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1. SUMMARY OF THE PROJECT

1.1 GOALS AND OBJECTIVES

The use of DNA evidence gathered from crime scenes to identify perpetrators (DNA profiling) is well established in the forensics community.[1] As DNA detection methods have increased in sensitivity and sophistication over the past 20 years, it has become possible to obtain DNA profiles from samples with very low DNA concentrations, including samples collected from touched objects (i.e. "touch DNA").[2] Despite the growing importance of touch DNA evidence in criminal investigations, there are many uncertainties regarding the basic properties of touch DNA. For example, it remains unclear what biological material is actually transferred by touch. Various studies have suggested that DNA may originate from shed keratinocytes from the outer layer of an individual's hand, nucleated epithelial cells from other fluids or body parts in contact with the hand (e.g., saliva, eyes, nasal fluids), or from cell-free DNA reserves either endogenous to the hands (e.g., sweat) or transferred onto the hands from aforementioned fluids.[3,4,5] However, the relative contributions of each component to the overall sample and to its relevant properties remain uncertain and likely vary by individual.

Because touch DNA deposited on a surface is not visible to the eye, it is difficult to link these samples to a crime scene with the same confidence as one might a DNA sample isolated from a visible blood stain. In order to evaluate the quality of touch DNA evidence, it is therefore critically important to understand the different factors affecting how a DNA sample could have arrived at a particular crime scene. These factors include (1) the background levels of human DNA in the environment, (2) the dynamics by which DNA may be transferred to a surface by touch or other relevant activities, and (3) the persistence of DNA samples under different environmental conditions.

A handful of studies have investigated how factors such as time, surface type, and environmental conditions (e.g., temperature, humidity, ultraviolet light) affect the quantity and stability of touch DNA (TABLE 1). These studies generally focus on a specific scenario (i.e. a particular surface type or simulated, crime-related activity) and involve relatively small sample sizes with limited or no statistical analysis. The focused scope of these studies combined with significant differences in experimental design between studies makes it difficult to draw generalizable conclusions. Another factor confounding quantitative studies of touch DNA persistence is the fact that the amount of DNA deposited by touch varies significantly between donors.[6] Several studies have circumvented this challenge by depositing controlled amounts of purified DNA or cell suspensions on surfaces. However, it is unclear whether the stability of actual touch DNA samples is similar to that of purified DNA or cell suspensions.

There is therefore a need for comprehensive well-controlled studies that provide practical data about the basic properties of touch DNA evidence in real-world environments to understand how long a touch DNA sample would be expected to persist in a particular environment. The current study was designed to address this need.

Touch DNA collected from donors and commercially purchased control DNA were both deposited on several representative surface types, and the impacts of temperature, humidity, ultraviolet light irradiation, and exposure time was determined on the DNA quantity and quality. DNA persistence was assessed by several metrics including (1) total DNA concentrations as measured by quantitative polymerase

chain reaction (qPCR), (2) degradation index as determined by the ability to amplify longer DNA targets, and (3) suitability for DNA typing analysis by short tandem repeat (STR).

TABLE 1. Summary of Literature on Persistence of Touch DNA

Category	Reference	Source(s) of DNA	Objects Tested	Number of Samples	Conditions tested
Sampling Time	Bille et al. 2009[7]	Controlled deposition of buccal cell suspension	Galvanized steel PVC	3 samples per condition	Time: up to 3 months
	Matte et al. 2012[8]	Controlled activity by volunteer (scratching)	Fingernails	60 volunteers 3 samples from each participant	Time: up to 8 hours
	Meakin et al. 2015[9]	Controlled use by volunteer	Knives	3 replicates per conditions	Time: up to 1 week
	Meakin et al. 2017[10]	Controlled handling by volunteer	Knives	4 volunteers, 3 knives per scenario	Time: up to 1 week
	Sethi et al. 2013[11]	Controlled activity by volunteer (grab and struggle model)	Cotton, Polyester Cotton/Polyester blend	4 volunteers 3 fabrics per conditions	Time: up to 7 days
Sampling Time Surface Type	Ostojic et al. 2017[12]	Controlled use by volunteer	Glass, Metal, Paper, Plastic	6 volunteers	Time: up to 40 days Surface type
Controlled Irradiation	Monson et al. 2018[13]	Fingerprints, Blood stains, Hair (no touch DNA)	Aluminum Paper	1 volunteer, 6 fingerprints per scenario 3 volunteers, 3 stains per scenario 3 volunteers, 5 hairs per scenario	Irradiation: alpha, beta, gamma radiation up to 9000 kGy
	Withrow et al. 2003[14]	Controlled licking by volunteer	Envelopes	4 volunteers	Irradiation: 0, 29.3, and 51.6 kGy
Controlled Temperature	Klein et al. 2017[15]	Controlled deposition of blood	Typical homes or garden shed objects (hammer, screwdriver, tiles, procelain, pipe, shoe, glass bottle)	11 objects	Temperature: 300°C,700°C,1000°C
	Kulstein et al. 2018[16]	Controlled deposition of seminal fluid	Cotton Synthetic fabric	2 or 4 replicates per condition	Washing temperature and conditions Sample type
Sampling Time & Controlled Temperature	Dissing et al. 2010[17]	Controlled deposition of blood	Paper	Not specified	Time: up to 12 months Temperature: 35°C, 45°C, 55°C, 65°C Humidity: 50%, 80%, 93%, 100%
	Harteveld et al. 2013[18]	Controlled mixture deposition of saliva and blood	Plastic tube	2 replicates per condition	Time: up to 20 days Temperature: -80°C, 20°C, 37°C, High humidity Irradiation: 254 nm up to 360 min
Sampling Time & Uncontrolled Environment	Helmus et al. 2018[19]	Cloth rubbed against neck Blood stains	Cloth	5 volunteers Each scenario repeated 2 times	Time: Up to 6 months Environment: tap water, bathtub, river, pond Season: Summer, Winter
	Mcleish et al. 2017[20]	Controlled use by volunteer	Traps, Rabbit Baits Corvid carcasses	2 replicates per condition	Time: up to 10 days Environment: outdoors, indoors
	Raymond et al. 2009[21]	Controlled deposition of buffy coat or naked DNA	Painted wood, Vinyl, Glass slides	3 replicates per conditions	Time: up to 6 wks Environment: outdoors, lab
Sampling Time & Uncontrolled Environment & Surface Type	Raymond et al. 2004[22]	Controlled use by volunteer	Aluminum, Glass, Paper Ahesive tape, Plastic	2 volunteers	Time: up to 3 days Environment: outdoor, indoor Surface type
	Templeton et al. 2015[23]	Controlled use by volunteer	Cartridge casings (brass, aluminum, nickel), Glass, Tape, Wood	3 replicates per condition	Time: up to 8 days Environment: outdoors, indoors Surface type

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1.2 RESEARCH QUESTIONS

- How do surface type, environmental condition, and exposure time affect the stability of touch DNA evidence?
- Does the stability of touch DNA deposits differ from control DNA deposits?

