

Touch DNA on Fabrics and Restraints

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Abstract

This study aims to discuss the effectiveness of DiamondTM Nucleic Acid Dye for visualization of DNA on surfaces. Touch DNA or touch deposits are important biological evidence for many types of crimes including criminal possession of weapons, robbery, and property crime as well as significant violent crimes against people. Although there is scientific research on touch DNA, there is still little literature regarding touch DNA deposition, adherence, and DNA recovery from fabrics and restraints. This article examines the visualization process of DNA deposits and the collection for downstream DNA processing by using a fluorescent dye called DiamondTM Nucleic Acid Dye that binds to the DNA and RNA molecules in the fingermark. DiamondTM Nucleic Acid Dye has an excitation maximum of 495 nm and a yellow-green emission maximum of 558 nm. DiamondTM Nucleic Acid Dye was successful to visualize DNA deposits on all types of restraints used in this study including nylon rope, cotton twine, polyester-cotton blend fabric, duct tape and plastic zip-ties. In addition, this study examines fabric composition and the ability to release DNA in a DNA quantitation experiment. Cotton and polyester fabric were found to yield more DNA than nylon fabric in an experiment using purified DNA showing that substrate composition can affect DNA recovery.

Keywords

DiamondTM Nucleic Acid Dye, Touch DNA, Microscopy, Fabric, DNA Visualization, DNA Yield

1. Introduction

Touch DNA refers to the invisible biological materials deposited when an individual touches a surface. Touch DNA is also called trace DNA and is comprised of water, salts, oils and nucleated and anucleate epithelial cells from the skin surface [1]. Free DNA molecules may also be present due to the breakdown of the

nuclear and cell membranes during the natural cycle of programmed cell death and rejuvenation of the epithelium. Prior to the development of nucleic acid visualization techniques such as Diamond™ Nucleic Acid Dye, confirmation of DNA before forensic evidence collection was limited and instead a process known as blind swabbing was commonly used. Blind swabbing is a technique where wet-dry swabs are used to gently collect from contact points on an object. The use of Diamond™ Nucleic Acid Dye to visualize latent DNA is beneficial to scientists as they can visually confirm the presence of DNA prior to extraction and quantification to produce DNA profiles [2]-[6]. Non-porous surfaces, such as plastics, metals, and polymers, frequently appear in forensic investigations as evidence. Fluorescent dyes such as Diamond™ Nucleic Acid Dye can bind to nucleic acids to visualize latent DNA on these non-porous materials [7]. Studies on Diamond™ Nucleic Acid Dye have shown that it is capable of successfully visualizing DNA deposits on a variety of substrates, including metal, glass, aluminum, acrylic, polypropylene, and other polymers [8]-[10]. Despite the effectiveness of Diamond™ Nucleic Acid Dye research on a range of non-porous materials, there is limited research regarding its effectiveness on porous fabrics [1] [11]. A limited number of research studies have been published examining the use of the forensic technique of vacuum metal deposition [12]-[14] and Nile Red dye fluorescence in grab studies on fabrics to enhance fingerprints [15].

This study focuses on the effect of fabric composition (100% polyester, 100% nylon, 100% cotton) on DNA recovery and, separately, the ability to visualize fingerprint DNA with Diamond™ Nucleic Acid Dye on other surfaces. Fibers are classified as natural or synthetic. Natural fiber examples are cotton, wool, silk or linen. Synthetic fibers are nylon, rayon, polyester or acrylic. Natural fibers are more absorbent than most synthetic fiber fabrics and therefore, there may be differences in DNA recovery based on absorbency and manufacturing characteristics. Some fibers that are woven are satin, denim and twill. Knitted fabrics are commonly composed of jersey, rib knit or fleece. Specialty fabrics include bamboo, velvet and chiffon. Due to the enormous variety of fabrics of various weaves and colors, it is challenging to cross-compare many of the published research studies for DNA recovery. In addition, many of the evidence collection methods, DNA sources, DNA extraction kits and evaluation methods are not comparable, making it more challenging in assessing which types of methods to use in the DNA typing process for biological evidence on fabrics. Fabrics are typically processed by cutting, intuitive swabbing, tape-lifting, vacuuming or scraping of the fibers and many studies have been performed to test the efficacy of methods [16]-[18]. In this study, we used a cutting method for DNA collection to compare DNA recovery of purified DNA from three different types of fabric. Statistical analysis was used to determine significance of DNA recovery for surfaces. In addition, evidence handling experiments were performed and analyzed for improvement of DNA collection and yield by using Diamond™ Nucleic Acid Dye enhancement.

