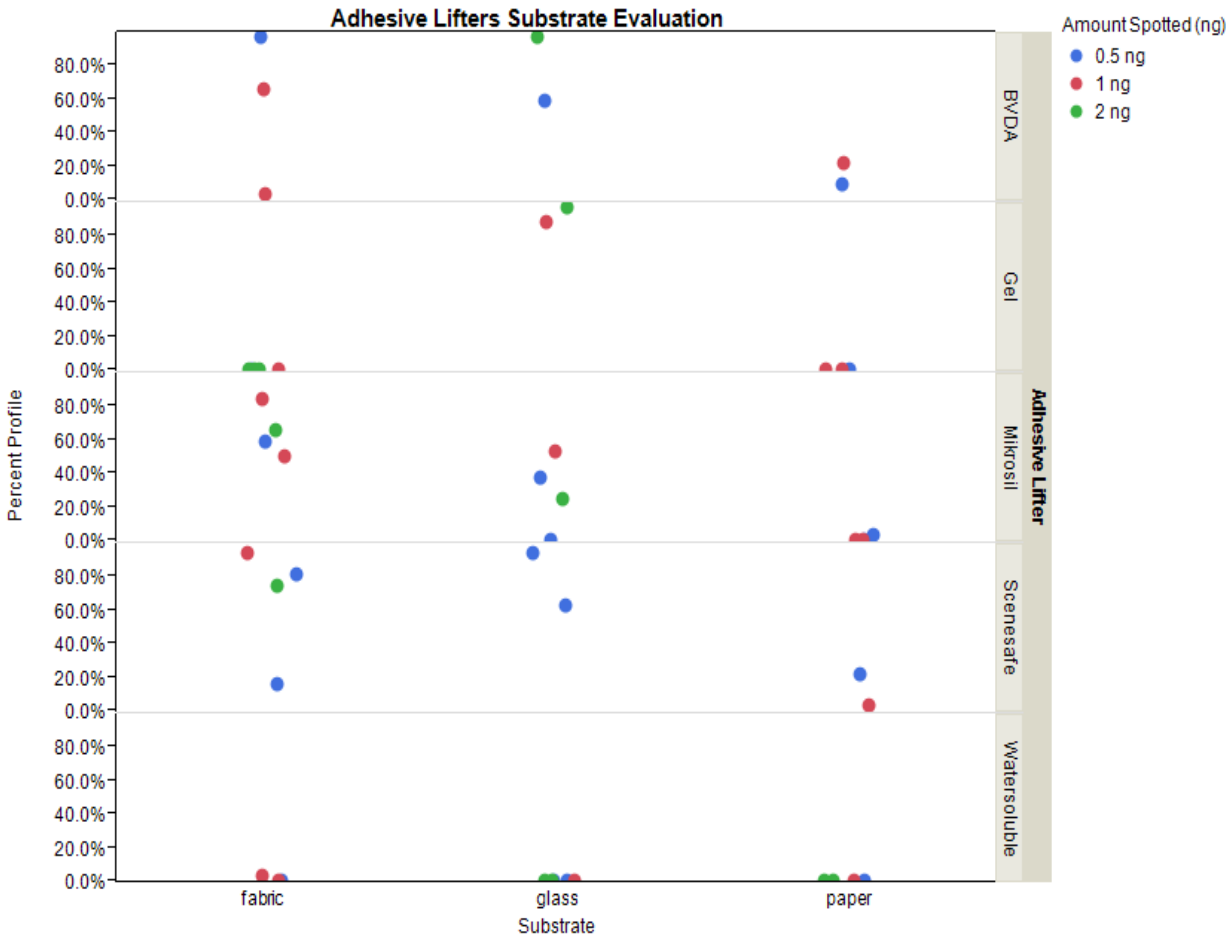

Figure 19: Summary of results obtained from 0.5 ng, 1.0 ng, and 2.0 ng buccal cells spotted on various substrates and collected using five different adhesive lifters.

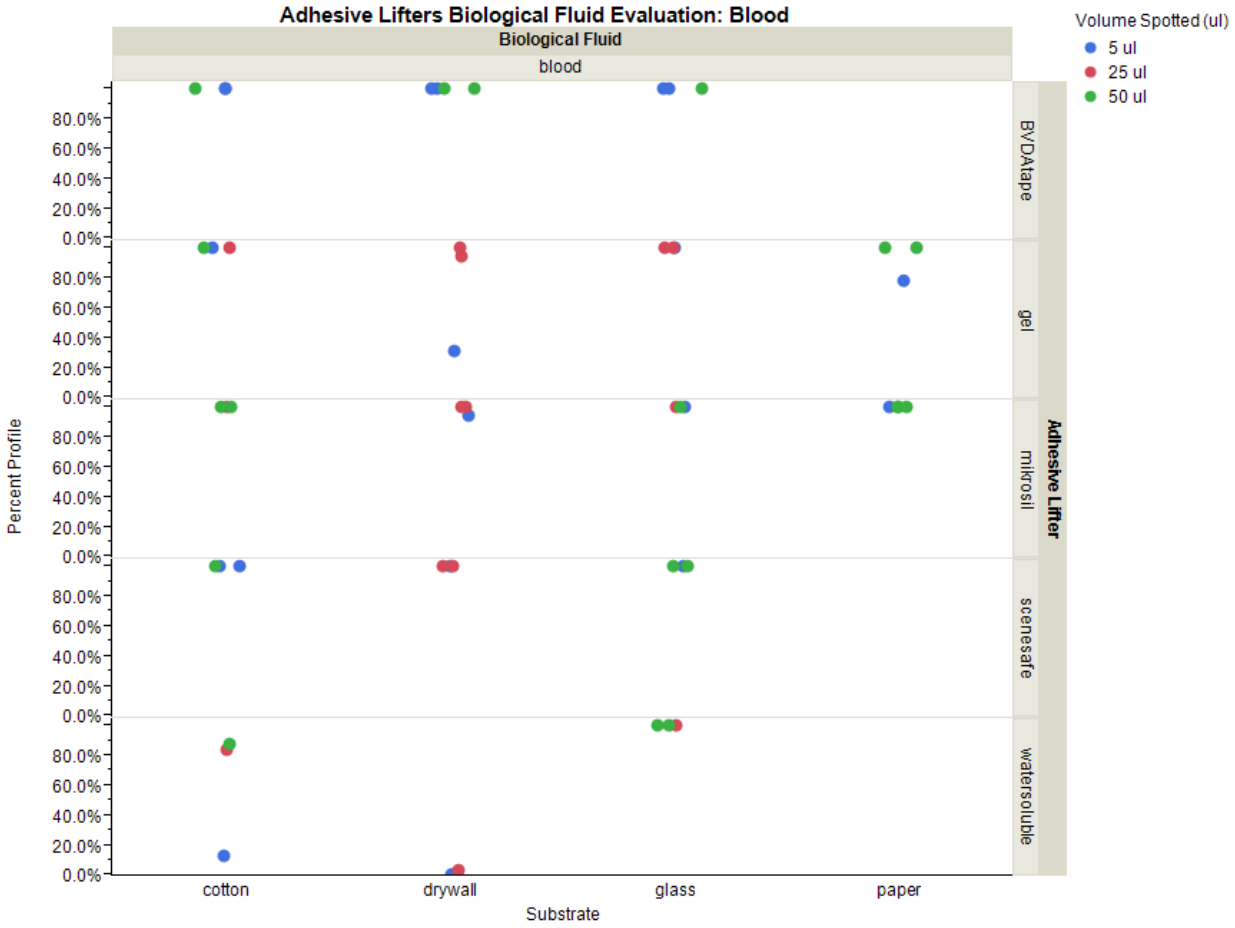

Figure 20: Summary of results obtained from 5 µl, 25 µl, and 50 µl of blood spotted on various substrates and collected using five different adhesive lifters.

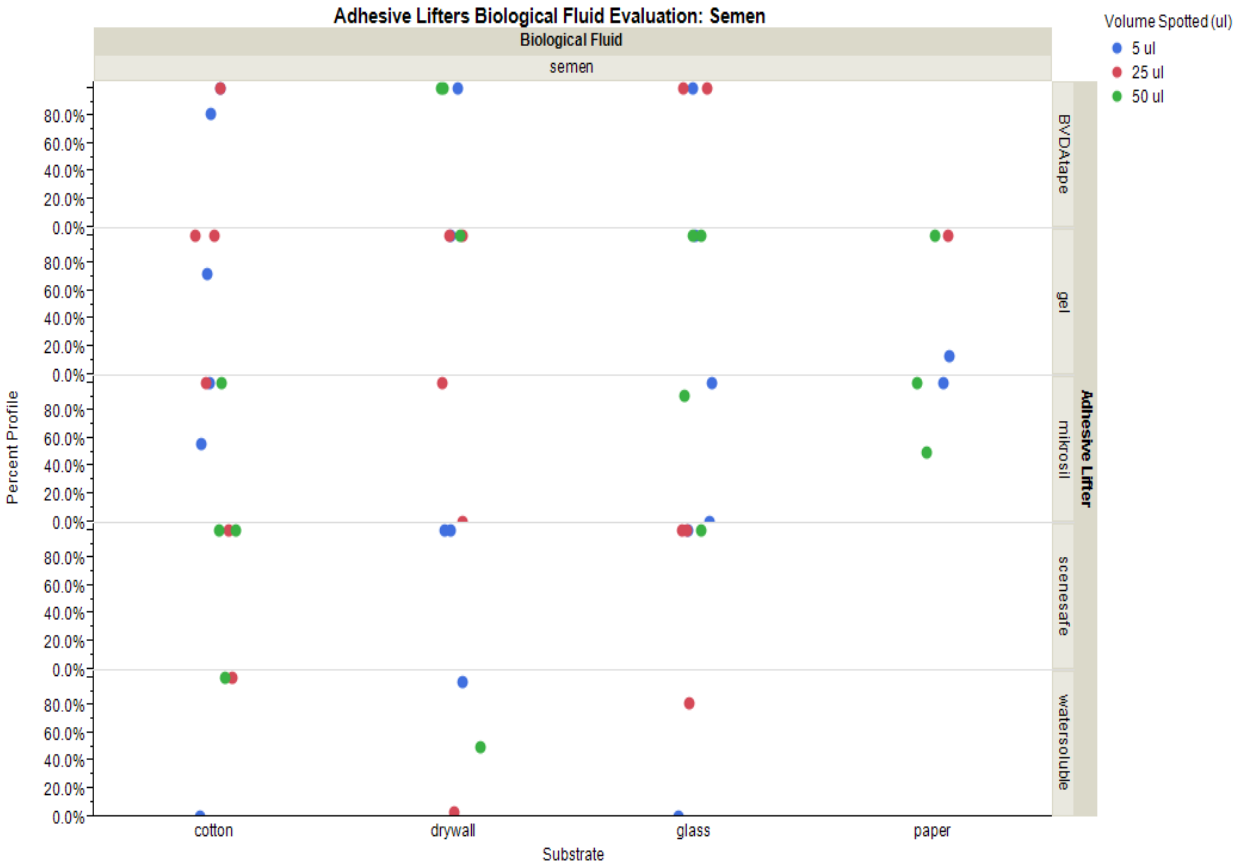

Figure 21: Summary of results obtained from 5 µl, 25 µl, and 50 µl of semen spotted on various substrates and collected using five different adhesive lifters.

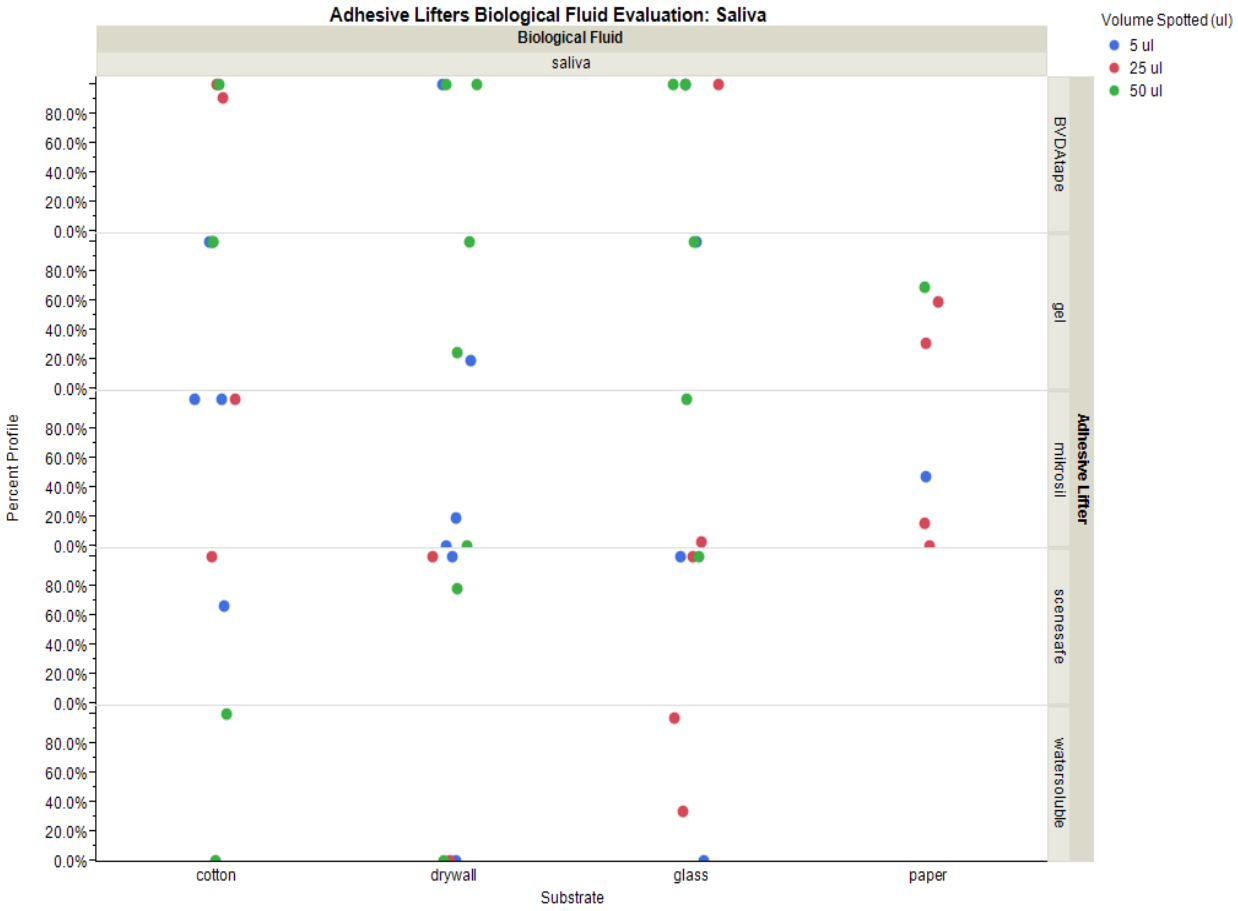

Figure 22: Summary of results obtained from 5 µl, 25 µl, and 50 µl of saliva spotted on various substrates and collected using five different adhesive lifters.