1.3 RESEARCH DESIGN

1.3.1 Sample Acquisition

Control human DNA was obtained from the Coriell Institute (several different samples from the Amyotrophic Lateral Sclerosis 1 family). DNA was quantified using a Nanodrop spectrophotometer and resuspended in TE buffer at a concentration of 5 ng/µl. A 10-µl volume (corresponding to 50 ng DNA) was deposited on objects of interest under sterile conditions and allowed to dry.

For experiments involving touch DNA, volunteers were asked to handle stainless steel bolts (McMaster-Carr, 3" long, partially threaded) or 1" ×1" cotton squares (cut from TexWipe TX304 cotton wipers). The bolts were selected as a representative smooth metal surface that was easy to handle, could be cleaned, and could be obtained in large quantities. Cotton fabric was selected as a representative rough/porous surface. Photographs of the substrates are shown in Figure 1. Bolts and fabric were cleaned prior to use to minimize background DNA contamination. Bolts were washed in sequential baths of 10% bleach and distilled water. Cotton squares were exposed to ultraviolet-C wavelength light for at least 20 minutes.

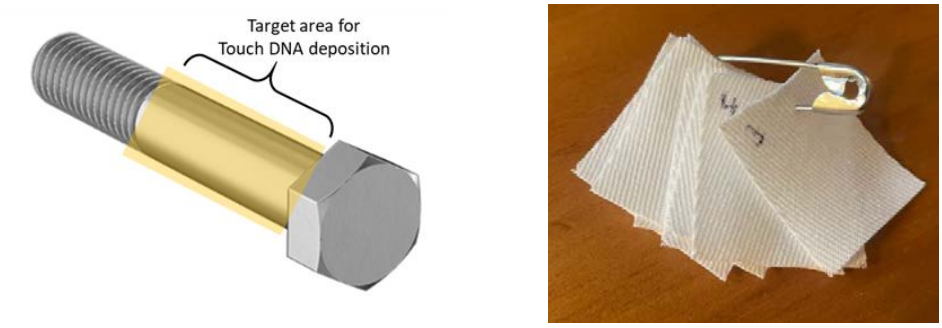

Figure 1. Images of the stainless steel bolts (left, partially threaded, 3" long) and cotton fabric squares (right, 1" × 1" cotton) used as substrates for DNA deposition.

Donors were instructed to (1) avoid washing their hands within 10 minutes of handling objects, (2) handle objects in sets of four, with at least 30 minutes in between rounds, and (3) handle each object for approximately 10 seconds in either their left or right hand. A formal randomization scheme was used to account for round order, order of handling within a round, and use of left vs. right hand when distributing

objects amongst the experimental conditions. After donors deposited touch DNA, the objects were stored in opaque boxes overnight at ambient temperature and were used for experiments within 24 hours of handling.

1.3.2 Maintaining Stable Environmental Conditions

Five different environmental conditions were established over the course of each experiment. Four of the environmental exposure conditions involved different pairwise combinations of temperature and relative humidity (Figure 2A). Environmental chambers were established in 3.75" × 7" × 8.5" watertight polycarbonate chambers (GSI Outdoors). Conditions are referred to as follows: low temperature and low humidity (LT-LH), low temperature and high humidity (LT-HH), high temperature and low humidity (HT-LH), and high temperature and high humidity (HT-HH). The specific temperature and relative humidity values for these conditions are provided in TABLE 2. Conditions remained very stable over the course of testing. The low humidity condition was established by first placing a small open container of desiccant (Drierite LTD, $CaSO_4$ CAS#7778-18-9, $CoCl_2$ CAS#7646-79-9) in the chamber for 2 – 3 hours. Once the humidity reached approximately 20% RH, the desiccant was removed and replaced by an open beaker containing a 50 ml volume of saturated (27.3 M) potassium acetate solution (Sigma Aldrich P1190). The high humidity condition was established by placing a 100 ml open beaker of potassium chloride (KCl) solution (Sigma Aldrich P9333) in the chamber. A 4.70 M KCl solution was made for the LT-HH condition, and a 5.37 M KCl solution was made for the HT-HH condition to account for the impacts of temperature on relative humidity.[24,25] The higher temperature condition was established by placing the environmental chamber inside a 37ºC incubator (ThermoFisher Scientific MaxQ 4000 Incubated Shaker Mod SHKE4000-7), while the lower temperature was established by maintaining the environmental chamber at ambient temperature in a controlled laboratory environment.

A. **B.**

Figure 2. Photographs of the enclosures used to control the environmental conditions for DNA sample exposure. These enclosures are simple and cost-effective to maintain. (A) Image of enclosure used to control temperature and relative humidity. This enclosure is shown with the stainless steel bolts in place. Bolts were spaced evenly on a peg rack. (B) Image of the enclosure used for ultraviolet (UV) light exposure. This enclosure is shown with the cotton fabric squares in place. Fabric squares were pinned to cork baseboard to enable even exposure of all surfaces to UV light. A light fixture containing UV bulbs was rested on support blocks at the top of the UV enclosure. Both enclosures housed glass beakers filled with saturated salt solutions to maintain constant humidity levels, and probes to monitor temperature, humidity, and UV light levels.