Research studies regarding fabrics are often focused on the fabric as the substrate surface or focused on the construction of the swabs used to collect the bio-

logical sample from the surface. Fabric or fiber constructed swabs retain DNA and determining the best method for extraction has been reviewed extensively [18]-[20]. Experiments adjusting time, temperature, and agitation for blood and semen samples on cotton and nylon swabs indicated that the most significant factor was time for the extractions which were recommended by the QIAmp DNA Investigator extraction kit manufacturer as “at least one hour” but DNA yield improved significantly for the three- and eighteen-hour incubation times [21].

Fabrics as substrates have been tested with blood and semen for samples stored up to three months. These body fluids were tested on black and white cotton and denim and leather fabrics. DNA was recovered from all surfaces for both body fluids, with a significant difference for time for blood samples that were deposited for 1 day, 30 days and 90 days. A different pattern was observed for the semen samples where time was not a significant factor, but the black colored fabrics resulted in reduced DNA yields [22]. DNA recovery has been tested for recovery rates from the side of deposit or the opposite underside of the fabric for blood, semen and saliva samples. The fabrics tested were viscose and polyester combined, cotton, polyester and denim [22]. Cotton is a natural fiber plant, and denim is derived as a twill weave of cotton fiber known for its durability. Viscose or rayon is classified as a semi-synthetic fiber made of regenerated plant cellulose but the process of manufacturing changes its chemical properties sufficiently that it is not considered a natural fiber. Polyester is a petroleum-based synthetic fiber that has durability and is wrinkle-free. All fabric types successfully yielded DNA and DNA profiles when collected using viscose swabs and a wet-dry swabbing method from the side of body fluid deposit (100% recovery) and there was 90% DNA recovery for the underside of the fabrics [22].

Fabric can also be useful as a medium for DNA transfer events. In one study, synthetic shirt cuffs were found to be a reasonable venue for DNA transfer, and this occurred more frequently than with natural fiber cotton or leather [23]. Everyday activities can result in accumulation of self-DNA in front of shirts and an accumulation of environmental DNA on the back of the shirts for an average of an 8-fold increase of DNA quantity accumulated throughout the day. DNA transfer events are common in crime scene scenarios such as domestic living arrangements, shared office space, commuter traffic in vehicles or trains where many DNA profiles can be detected on evidentiary items but may have no relevance to the crime itself. DNA transfer has also been documented in laundry washes and is persistent in water [24] [25].

2. Materials and Methods

2.1. DNA Recovery Experiment

Fabric Preparation. Fabrics were purchased as labeled remnants from JoAnn Fabrics (Danbury, CT). All items were UV sterilized using a Stratagene UV Cross-linker (Agilent Technologies, Santa Clara, CA) prior to use. To examine the effect of dye on DNA recovery, we applied Diamond™ Nucleic Acid Dye (Promega

Corp., Madison, WI) to each fabric surface and used microscopy and imaging techniques to sample the fabric to capture the details of cell adherence in the handling experiment or DNA in solution prior to DNA recovery and in comparison, to undyed fabric.

