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Abstract

Wearer DNA is the deposit of epithelial cells on clothing worn by an individual. Detection of the last individual to handle or wear an item is often an important and desirable determination in forensic science. The most commonly used collection methods for wearer DNA include swabbing and scraping. These often result in mixture profiles. Recently, adhesives have been introduced as a possible reliable method for the collection of biological evidence. The goal of the research was to compare the current collection methods of swabbing and scraping with a gel film called Gel-Pak '0' which shares similar properties with adhesives. Gel-Pak '0' has been previously studied in comparison to other adhesives for the collection of epithelial cells, and was shown to recover the top layer of loose particulate. This particulate had a tendency to be deposited by the individual who last came in contact with an item. Therefore, in comparison to the other two collection methods, Gel-Pak '0' was

hypothesized to recover single source profiles on clothing items from the most recent wearer. DNA analysis was performed on samples collected by the three methods from various clothing items including baseball hats, t-shirts, sweatpants, socks, and other items commonly submitted to crime labs for DNA analysis. The habitual wearer and the second/last wearer wore each item for a predetermined amount of time. The results of the research showed that Gel-Pak '0' recovered a similar number of CODIS (local and national) eligible profiles as swabbing. However, coupled with the fact that it is time consuming, costly, and cannot be used on all surfaces, Gel-Pak '0' was determined to not make for an effective collection method of the most recent wearer's DNA. Therefore, Gel-Pak '0' will not be considered for casework. Although Gel-Pak '0' will not be further used, the results did reveal some trends that may shed light on how DNA analysts may approach wearer DNA cases. Swabbing had a tendency to yield smaller amounts of DNA in comparison to scraping, but obtain DNA from the last wearer of the piece of clothing more effectively than the other two methods. Scraping had a tendency to yield greater quantities of DNA, recovering more DNA from the habitual wearer due to its invasive nature. Revealing individuals who last wore an item can be of great importance in forensic science, and therefore, further research with various adhesives and gel films could be vital for solving forensic investigations.

Keywords

DNA, forensic science

Comparing Wearer DNA Sample Collection Methods for the Recovery of Single Source Profiles

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Abstract

Wearer DNA is the deposit of epithelial cells on clothing worn by an individual. Detection of the last individual to handle or wear an item is often an important and desirable determination in forensic science. The most commonly used collection methods for wearer DNA include swabbing and scraping. These often result in mixture profiles. Recently, adhesives have been introduced as a possible reliable method for the collection of biological evidence. The goal of the research was to compare the current collection methods of swabbing and scraping with a gel film called Gel-Pak '0' which shares similar properties with adhesives. Gel-Pak '0' has been previously studied in comparison to other adhesives for the collection of epithelial cells, and was shown to recover the top layer of loose particulate. This particulate had a tendency to be deposited by the individual who last came in contact with an item. Therefore, in comparison to the other two collection methods, Gel-Pak '0' was

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1. Introduction

1.1 Forensic Science and Wearer DNA

The collection, analysis, and interpretation of DNA are imperative in forensic science for solving criminal

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investigations. DNA can be found on all items that are handled, worn, and touched by individuals. Determining who last wore an item of clothing is extremely valuable.

Wearer DNA is the deposit of DNA on clothing worn by an individual. This occurs when epithelial cells from skin come in contact with clothing. If more than one person wears at item, a DNA mixture may be detected. Often, the major contributor's profile is that of the habitual wearer. A minor contributor's profile may be detected from those who have borrowed or most recently worn the item of clothing (Taupin et al., 2011).

1.2 Current Collection Methods

The current collection methods used for the recovery of wearer DNA include swabbing, scraping, and tape lifting. Each method has a disadvantage. When swabbing an item of clothing, it is unknown how many cells are collected by observing the swab. Scraping is the most invasive method and can be destructive to the clothing. Tape lifting with various adhesives has a tendency to collect more particulate than desired and can inhibit PCR (Taupin et al., 2011). All methods may result in complicated mixtures which can make interpretation difficult or impossible.

1.3 Mixtures

Mixtures are the result of more than one person's DNA contributing to a sample and observed when more than two alleles are present at each locus. The detection of a profile belonging to the minor contributor is usually difficult compared to the major contributor (Butler, 2010). Some samples may

include more than two people's DNA, and therefore, have more than one minor contributor. These complex mixtures are often times uninterpretable in that the major and/or minor contributor(s) cannot be detected.

