Journal of Forensic Research
ISSN: 2157-7145
Snyder and Aldredge, J Forensic Res 2016, 7:5
DOI: 10.4172/2157-7145.1000352
Research Article
OMICS International

# Trial by Fire: Comparing DNA Degradation in Blood versus Semen after Fire Exposure

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## Abstract

Criminals have long used fire to try to destroy evidence of other crimes. Historically this cover-up attempt has had enough success that today investigators may assume evidence has either been consumed by the fire or ruined by the fire-fighting efforts. Yet research indicates evidence can endure fire and still yield partial or even full DNA profiles. However, there has been little investigation into the robustness of DNA from one biological fluid compared to another. This study examines whether fire degrades the DNA from blood more than the DNA from semen. The two biological fluids were exposed to fire, the DNA from these samples was extracted using Qiagen kits, and DNA degradation was quantified using a real-time polymerase chain reaction. DNA was quantified from 59 post-fire samples plus 10 positive controls. Data analysis indicated the DNA degradation levels were not significantly different between blood samples and semen samples. However, DNA degradation levels varied significantly between samples on different substrates (nylon versus polyester).

**Keywords:** DNA; Degradation; Arson; Fire investigation; CSF1PO; TH01; Real-time PCR; Forensic science

## Introduction

### DNA backlog

According to a 2010 report by the National Institute of Justice (NIJ), the number of DNA samples submitted to crime labs is outpacing the number of DNA cases these crime labs can process [1]. As a result, the national level of backlogged DNA cases has been increasing every year. In particular, the NIJ reports that the number of samples from property crimes has been "skyrocketing" and that this increase has significantly contributed to the DNA backlog [1]. In response, some crime labs now limit the number of evidence samples they will accept per case. For example, starting in 2012, North Carolina crime labs began accepting no more than two evidence samples per property crime case [2].

Fire, including arson (a common property crime), is notorious for complicating evidence collection. Both the fire itself and the fire-fighting efforts can destroy evidence. Investigators may be skeptical that any biological evidence they collect will prove useful, and so may be tempted to collect and submit many different samples to increase the chances of recovering useful evidence. However, investigators working within evidence submission limits will not have that option; they will have to be more discerning, submitting the "best chance" evidence they can collect. For DNA, "best chance" evidence is evidence most likely to contain complete DNA profiles. Since DNA degradation decreases the odds of getting a complete profile, it would be helpful to understand how fire degrades DNA in different types of samples. This study compared DNA degradation in blood to that in semen after the samples were exposed to fire.

### DNA recovery

Multiple studies have indicated evidence can endure fire [3-7]. Post-fire biological fluids have yielded partial or even full DNA profiles [3,6,7]. There have been a variety of studies investigating how degradation affects DNA samples and how to get useful information from the degraded samples. Alonso et al. [8] developed a highly sensitive and specific method for detecting DNA from 4- to 5-year-old bone samples using fluorescent probes. Swango et al. and Nicklas et al. [9,10] both found degraded DNA contains proportionally greater quantities of shorter loci compared to longer loci.

Rahikainen et al. [11] were able to obtain STR profiles from bloodstain samples stored for up to 16 years. Researchers have also demonstrated that DNA evidence can be recovered after fire or other extreme heat. Tontarski et al. [7] found they could recover DNA from post-fire bloodstains even in some cases where presumptive testing was negative. Rees et al. [12] were able to recover DNA from the dental pulp of wild boar after the jaw bones were heated to an average of 625°C. Hoffman et al. [13] got correct DNA profiles off of backpacks destroyed by pipe bombs. Bús et al. [14] developed a mitochondrial DNA profile from a post-arson burned paper towel that was consistent with the case's suspect profile. In summary there's been significant work on DNA recovery both from degraded samples and from samples exposed to fire or other extreme heat. However, there has been little investigation into how fire affects DNA degradation in blood versus semen.