TABLE 2. Temperature and Relative Humidity Levels for Environmental Exposures Over the Course of All Experiments

Environmental Condition	Temperature (°F)	Relative Humidity (%)
LT-LH	70.76 ± 0.74	27.64 ± 2.23
LT-HH	70.72 ± 0.75	78.99 ± 3.61
HT-LH	97.72 ± 0.77	27.22 ± 1.79
HT-HH	97.15 ± 0.97	83.11 ± 3.11
UV	50.98 ± 2.62	39.96 ± 7.05

LT-LH: Low temperature and low humidity, LT-HH: low temperature and high humidity, HT-LH: high temperature and low humidity, HT-HH: high temperature and high humidity, UV: ultraviolet light

The fifth environmental exposure condition involved exposure to ultraviolet (UV) light in the UV-AB range (280 – 400 nm). For the UV exposure condition, temperature and relative humidity levels were maintained close to the LT-LH conditions. Key features included a 20.25” × 10.5” × 12.5” glass aquarium (Aqueon), a laser-cut plexi-glass door, and two UV-B bulbs (Exo Terra high output reptile fluorescent bulb UVB 200 14 Watt, 15”, Figure 2). UV-B bulbs were selected to mimic real-world levels of UV radiation from sunlight. Ultraviolet light is subdivided into several ranges: UV-A (315 – 400 nm), UV-B (280 – 315 nm), and UV-C (100 – 280 nm).[26] Of the three ranges of light, UV-A has the lowest efficiency of inducing DNA damage, while both UV-B and UV-C are known to cause a range of different DNA lesions.[27] The sun

generates UV radiation across all wavelengths, but the Earth's atmosphere absorbs almost all light in the UV-C range. Thus, the vast majority of UV light reaching Earth's surface is UV-A and UV-B. The amount of UV light reaching the earth's surface can differ as a function of several variables, for example, season, geographical location, ozone levels, and aerosol patterns.[26] The spectrum of the bulbs used during testing is provided in Figure 3. These bulbs provided an average UV-B intensity of 155.99 ± 24.78 μW/cm^2 over the course of our testing, which is consistent with reported irradiance values in the northern and southern hemispheres. Reference values were determined via literature review of previous irradiance studies in Argentina and through conversion of erythemal irradiance measurements taken on August 1, 2020 in Geneva, NY from the USDA UV-B Monitoring and Research Program (UVMRP) to UV-B irradiance values in the 280 – 315 nm range.[26,28,29]

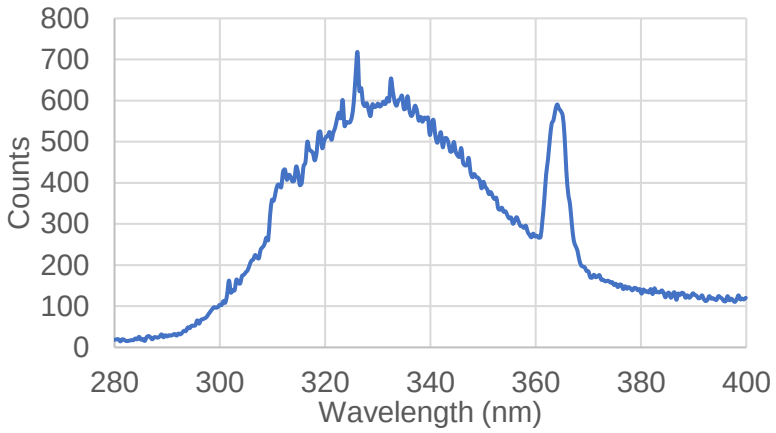

Figure 3. Ultraviolet spectrum of the UV-B bulb used for DNA exposure experiments.

Environmental conditions were monitored throughout the course of experiment via HOBO External Temp/RH Data Loggers (Onset UX100-023A). UV levels were monitored using a real-time illuminance UV recorder (TandD TR-74Ui) and taken intermittently with an ultraviolet UV-AB light meter (General Tools UV513AB Digital UVA/UVB light meter) over the course of the experiment. All chambers were allowed to equilibrate for at least 24 hours prior to introducing samples.

1.3.3 Sample Collection & DNA Purification

DNA samples were collected from the stainless steel bolts by swabbing the area of target deposition with BODE swabs (BODE SecurSwab2 DNA Collection System, P13D04). Swab tips were placed into Lyse and Spin baskets, and samples were lysed and collected from the swabs using the Qiagen Lyse and Spin kit (#19598). The cotton fabric squares were placed directly into the Lyse and Spin baskets for direct sample lysis. Briefly, a mixture of 475 μl ATL buffer and 25 μl proteinase K was added to each sample. Samples were mixed for 5 seconds by vortexing and subsequently incubated in a 56°C thermomixer shaking

at 900 RPM for 1 hour. Samples were centrifuged at 15,000×*g* for 1 minute, and the basket was discarded. A 1-µl volume of 1 µg/µl carrier RNA was then added to the lysates. DNA was purified using the QIAmp DNA Investigator Kit (Qiagen cat #56504) and the QIAvac vacuum manifold (Qiagen). A 475-µl volume of Buffer AL was added to lysates, which were then vortexed for 15 seconds and incubated in a 70°C thermomixer shaking at 900 RPM for 10 minutes. A 238-µL volume of 100% ethanol was added to samples which were then vortexed for 15 seconds and centrifuged. Lysates were then transferred to Qiagen MinElute columns on the QIAvac manifold. Buffer AW1 (500 µl), buffer AW2 (650 µl), and 100% ethanol (650 µl), were added sequentially to each column with the vacuum on. MinElute column membranes were then allowed to dry by sitting on the QIAvac at room temperature for 10 minutes or via centrifugation. A 100-µl volume Low TE/ATE buffer was added to the center of the membrane, and tubes were allowed to incubate at room temperature for 1 minute. DNA was eluted off the membrane into a DNA LoBind 1.5 ml Eppendorf tube (USA Scientific 22431021) by centrifuging at full speed (20,000×*g*) for 1 min. Purified DNA was stored at 4°C until analysis.