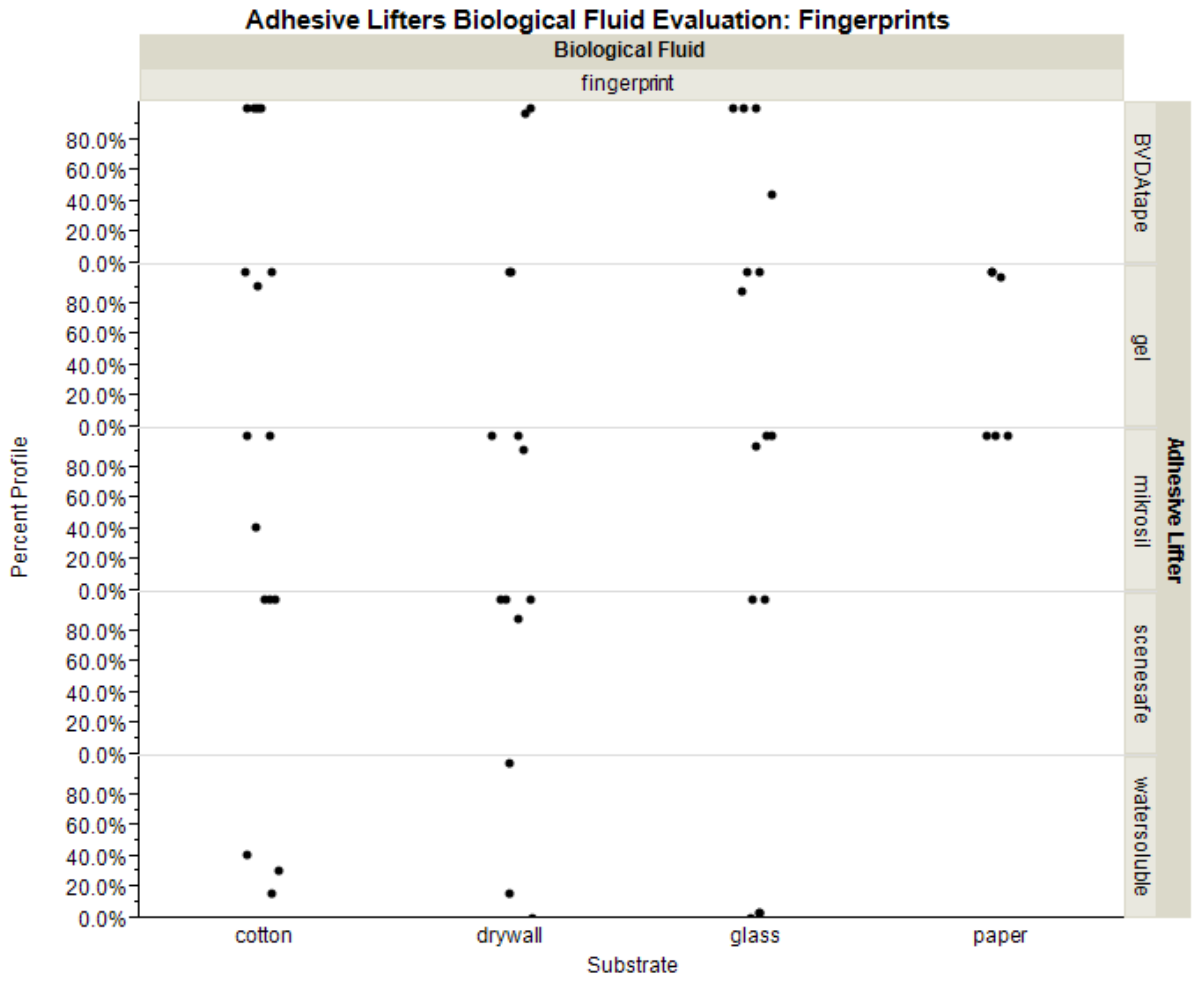

Figure 23: Summary of results obtained from fingerprints spotted on various substrates and collected using five different adhesive lifters.

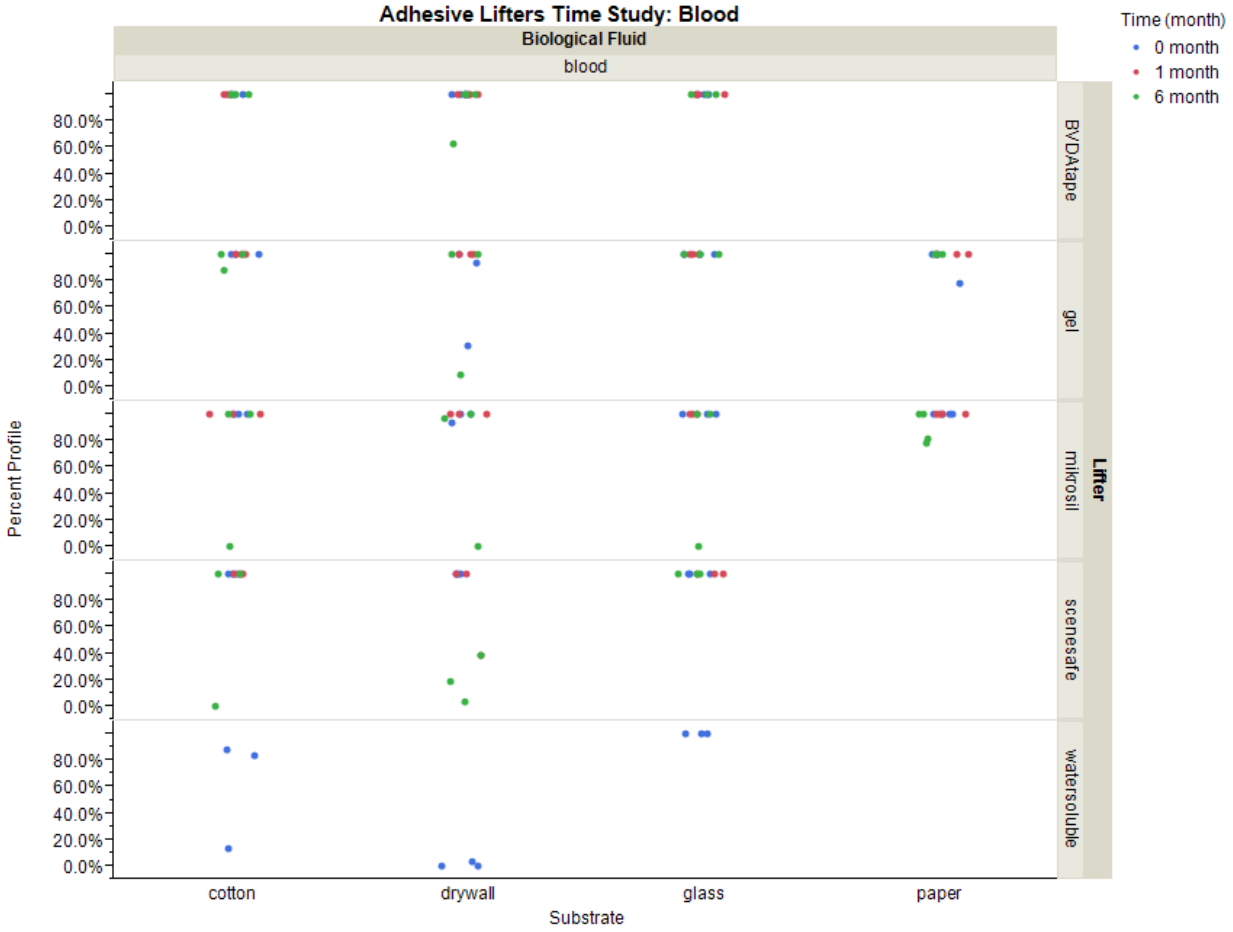

Figure 24: Summary of results obtained from blood spotted on various substrates and collected after 0, 1 month, and 6 month time points using five different adhesive lifters.

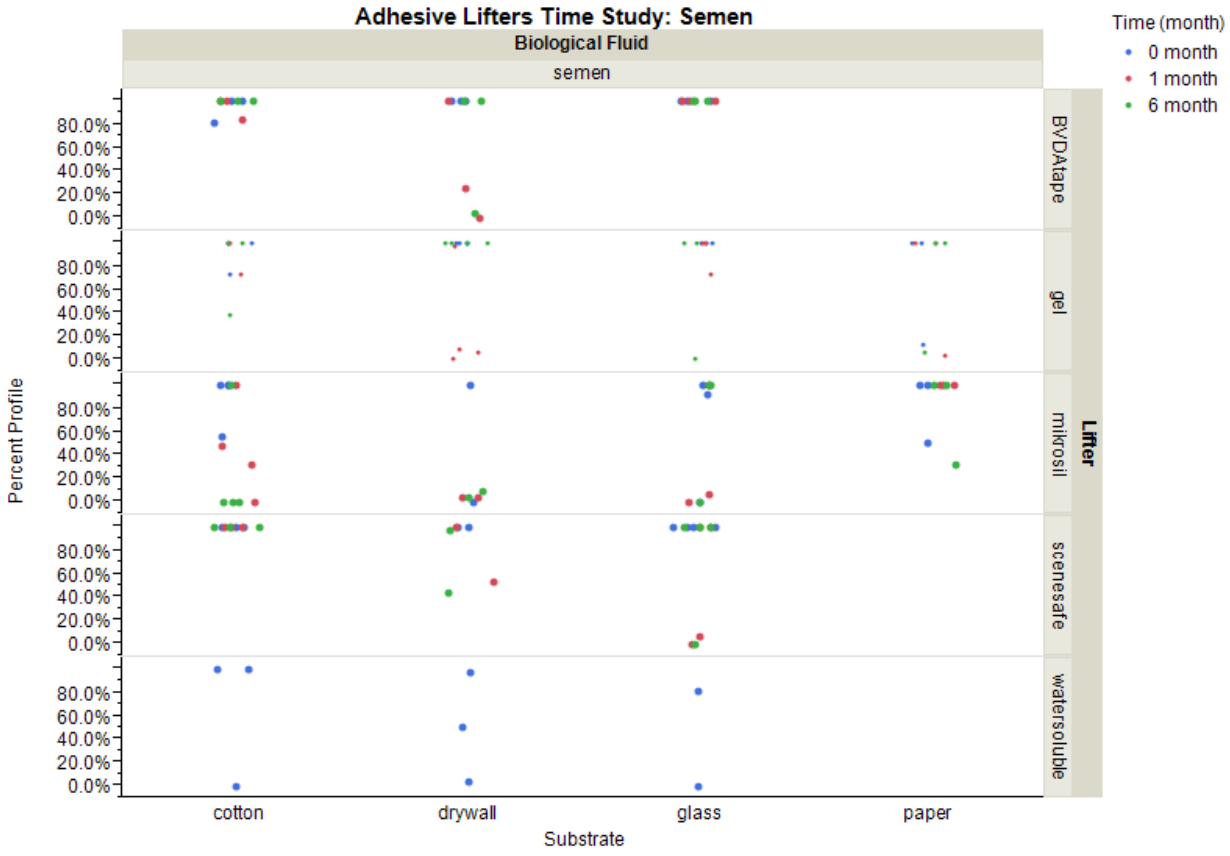

Figure 25: Summary of results obtained from semen spotted on various substrates and collected after 0, 1 month, and 6 month time points using five different adhesive lifters.