## Background

### DNA degradation

One form of DNA degradation is hydrolytic cleavage of the phosphodiester bonds. Hydrolytic cleavage increases with heat [15], so fire exposure would likely increase DNA degradation. When the phosphodiester bonds break in the PCR primer's binding region, the primer cannot bind efficiently during PCR and so the DNA strand will amplify poorly or not at all. Similarly, if the phosphodiester bonds

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**Received** October 27, 2016; **Accepted** November 20, 2016; **Published** November 25, 2016

**Citation:** Snyder ML, Aldredge RC (2016) Trial by Fire: Comparing DNA Degradation in Blood versus Semen after Fire Exposure. J Forensic Res 7: 352. doi: 10.4172/2157-7145.1000352

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break anywhere within the target DNA region, when the polymerase reaches that break it will fall off the DNA and the DNA will not amplify [15]. The longer the strand of DNA, the more places there are to cleave the phosphodiester bond, so longer strands degrade more readily and become harder to amplify than shorter strands [15]. Degradation can therefore be approximated by trying to amplify both long and short loci and comparing the resulting amounts [9]. More degraded samples will produce more short DNA amplicons and fewer long ones.

### Wood fires

Because wood is the most common fuel source for structural fires, all of the fire trials in this study were wood-based. Wood-based fires are highly variable. Wood is composed primarily of cellulose, followed by hemicellulose and lignin, and smaller amounts of resins, salts, and water. These components undergo chemical decomposition at different temperatures, ranging from as low as 204°C for hemicellulose to as high as 499°C for lignin [5]. Additionally, some portions of a piece of wood will ignite more easily than others based on the wood's orientation, which affects thermal conductivity and the permeability and natural convection of air. While wood will quickly char (reduce to charcoal) at temperatures above 204°C, long-term exposure to temperatures as low as 80°C can also cause charring [5]. Charred wood heats more quickly than uncharred, which means as a piece of wood chars its ignition temperature changes. This variability is compounded by the inconsistency in wood's moisture content, thickness, and oxygen concentration [5].

The flames produced in wood-based fires are also highly variable. There are many factors that affect flame temperature, but oxygen content and the degree of carbonization are especially significant [5]. The amount of heating provided by a flame varies not only with temperature but also with the flame's position, which fluctuates rapidly. Additionally, samples exposed to wood-based fire experience both radiant and convective heat. The turbulent nature of these fires means their high temperature zones flicker back and forth and up and down, with an average temperature over time of 500°C to 600°C in the flames themselves. Only some of this heat energy would be transferred by convection to a target surface such as carpet swatches. Additionally, any smoke or gas plumes above the flames will give off heat, although the temperature drops as a function of height above the burning fuel. All of these factors combined create different and more variable effects than if the samples were placed in a more consistent heat environment, such as an oven.

### DNA amplification and quantification

This study used quantitative PCR, or qPCR, to determine the amount of DNA in each sample. In addition to exponentially amplifying the original DNA, qPCR includes a method for detecting each new amplicon, making it possible to back calculate the amount of DNA in the original sample. The detection method is fluorescence-based and uses a Taqman™ probe to hybridize with the template DNA in a region between the PCR primers [16]. The probe has a fluorescent dye attached to its 5' end and a fluorescent quencher on its 3' end; the quencher prevents the dye from being detected [17]. During PCR, Taq polymerase synthesizes amplicons. It begins at the primer, and when it reaches the Taqman™ probe, it digests the probe and continues synthesizing. When the probe is digested the quencher and the fluorescent dye separate; now the dye is detectable [17].

The total number of amplicons at the end of PCR depends on how much template DNA was present in the original sample. Because the fluorescent dye attaches to each amplicon, and because the number of amplicons indicates the amount of initial DNA, the final level of fluorescence can be used to calculate the amount of initial DNA. Additionally, the Taqman™ probe is available in different dye colors that will bind to different target sequences, which means the probe can help quantify different DNA sequences in a single sample. This study involved targeting three different sequences using probes with either red, blue, or yellow dyes (Table 1).