1.3.4 Sample Concentration and DNA Quantification

In order to ensure adequate DNA concentrations, samples were concentrated using Amicon Ultra 0.5 Centrifugal Concentration Filters (30K MWCO, Fisher UFC 503096). Filters were pre-rinsed prior to introducing samples by adding 400 µl of Qiagen ATE buffer to the membranes and centrifuging for 4 min at 14,000×*g*. Samples were then added to the columns and centrifuged for 10 mins at 14,000×*g* to concentrate. Samples were removed by inverting the filter and centrifuging into a new collection tube for 2 min at 1,000×*g*. The volumes of the concentrated samples were determined by weighing collection tubes with and without the sample. DNA content in concentrated samples was determined via quantitative polymerase chain reaction (qPCR) using the QuantStudio6 Flex instrument. The Quantifiler HP kit (ThermoFisher Life Technologies #4482911) was used to set-up the assay. Samples were quantified relative to a five-point standard curve made from AVIVA Human Genomic DNA (Aviva Systems Biology) which was kept consistent throughout the experiments (DNA quantities ranged from 0.005 – 5 ng/µl). The Quantifiler kit measures both a large and a small autosomal human DNA target sequence. Quantification of the large DNA sequence was used to determine DNA concentration, while the ratio of the quantity of small:large sequences was used to determine a degradation index (DI).

The DI is a qualitative descriptor of DNA quality based on the relative efficiency of amplification of a large vs. a small autosomal DNA target sequence. In samples that are not degraded, both large and small target sequences amplify with equal efficiency and the DI value is approximately one. In a degraded sample, fewer large targets will be amplified. The ratio of small:large targets thus increases, and the DI value increases. Samples with a DI value <1 are considered to not be degraded, samples with values between 1-10 are slightly to moderately degraded, and samples with values >10 are significantly degraded.

1.3.5 Statistical Analysis

For control DNA datasets, a t-test with a pooled variance estimated across all days and test conditions was used to evaluate the null hypothesis that the mean log_{10} of the recovered DNA quantity at day 0 was equal to the mean log_{10} recovered DNA quantity after one day or seven days of exposure to a particular

environmental condition. One-sided p values are reported below to test for a decrease in DNA quantity at day 1 or 7 compared to day 0.

For touch DNA datasets, a simple linear random effects model was used to fit the data in order to take into account the repeated measures on each donor. Analysis was performed using the "lme4" package version 1.1-27.1 in R. A contrast was then performed to evaluate the null hypothesis that the mean log_{10} of the quantity of DNA recovered at day 0 was equal to the mean log_{10} of the quantity of DNA recovered after a particular environmental exposure. Reported one-sided p values are for a Wald type test of the null hypothesis.

A multiple testing correction was not deemed necessary, due to the small number of tests performed in each experiment.

1.4 APPLICABILITY

Collectively, these studies provide the most comprehensive information to date regarding basic properties of touch DNA evidence and enable improved recommendations to be made to the forensic science community regarding best practices for the interpretation and evaluation of touch DNA evidence. These studies also generated a number of recommendations for best-practices when working with touch DNA samples in a laboratory setting.

2. PARTICIPANTS AND COLLABORATORS

This two-year applied research project was a collaboration between researchers at Massachusetts Institute of Technology Lincoln Laboratory (MIT LL) and South Dakota State University (SDSU). The following individuals contributed to the project:

Dr. Meghan Ramsey (PI), MIT LL
Dr. Natalie Damaso, MIT LL
Dr. Joshua Dettman, MIT LL
Elena Parsons, MIT LL
Dr. Martha Petrovick, MIT LL
Isabel Smokelin, MIT LL

Dr. Christopher Saunders, SDSU
Cami Fuglsby, SDSU

3. CHANGES IN APPROACH

In general, this project was executed in accordance with the original project proposal. The six major conditions of interest originally listed in the proposal were addressed in the research project, including: DNA source (control DNA and touch DNA), surface type, temperature, humidity, UV light exposure, and time. Minor modifications were made to the specific variables examined within each category, including a down-selection of the number of substrates examined (wood surfaces were not examined). The original proposal specified independent completion of control DNA and touch DNA studies. During the actual research project, it was decided to run both studies in parallel so that control DNA results could be used to benchmark and cross-compare experiments. We had also originally proposed evaluating DNA stability using single nucleotide polymorphism (SNP) sequencing analysis. However, due to lengthier than expected pilot/optimization studies, and in order to prioritize resources to accommodate larger sample sizes, SNP sequencing analysis was not pursued.

The COVID-19 pandemic impacted laboratory research and human subjects research and significantly slowed down progress between Spring – Fall 2020. As a result, a six-month no-cost extension was requested and granted.

4. OUTCOMES

4.1 ACTIVITIES/ACCOMPLISHMENTS

- Analyzed approximately 220 control DNA samples and 408 touch DNA samples collected from 8 different donors.
- Delivered a presentation entitled "Environmental Persistence of Touch DNA" at the 2021 NIJ Forensic Science R&D Symposium (February 16, 2021).
- Delivered a presentation entitled "Stability and Persistence of Touch DNA for Forensic Analysis" for the Forensic Technology Center of Excellence Webinar series (March 11, 2021). This webinar had 556 attendees, with participants identifying as DNA analysts/technicians, forensic DNA specialists, and forensic professionals. This presentation included experimental results as well as a number of lessons-learned regarding laboratory analysis of touch DNA.
- Publication in preparation
- Training opportunities for an intern (MIT LL) and graduate student (SDSU)

4.2 RESULTS AND FINDINGS

Control DNA and touch DNA samples were exposed to five different environmental conditions. The quantity of DNA, the degradation index, and the ability to obtain a short tandem repeat (STR) profile was determined for samples after different durations of exposure.

4.2.1 Control DNA

A 50-ng quantity of control DNA was deposited onto stainless steel bolts or cotton fabric. A subset of samples was set aside on day 0 for immediate extraction. For the day 0 samples, recovery efficiency was 38-60% from stainless steel substrates and 19-24% for fabric substrates. The remaining samples were distributed between the five different environmental enclosures. Samples were removed from the enclosures for analysis after one day or seven days. DNA quantity and degradation index were then determined by qPCR.

To identify significant differences in DNA quantity, a t-test was performed to evaluate the null hypothesis that the mean log_{10} of the recovered DNA quantity on day 0 was equal to the mean log_{10} recovered DNA quantity after environmental exposure. There were 49 degrees of freedom for both fabric and stainless steel datasets. Based on the standard error of the contrasts calculated using the t-test, and assuming that any difference would need to exceed two standard errors to be deemed significant, a difference in DNA quantity between 1.4 – 1.8-fold would be deemed significant given our datasets.