Purified DNA Recovery from Fabrics. AmpFlSTR DNA Control 007 human male genomic DNA (Thermo Fisher Scientific, Waltham, MA) was applied to 100% nylon, 100% polyester and 100% cotton fabrics. One microliter of purified DNA (2 ng/ul) was applied by micropipette to the surface of the fabric and air dried. After drying, 5 × 5 mm samples were cut, and DNA was extracted with a Quick-DNA/RNA™ Microprep Plus kit (Zymo Research, Irvine, CA) using manufacturer instructions. DNA extracts were quantified using the Quantifiler™ Trio DNA Quantification Kit (Thermo Fisher Scientific, Waltham, MA) per manufacturer instructions. Samples were tested as duplicates for replication. This experiment was repeated with Diamond™ Nucleic Acid Dye in addition to comparing DNA yield to untreated (without dye) samples. Microsoft Excel software was used to calculate statistical significance using an independent two-sample equal variance test with a 95% confidence interval (Student's t-test, n = 8 per treatment) to compare differences between fabric type.

Preparation of Diamond™ Nucleic Acid Dye. 20X Diamond™ Nucleic Acid Dye solution was prepared after storage at -20°C by thawing, diluting in ethanol (nonporous surfaces) or sterile water (porous surfaces) for the spray misting on the respective surfaces. Unused solution was properly stored in a freezer without any light for up to 2 weeks, thawed and reused successfully.

2.2. Evidence Handling Experiment

Sample Preparation. Prior to sample collection, the surfaces and restraint samples were prepared by UV crosslinking in a SpectroLinker XL-1500 UV Crosslinker (Spectronics Corp, Melville, NY) to remove any environmental DNA contamination. Full size pool noodles were cut to create 5" long pieces for three male participants to use as a base to apply restraints in the tying experiment. The examiner was female, so the selection criterion of male donors was designed to detect any differences in total versus male DNA quantities if contamination were present. Individuals were asked to wash hands and wear latex gloves for 15 minutes prior to handling each surface to provide some uniformity in DNA application although differences in manner of tying and shedder status of the donor were uncontrolled variables. The collection order for each sample type was randomized for each donor by method. Dyed samples were processed for each donor followed by the blind swabbing method samples for the same donor. Each donor's samples by method were processed on different days. The nonporous restraints were nylon rope, duct tape and plastic zip-ties. The porous restraints were cotton twine and fabric (68% polyester and 32% cotton blend). This variety of restraint surfaces were used to compare with the effectiveness of Diamond™ Nucleic Acid Dye to the selected polyester-cotton blend fabric. The same donors participated in both types of processing methods.

Evidence Processing. One of each sample material type was processed with two types of evidence processing methods: blind swabbing and Diamond™ Nucleic Acid Dye enhancement for each donor resulting in ten total samples per donor. In total, with three donors, thirty samples were processed. Evidence was stored at 25°C and processed after all the donors had participated. The main steps in evidence processing included swabbing using a wet-dry combined swab method, DNA extraction and quantification. Blind swabbing occurred using Copan FLOQSwabs (nylon swabs), following a wet/dry swabbing method. DNA extraction was performed using the manufacturer instructions for the PureLink Genomic DNA Mini Kit (Thermo Fisher Scientific, Waltham, MA). DNA quantification was performed by manufacturer instructions for the Quantifiler™ Trio DNA Quantification Kit. Results were examined for statistical difference between methods using an independent two-sample equal variance test with a 95% confidence interval (Student's t-test, $n = 15$ per method). In addition, this analysis was also performed for each type of restraint (Student's t-test, $n = 3$ per restraint type).