## Materials and Methods

### Sample preparation

This study involved fluids on two common types of carpet: polyester (Beaulieu's Value Added™, color: Blank Canvas) and nylon (Shaw Expressive™, color: Ivory Tusk). A Fischer Scientific SterilElite24™ autoclave (Fisher Scientific, Petaluma, CA) set to 121°C destroyed any latent DNA present on the carpets. The carpets were then cut into 3" × 3" swatches and sterile scalpels were used to create one hole in each swatch, which would later be used to thread galvanized steel wire.

The 1mL aliquots of both blood and semen thawed on the lab bench at room temperature. Two 30 µL aliquots of either one fluid or the other were added to the carpet swatches. While the polyester fibers quickly absorbed the biological fluids, the nylon fibres were more resistant. Instead of getting absorbed, the fluid drops rested on top of the nylon fibres. The drops were broken up with a sterile scalpel before the fluids air-dried. Each swatch was packaged separately in sealed and labelled paper bags for transportation to the fire station. Some of the packages included negative controls: carpet swatches with no biological fluids on them.

### Fire trials

Fire fighters supervised all fire trials. For each fire trial, a fire fighter built a fire in the center of a 31 gallon galvanized steel trash can using crumpled paper, kindling (StarterStikk™, Fatwood Firestarter™), parts of a log, and one or more matches. The fire fighter then added some charcoal (Frontier, hardwood lump) and waited for the coals to heat up before adding any samples to the trash can.

The pre-made holes in each carpet swatch were looped with galvanized steel wire with a small weight tied to the end to ensure the swatches would not fall directly into the fire. The fire fighter hung the swatches a few inches from the top of the trash can and equidistant from each other around the circumference of the can. The semen and blood swatches were alternated so that both types of biological fluids experienced the effects of the fire even if the fire tended toward one part of the can over another. Every trial included only one type of carpet or the other and samples from only one donor or the other. Every trial also included one negative control: a polyester or nylon swatch without any blood or semen on it.

| Dye | Locus | Color | Sequence, 5'--> 3' | Position |
|---|---|---|---|---|
| Cy5 | nuTH01 | Red | CY5-ATT CCC ATT GGC CTG TTC CTC CCT T-BHQ2A | Chr.11 (11p15.5) 1138-1162 |
| FAM | nuCSF | Blue | FAM-CAA CCT GCT AGT CCT T-MGB-NFQ | Chr.5 (5q33.3-34) 11,862-11,877 |
| NED | IPC | Yellow | NED-TAC CAT GGC AAT GCT-MGB-NFQ | N/A |

**Table 1:** Taqman™ probe dyes; Adapted from Invitrogen Inc. "Real-time PCR: from theory to practice" (2008).

As expected, these wood-burning fires were highly variable. For each trial, we aimed to leave the swatches exposed to fire long enough to be physically deformed but not so long as to melt the carpet fibers specifically holding the biological fluids (making DNA extraction impossible). Signs of physical deformation included soot-covered or melted carpet fibers, warped carpet backing, or pieces of carpet detaching and falling into the fire.

Despite the fires being built the same way each time, the swatches physically deformed at very different rates: as quickly as 2.5 min to as long as 18 min. The temperatures on the surfaces of the swatches also varied considerably. The fire fighters provided a Thermal Imaging Camera (ISG INFRASYS Elite XR High Resolution, MicroFinity, Chilliwack, BC, Canada) to take swatch surface temperature readings. The TIC reported temperature variations of up to 117°C during a single time point (the surface of one swatch was 121°C at the same time the surface of another swatch was 238°C). After each trial the exposed samples were packaged in new labelled paper bags. Positive controls (swatches that were not exposed to the fire but that had biological fluid on them) were also packaged.