UV light exposure consistently had the biggest effect on the degradation of control DNA deposited on either fabric and stainless-steel substrates (Figure 4). The quantity of control DNA recovered from either

fabric or stainless steel substrates after both one day and seven days of UV light exposure decreased significantly compared to starting quantities of DNA on day 0 (one-sided $p<7.048\times10^{-26}$ based on a t-test comparison). By day 7, DNA quantities on stainless steel had decreased 306.9-fold, and DNA quantities on fabric had decreased 4448.4-fold compared to day 0 (TABLE 3). The DNA quantities for seven of the eight samples exposed to UV light were below the limit of quantification (LOQ) of the qPCR assay by day 7.

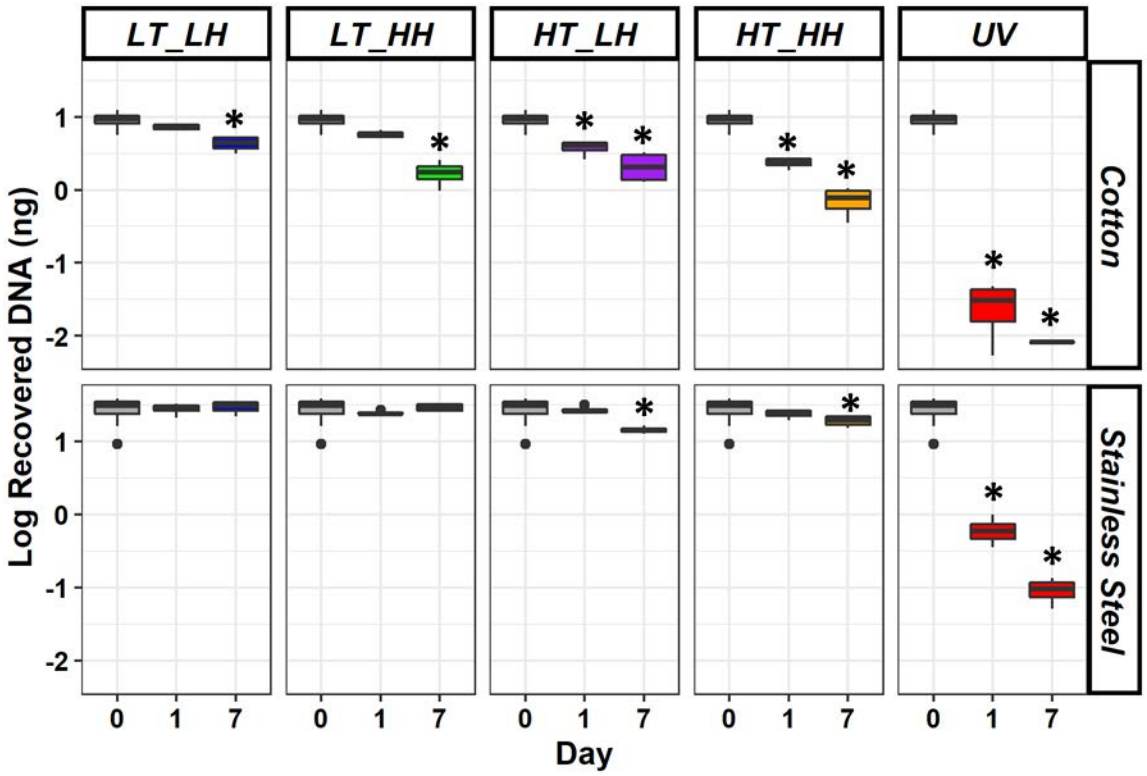

Figure 4. Quantity of control DNA recovered from cotton fabric (top row) or stainless steel bolts (bottom row). Control DNA was deposited on either cotton or stainless steel. Box plots show the logarithm of the quantity of recovered DNA (ng) following exposure to five different environmental conditions at three different timepoints (day 0, day 1, and day 7). Asterisks indicate significant decreases in the mean log_{10} of DNA quantity compared to the mean log_{10} quantity recovered on day 0. See TABLE 3 for more details. LT-LH: Low temperature and low humidity, LT-HH: low temperature and high humidity, HT-LH: high temperature and low humidity, HT-HH: high temperature and high humidity, UV: ultraviolet light.

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TABLE 3. Fold Decrease in Control DNA Quantity After Environmental Exposure

Environmental Condition		Stainless Steel		Fabric	
		Fold reduction* in DNA quantity	One-sided p-value	Fold reduction* in DNA quantity	One-sided p-value
LT-LH	1 day	1.0	0.5372	1.2	0.2331
	7 days	0.9	0.6662	***2.1***	***0.0071***
LT-HH	1 day	1.1	0.2362	1.6	0.0661
	7 days	0.9	0.6634	***5.4***	***2.944×10^{-7}***
HT-LH	1 day	1.0	0.4377	***2.4***	***0.0023***
	7 days	***1.9***	***0.0001***	***4.4***	***3.286×10^{-6}***
HT-HH	1 day	1.2	0.1787	***3.8***	***1.719×10^{-5}***
	7 days	***1.5***	***0.0097***	***13.1***	***7.697×10^{-12}***
UV	1 day	***46.6***	***1.047×10^{-29}***	***410.0***	***7.048×10^{-26}***
	7 days	***306.9***	***9.005×10^{-38}***	***4448.6***	***1.972×10^{-32}***

** Values reflect the difference between the mean $\log_{10}$ of recovered DNA quantity on day 0 and the mean $\log_{10}$ of recovered DNA quantity after exposure to the particular test condition. Significant changes are shown in bold italics ($p<0.05$ based on a one-sided t-test).*

The stability of control DNA under the other temperature/humidity combinations varied depending on the substrate (i.e. cotton vs. stainless steel, Figure 4). Very little DNA degradation was observed for control DNA deposited on the stainless steel under any of the temperature/humidity conditions evaluated. The only significant decreases in DNA quantity were for the HT-LH and HT-HH conditions after seven days of exposure (TABLE 3). In contrast, control DNA deposited on the fabric substrates did degrade after seven days of exposure to all four temperature/humidity conditions. Significant degradation was also observed after one day of exposure to the HT-LH and HT-HH conditions. The fold-changes in DNA quantity under each condition as well as one-sided p-values from the t-tests are shown in TABLE 3. Collectively, these results suggest that UV light exposure and high temperature conditions are most likely to result in degradation of control DNA. Low temperature environments are more conducive to DNA stability. Control DNA also appears to be more stable on stainless steel substrates compared to fabric.