Diamond™ Nucleic Acid Dye. Evidence was unsealed and documented through photography, before dye was applied to the evidence. To create an even application of dye on the surface, a solution of dye with either ethanol or water, dependent on the porosity of the material, was added to a small spray bottle. The solution was 9.5 mL of ethanol (nonporous surfaces) or water (porous surfaces), combined with 50 uL of 20X Diamond™ Nucleic Acid Dye. The ethanol was 200 proof, molecular biology grade, absolute ethanol (Fisher Scientific, Waltham, MA). Similarly, the water used for the solution was molecular biology grade to prevent DNA degradation. For safety, dye was always applied in a large hood for ventilation, with a plastic guard in front of the evidence, to catch overspray. To reduce variability, dye was sprayed 5 inches from the surface. After application, the evidence was set within the hood to allow the dye to fully dry on the item before it was removed for visualization. The dye and DNA were documented using UV light to amplify fluorescence and then visualized under a stereomicroscope set to 40X magnification. The image could easily be photographed with a standard iPhone held to the eyepiece of the stereomicroscope. The evidence item was fully examined. After visualization, swabs were collected using the same swabbing method as described above.

3. Results

3.1. DNA Recovery Experiment

In this experiment, polyester fabric yielded the greatest amount of DNA recovered after DNA extraction (**Figure 1**). This is consistent with scientific literature that up to 50% of DNA on cotton swabs is not recovered in the first extraction step [21]. However, the polyester yield was variable between the replicates indicating some variability in yield recovery from sample to sample. This also could reflect slight variability in pipetting in application of DNA to the fabric surface. The addition of Diamond™ Nucleic Acid Dye reduced the yield of DNA by 50% or more in our

study; however, PCR amplification was not inhibited as evidenced by the quantitation results which require a PCR step (Figure 2). This agrees with published literature regarding the use of Diamond™ Nucleic Acid Dye as an enhancement reagent that does not affect downstream STR analysis. However, the dye enhancement method may need to be carefully considered when quantities are expected to be very low. Although the DNA yields were different with and without dye treatment, there was no statistical significance using the Student's t-test (p-value of 0.4113).

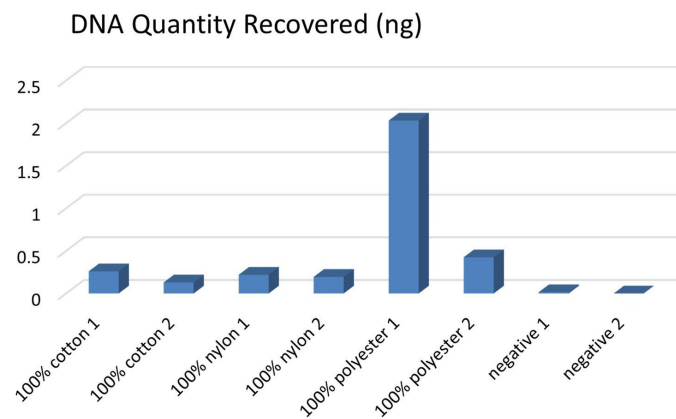


Figure 1. DNA recovery of purified DNA from fabrics from 5 × 5 mm sample. Samples 1 - 2 (100% cotton), samples 3 - 4 (100% nylon) and samples 5 - 6 (100% polyester) and samples 7 - 8 (negative controls). DNA was recovered from all sample types, but yield was greatest from polyester.

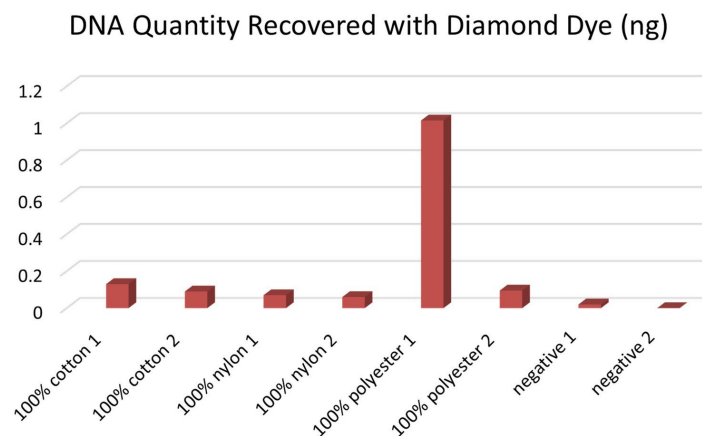


Figure 2. DNA recovery of purified DNA from fabrics from 5 × 5 mm sample with Diamond Dye applied. Samples 1 - 2 (100% cotton), samples 3 - 4 (100% nylon) and samples 5 - 6 (100% polyester) and samples 7 - 8 (negative controls). DNA was recovered from all sample types, but yield was greatest from polyester.