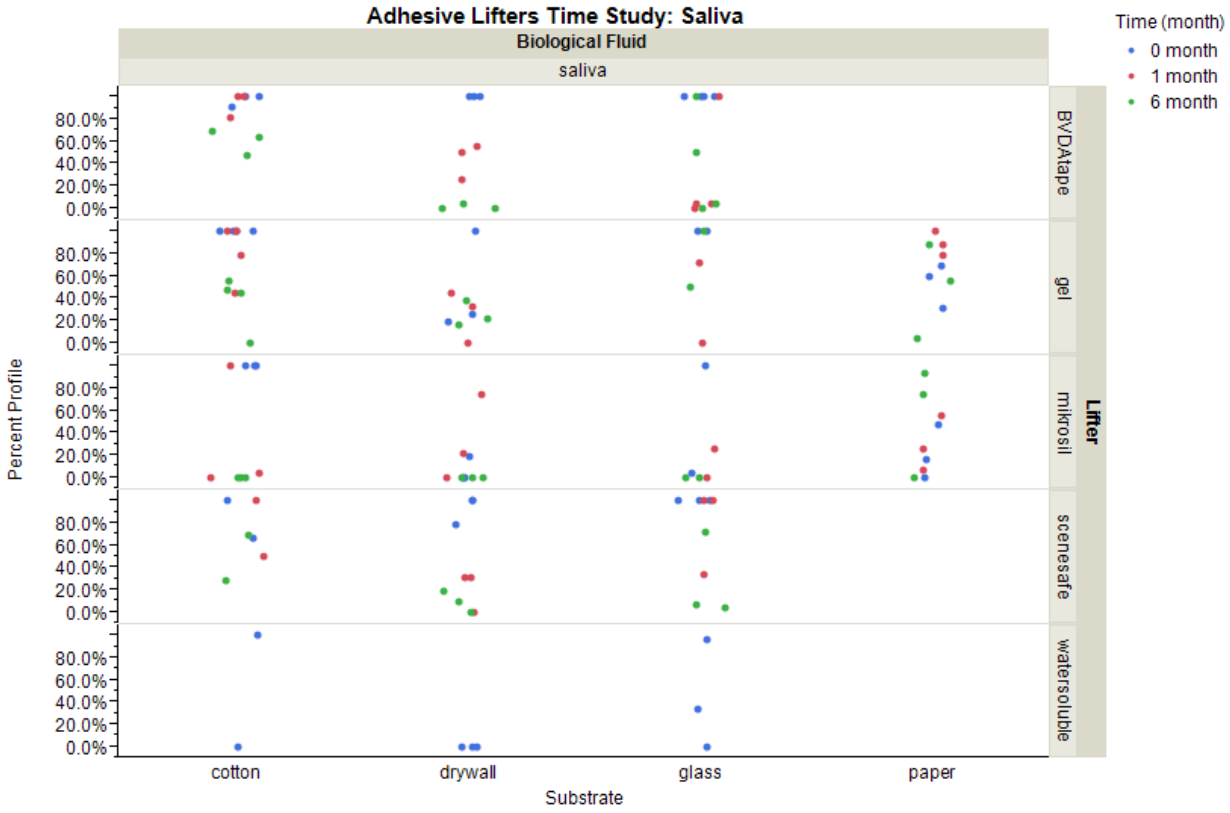

Figure 26: Summary of results obtained from saliva spotted on various substrates and collected after 0, 1 month, and 6 month time points using five different adhesive lifters.

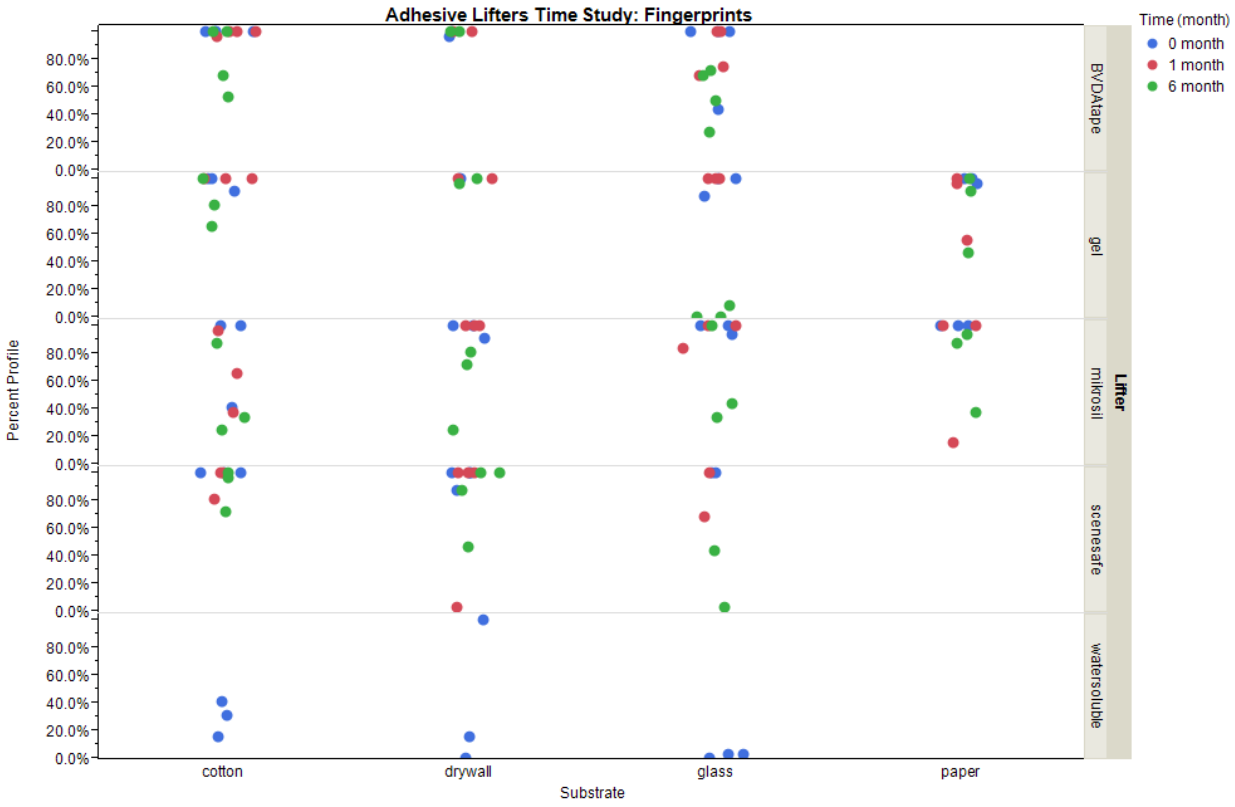

Figure 27: Summary of results obtained from fingerprints spotted on various substrates and collected after 0, 1 month, and 6 month time points using five different adhesive lifters.

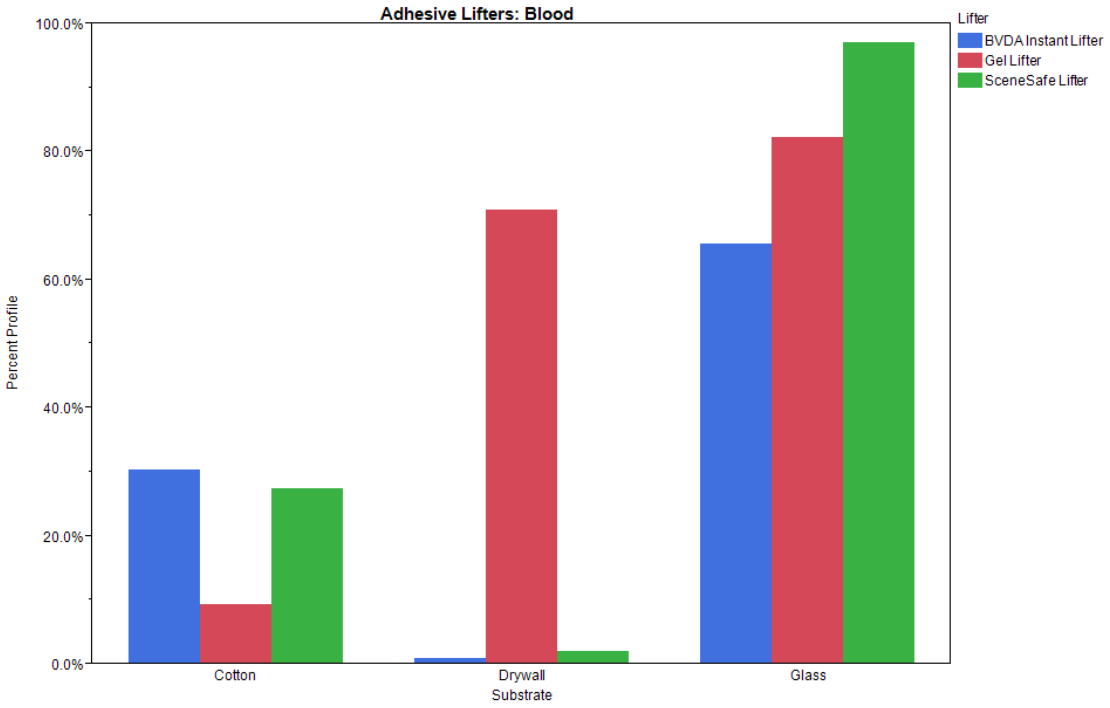

Figure 28: Summary of results obtained from 1.0 ng of blood spotted on various substrates and collected using the three best performing lifters determined from the previous tasks.

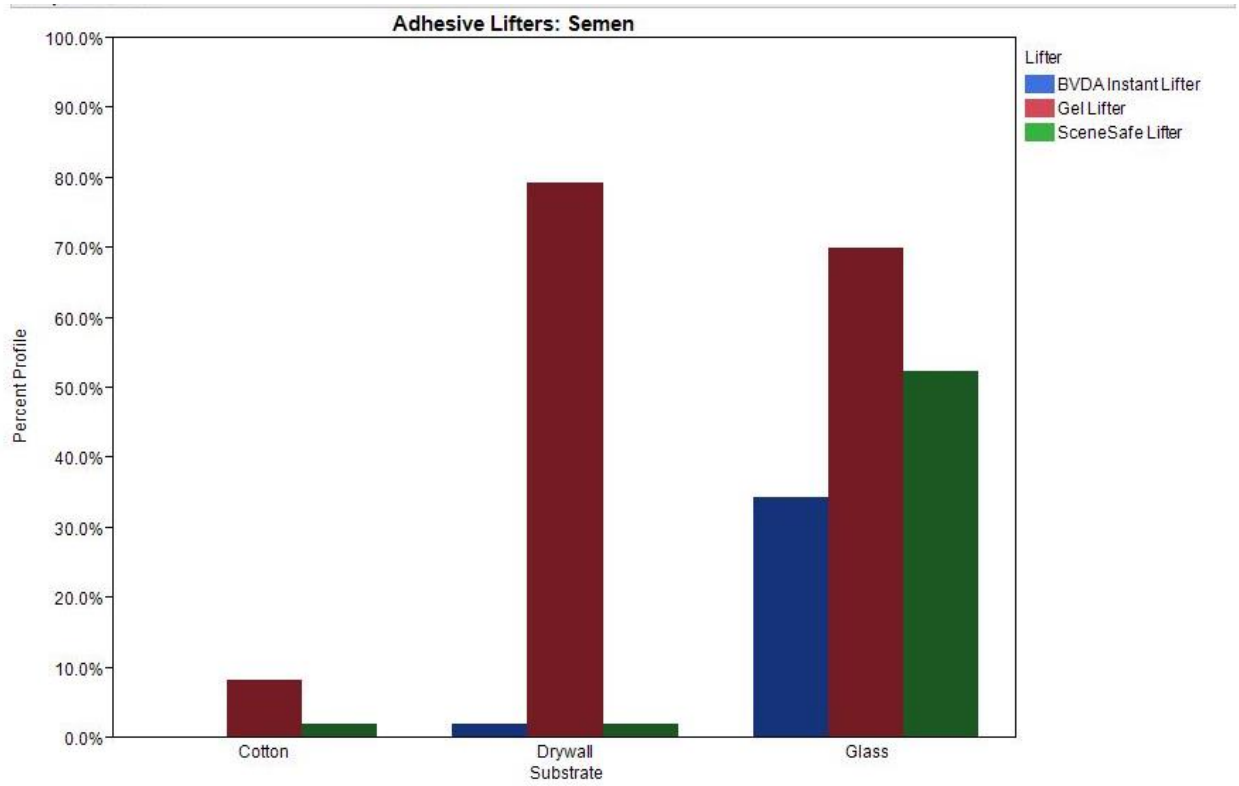

Figure 29: Summary of results obtained from 1.0 ng of semen spotted on various substrates and collected using the three best performing lifters determined from the previous tasks.