A degradation index (DI) was also determined for all samples. The average DI for the day 0 samples deposited on fabric and stainless steel substrates was 0.92 ± 0.19 and 0.99 ± 0.55, respectively, indicating that the starting DNA was not degraded. The average DI did not increase above 2.0 for samples exposed to any of the environmental conditions, except for UV light exposure. After one day of UV exposure, the average DI for control DNA samples on fabric had significantly increased to 9.2 ± 5.1 ($p < 0.0474$), and the average DI for control DNA samples on stainless steel had significantly increased to 15.1 ± 6.7 ($p < 0.025$). By day 7, DNA concentrations in several of the UV samples were undetectable, so a DI was not determined. These results are consistent with measures of DNA quantity and suggest that UV light exposure results in the most significant degradation of touch DNA samples of all the conditions tested.

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4.2.2 Touch DNA

Because of the expected variability in the amount of touch DNA deposited by the donors, a formal randomization scheme was implemented to account for variables related to object handling, and to randomly distribute any effects from these variables across experimental conditions. The formal randomization removed the cofounding variable of touch order in particular.

Touch DNA samples were collected onto cotton squares from five donors. The average amount of touch DNA recovered from the fabric squares on day 0 was 19.3 ± 33.7 ng and ranged from 2.4 ng to 215.2 ng. Samples were then exposed to the four temperature/humidity combinations and to UV light. DNA concentrations were determined after one or seven days (Figure 5). A simple linear random effects model was used to fit the data in order to take into account the repeated measures on each donor. A contrast was then performed to test whether the mean log_{10} of the quantity of DNA recovered after a particular environmental exposure was less than or equal to the amount of DNA recovered at day 0.

For samples deposited on fabric, the quantity of touch DNA decreased significantly after UV light exposure for both one day (16.3-fold decrease, with an observed Wald type p-value of 3.6×10^{-17}) and seven days (50.1-fold decrease, with a Wald type p-value of 6.0×10^{-32}, Figure 5). These results are consistent with those obtained for control DNA: both touch DNA and control DNA are very susceptible to degradation following UV light exposure (Figure 4, Figure 5). None of the other environmental conditions that were tested resulted in a significant decrease in touch DNA quantity (Figure 5). These results differ from those observed with control DNA (Figure 4), and suggest that, at least on fabric substrates, touch DNA may be considerably more stable than control DNA following exposure to a range of temperature and humidity conditions.

Touch DNA samples were also collected onto stainless steel bolts from eight donors. In initial studies, a swab was used to collect touch DNA from a single bolt. However, the average quantity of DNA recovered from single bolts was only 0.35 ± 0.66 ng, and DNA quantities for 32 of the 50 samples were below the LOQ of the qPCR assay on day 0. With starting DNA quantities this low, it was impossible to measure DNA degradation due to environmental conditions. In subsequent studies, one swab was used to collect a "pooled" touch DNA sample from two bolts in order to increase starting quantities of touch DNA. This method, in combination with screening for donors that shed higher quantities of DNA, resulted in significantly increased DNA recovery amounts: average 1.8 ± 2.4 ng ($p<0.0004$), with the majority of samples above the LOQ of the qPCR assay (38 out of 40 samples). Note that this DNA quantity is still significantly less than the amount recovered onto the fabric squares ($p<0.002$). This observation is consistent with prior studies showing higher quantities of touch DNA transfer onto porous or rough surfaces.[30,31]

Data gathered using this "double-swab" method are shown in the lower panel of Figure 5. Consistent with prior results, UV light exposure had the biggest impact on DNA quantity. A linear random effects model was used to fit the DNA concentration measurements collected under the different conditions. DNA quantity decreased significantly after UV light exposure for both one day (3.4-fold decrease, with a Wald type p-value of 3.9×10^{-4}) and seven days (25.7-fold decrease, with a Wald type p-value of 8.7×10^{-20}). A significant decrease in DNA quantity was also observed for samples exposed to the HT-HH condition after seven days (5.0-fold decrease, with a Wald type p-value of 3.7×10^{-6}). These results are generally consistent with those observed for control DNA deposited on stainless steel (Figure 4), and suggest that

touch DNA samples gathered from surfaces like stainless steel may exhibit high levels of stability under most conditions, except for UV light exposure.