3.2. Evidence Handling Experiment

It was hypothesized that there would be an increase in the quantity of the DNA yield, when using dye to visualize DNA before collection. However, the results show no statistically significant difference between the mean values for the DNA quantity for the Diamond™ Nucleic Acid Dye and blind swabbing methods (p-

value of 0.3716) using a Student's t-test when all data for donors and sample type were combined and the mean values compared. Results were examined for statistical difference using an independent two-sample equal variance test (Student's t-test, $n = 15$ per method). No significantly different mean values ($n = 3$) were obtained based on type of restraint used in the handling process when the three donor DNA quantity values were combined and compared. The sample size was small but method (blind-swabbing) to method (dye) comparison and Student's t-test resulted in p-values of 0.0798 for fabric, 0.7434 for twine, 0.3855 for rope, 0.3280 for duct tape and 0.3840 for zip tie. The fabric method quantity values though not significant were closest to the p-value of 0.05 for significance between the two methods. If the sample size and number of donors had been larger, it is possible a difference could have been observed but more research would need to be done to verify. Diamond™ Nucleic Acid Dye is a useful visualization tool for determining the location of where to swab on a touched surface. The evidence handling experiment was performed in the blind such that the tied surface was not viewed, and the examiner used the dye to locate the handled areas independently. This simulates crime scene evidence that arrives at the forensic laboratory without prior knowledge of how the item was used (**Figure 3** and **Figure 4**).

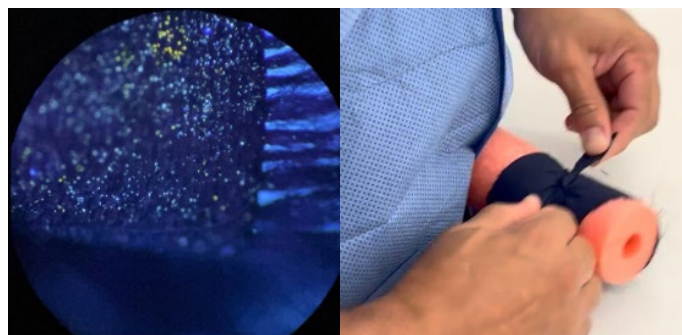


Figure 3. Touched porous fabric surface enhanced with Diamond™ Nucleic Acid Dye under 40X magnification (left) and fabric handling showing the fabric being tied around a support prior to dye enhancement (right). The pale green areas of the magnified dyed fabric are cell nuclei that contain DNA useful for forensic analysis.

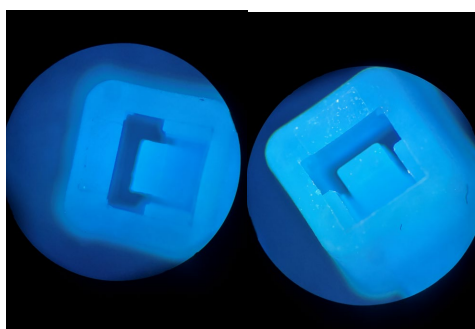


Figure 4. Negative control nonporous zip-tie without fingermark (left); zip-tie with fingermark and Diamond™ Nucleic Acid Dye applied and viewed under 40X magnification. Diamond™ Nucleic Acid Dye defines the touched surface area by enhancing the fluorescent DNA containing cells.

