

## Changes in Yield of Contact DNA over Time after a Physical Assault

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### Abstract

Touch DNA evidence can be defined as DNA resulting from the transfer of epithelial cells from skin to an object. Due to its high success rate in recent years, such as the JonBenet murder case and Waldo rape case, touch DNA evidence has been increasingly studied. Sexual assaults, which generally involve unwanted sexual contact between the victim and the offender commonly go unreported and often the perpetrator cannot be identified because of lack of evidence. This study aimed to determine how touch DNA collected from a victim's wrist after a simulated sexual assault changed over time. Sexual assaults were simulated with male "assailants" grabbing the left wrists of female "victims" while they struggled to free themselves from the grip. Samples were collected immediately and at 15-minute intervals thereafter for 120 minutes after assault and then quantified using the Quantifiler® Duo DNA Quantification kit. The maximum amount of male DNA obtained at time 0 was 10 pg/μL, which is below the quantification threshold of the Quantifiler® Duo DNA Quantification kit and detection limit of most commercial human STR identification kits. Although results here were limited by low DNA quantities and assay sensitivity, significant improvements can be made to better assay low template DNA.

**Keywords:** Touch DNA; Sexual assault; Shedder status; DNA quantification

### Introduction

DNA typing is a technique commonly used to genetically identify individuals. DNA can be obtained from samples in the form of blood, saliva, or semen or from materials such as bone, hair, or skin [1-4]. In criminal cases, evidentiary samples from a crime scene are compared to a "reference" sample to determine if a person of interest may have been the source of the evidentiary samples. Conversely, DNA typing can also be used to rule out persons of interest if their DNA does not match the evidentiary sample or exonerate wrongfully convicted individuals.

Sexual assaults, which are any type of sexual activities that a person does not agree to, occurs an average of 237,868 times a year in the United States [5]. Out of the large number of victims who were sexually abused, 84% reported the use of physical force only [5]. But even when the crime is reported, it is unlikely that it will lead to an arrest and prosecution due to lack of evidence. However, investigators are now able to use "touch DNA" evidence to link individuals to a crime, such as a sexual assault.

Since its introduction in the mid-1980s, DNA analysis has allowed law enforcement to link perpetrators with crime scenes. Initially, DNA analysis required blood or semen stains about the size of a quarter to generate sufficient profiles for identifying individuals. However, with the rise of new technology, DNA samples no longer need to be visible to analyze. "Touch" or "contact" DNA is DNA that is transferred via skin epithelial cells to an object that is handled or touched [6]. According to Edmond Locard's Exchange Principle, when two items come into contact with each other, there will always be an exchange of material. With humans shedding roughly 400,000 skin cells per day [3], epithelial cells are easily transferred to surfaces with which skin comes into contact with. Van Oorschot and Jones [7] reported that DNA profiles could be obtained from items that had been handled, even if only briefly. Therefore, the simple act of picking up an object or touching a surface can lead to the identification and apprehension of a criminal.

Even though touch DNA has been studied for over a decade, its true potential only became evident in the JonBenet Ramsey murder case where it was used to exonerate the parents as the perpetrators [8]. Since then, amplifiable DNA has been collected from countless items including the interiors of latex gloves, grips and shafts of tools, drinking glasses, steering wheels, cellular phones, remote controls, and human skin [3,4,6].

Though touch DNA analysis is a promising technique with great potential for forensic applications, it currently has limitations that prevent its use. The quantity and quality of DNA profiles recovered from items depend on the shedder status of individuals that deposited the DNA [6,9]. Good DNA shedders might leave behind a full DNA profile after handling an object, whereas poor shedders may leave a partial or no profile at all. Lowe et al. [1] suggested that determining the shedder status of individuals and the distribution of shedders in the wider population could help increase the understanding of factors that affect the transfer of trace DNA. In addition to shedder status, the type of contact, length of contact, type of substrate, amount of pressure applied, previous hand washing, personal habits, and perspiration also impact trace DNA transfers [6,9].

Many investigators are faced with the daunting task of determining what samples are suitable for collections and if a profile can be obtained. The key to obtaining successful touch DNA results depends on

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recognizing items that are suitable for touch DNA analysis and applying proper techniques to recover the highest amount of DNA [3,6,9]. For sexual assault cases, it is vital for forensic examiners to take very careful histories from victims describing in detail the sites of contact, nature of struggles, and degree of force involved in order to evaluate appropriate sites for collection. During an assault investigation, contact DNA is typically collected by swabbing surfaces an assailant is thought to have touched or held, even without any visible signs.