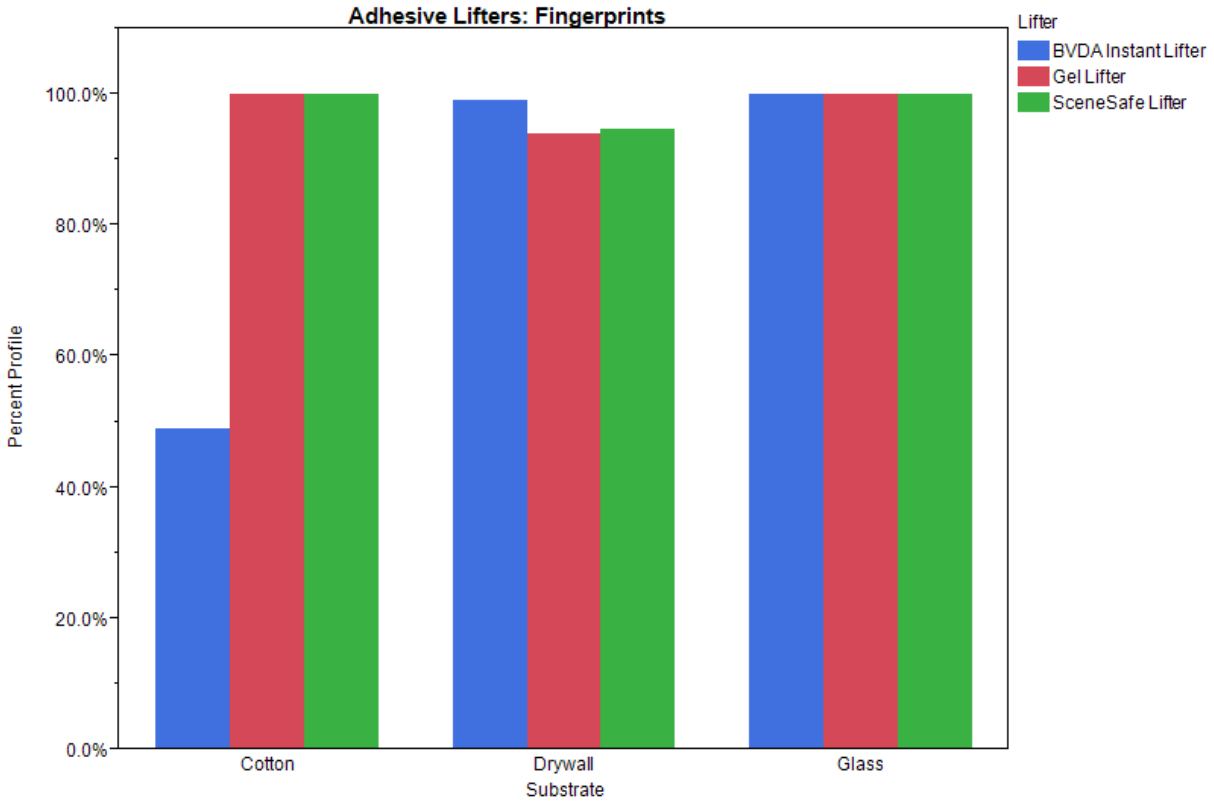

Figure 30: Summary of results obtained from fingerprints spotted on various substrates and collected using the three best performing lifters determined from the previous tasks.

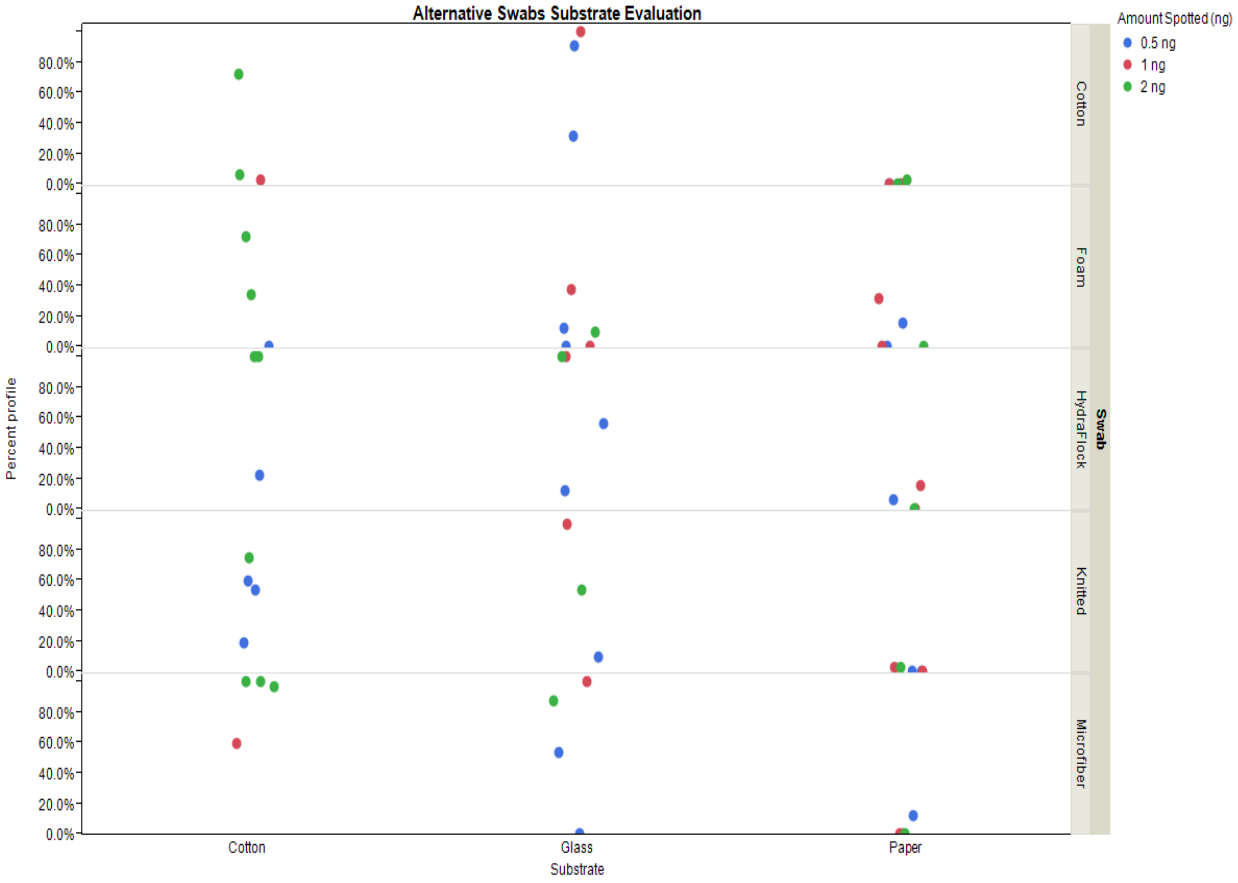

Figure 31: Summary of results obtained from 0.5 ng, 1.0 ng, and 2.0 ng buccal cells spotted on various substrates and collected using four different alternative swabs and the more commonly used cotton swab.

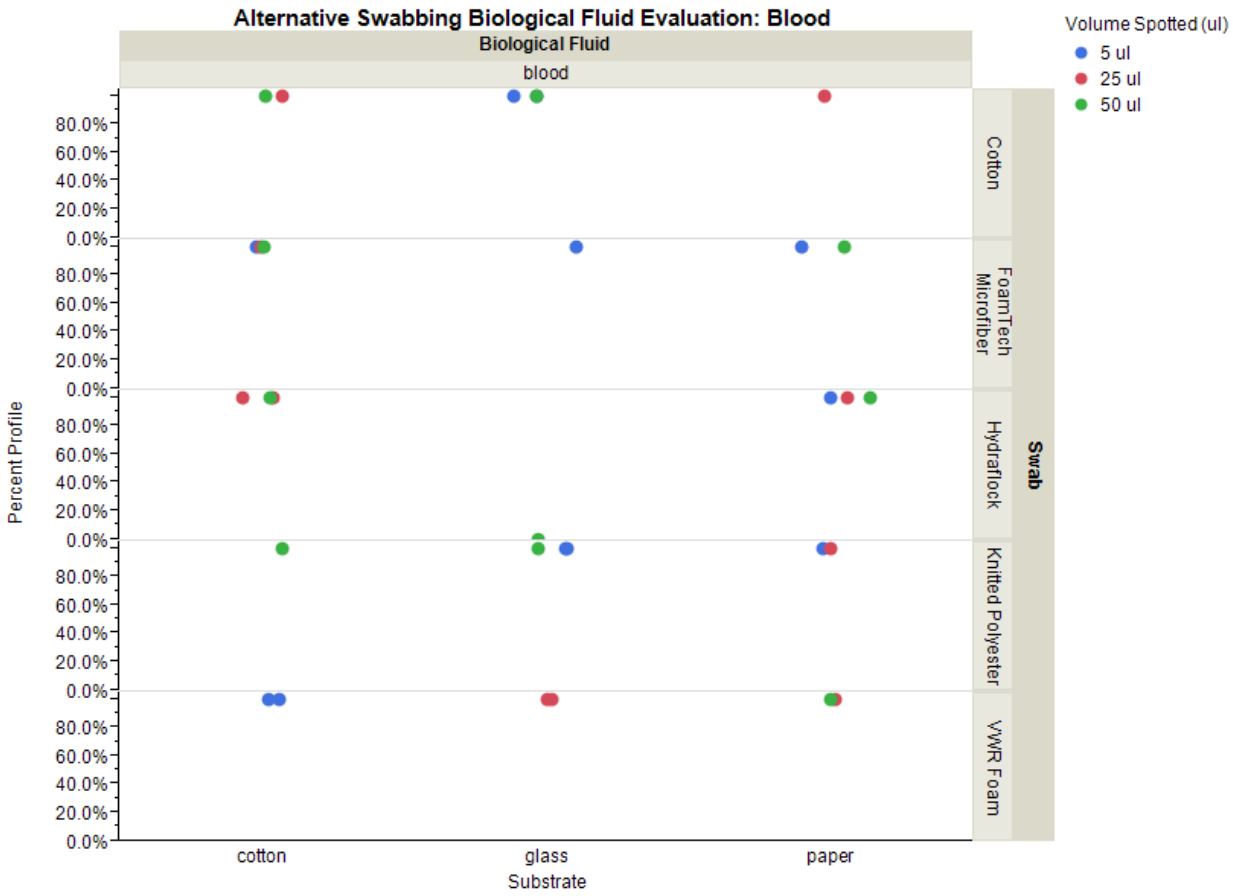

Figure 32: Summary of results obtained from 5 µl, 25 µl, and 50 µl of blood spotted on various substrates and collected using five different swabs.

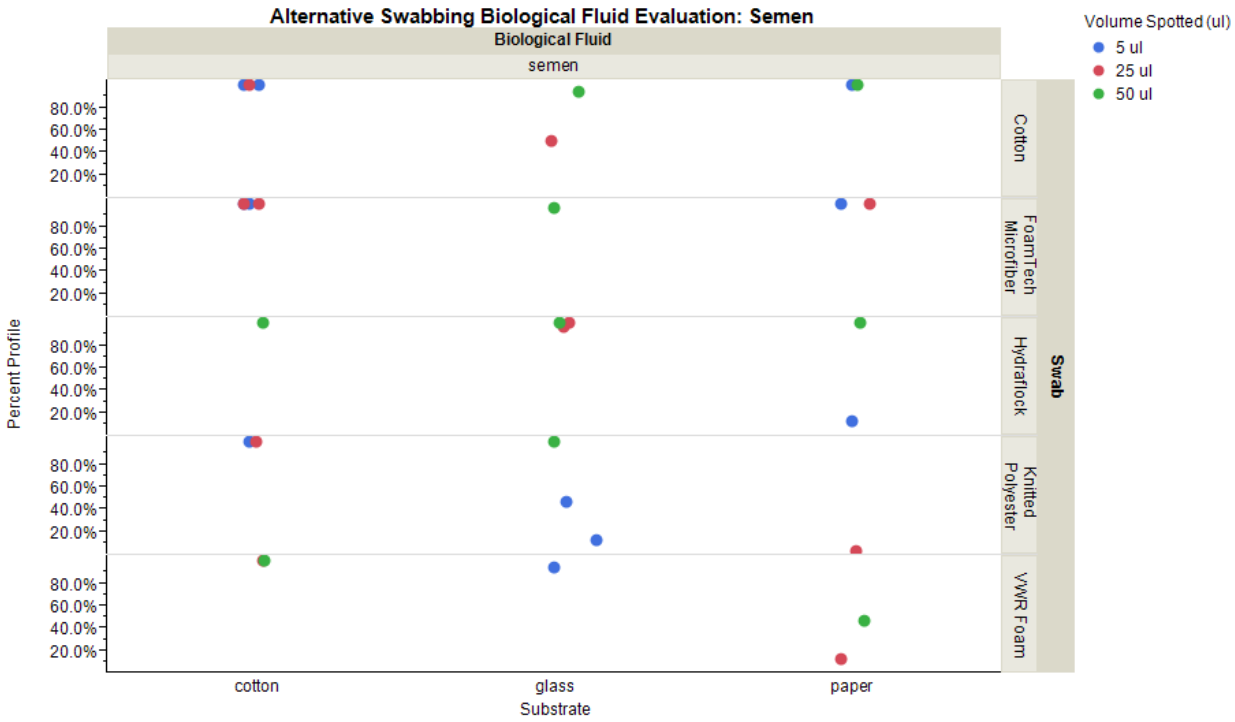

Figure 33: Summary of results obtained from 5 µl, 25 µl, and 50 µl of semen spotted on various substrates and collected using five different swabs.