## DNA quantification

All swatches were extracted using Qiagen QIAamp DNA Investigator kits according to Qiagen's protocol "Isolation of Total DNA from Body Fluid Stains" (April 2010) with some minor modifications. This study did not involve using a commercially produced kit to quantify the amount of autosomal DNA. Instead, we used reagents based on a qPCR degradation assay developed by Margaret Aceves: a blend of primers and probes ("Primer-Probe Mix") (Table 2) and a Reaction Mix (Table 3) [18]. The Primer-Probe Mix uses Taqman™ probes (Table 1) to target a mitochondrial DNA location, an internal PCR control (IPC), and two nuclear DNA loci. The information from the mitochondrial location was beyond the scope of this study. The IPC is a 77 base pair (bp) template of synthetic DNA which can indicate PCR inhibition. The nuclear loci are CSF (a 67 bp span of the CSF1PO locus) and TH01 (an approximately 170 bp to 190 bp span of the TH01 locus). Because degraded samples will have proportionally more short loci than long loci, the ratio of CSF to TH01 can indicate levels of degradation. The Reaction Mix is a combination of the Primer-Probe Mix, a 2X Taqman™ Universal Master Mix (Applied Biosystems), and 5 U/µL AmpliTaq Gold® enzyme. The Master Mix includes a ROX-labeled reference to correct for background fluorescence.

In order to calculate the DNA quantities in each sample, we generated standard curves for each nuclear locus using standards created by serially diluting pre-quantified human male DNA (Promega, Sunnyvale, CA) in Tris EDTA buffer (TE-4) (Figure 1).

All samples were quantified on an Applied Biosystems 7500 Fast Real-Time PCR platform (Fisher Scientific, Petaluma, CA) according to the following protocols: 20 µL sample volumes, 9600 emulation mode, a polymerase activation step at 95°C for 10 min, and a two-step qPCR process repeated 45 times (melt the double-stranded DNA at 95°C for 15 sec and anneal primers and extend complementary DNA at 60°C for 60 sec). Every run included a set of standards (16 µL of Reaction Mix plus 4 µL of serially diluted standard), extracted samples (16 µL of Reaction Mix plus 4 µL of sample), and negative controls (16 µL of Reaction Mix plus 4 µL of TE-4 buffer).

## Data analysis

The 7500 software produces amplification curves (Figure 2) and standard curves (Figure 3) for each qPCR run. The amplification curves are a plot of the fluorescence intensity vs. the PCR cycle number. A sample's cycle threshold ($C_T$) is the number of PCR cycles the sample goes through before it fluoresces at an empirically determined threshold.

| Reagents | Volume/Sample (µL) |
|---|---|
| 2x Taqman Universal Master Mix | 10 |
| 5 U/µL Amplitaq Gold enzyme | 0.5 |
| Primer-Probe Mix | 5.5 |
| Total | 16 |

**Table 3:** Composition of Reaction Mix.

| Reagents* | Initial Conc. (µM)† | First Dilution | | | Final Primer-Probe Mix | |
|---|---|---|---|---|---|---|
| | | Reagent Volume (µL)‡ | TE-4 Volume (µL)§ | Concentration (µM) | Reagent Volume (µL) | Concentration (µM) |
| Sterile water | N/A | N/A | N/A | N/A | 52.6 | N/A |
| BSA (3.2µg/µL) | 4.6 | N/A | N/A | N/A | 277.4 | 0.58 |
| nuTH01-probe-Cy5 (8000 nM) | 100 | 5 | 45 | 10 | 44 | 0.2 |
| nuTH01-F primer (120,000 nM) | 500 | 10 | 40 | 100 | 13.2 | 0.6 |
| nuTH01-R primer (120,000 nM) | 500 | 10 | 40 | 100 | 13.2 | 0.6 |
| nuCSF-probe-FAM (10,000 nM) | 100 | 5 | 45 | 10 | 22 | 0.1 |
| nuCSF-F primer (200,000 nM) | 500 | 10 | 40 | 100 | 8.8 | 0.4 |
| nuCSF-R primer (200,000 nM) | 500 | 10 | 40 | 100 | 8.8 | 0.4 |
| IPC-probe-NED (12,000 nM) | 100 | 5 | 45 | 10 | 44 | 0.2 |
| IPC-F primer (18,000 nM) | 500 | 4 | 96 | 20 | 5.5 | 0.05 |
| IPC-R primer (18,000 nM) | 500 | 4 | 96 | 20 | 5.5 | 0.05 |
| IPC-oligo (50 nM --> 500 fM) | Variable | N/A | N/A | N/A | 33 | 90,000 copies |
| 283bp mtprobe-VIC (8000nM) | 100 | 5 | 45 | 10 | 44 | 0.2 |
| 283bp mito-F primer | 500 | 4 | 96 | 20 | 16.5 | 0.15 |
| 283bp mito-R primer | 500 | 4 | 96 | 20 | 16.5 | 0.15 |