1.4 New Collection Method: Gel-Pak '0'

Revealing who wore an item of clothing during a crime can be extremely beneficial to solving a forensic investigation. Current collection methods and their tendency to recover complicated mixtures cannot always provide such information when clothing has been worn by multiple individuals. Therefore, a new method similar to adhesives and tends to only recover the last or most recent wearer's DNA raised interest.

Gel-Pak '0' and the recovery of the last wearer's DNA from clothing is the focus of this research. Gel-Pak '0' is made from a proprietary elastomeric material. The current use is for the safe transportation of small devices (Gel-Film®). There are various advantages of using Gel-Pak '0' instead of adhesives and the other methods. Gel-Pak '0' is less tacky than most, if not all, adhesives, and therefore, may collect fewer cells. In addition, Gel-Pak '0' is a gel film that does not inhibit PCR unlike adhesives, and is less invasive than the swabbing and scraping methods (Kelley-Primozic et al., 2010). Lastly, after collecting cells from clothing with Gel-Pak '0', the cells can be directly observed under a microscope and then easily removed from Gel-Pak '0' with a wet swab.

Previous research showed that Gel-Pak '0' had a tendency to recover the loosest layer of particulate and collected little extraneous particulate (Vigil et al., 2010). The purpose of this research is to compare the current collection methods of

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swabbing and scraping with Gel-Pak '0' to determine which method is best for the recovery of single source profiles of the last wearer. Based on previous research, it was hypothesized that Gel-Pak '0' would best recover the loosest layer of particulate on clothing, resulting in a single source profile of the last wearer. By recovering only the DNA from the last wearer, it was also hypothesized that Gel-Pak '0' would recover the least quantity of DNA in comparison to the other methods. In forensic investigations, these profiles are desired because they can be uploaded into the Combined DNA Index System (CODIS) to identify potential suspects.

2. Methods and Materials

2.1 Sample Selection and Sample Sets

Common clothing items submitted to crime labs as evidence were chosen for sampling. A total of twenty clothing items were sampled in four sets using all three methods except for sample set three in which only swabbing and Gel-Pak '0' methods were used. Sample set one consisted of three jackets. Sample set two consisted of two pairs of socks, one pair of sweatpants, one t-shirt, and two baseball hats. Sample set three consisted of one pair of sweatpants, one t-shirt, three baseball hats, and one glove. Lastly, sample set four consisted of one pair of sweatpants, three t-shirts, and one bandana.

2.2 Sample Preparation

Each item was worn by the habitual wearer of that item overnight or for several hours. Then, a second wearer, also referred to as the last wearer or most recent wearer, wore the

item for about one hour. The amount of time in which the items were worn by both wearers was varied in an attempt to best replicate items submitted to crime labs where wear time is not consistent or known.

2.3 Sample Collection

With the exception of sample set three, the area of each clothing item in which skin comes in contact the most was divided into three equal sections. Each section was assigned one of the collection methods: Gel-Pak '0', swabbing, and scraping.

After obtaining results from sample set one and two, it was found that scraping better recovered DNA from the habitual wearer and not the last wearer. Therefore, the scraping method was eliminated from sample set three, and only Gel-Pak '0' and swabbing samples were taken. The two sample sections were chosen by dividing the area of each item where skin comes in contact most frequently into two sections. However, it was later determined that scraping revealed valuable information pertaining how to best approach casework related to wearer DNA. Therefore, scrapings were collected from items in sample set four.

To collect cells with Gel-Pak '0', Gel-Pak '0' gel film was mounted on clean microscope slides. Gel-Pak '0' was then UV cross-linked at 250,000μJ for 12 minutes as a precautionary step to avoid contamination. The clear cover was taken off and Gel-Pak '0' was firmly pressed onto the section of the clothing. Gel-Pak '0' was then placed under a Leica compound microscope to confirm the collection of cells. A picture was taken at 100X magnification as seen in Figure 1. A wet Puritan® cotton swab was used to wipe and remove almost all the cells off Gel-Pak '0'.