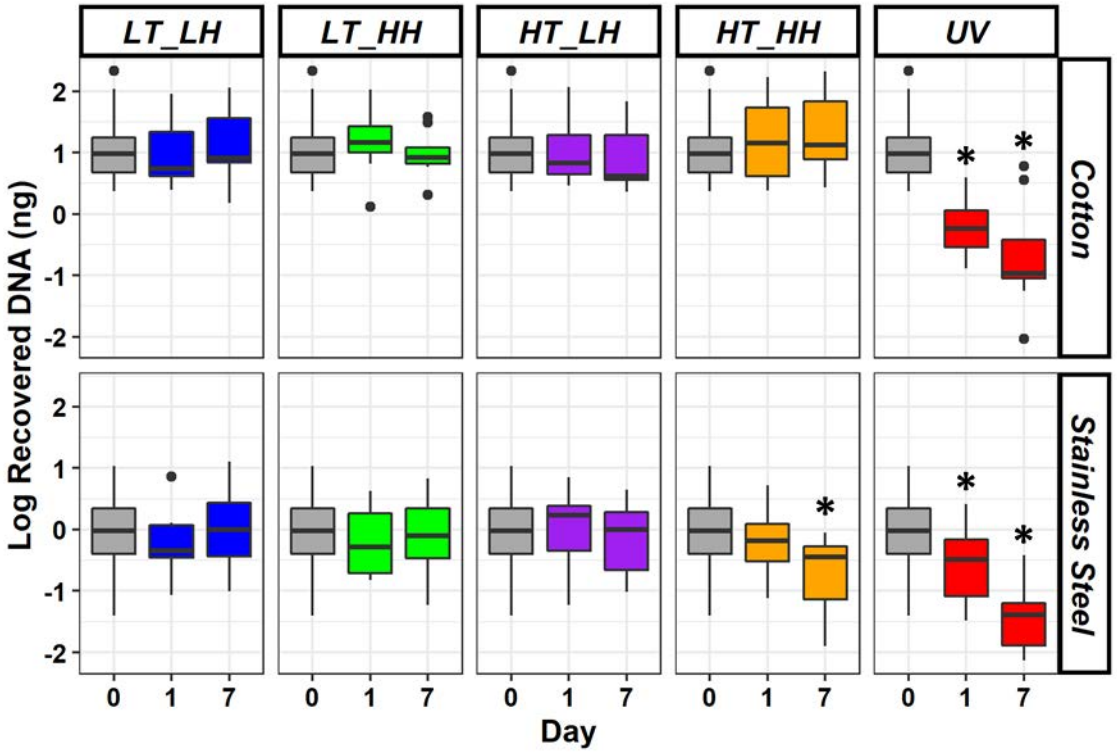

Figure 5. Quantity of touch DNA recovered from cotton fabric (top row) or stainless steel bolts (bottom row). Control DNA was deposited on either cotton or stainless steel. Box plots show the log_{10} quantity of recovered DNA (ng) following exposure to five different environmental conditions at three different timepoints (day 0, day 1, and day 7). Asterisks indicate significant decreases in the mean log_{10} of DNA quantity compared to the mean log_{10} quantity recovered on day 0. LT-LH: Low temperature and low humidity, LT-HH: low temperature and high humidity, HT-LH: high temperature and low humidity, HT-HH: high temperature and high humidity, UV: ultraviolet light.

Because of the variability in the starting quantity of touch DNA deposited by each donor and the inability to standardize starting quantities, a potential caveat to our results is that variation in touch DNA quantity would mask small effects on DNA degradation. Based on the standard error of the contrasts calculated using the linear random effects model, and assuming that any detected difference would need to exceed two standard errors to be deemed significant, a difference in DNA quantity greater than approximately 2-fold would be deemed significant given our current dataset.

The DI measurements for the touch DNA samples were consistent with DI measurements for the control DNA. Touch DNA samples collected on day 0 from stainless steel bolts (using the "double bolt" method) or from fabric had average DI values of 2.0 ± 0.66 or 1.38 ± 0.57, respectively, indicating that the touch DNA starting material was not degraded. Exposure to UV light resulted in a significant increase in the DI value for touch DNA samples on both stainless steel and fabric after one day of exposure: 5.2 ± 1.8

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for stainless steel and 5.7 ± 2.9 for fabric (p=0.001 in both cases). By day 7, 12 of the 18 touch DNA samples exposed to UV light had DNA quantities below the LOQ of the qPCR assay and a DI was not determined. Exposure of touch DNA to any of the other temperature/humidity combinations did not have any substantial impacts on DI, regardless of substrate or exposure time.

Short tandem repeat (STR) analysis was performed for a set of touch DNA samples recovered from single stainless-steel bolts. Analysis was performed by Signature Science using the GlobalFiler STR kit. A total of 39 touch DNA samples were submitted from two independent donors, along with reference buccal samples from each donor. A set of negative controls were also submitted; none of the 24 alleles in the GlobalFilter kit were detected on any of the negative control samples. Because the touch DNA samples submitted for STR analysis were collected from single stainless steel bolts, the amount of starting DNA material was very low (ranging from 0.02 – 4.1 ng). The percentage of the full 24-allele STR profile that was determined correctly relative to the reference buccal sample was determined for each sample. The completeness of the STR profile was positively correlated with the quantity of DNA in the sample (Pearson correlation coefficient $\rho = 0.48$). The mean percentage of full STR profiles reported for each environmental condition is shown in Figure 6. There was substantial variability in the completeness of the profiles determined for samples across all environmental conditions, likely related to the overall low quantities of DNA in these samples. Notably, however, profiles were completely undetermined for all samples exposed to UV light. These results are consistent with DNA quantity and DI measurements, which collectively suggest that samples exposed to UV light are highly degraded.

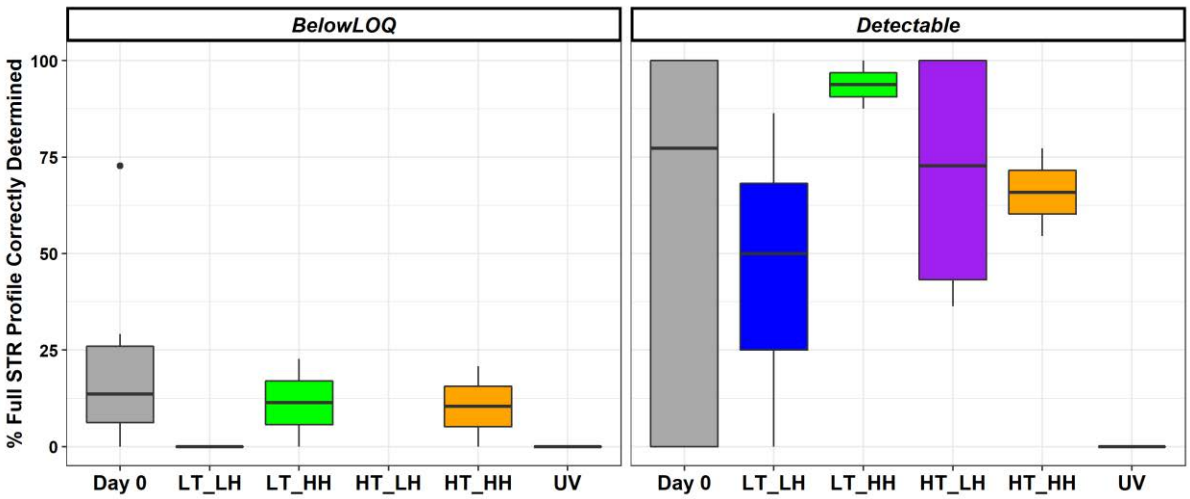

Figure 6. Percent of complete STR profile determined for touch DNA samples exposed to different environmental conditions. Results are shown separately for samples with DNA quantities below the limit of quantitation (LOQ) for the qPCR assay, and for those that are above the LOQ (Detectable). The following numbers of samples were included in the analysis. For BelowLOQ samples: day 0 (n=7), LT-LH (n=1), LT-HH (n=2), HT-LH (n=0), HT-HH (n=2), UV (n=2). For Detectable samples: day 0 (n=13), LT-LH (n=3), LT-LH (n=2), HT-LH (n=4), HT-HH (n=2), UV (n=1). Touch DNA samples were exposed to the temperature/humidity combinations for 7 days. The UV light exposure was for 1 day.