* "F primer" and "R primer" are the forward and reverse primers.
† Concentration of stock solutions.
‡ Volume of stock solution used for first dilution.
§ Volume of Tris EDTA buffer used as diluent in first dilution.
(( If the reagents have been diluted, take the volume from the diluted version.
Otherwise take the volume from the initial solution.

**Table 2:** Composition of Primer-Probe Mix.

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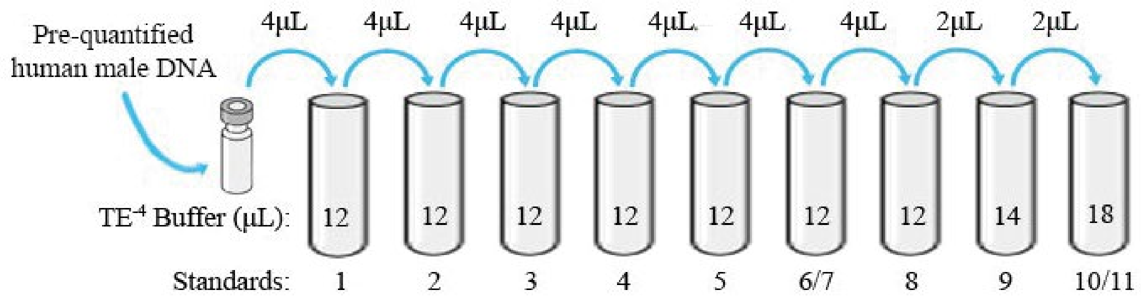