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Another picture of Gel-Pak '0' was taken to confirm the removal of the cells.

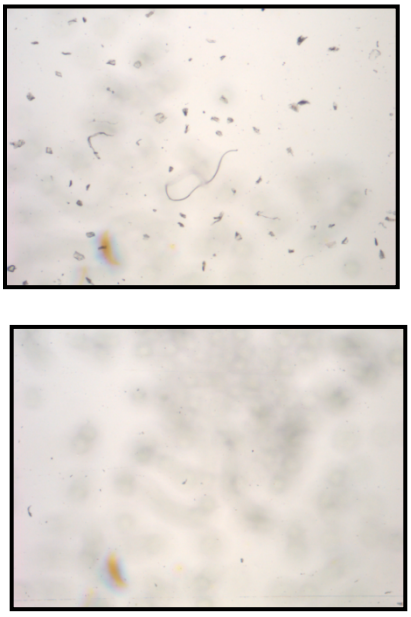

Figure 1:
Pictures of Gel-Pak '0' slide from t-shirt in sample set three taken at 100X magnification.

Top-Gel-Pak '0' before swabbed.

Bottom-Gel-Pak '0' after swabbed.

The swab used to remove the cells from Gel-Pak '0', the swab used directly on the clothing, and the scrapings collected using a sterile disposable scalpel were placed into clean UV cross-linked (250,000μJ for 12 minutes) labeled 2mL tubes. Therefore, there were three samples for each clothing item: one from Gel-Pak '0', one from swabbing, and one from scraping.

2.4 Organic DNA Extraction

Biological samples cannot be analyzed until DNA molecules have been isolated because many cellular proteins and other

materials within cells can inhibit PCR. For this reason, DNA molecules must be extracted from cells before further analysis. Organic DNA extraction separates proteins and other cellular materials from DNA molecules (Baker, 2010).

The "Organic DNA Extraction" procedure in the Santa Clara County Crime Laboratory Forensic Biology Procedures Manual (2011) was followed. Each sample, along with two extraction controls, was placed into a heat block set a 56ºC for a minimum of 6 hours for complete digestion. Two washes were performed using a total of 800 µL of Teknova TE buffer. An elution volume of 25 µL of Teknova TE buffer was added to the Microcon® YM-100 concentrators.

2.5 DNA Quantification

The "DNA Quantification" procedure in the Santa Clara County Crime Laboratory Forensic Biology Procedures Manual (2011) was followed. The Quantifiler® Duo Quantitation Kit (Applied Biosystems) was used to quantify the amount of DNA in ng/µl, with the aid of a ABI PRISM® 7500 instrument through the process of real-time PCR. The eight human DNA standards used ranged from concentrations of 50ng/µL to 0.023ng/µL and were ran in duplicate. Samples included the standards, a TE blank sample, two extraction controls, and all item samples.

The samples were placed in the MicroAmp® Optical 96-Well Reaction Plates were loaded into the ABI 7500 instrument and quantified using the HID Software version 1.1.

2.6 Amplification

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The "Autosomal STR amplification using the Identifiler® Plus kit" procedure in the Santa Clara County Crime Laboratory Forensic Biology Procedures Manual (2011) was followed.

A total of 15 STR loci (D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, D5S818, and FGA) and a gender marker (Amelogenin) were amplified using the Identifiler® Plus Amplification Kit (Applied Biosystems).

Each sample set included item samples, one extraction control, a positive amplification control, and a negative amplification control. The target DNA quantity was 0.7 to 0.8ng. AmpF*l*STR® Control DNA 9947A was used for the positive control sample and Teknova TE buffer was used for the negative control. Samples were placed in the programmed thermal cycler and set to run. The 9700 silver block Thermalcycler (Perkin Elmer) was set with the following parameters: initial incubation at 95°C for 11 minutes, step cycle (28 cycles) which included denaturation at 94°C for 20 seconds and annealing at 59°C for 3 minutes, final extension at 60°C for 10 minutes, and hold temperature at 4°C.