4. Discussion

DNA recovery from surfaces can be performed using wet-dry swabbing, micro-vacuuming, scraping, and adhesive tape lifts. Studies have shown that in most cases, sufficient DNA can be recovered from most fabric fiber types. The recovery of high quantity and high-quality DNA from fabrics is important, otherwise, no DNA profile or partial DNA profiles with a high degree of allele drop out might occur making the data interpretation challenging. There is currently no standard best practice for recovery of touch DNA deposits for specified surfaces or for specific fiber types in most forensic laboratories. Evidence collection protocols state that a variety of methods can be used and it is up to analyst discretion to select an appropriate method.

DNA transfer events can also complicate scene interpretation as the context of the DNA may be in question. In a study by Henry and Zeiger (2024), shirt cuffs were sampled with SceneSafe Fast™ minitapes (SceneSafe, UK) and stored at room temperature before DNA processing [23]. This study detected more non-self than self-DNA in 15.8% of samples, with four samples being suitable for database search. This finding illustrates the possibility of transfer of non-self-DNA to a potential crime scene via clothing transfer and could possibly implicate an individual. The possibility of DNA transfer events via handling or as DNA collected from the environment on restraints is also an issue for casework analysts to resolve when evaluating the contextual information of the case.

Casework in forensic biology is quite variable and there are many factors that contribute to the successful recovery of DNA from items constructed of fabric or other materials used as restraints. These factors include exposure to the environment, the initial quantity and quality of the DNA deposited, time since deposit, type of fabric or surface, presence of PCR inhibitors and choice of DNA recovery method. Common types of evidence that are fabric include clothing such as shirts, pants, socks, shoes, belts, scarves, baseball caps, masks, and gloves as well as purses, backpacks, luggage, furniture and vehicle upholstery and carpets. Since these items are so common and routinely processed for DNA, a better understanding of the placement of DNA is useful using enhancement dyes such as Diamond™ Nucleic Acid Dye to capture the greatest quantity of DNA possible on the swab during the evidence collection step.

Fabrics are an integral part of many types of forensic cases including homicides, sexual assaults, kidnappings and property crimes. DNA deposited during a crime is often invisible and having nucleic acid binding dyes fluoresce is an aid to processing fabrics and other restraints that have large surface areas but limited and finite contact points that can provide critical information regarding candidate perpetrators and victims. The types of restraints that we used were selected for their difficulty in evidence processing and included lengths of rope, twine, fabric strips, duct tape or conjoined zip-ties. One limitation of this study is that it did not control how participants handled the material to restrain the base. It was expected that participants would use the restraints in a fairly similar manner. How-

ever, it was determined from the analysis of video data during the collection step that there was substantial variability in how the participants used restraint materials, therefore, the contact points were different. In future studies, controlling the way that restraint materials were applied to the base may create more uniformity in the data. In addition, gaps in many published fabric research studies involving DNA include a lack of consistent analysis of weave and manufacturing patterns for fabric surface textures and the impact on DNA adherence and recovery, a lack of controlled environmental studies examining independent variables for temperature and humidity exposure prior to collection as well as for during storage, and a lack of a comprehensive survey of DNA data from fabric types used in actual forensic casework.

Overall, Diamond™ Nucleic Acid Dye was an effective visualization tool for highlighting DNA on various touched sample materials, with a variety of absorbency and porosity, that could all be used as restraint evidence in real forensic casework. Diamond™ Nucleic Acid Dye has been shown to be useful on a variety of surfaces but optimization of evidence collection from nonporous fabric surfaces is still being researched to maximize DNA recovery and yield. The enormous variety of fabrics used in domestic living spaces, offices, vehicles and for clothing, luggage, footwear, masks, hats and gloves suggest that there may ultimately be useful but different approaches and recommendations for various fabrics and restraints based on porosity, absorbency, color, weave and fiber type to optimize the evidence collection process.

Author Contributions

The co-authors contributed equally to the manuscript. E. Gallardo performed the evidence handling research and wrote portions of the text. H. Miller Coyle wrote portions of the text, performed the DNA recovery research, reviewed the data, and edited the manuscript for publication.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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