Many studies have tested the effectiveness of touch DNA on different surfaces [10-12], but few have explained the persistence of Low Copy Number (LCN) DNA and how these samples should be processed. In a recent study, Sandoval et al. [9] determined that male touch DNA evidence after a five second simulated sexual assault is present 30 minutes but absent after 2 hours post contact. Since Sandoval et al.'s study failed to obtain any DNA 2 hours post contact, we tried to determine at what point touch DNA is completely absent from the skin after a sexual assault. Thus, this current research proposal aims to look at how touch DNA yield collected from female victims after simulated sexual assaults change over time.

## Materials and Method

The study was performed using university approved procedures (IRB Protocol Number 385320-1).

Ten random subjects, five males and five females, were recruited for this study. Before obtaining consent, subjects had to meet four conditions: range from 18 to 30 years old, not have any skin diseases, not be pregnant, and not be taking any medications. The ten subjects were then paired into groups consisting of one male and one female to model common physical assault scenarios. The males were referred to as "assailants" and females as "victims".

The shedder statuses of male subjects were determined based on the methodology of Lowe et al. [1] to assess their ability to deposit DNA on an item. All male subjects were asked to wash their hands 15-minutes prior to gripping a sterile 50 mL conical tube for 30 seconds. Tubes were swabbed before (negative control) and after contact with a sterile swab (Epicenter Biotechnologies, Madison, WI).

For the study, male subjects were directed to wash their hands thoroughly using the provided soap and water and to dry their hands with fresh paper towels 15 minutes prior to the study while female subjects were asked to shower one hour before to avoid contamination due to secondary transfer of DNA from people outside of the experiment. After washing, subjects were asked to continue with their 'normal' activities during the waiting period but to avoid eating or touching other people. According to Lowe et al. [1], a 15-minute interval between hand washing and touching defined a 'good' and 'bad' shedder and had the greatest variation in DNA shedding between volunteers.

To simulate a sexual assault, the male "assailants" were asked to perform a grab/hold maneuver on the left wrist of the female "victims" for 30 seconds while the victim struggled to release the grip. Using sterile swabs, the grabbed area was immediately swabbed after the assailant-victim interaction using moderate pressure. The double swab technique, as described by Sweet et al. [10], was used to maximize DNA yield. The first sterile swab in the double swab method was moistened with sterile water and used to swab the skin. A second dry, sterile swab was used to recover the remaining moisture on the skin's surface. A sterile swab moistened with sterile water was also collected as a negative control. All swabs were allowed to air dry completely for 30 minutes

before extraction. Since both collection swabs were used to collect from the same contact site, they were pooled together into a single sample.

Depending on the DNA yields at 0 hours, samples were then collected at 15 minute intervals up to 120 minutes after exposure. Each collection time was performed on different days. Buccal mouth swabs from each volunteer were also collected as reference samples.

After air drying for 30 minutes, swab tips were cut into 1.5 mL micro centrifuge tubes using sterile scissors. The samples were extracted using the QIAamp DNA Investigator kit (Qiagen Inc., Valencia, CA) according to the protocols set forth in the DNA Investigator kit handbook [13-15]. In order to obtain a very concentrated sample, samples were eluted with 30  $\mu$ L buffer ATE. Extractions were performed in ideal laboratory conditions that minimized the potential for contamination. The DNA extracts were stored at -20°C until quantification.

Extracted DNA samples were quantified using the Quantifiler<sup>®</sup> Duo DNA Quantification Kit (Applied Biosystems, Carlsbad, CA) according to manufacturer's protocols [16] on an Applied Biosystems 7500 Fast Real-Time PCR system. The kit simultaneously amplifies a region from the human ribonuclease P RNA component H1 (RPPH1) and Sex Determining Region Y (SRY) genes, which quantify the total amount of amplifiable human and male DNA, respectively, and allows for the determination of relative human male and female DNA quantities in the samples.

## Results

Quantification of the reference buccal samples showed that an ample amount of DNA was collected. The reference sample yield ranges for males and females were between 6.3-11.0 ng/ $\mu$ L and 6.63-13.4 ng/ $\mu$ L of DNA, respectively.