2.7 Capillary Electrophoresis

Capillary electrophoresis is a DNA fragment separation technique that separates DNA by size and charge. When the fluorescent dye-labeled DNA fragments pass through the capillary and reach the window, the fluorescent dyes are excited by the laser and emit a specific wavelength of light for each dye as seen in Figure 2 (Applied Biosystems, 2004). The five dyes used during capillary electrophoresis include 6-FAM™ (blue), VIC® (green), NED™ (yellow), PET® (red), and LIZ™

(orange). LIZ™ is used as the internal size standard. The 15 STRs and Amelogenin primers are labeled by one of the four dyes. There is a specific spectral range in which each dye fluoresces, making it possible to simultaneously detect many DNA fragments (Baker, 2010).

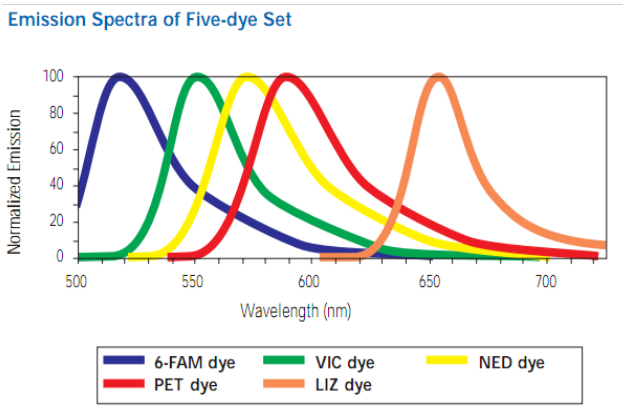

Figure 2: Above is the emission spectra of dyes utilized with the AmpF*l*STR® Identifiler® Plus Kit.

The "Running samples on the 310 and 3130 Genetic Analyzers" procedure in the Santa Clara County Crime Laboratory Forensic Biology Procedures Manual (2011) for the 310 Genetic Analyzer was followed.

Additional samples for each sample set included a negative control consisting of formamide and another sample containing AmpF*l*STR®Allelic Ladder for sizing. All samples were placed in a 48-well sample tray and loaded into an ABI PRISM® 310 instrument. The program used was the ABI PRISM® 310 Collection Software version 3.1.0. Item samples and extraction control samples were injected for 5 and 10 seconds. Injections

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which called more alleles were used for data collection. Negative amplification controls, positive amplification controls, formamide samples, and allelic ladder samples were injected for 5 seconds. Due to the low quantity of DNA recovered from the clothing items, item samples and extraction control samples were analyzed at an analytical threshold of 50 relative fluorescence units (RFU). Positive amplification controls, negative amplification controls, formamide samples, and allelic ladder samples were analyzed at an analytical threshold of 150 RFU.

GeneMapper® version 3.2 software was used to analyze the results and the GeneScribe Excel Workbook (Trowbridge, 2011) was used to organize the results.

3. Results

3.1 Mixture Profiles

Gel-Pak '0' collected DNA from both the habitual and last wearer from each item resulting in DNA mixtures. For a number of samples, other minor contributors were also detected. These minor contributors were found to be family members of the wearers. Swabbing and scraping methods also resulted in mixtures.

3.2 Quantity of Collected DNA

Gel-Pak '0' recovered quantities of DNA that ranged from 0.0338ng to 4.307ng. Swabbing recovered quantities of DNA that ranged from 0.1625ng to 4.924ng. Lastly, scraping recovered quantities of DNA that ranged from 0.101ng to 4.703ng. The large variability in ranges of collected DNA belonging to both the habitual wearer and most recent wearer

were due to many factors. Factors that may have contributed to this variability included the type of clothing material, the amount of time since the item had last been washed, the way in which wearers wore the clothing, what the wearers were doing in the clothing, and the amount of DNA that the wearers deposit.

Although Gel-Pak '0' did not recover single source profiles from the last or most recent wearer, Gel-Pak '0' had a tendency to recover the least quantity of total DNA. This shows that Gel-Pak '0' had a tendency to be less invasive than the swabbing and scraping methods. Figure 3 below also shows that scraping was most effective in recovering the greatest amount of total DNA given the number of items sampled with the scraping method.