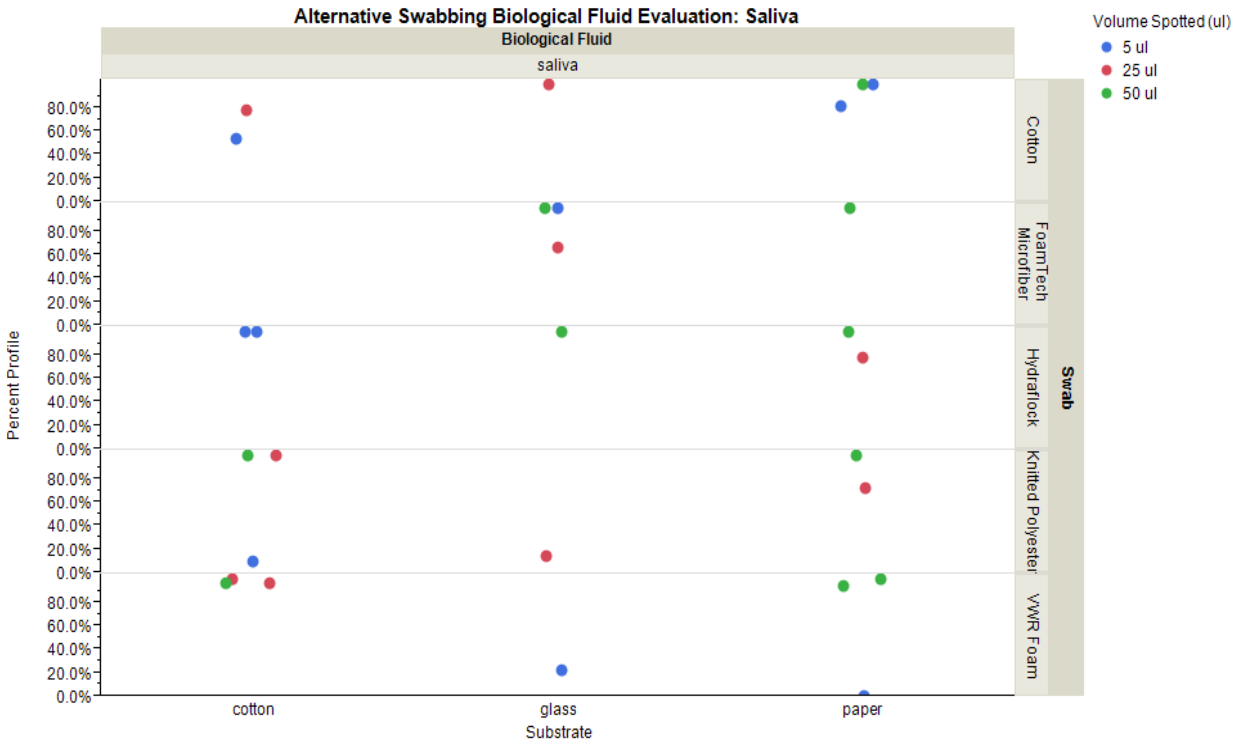

Figure 34: Summary of results obtained from 5 µl, 25 µl, and 50 µl of saliva spotted on various substrates and collected using five different swabs.

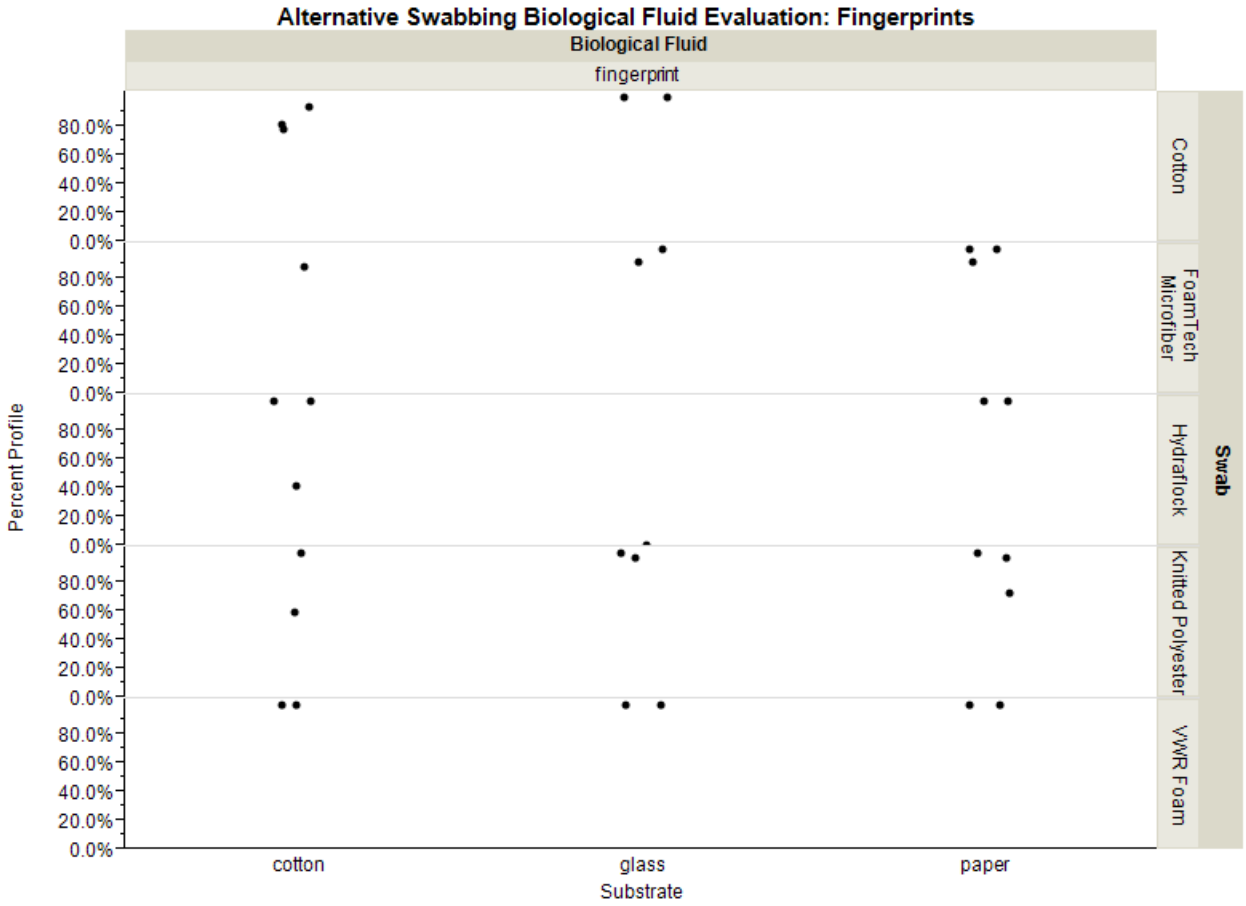

Figure 35: Summary of results obtained from fingerprints spotted on various substrates and collected using five different swabs.

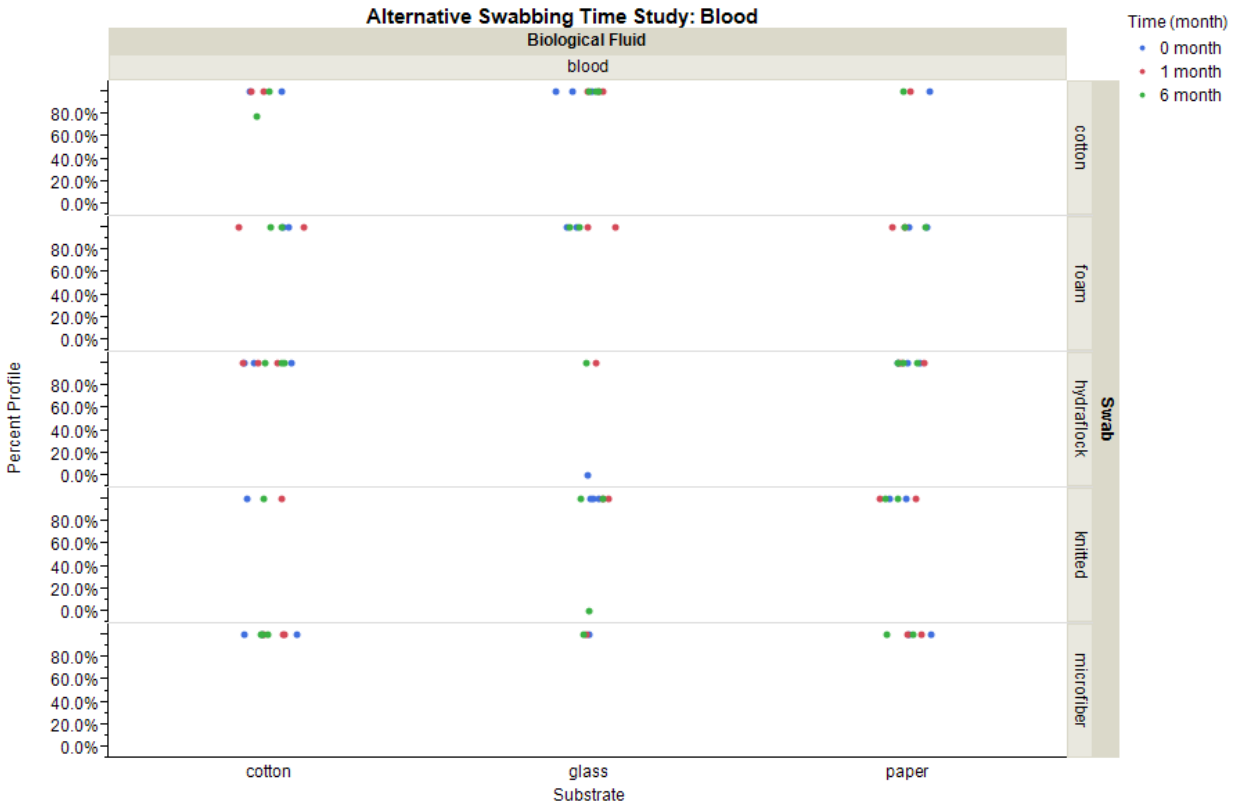

Figure 36: Summary of results obtained from blood spotted on various substrates and collected after 0, 1 month, and 6 month time points using five different swabs.

Figure 37: Summary of results obtained from semen spotted on various substrates and collected after 0, 1 month, and 6 month time points using five different swabs.

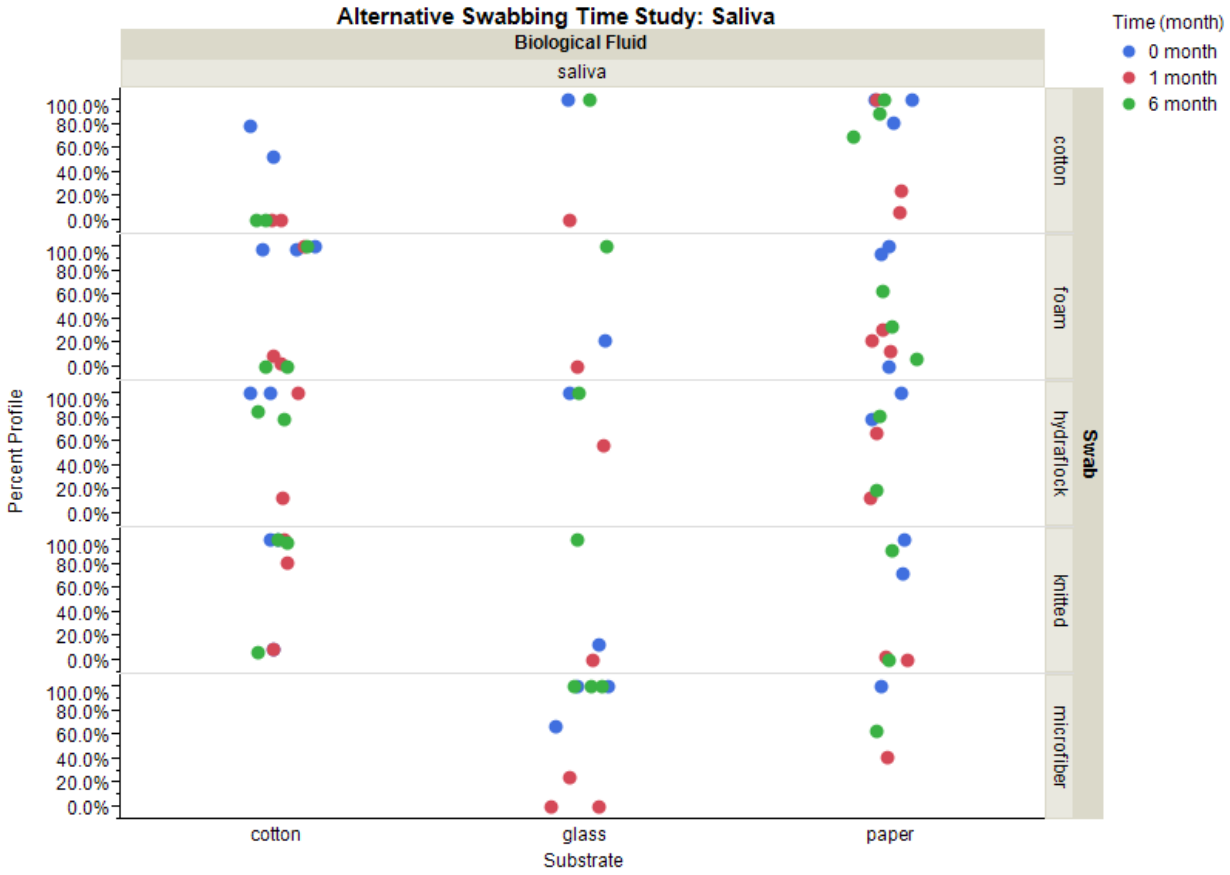

Figure 38: Summary of results obtained from saliva spotted on various substrates and collected after 0, 1 month, and 6 month time points using five different swabs.