Results from the shedder status study showed that neither human nor male DNA was recovered from all five male volunteers (Table 1). The Internal Positive Control (IPC) showed consistent uninhibited amplification indicating that reaction failure was due to insufficient template DNA.

In the simulated assault samples, two samples, collected immediately post contact, showed slight amplification for male DNA. A closer look at the two samples (Figures 1 and 2) showed  $C_T$  values of 39 for both reactions. Thus, this could imply that exceedingly low quantities of male DNA were present (<3 copies). The maximum concentration of male-specific DNA detected for both samples was 10 pg/ $\mu$ L but did not show any total human DNA (Table 2). The amounts of male DNA relative to that of total DNA may be due to preferential PCR amplification of smaller SRY (130 bp) compared to those of RPPH1 (140 bp) [13]. The amount of DNA present in the sample is below the optimum working range of the AmpFISTER<sup>®</sup> Identifier<sup>®</sup> kit (0.5 to 1 ng). Thus, STR analysis was not performed.

Subjects	DNA Concentration (pg/ $\mu$ L)	
	H	M
Assailant 1	UD	UD
Assailant 2	UD	UD
Assailant 3	UD	UD
Assailant 4	UD	UD
Assailant 5	UD	UD
Control	UD	UD

H/M: total human DNA/male-specific DNA; UD: undetermined.

**Table 1:** Shedder status quantification results for male (assailant) volunteers and negative control.

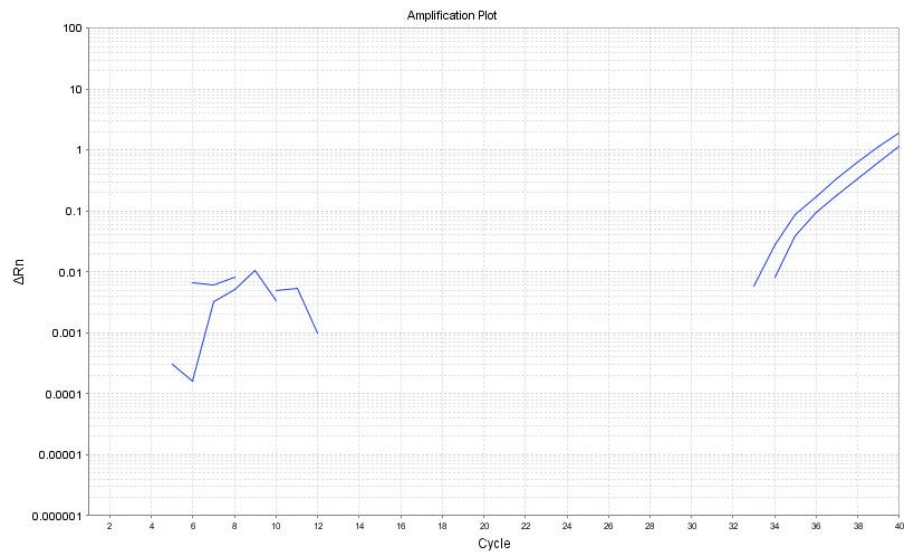


Figure 1: Amplification plot showing slight amplification around cycle 39 for one of two samples.