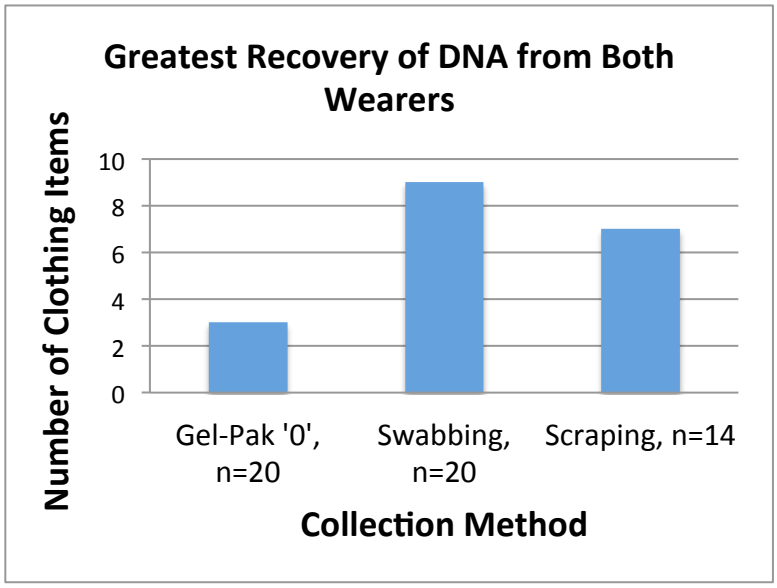

Figure 3: Gel-Pak '0' had a tendency to recover the least amount of total DNA while scraping had a tendency to recover the greatest amount of total DNA. Scrapings were not

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collected from items in sample set three, therefore, scrapings were collected from only 14 out of the 20 items sampled.

3.3 Quality of Collected DNA

Results from sample sets one and two showed that scraping has a tendency to recover more of the habitual wearer's DNA than the last wearer's DNA. It was then decided to only take Gel-Pak '0' and swabbing samples from items within sample set three. Scraping was eliminated because the purpose of the research was to recover DNA from the last wearer. However, scrapings were collected for sample set four after determining that the scraping results help reveal how a criminalist may best casework in which habitual wearer DNA must be recovered.

As seen in Figure 4, Gel-Pak '0' and swabbing recovered the greatest amount of the last wearer's DNA from the same number of items.

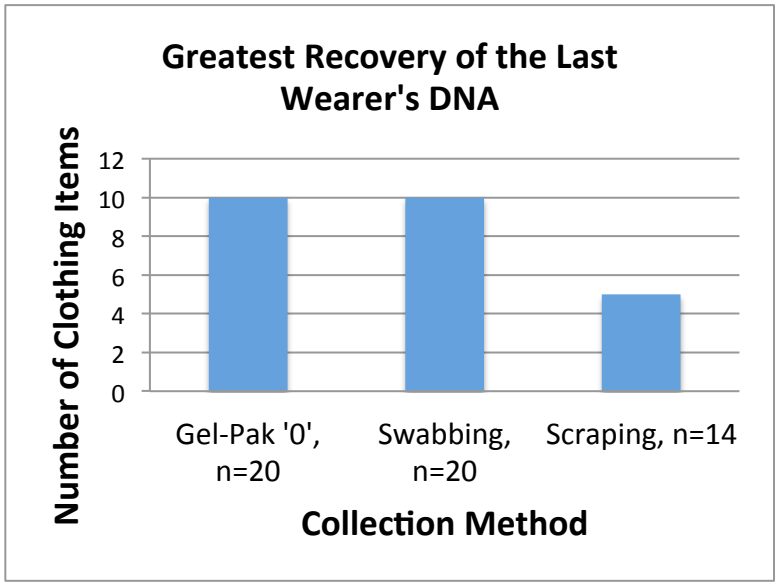

Figure 4 (previous page): Gel-Pak '0' and swabbing recovered the most DNA from the last wearer from 10 items while scraping only recovered the most DNA from the last wearer from 5 items. Note: For some items, multiple methods recovered equal proportions of DNA from the last wearer so, they were counted more than once.

3.4 CODIS Eligible Profiles

Low levels of DNA associated with wearer DNA often resulted in peak height imbalance and stochastic effects. This often times resulted in uninterpretable profiles from both the habitual and last wearers. An interpretable CODIS profile is one that can be searched and will hit to the offender who left the DNA if the matching offender is in the database, while not hitting to multiple offenders by chance alone (Barloewen, 2011). Seven interpretable CODIS core loci allows a profile to be eligible for the local database while ten interpretable CODIS core loci allows a profile to be eligible for the national database. Having profiles for the local database is very important since most repeat offenders tend to not move, and commit crimes in the same general area.