Figure 39: Summary of results obtained from fingerprints spotted on various substrates and collected after 0, 1 month, and 6 month time points using five different swabs.

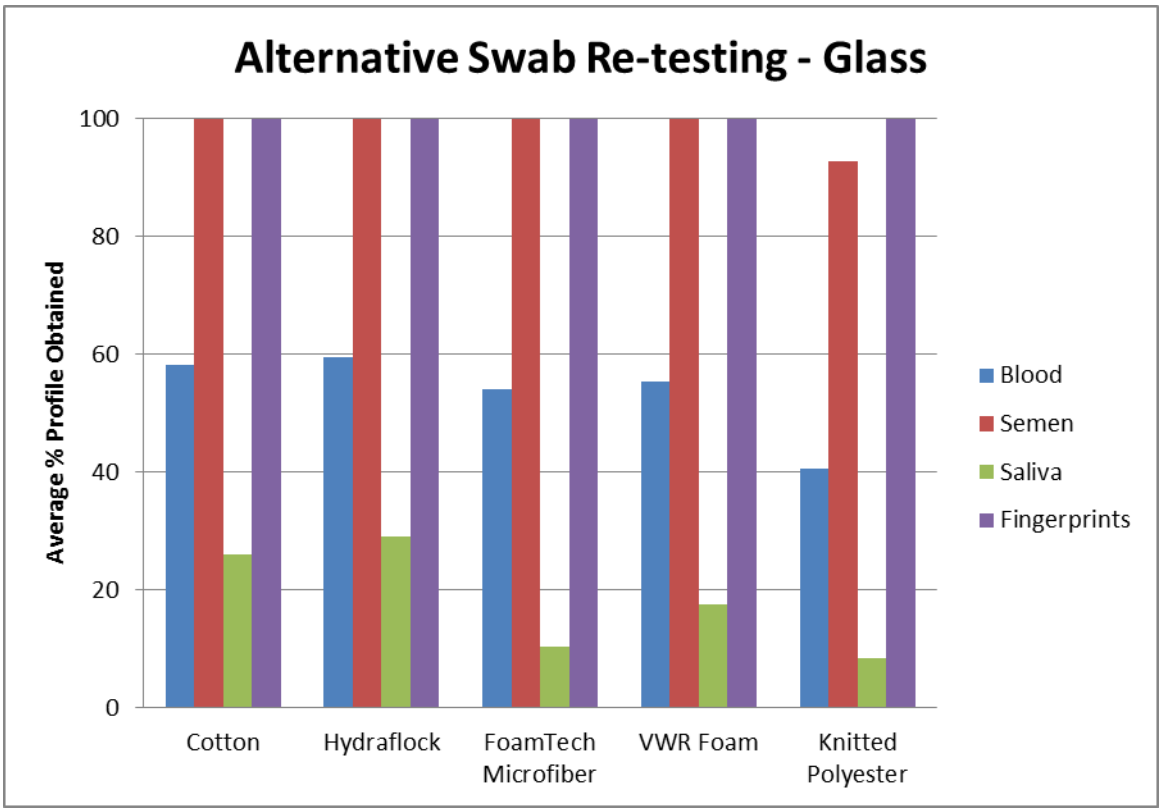

Figure 40: Summary of results obtained from 0.1 ng of semen, 0.5 ng blood, 1.0 ng saliva, and fingerprints spotted on glass and collected using five different swabs.

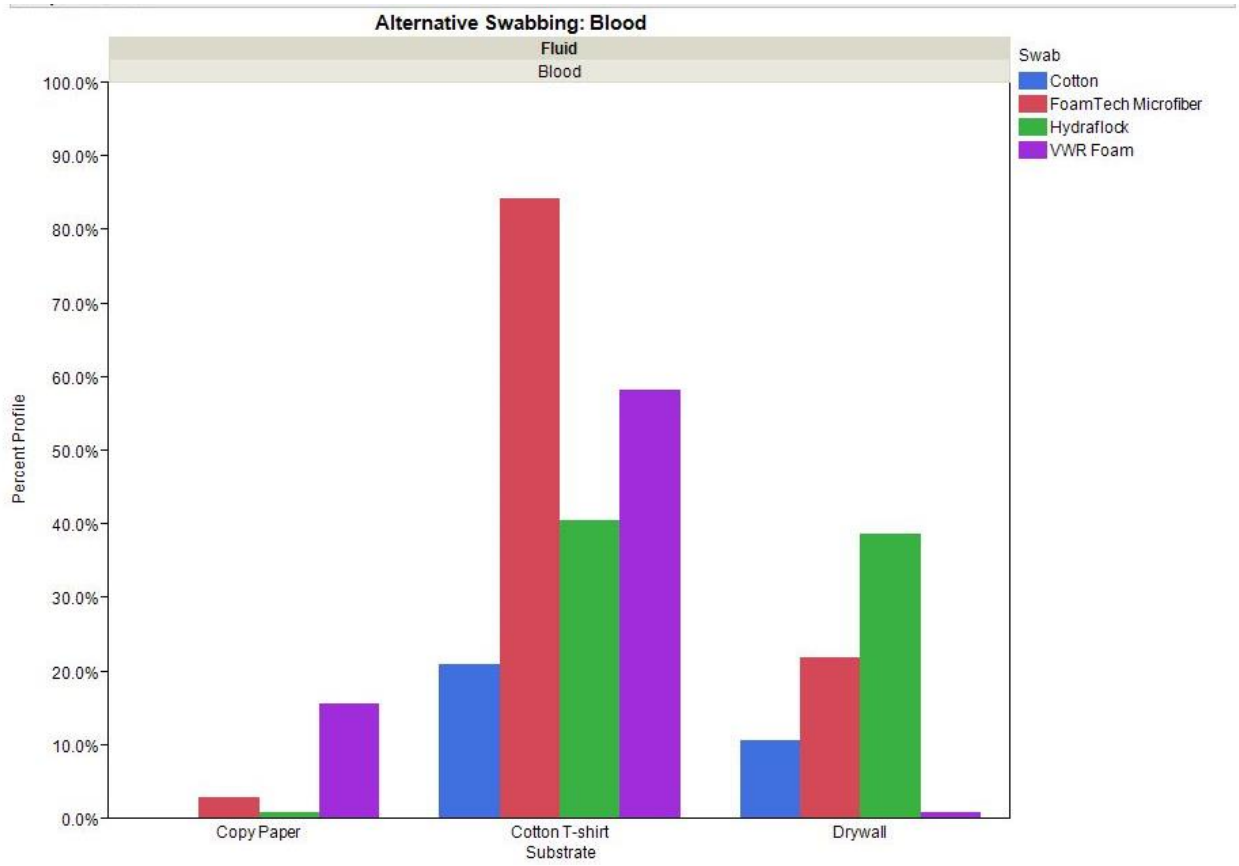

Figure 41: Summary of results obtained from 1.0 ng of blood spotted on various substrates and collected using the three best performing swabs determined from the previous tasks and the cotton swab for comparison purposes.

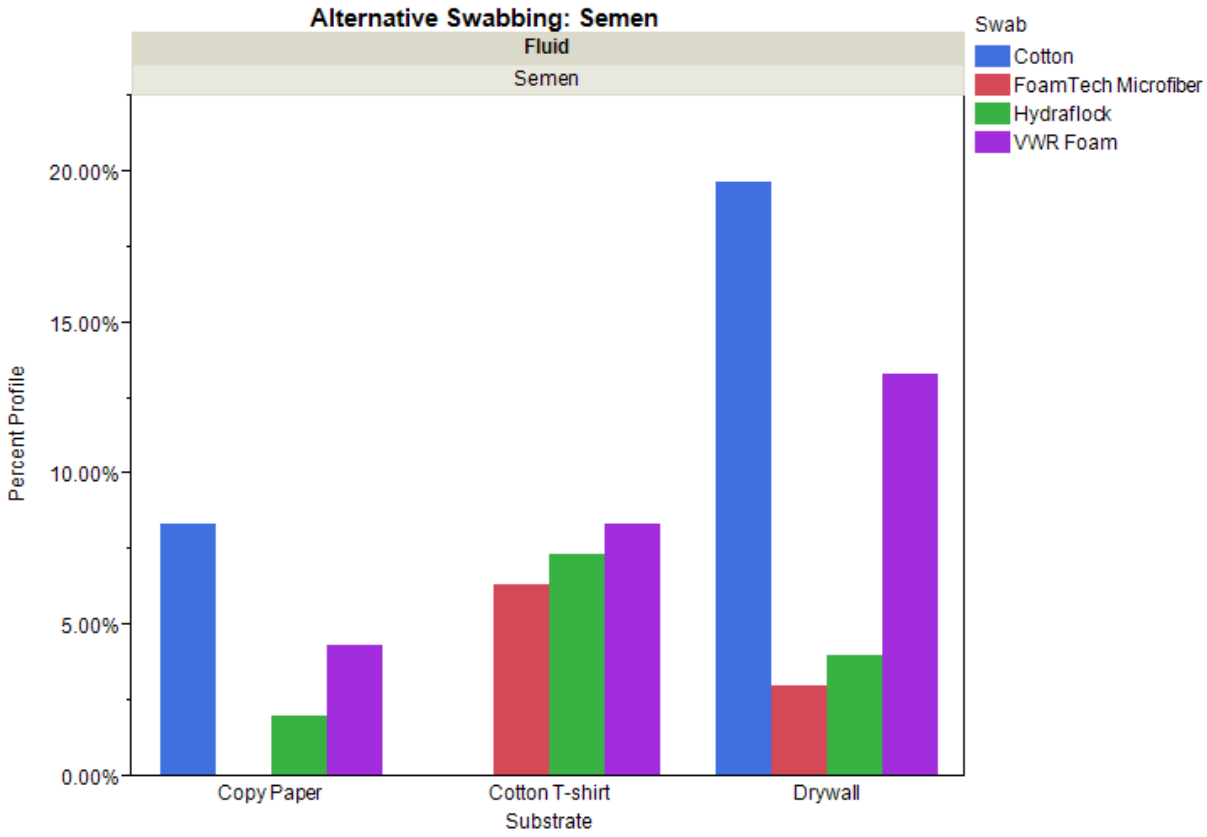

Figure 42: Summary of results obtained from 1.0 ng of semen spotted on various substrates and collected using the three best performing swabs determined from the previous tasks and the cotton swab for comparison purposes. *Note: The Y-axis of this figure is not scaled to 100% for enhanced data viewing*

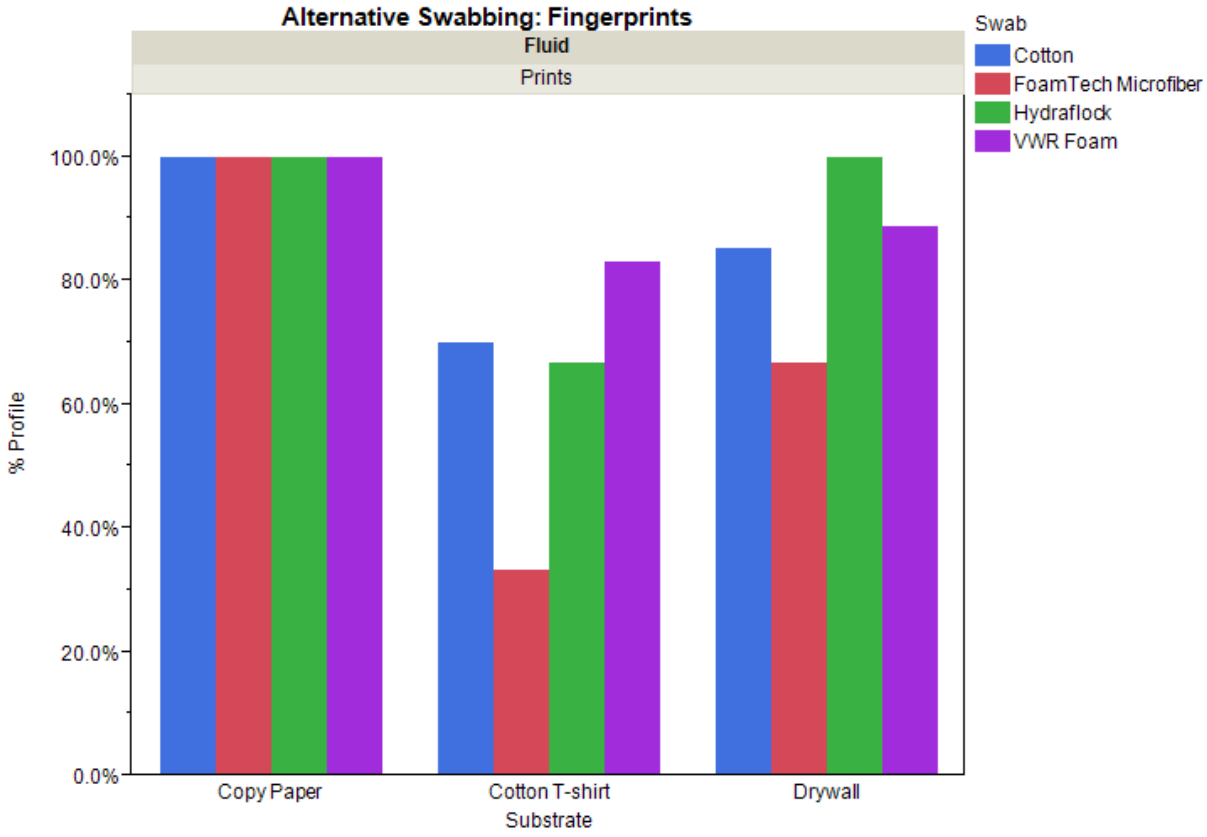

Figure 43: Summary of results obtained from fingerprints spotted on various substrates and collected using the three best performing swabs determined from the previous tasks and the cotton swab for comparison purposes.