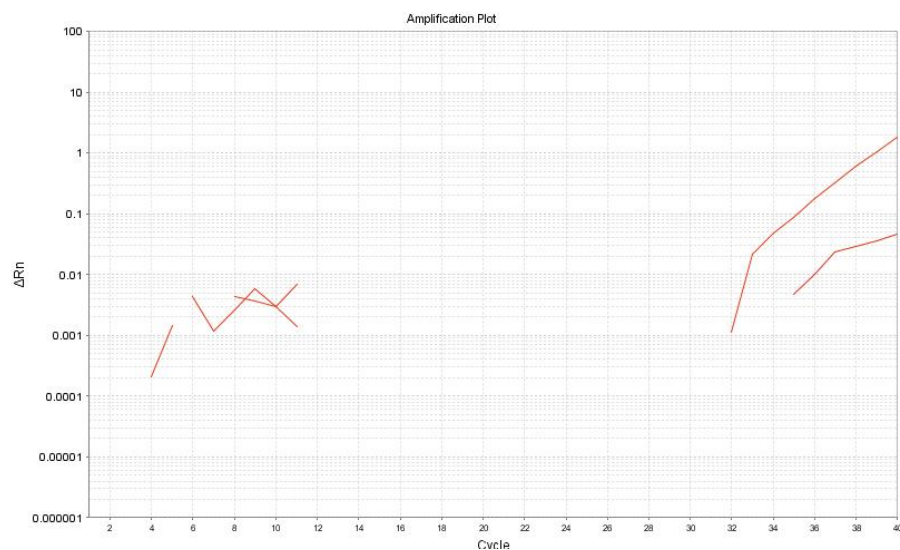


Figure 2: Amplification plot showing slight amplification around cycle 39 for one of two samples.

Since male DNA quantities were below the minimum detection levels of the Quantifiler® Duo kit at the initial sampling period (0 minutes) after the simulated sexual assault, later sampling for contact DNA was not performed.

## Discussion

In the United States, someone is sexually assaulted every 2 minutes [5]. Sexual assaults can vary from groping, forced kissing, unwanted touching, or even rape. Following a sexual assault, many investigators are just left with vague descriptions of the assailants and with little to no physical evidence that can lead to the apprehension of the culprit. Touch DNA analysis from sexual assault cases can be critical during an investigation when not much visible evidence is left at the scene. A few studies [13,17,18] have focused on sexual assault relevant to contact

DNA, but none have studied the persistence of DNA within the context of forcible interactions such as struggling.

Currently, microscopic skin cells containing DNA that naturally rub off when touched can be used as evidence [9,19]. Edward Locard's exchange principle states that the perpetrator of a crime will take something from and leave something at a crime scene. Thus, there is always an exchange of material when two items come into contact with each other. Therefore, sometimes it is what investigators cannot see that helps solve crimes.

Although DNA testing is typically reserved for more severe crimes where blood or semen samples are obtained, touch DNA testing can be a useful tool. After the JonBenet Ramsey murder case, touch DNA testing has increased and numerous studies [1,4,10] have been done yielding successful results. However, very few studies have assessed



Subjects	DNA Concentration (pg/ $\mu$ L)
	H/M
Couple 1	0/10
Couple 2	UD
Couple 3	UD
Couple 4	0/10
Couple 5	UD

H/M: total human DNA/male-specific DNA; UD: undetermined.

**Table 2:** Quantification results after a simulated sexual assault 0 minutes post contact. Amount of male DNA relative to that of total DNA may be due to preferential amplification of the smaller SRY (130 bp) gene.

the persistence of touch DNA. This study set out to determine how touch DNA changed over time after a simulated sexual assault. Since shedder status plays a significant role in determining whether an individual would leave DNA behind or not, we tried to determine the shedder status for each male assailant. Our results showed that no DNA or undetermined amounts of DNA was present for each individual. According to Lowe et al. [1], 'good shedders' are classified as individuals who produce full DNA profiles after handling an item and 'bad shedders' are those who produce no or partial profiles. Since no DNA was detected in our shedder status experiment (Table 1), all individuals were classified as 'bad shedders'. It should be noted that the shedder status of an individual could fluctuate as repeated tests on the same person on different days can give very different results [9,11]. Thus, the same people were used in the experiment even though initial results classified them as 'bad shedders'.

Our initial sampling period (0 minutes) results showed that after a 30-second grab/struggle situation, zero to very low quantities of DNA was collected (Table 2). Amplification of the Internal Positive Control (IPC) had  $C_T$  values of roughly 29 cycles, indicating that no PCR inhibitors were present, instrumentation did not fail, and the assays were set up correctly since the reactions showed normal IPC amplification across a broad range. Therefore, the failure of the human and male target amplification is likely due to no or extremely low amounts of human DNA.

Real-time PCR assays are extremely sensitive and detection of DNA at a high  $C_T$  value ( $>35$ ) indicates the presence of exceedingly low quantities of DNA [12]. Two of our samples showed slight amplification around cycle 39, indicative of low DNA quantities. The Quantifiler<sup>®</sup> Duo DNA Quantification kit is highly sensitive and can quantify 23-pg/ $\mu$ L of human genomic DNA from a 2  $\mu$ L sample volume [16]. Even though none of our samples fell within the range of the standard curve, extrapolation of the standard curve would yield 10 pg/ $\mu$ L of male DNA for the two amplified samples.