The results of the research showed that Gel-Pak '0' recovered a similar number of CODIS (local and national) eligible profiles as swabbing from both the habitual and most recent wearer. Swabbing resulted in 25 interpretable profiles compared to the 23 recovered with Gel-Pak '0'. In addition, swabbing resulted 4 more CODIS (local and national) eligible profiles belonging to the most recent wearer compared to Gel-Pak '0'. Lastly, scraping resulted in more interpretable profiles from the habitual wearer than the last wearer as seen in Table 1.

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At Least 7 Interpretable CODIS Core Loci (local)		At Least 10 Interpretable CODIS Core Loci (national)	
1st / habitual wearer	2nd / most recent wearer	1st / habitual wearer	2nd / most recent wearer
Gel-Pak '0' 10	Gel-Pak '0' 4	Gel-Pak '0' 5	Gel-Pak '0' 4
Swabbing 9	Swabbing 6	Swabbing 4	Swabbing 6
Scraping 6*	Scraping 2*	Scraping 5*	Scraping 2*
Total: 25	Total: 12	Total: 14	Total: 12
Total number of profiles from both wearers: 37		Total number of profiles from both wearers: 26	

Table 1: Gel-Pak '0' and swabbing recovered more profiles from the last wearer in comparison to scraping. Swabbing resulted in the most overall CODIS eligible profiles and the greatest number of CODIS eligible profiles belonging to the most recent wearer.

*As previously mentioned, scrapings were collected from only 14 out of the 20 items sampled.

4. Discussion

The results proved part of the hypothesis to hold true. While Gel-Pak '0' recovered the least amount of total DNA compared to the swabbing and scraping methods, it did not succeed in obtaining single source profiles from the last wearer.

Gel-Pak '0' was not as selective as expected. Although Gel-Pak '0' is a low tack adhesive and collects the loose cells that are not imbedded within the material of clothing, DNA from the

habitual wearer was also collected. This suggests that when an individual other than the habitual wearer, wears an item of clothing, a full layer of their DNA is not deposited on top of the habitual wearer's DNA. Therefore, the loosest layer of particulate likely consists of DNA from both the habitual wearer and most recent wearer, resulting in a mixture profile.

In comparison to Gel-Pak '0', swabbing appears to be the most reliable and convenient collection method for recovering the last wearer's DNA for a number of reasons. One, swabbing tended to recover the greatest amount of total DNA. Two, the method resulted in slightly more CODIS eligible profiles belonging to the most recent wearer. Three, swabbing is more cost effective. The major materials needed for swabbing include swabs and sterile scalpels while the major materials needed for Gel-Pak '0' include the gel film, microscope slides, a compound microscope (optional), swabs, and sterile scalpels. Four, swabbing is less time consuming than sampling an item with Gel-Pak '0' in which the gel film is cut, the film is then mounted on a microscope slide, the cells are observed under a microscope (optional), and the cells are removed with a wet swab. Five, since Gel-Pak '0' is mounted on a microscope, not all materials and items such as baseball hats are not easily sampled. For these reasons, Gel-Pak '0' will not be considered for casework.

Although Gel-Pak '0' will not be further used, the results of the research did reveal some trends that may shed light on how DNA analysts may best approach their casework involving wearer DNA. Results show that swabbing should be highly considered when attempting to identify the most recent wearer. Also, scraping had a tendency to recovery more of the habitual wearer's DNA. This reveals that scraping may be the best method for detecting the habitual wearer.

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Wearer DNA is vastly varied and unpredictable. There are many variables that are difficult to control for including how individuals wear items of clothing, the wearer's level of physical activity, and the degree in which individuals shed their DNA.

Future research aimed at recovering the last wearer's DNA should include more data by sampling from a large number of clothing items and better control of the deposit of DNA. In addition, various other adhesives and gel films not mounted on microscope slides could be compared to the swabbing method. Research pertaining to wearer DNA and using techniques to reveal those who last wore an item of clothing can be a crucial step in solving forensic investigations.

5. Acknowledgments

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