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4.2.3 Conclusions

In conclusion, this study represents the largest systematic study to date of touch DNA persistence under controlled representative environmental conditions. The quantity and quality of touch DNA samples was tracked over time after exposure to different pairwise temperature and humidity combinations as well as exposure to realistic levels of ultraviolet light. These metrics were compared to those for control DNA samples exposed to the same conditions.

Overall, exposure to UV-B light had the biggest impact on the stability of control and touch DNA samples, resulting in decreased DNA quantities and increased degradation indices by 24-hours of exposure. In the absence of UV light, touch DNA samples were generally stable under the different temperature/humidity combinations up to a period of 7 days, regardless of substrate. Some degradation of touch DNA samples was apparent on stainless steel substrates exposed to high temperature and high humidity for seven days. Control DNA samples deposited on stainless steel were also generally stable across different temperature/humidity conditions in the absence of UV light. In contrast, control DNA samples deposited on fabric degraded after exposure to all four temperature/humidity conditions, sometimes after only one day of exposure. These results suggest that while the use of control DNA as a mimic for touch DNA in laboratory experiments has significant advantages in terms of reproducibility and experimental design, it may not be an appropriate mimic for touch DNA under all conditions. These results also provide further motivation for in-depth studies to characterize the relevant constituents and properties of touch DNA so that it can be realistically mimicked in a more controlled way.

These results provide insight into the expected stability of touch DNA evidence gathered in different environments. Touch DNA samples collected outdoors that have been exposed to sunlight are more likely to be degraded than those collected indoors and should be collected well within 24 hours of deposition for the best likelihood of obtaining a useable sample. Generally speaking, these results suggest that touch DNA samples in clean environments are likely to remain intact over a range of ambient conditions. Prolonged exposure to high temperature and high humidity are likely to result in degradation of the sample. Surface type may also influence the properties of the collected evidence, both in terms of the likely amount of material deposited and the stability of the sample over time.

From an experimental design perspective, our findings provide a number of lessons-learned to forensic scientists. Although using control DNA (purified DNA or cell deposits) as a stand-in for touch DNA is advantageous in terms of reducing variability and enabling the use of smaller sample sizes, control DNA cannot be expected to act as a surrogate for touch DNA in all circumstances. Efforts should be made to control for and randomize as many sources of variability in touch DNA deposition as possible. Finally, methods were developed as part of this work to mimic real-world environments in a highly controlled manner. Minimizing variability in the environmental exposure conditions is critical to being able to draw conclusions about the effect of environmental conditions on touch DNA stability. The methods described here are cost-effective and easy to implement, and we hope they will be useful for future efforts.

4.3 LIMITATIONS

Given the high degree of variability in the amount of touch DNA deposited by different donors, and even by a single donor over different touches, obtaining large enough sample sizes for statistical significance is the biggest limitation for studies such as the one described here. Implementation of formal randomization procedures to distribute any effects of variables related to DNA deposition was an important aspect of randomizing sources of noise in this experiment.

The high degree of effort associated with collecting and processing touch DNA samples presented limitations on sample size and limited the ability to build a full predictive responsive surface for how touch DNA degraded over time. For cases where there is similarity in the results of the significance between control DNA and touch DNA, it may be feasible to build these response surfaces with the control DNA experiments and then verify the results with the touch DNA experiments. This is an ongoing effort. Current work is focused on building formal Bayesian predictive models for the amount of recovered DNA under the various conditions over time. Regardless of the type of predictive models built, the presence of three time points (Day 0, Day 1, and Day 7) will limit the type of models that can be fit.

Experimental design is also critical to ensuring that enough starting material is obtained to effectively measure changes in quantity over time. Initial studies using touch DNA harvested from single stainless steel substrates resulted in initial touch DNA quantities near or below the LOQ of the qPCR assay. Factors that were optimized in this study to maximize touch DNA recovery include the following: (1) swabbing multiple stainless steel objects to obtain "pooled" touch DNA samples, (2) using different substrate types such as fabric that result in higher initial touch DNA deposition, (3) direct extraction from fabric substrates to reduce loss and variability due to swabbing and subsequent extraction from the swab, (4) screening for individuals that are "high shedders," and (5) concentrating DNA samples post-extraction. Screening for "high shedders" is a common practice in touch DNA studies, but it remains unclear why some individuals may shed more DNA than others and whether there are associated differences in touch DNA properties for "high" versus "low" shedders. The extent to which the properties of touch DNA differ between individuals is also unknown. Selection for "high shedders" thus not only limits the overall donor pool, it may also bias the properties of the collected samples in unknown ways. Further characterization of touch DNA composition and properties is essential in order to move toward more controlled, systematic studies of touch DNA persistence over time.

5. ARTIFACTS

5.1 LIST OF PRODUCTS

- Presentation delivered at the 2021 NIJ Forensic Science R&D Symposium
- Webinar delivered for the Forensic Technology Center of Excellence webinar series

5.2 DATA SETS GENERATED

The following datasets were generated:

- Quantity and DI of control DNA on fabric
  - 5 environmental conditions, 3 timepoints
  - n = 60
- Quantity and DI of control DNA on metal
  - 5 environmental conditions, 2 or 3 timepoints
  - n = 87
- Quantity and DI of touch DNA on fabric
  - 5 environmental conditions, 3 timepoints, 5 donors
  - n = 150
- Quantity and DI of touch DNA on metal
  - 5 environmental conditions, 3 timepoints, 8 donors
  - n = 258
  - STR analysis of 40 samples

5.3 DISSEMINATION ACTIVITIES

Data and lessons-learned were shared in a presentation and a webinar, as discussed above. A manuscript is in progress and will be submitted for publication.

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