When working with very low copies of DNA, it is important to keep in mind that qPCR is subject to stochastic variation. Stochastic variation occurs when a limited number of DNA target molecules exist in a sample, resulting in unequal sampling of the alleles due to preferential amplification [9,16]. Detection of low DNA quantities can vary from amplification to amplification making results unreliable.

A sensitivity study using the Quantifiler<sup>®</sup> Duo assay determined that quantities as low as 11.5 pg/ $\mu$ L of human DNA were reliably and reproducibly quantified and detected [16]. Additionally, concentrations of human DNA at or below 5.75 pg/ $\mu$ L were determined to be irreproducible due to stochastic variation [16]. Therefore, our samples with 10 pg/ $\mu$ L of male DNA quantified by the kit could possibly be male DNA. However, since amount of DNA detected was outside the standard curve quantification range of 23 pg/ $\mu$ L to 50 ng/ $\mu$ L, it is

difficult to reliably conclude whether male DNA was detected or not. Until more sensitive assays with lower limits of detection are produced, our results will be 'undetermined' since we cannot reliably quantify the DNA.

The assays used to study contact DNA have valuable uses and have been instrumental in solving many cases, but improvements still need to be made. In regards to this study, samples could have been more reliably quantified if assay sensitivity was higher. The lowest amount of DNA the Quantifiler<sup>®</sup> Duo DNA Quantification kit can reliably quantify is 23 pg/ $\mu$ L. If an assay can be developed with a standard curve that quantifies down to 1 pg/ $\mu$ L of DNA, touch DNA testing would produce more reliable and consistent detection and quantification results. In addition to quantifying touch DNA, downstream analyses would involve analyzing touch DNA samples with STR profiling kits such as the AmpFISTR<sup>®</sup> Identifiler<sup>®</sup> PCR Amplification kit to obtain genetic profiles. A majority of common commercial STR kits require a minimum of 0.1-0.5 ng of DNA to obtain full STR profiles. Below this range, standard methods tend to provide only partial profiles. Like the Quantifiler<sup>®</sup> Duo DNA Quantification kit, the sensitivity of most commercial STR kits need to be improved to better detect exceedingly low quantities of DNA present in touch DNA samples.

Even if detection techniques can be improved, a better understanding of touch DNA properties and transfer is needed to improve collection of this sample type. A study by Raymond et al. [20] on the success rate of profile generation noted that from 252 trace casework samples (all from surfaces touched by hands), 111 (44%) did not produce a profile. However, another study by Bright & Petricevic [12] found that 0.16-6.4 ng of DNA was obtained from swabbing the hands of volunteers. This shows that touch DNA is often present on the hands of individuals, but the mechanism behind why DNA is occasionally transferred to an object via touch still has to be determined. Once we know why and how touch DNA is transferred to objects, we will be able to better determine what pieces of evidence are suitable for collection and if touch DNA testing should be performed. Zoppis et al. [21] determined that sebaceous fluid represents an important vector responsible for DNA transfer, therefore "touch DNA" secondary transfer is indeed possible depending on the specific sebaceous or non-sebaceous skin area previously touched. Cutaneous areas previously touched by our volunteers were not recorded in this experiment but could be something to take into consideration in future experiments. In addition to sebaceous fluids, Quinones and Daniel [22] showed that sweat contains epithelial cells and cell-free nucleic acids, which represent additional DNA available for transfer to an object. The incorporation of perspiration to this study may possibly yield higher DNA concentrations than the ones found here. The results obtained in the present study are preliminary, but imply that further investigations for improving the recovery and amplification techniques of contact DNA should be sought to increase the chance of providing probative value of this evidence in sexual assault investigations.

When there is no visible evidence, sexual assault cases can be difficult to investigate and prosecute. In many instances, investigators are faced with the daunting task of determining if there was actually contact between the parties. Furthermore, after determining that there was contact, investigators have to determine if it was consensual or not. Contact DNA could provide this but the examiner needs to take a careful history of the events that transpired to determine what sites (if any) are suitable for contact DNA collection. We are currently in the early stages of understanding touch DNA analysis, however with further studies, novel techniques can be developed to improve recovery and analysis of touch DNA evidence.

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