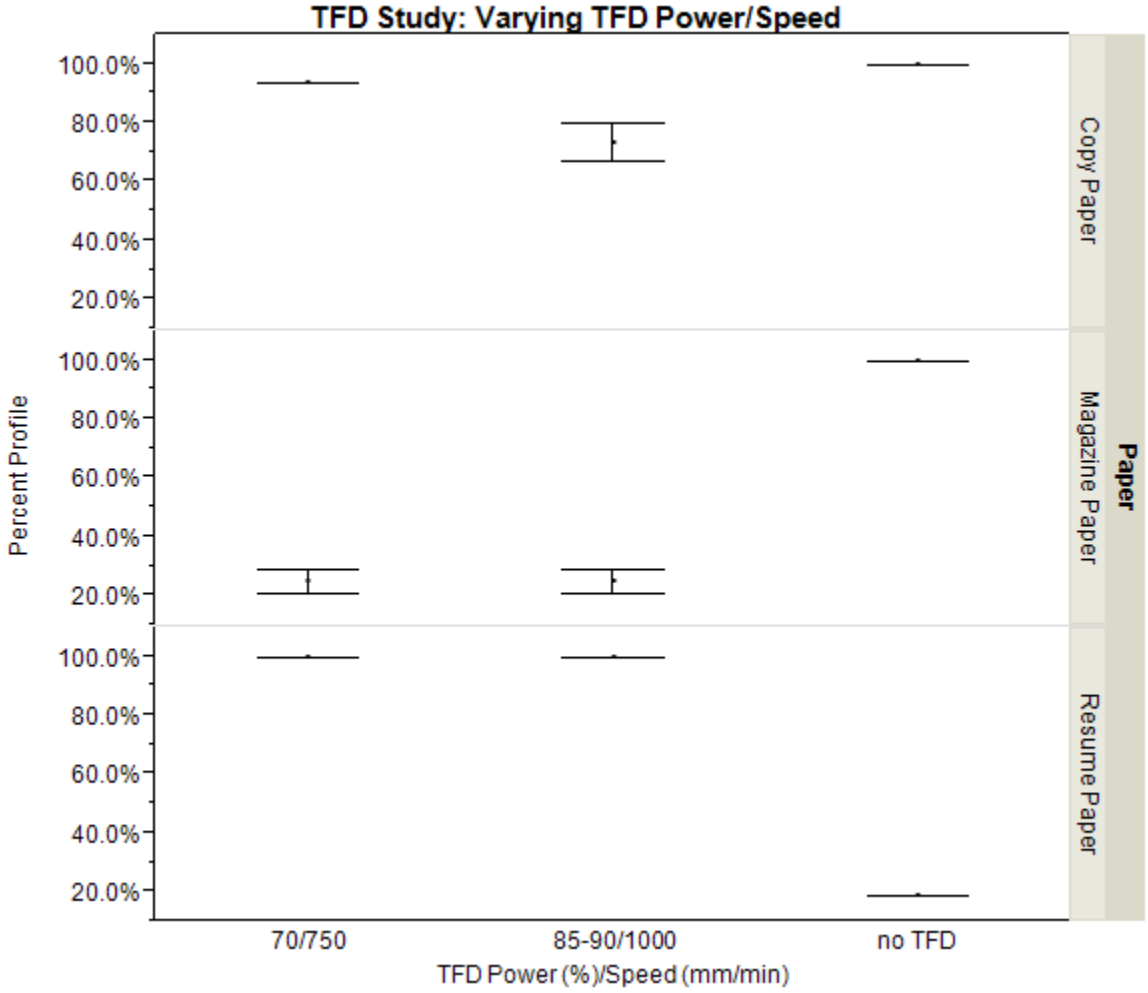

Figure 44: Summary of results obtained from 5.0 ng spotted on various substrates and detected with one of two intensity settings using TFD or collection not utilizing TFD.

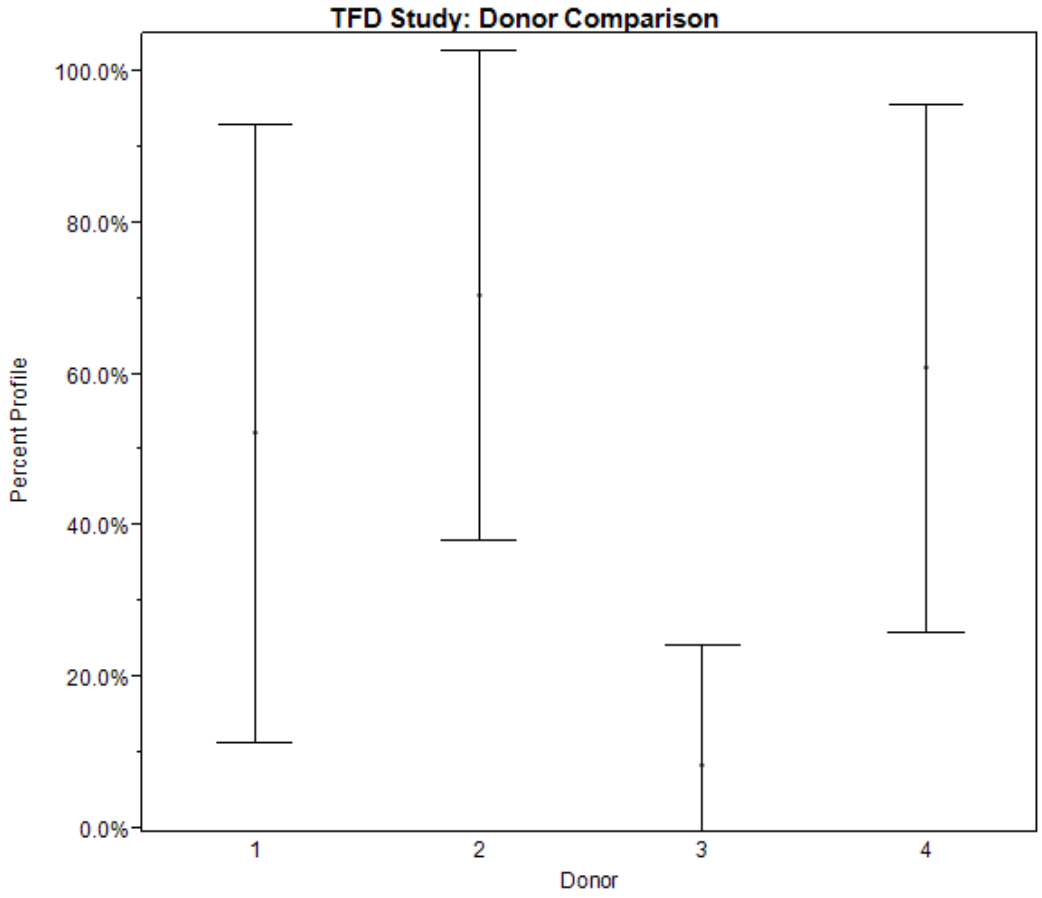

Figure 45: Overall performance of each of the four donors throughout the TFD study.

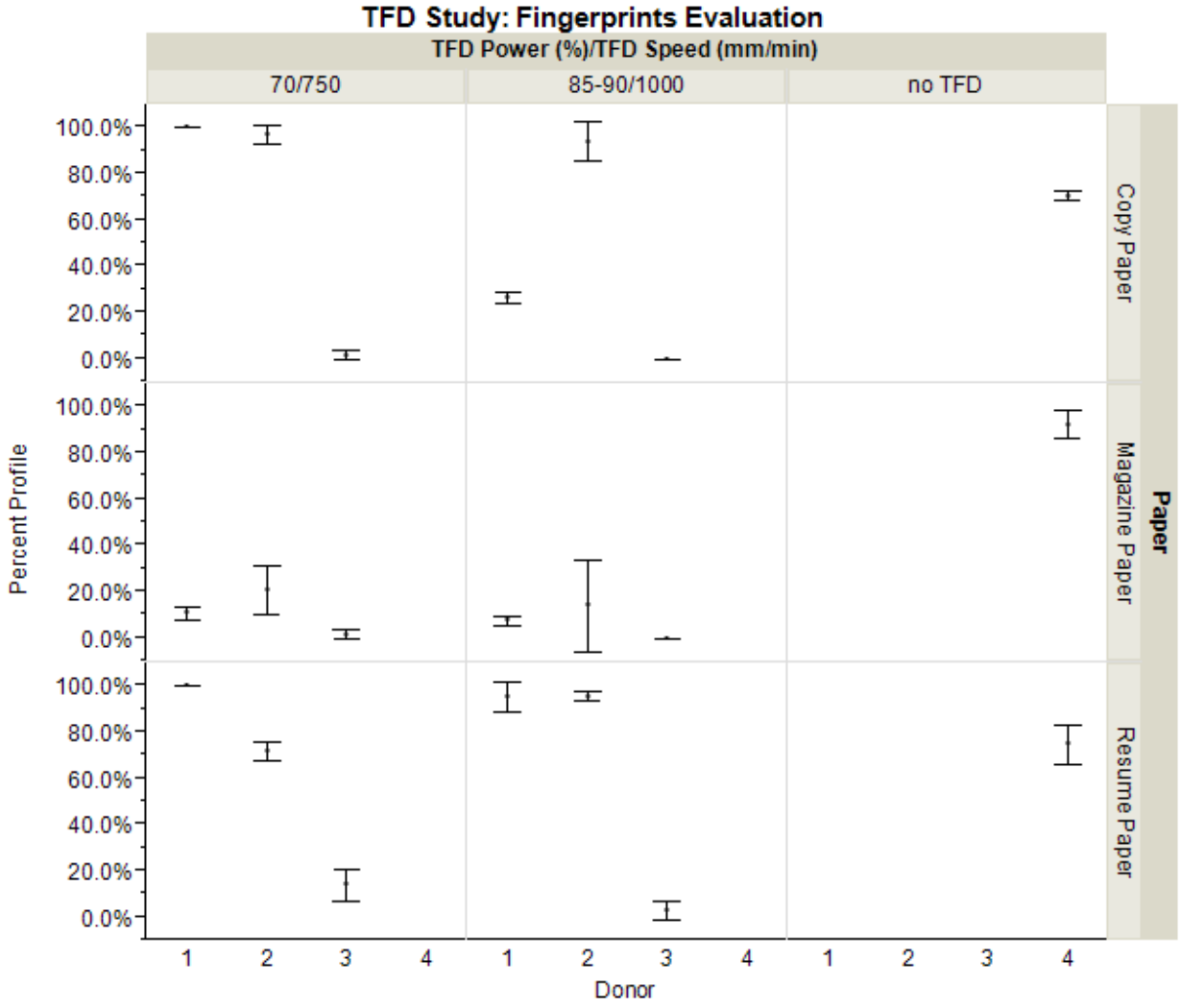

Figure 46: Summary of results obtained from four donors spotting fingerprints onto various substrates and detected with one of two rates using TFD or collection not utilizing TFD.

4.4 Conclusions

4.4.1 Discussion of Findings

Phase I- ESDA

Initial results indicated that the ESDA-Mylar film technique is not a reliable and effective collection method for buccal cell samples re-suspended in 1X PBS particularly for low cell amounts on porous substrates suggesting that the buccal cells did not transfer to the Mylar film after ESDA processing. When investigating the effect of the ESDA-Mylar film technique on a variety of biological fluids, results showed greater recovery from a non-porous glass substrate for blood, semen, and saliva than when these fluids were spotted on porous substrates. As expected, DNA from fingerprint samples was best recovered from copy paper exposed to ESDA processing than from non-porous substrates. Though results varied, this can likely be attributed to the use of three different donors and the nature of fingerprint deposition. The amount of DNA present in a given fingerprint can vary greatly between donors and throughout a day based on the sloughing of cells upon fingerprint deposition. Although variation is observed, it has been shown that useful DNA profiles (full or high partial) can be obtained by swabbing the Mylar film after ESDA processing.

Based on the distribution of the data collected in the time study, there did not appear to be any trends indicating that DNA profile recovery for the biological fluids decreased over time. This is not true for the fingerprint data, with the majority of the six month time points falling to under 40% profile recovery. Additionally, basic trends indicate that DNA profiles obtained from glass for blood, semen, and saliva were typically higher than those profiles obtained from cotton or paper substrates. However, due to the initial screening experimental design produced using Design of Experiments, there is not an appropriate distribution or number of replicates for the variables designated. The number of samples for each fluid, volume, and substrate are greatly varied, and thus no sound conclusion can be drawn from the time study data.

Evaluation of the ESDA-Mylar film technique to collect fingerprints from a variety of paper substrates indicated that full and partial STR profiles could be recovered. Amongst all three donors, a higher percent average profile was consistently associated with the cotton paper. Initially, for all types of paper substrates, directly cutting the paper substrate area where the fingerprints were applied produced results similar to the ESDA-Mylar film technique, suggesting the use of a non-destructive collection technique over a destructive one could be employed in this scenario. Further testing comparing three different collection techniques used to collect fingerprints from various paper substrates show that non-destructive dry swabbing outperformed both the Mylar-film technique and the direct cutting technique. However, the Mylar-film technique still outperformed direct cutting on most types of paper substrates, giving an overall higher percentage of full to high partial profiles obtained. Both ESDA paper evaluations follow the same trend in that higher average percent profiles were obtained from fingerprints on thicker paper substrates such as the cotton paper and resume paper. This may be why direct cutting was better for newspaper substrates and why, at times, it was seen that wet/dry swabbing the currency was comparable to the ESDA-Mylar film technique results.

These results indicate that the ESDA-Mylar film method can be utilized as a non-destructive collection technique to recover useful STR profiles from fingerprints on a variety of paper. The ESDA is generally used to analyze written documents which may contain fingerprints; however, the Mylar film is typically disposed of following this analysis. With optimization of the process, these results indicate that the Mylar film could be swabbed and processed for DNA results after ESDA analysis is performed. This would maximize the information obtained from a single piece of evidence, while preserving the integrity of the evidence for further processing.

Phase II- Adhesive Lifters

The results of buccal cell collection on various porous and non-porous substrates initially indicated that the Scenesafe FAST tape and BVDA Instant lifters were the top contenders, having recovered more full to high partial profiles across a variety of substrates. While Gellifters only performed well on glass in the initial buccal cell collection testing, they worked exceptionally well on all substrates when lifting blood, semen, saliva, and fingerprints in Task 2, likely due to optimizing the use of the lifter. Scenesafe FAST tape and BVDA Instant lifters performed equally well to Gellifters on all substrates for all fluids in Task 2 as well. Though Mikrosil performed well on certain substrates, the Mikrosil itself was difficult to work with in a timely fashion, with the pastes often hardening before effectively being applied to the sample area, and once dried, it was difficult to remove from most substrates in a non-destructive manner. Water-soluble tape was eliminated from further testing due to its poor performance across all substrates for all types of fluids and handling difficulties that were encountered during its use. Painted drywall was added as an additional non-porous substrate for testing, because many of the lifters were destructive to paper.

In an attempt to optimize the time study, DOE software was utilized to generate the experimental design in order to maximize the statistical power of the data while minimizing the number of replicates to be completed. This resulted in more of a screening experimental design which can be typical of DOE applications. As a result, the distribution of replicates for the time study variables did not produce statistically significant data. The number of samples for each fluid, volume, and substrate was greatly varied, and thus only weak conclusions could be drawn from the data. The data presented in Figures 24-26 is a combination of all three volumes spotted (5 µl, 25 µl, and 50 µl). Preliminary data analysis of the volumes independently resulted in certain gaps in the data indicative of a DOE screening experiment; i.e. no data points for a certain volume of a fluid on various substrates. Therefore, independent analysis of the volumes could not be completed, and evaluating a mixture of volumes of bodily fluids across time points would not provide any statistical significance. While the biological fluid data was only useful for trend analysis, the fingerprint time study did not indicate a reduction in the ability to obtain STR profiles from forensically relevant substrates with adhesive lifters. The only exception seen was the Gellifters on glass replicate set which displayed a wide reduction of percent profile recovery at the six month time point. Additional time studies should be evaluated with revised systematic sample set-up as well as elongated time points of one year or longer.

Additional testing using the three best performing lifters from earlier tasks (Scenesafe FAST™ tape, Gellifters, and BVDA Instant lifters) showed that Gellifters performed as well as or better than the other two lifters on all substrates for all fluids, with the most striking difference

occurring when lifting blood and semen from drywall. With the exception of blood and semen on cotton, which no lifter obtained useful profiles for, the use of Gellifters obtained full to high partial profiles no matter the substrate or fluid.

Adhesive lifters are a feasible method of non-destructive DNA collection. While many of the lifters were destructive to paper, no visible difference was observed before and after processing for any other substrates. The reduced cost and commercial availability of these lifters make them a favorable tool for law enforcement and crime scene investigators. Further studies can be performed to test the effectiveness of adhesive lifters on additional types of substrates.

Phase III- Alternative Swabs

Initial results indicated that paper was not an optimal substrate for dry swab collection of buccal cell samples, as no useable profiles were obtained from any swab. Generally, higher concentrations of spotted cells correlated to the generation of higher quality profiles when samples were collected with any type of swab. When comparing average profiles obtained from buccal cells spotted on various substrates, Hydraflock and Microfiber swabs outperformed the other swab types. Likewise, when lifting blood, semen, saliva, and fingerprints across all substrates, Hydraflock, Microfiber, and cotton swabs performed equally well. Although these general conclusions were made, the results seemed variable, perhaps due to the differing volumes of fluids spotted. In an effort to streamline the results, additional substrate testing was done where the same concentration and volume of fluid was spotted onto a non-porous glass substrate. The results confirmed Hydraflock as the best performing swab, with cotton and VWR Foam having slightly less successful but comparable results across all fluids on all substrates.

In an attempt to optimize the time study, DOE software was utilized to generate the sample set-up in order to maximize the statistical power of the data while minimizing the number of replicates to be completed. This resulted in more of a screening experimental design which can be typical of DOE applications. The number of samples for each fluid, volume, and substrate was greatly varied, and thus did not produce statistically significant data. The data presented in Figures 36-38 is a combination of all three volumes spotted (5 µl, 25 µl, and 50 µl). Preliminary data analysis of the volumes independently resulted in certain gaps in the data indicative of a DOE screening experiment;; i.e. no data points for a certain volume of a fluid on various substrates. Therefore, independent analysis of the volumes could not be completed, and evaluating a mixture of volumes of bodily fluids across time points would not provide any scientific significance. While the biological fluid data was only useful for trend analysis, the fingerprint time study did not indicate a reduction in the ability to obtain STR profiles from forensically relevant substrates with alternative swabs. The only exception was the cottons swabs on cotton substrate replicate set which displayed a gradual reduction of percent profile recovery over time. Additional time studies should be evaluated with revised systematic sample set-up as well as elongated time points of one year or longer.

Additional testing using the three best performing swabs from earlier tasks (Hydraflock, Microfiber, and VWR Foam) and cotton swabs for comparison purposes yielded promising results. As previously mentioned, the semen results including positive controls displayed much lower values than anticipated. The semen testing was repeated and the same low level results

were obtained, thus the results from the semen portion of this study could not be reliably analyzed. For the blood and fingerprint samples, all three alternative swabs outperformed the standard cotton swab. Out of the six substrate and fluid/print combinations/trials, the Microfiber swabs equaled or outperformed the cotton swabs in four out of six trials, while the VWR foam swabs and Hydraflock swabs outperformed cotton in five out of six trials each. Furthermore, both the Hydraflock and VWR Foam swabs equaled or outperformed the Microfiber swabs in four out of six trials each. The Hydraflock swabs performed at or equal the level of the VWR foam swabs in three out of six trials. These results, in combination with the Hydraflock's top performance on the glass substrate, indicated that it is the best performing alternative swab matrix. However, all three alternative swab matrices have displayed the potential to outperform standard cotton swabs on forensically relevant samples.

Certain alternative swabs have proven efficient in obtaining useful DNA profiles from a variety of substrates. Though the wet/dry swab technique is typically used to recover DNA from samples, it can be destructive to evidence and should not be used when integrity must be maintained. None of the alternative swabs tested here were destructive to any of the substrates and can potentially be an inexpensive and greater performing substitute to the commonly used cotton swab as a non-destructive collection technique. Further studies can be performed to test the effectiveness of alternative swabs on additional types of substrates.

Phase IV- TFD

Results using TFD to detect buccal cells and fingerprints on various paper substrates indicated virtually no difference between the two intensities at which the TFD was used. Excluding results obtained from the consistently outlying donor three, no difference was seen between any of the collection methods when collecting either buccal cells or fingerprints from copy paper, with all samples producing full to high partial profiles. Similar trends can be seen with collection from resume paper of both buccal cells and fingerprints, although with the buccal cell results it was observed that the use of TFD for detection and subsequent dry swabbing ultimately obtained higher profiles than when TFD was not used. Conversely, collection of fingerprints and buccal cells from magazine paper resulted in drastically lower profiles when TFD was used whereas full to high partial profiles were obtained when TFD was not used for detection. A plausible explanation for this is the heat applied to the magazine paper when using the TFD may make the ink on the magazine paper run, thus being collected along with the DNA when dry swabbed. Even after purification steps, trace amounts could be causing inhibition in downstream DNA processing. Another likely scenario is that due to the thin nature of magazine paper, it could become more heated than thicker paper substrates when processed with TFD, causing DNA degradation even before collection.

These initial experiments demonstrated that STR profiles can be recovered from latent prints placed on various paper substrates visualized with TFD and subsequently dry swabbed and, in some cases, this technique may produce better results than sole dry swabbing. Visualizing fingerprints on paper with the TFD can greatly reduce the sample area that is swabbed, as it concentrates the area of collection to any potential prints that are detected. Further studies need to be performed to determine the effect(s) that TFD has on recovery of an STR profile.

Summary

This study displayed the potential of the ESDA, TFD, adhesive evidence lifters, and alternate swab matrices to perform non-destructive DNA collections across a variety of forensically relevant substrates, biological fluids, and fingerprints. Table 1 summarizes the non-destructive processing technique recommendations for each substrate and biological sample type based on the complete results this study. In the cases of the TFD and the ESDA, the results have shown that these devices can aid in non-destructively locating fingerprints on paper substrates to enable analysts to target sampling areas and achieve high quality DNA profiles. The adhesive lifters and alternate swab matrices provide alternative methods to non-destructively collect sample and obtain DNA profiles at higher rates of success compared to standard cotton swab collections. The potential improvement in DNA results compared to standard methodologies and the non-destructive operational nature of the techniques, which allows for multiple forensic discipline examinations, make the non-destructive DNA collection techniques evaluated in this study a valuable forensic methodology.

4.4.2 Implications for Policy and Practice

Impact of the project on the criminal justice system

The ability to detect and collect biological samples from evidence items without causing structural damage continually proves to be a difficult challenge in the field of forensics. The damages inflicted to evidence items during normal sample collection will typically inhibit and/or prevent additional evaluations of the object. This research outlines methods to successfully detect, collect, and process individual biological samples from various evidence substrates without causing integral surface damage.

Having the ability to effectively detect and collect biological samples while allowing other specialists the opportunity to perform thorough forensic investigations of evidence items would greatly benefit the field. The additional information gained from items processed in this manner could convict or exonerate individuals associated with questioned documents, entry point surfaces, various clothing items, and other touch type evidence materials. Evidence processed in a non-destructive manner would also remain available for future evaluations which could prove pivotal to the outcome of a cold case investigation and/or criminal retrial. All of the tools and techniques suggested are relatively inexpensive or are already available in crime labs and could easily be incorporated into standard laboratory operating procedures.

Contributions to crime laboratories

The outlined techniques would not only benefit DNA analysts, but also questioned document examiners, trace evidence analysts, and forensic chemists. Forensic DNA analysts would have the ability to detect and collect biological materials from an item without damaging the structural integrity of evidence items and/or interfere with any subsequent examinations.

The newly developed techniques could allow DNA analysts access to evidence items prior to the performance of any other forensic type examination. This is a critical point in regards to touch type evidence items. It is possible that vital DNA evidence is lost when fingerprint, trace evidence (fibers, hairs, etc.), and/or chemical examinations are performed in advance of

biological inspections. By using these proposed techniques, the analysts examining the evidence post-biological collection would receive the processed items in a seemingly untouched state. For example, the use of an ESDA instrument to collect biological deposits from a handwritten document would allow a DNA analyst to collect biological material during the initial processing of the item and it would not interfere with any subsequent fingerprint development and/or handwriting analysis. The implementation of these proposed techniques would afford forensic scientists the ability gain more information from sensitive evidence type items by allowing multiple full-scale examinations to be performed.

4.4.3 Implications for Further Research

This study has demonstrated the potential of non-destructive DNA sampling techniques of the ESDA, alternative swab, adhesive evidence lifters, and the TFD. As alternatives to the destructive or standard non-destructive techniques (cotton swab), these techniques have displayed not only the ability to non-destructively obtain full DNA profiles from a variety of substrates, but in the case of the ESDA, to be able to non-destructively locate areas to sample on a large forensic substrate. Further research should be performed examining the ESDA as a viable non-destructive method of exploiting DNA from fingerprints from documents.

With the majority of document exploitation (DOMEX) techniques for fingerprints/DNA being of a destructive or document altering fashion (powders, ninhydrin, etc.), the viable non-document altering alternative for DNA exploitation is to take numerous, independent samplings from various sections of the document. The ESDA offers not only a non-destructive technique to visualize the areas to target when performing DNA collections, but also allows for the DNA sampling to take place on the mylar contact film; thus, no direct samplings are ever taken from the document, leaving it completely intact. Additional research on the ESDA's ability to aid in non-destructive processing of DNA from latent fingerprints on documents could be extremely valuable for intelligence, law enforcement, and crime laboratories.

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4.6 Dissemination of Research Findings

The poster entitled “Evaluation of ESDA and Other Non-Destructive DNA Collection Methods” was presented at the 2012 NIJ Conference in Arlington, Virginia.

The poster entitled “Non-Destructive DNA Collection from Handled Documents Using an Electrostatic Detection Device” was presented at the 2013 AAFS Conference in Washington, DC.

This study will also be published in peer-reviewed journals such as The Journal of Forensic Science and/or Forensic Science International.