**Figure 1:** Method for creating serially diluted standards.

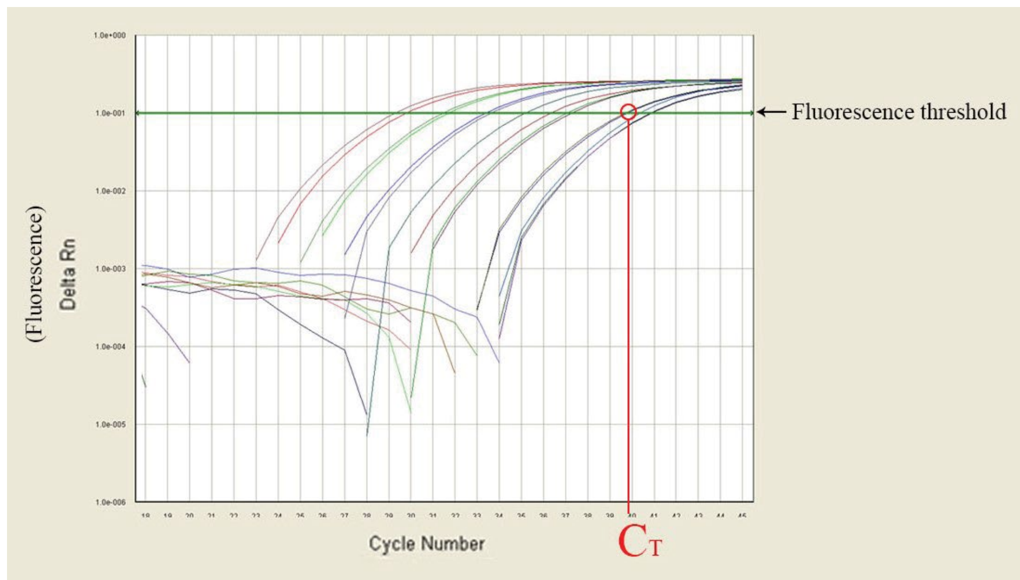


**Figure 2:** An example of a 7500 amplification curve.

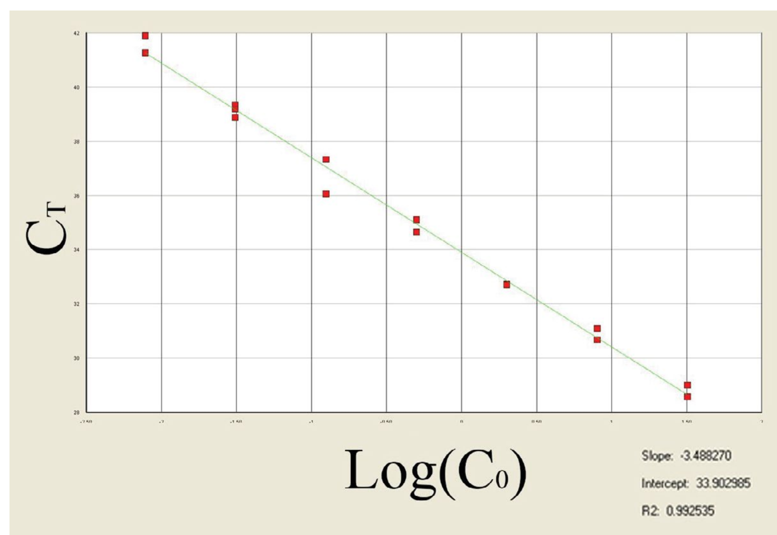


**Figure 3:** An example of a 7500 standard curve.

In this study, the thresholds for IPC, CSF, and TH01 were 0.025, 0.05, and 0.15, respectively.

The standard curves are plots of a standard's $C_T$ versus the log of $C_0$ (the initial concentration of the standard). An acceptable standard curve has a slope between -3.1 and -3.58 and an $R^2$ value greater than 0.985. The slope is a measure of the run's efficiency; a 100% efficient run will have a slope of -3.32, which indicates the DNA quantity has doubled during each cycle of PCR. The $R^2$ value indicates the assay's linearity; a 100% linear run will have an $R^2$ value of 1.000, which means the relationship between the $C_T$ and the log of $C_0$ is perfectly linear. The results from each 7500 run were exported as CSV files (comma delimited) to Microsoft® Excel 2010 spread sheets.

Replicate measurements of all samples were collected in order to identify outliers. In most cases samples were quantified in triplicate during the same run, but for some samples replicate measurements were taken during different runs. Results can be compared across different runs as long as the results fulfil the assay's requirements for acceptable standard curves, amplification thresholds, and amplification cycle numbers. To identify outliers for a given loci, we looked for replicates that crossed the amplification threshold more than 0.5 cycles ($C_T$) apart. For samples measured in triplicate, if two of the measurements were within 0.5 $C_T$, those two values were kept and the third measurement was disregarded as the outlier. If samples were measured in duplicate and they were more than 0.5 $C_T$ apart, both values were disregarded.

After eliminating replicate outliers, we calculated the ratios of CSF concentrations to TH01 concentrations for each sample. The DNA samples were extracted using the same volume of elution buffer (40 µL) for every extraction. Each aliquot could still contain varying amounts of DNA, as DNA concentration is not always uniform within a sample; however, using the same volume of elution buffer was intended to keep the DNA quantities as similar as possible between loci.

The purpose of the study was to determine whether the semen ratios were significantly different from the blood ratios. Before choosing a statistical tool for this process, Shapiro-Wilks tests were performed to determine whether the ratios are normally distributed. These tests assign a critical W score ($W_c$) for a given sample size and p-value. Normally distributed data will have a Shapiro-Wilk Score (W) greater than $W_c$. Additionally, we created histograms to visually confirm data distribution. The bins for the histograms were determined based on the minimum and maximum values in each dataset. The bin sizes were chosen such that all bins were the same size and they encompassed the entire dataset. Because each dataset is a set of degradation ratios, the bins numbers also represent ratios. For example, a bin marked "2" includes all degradation ratios higher than 1 but lower than 2.

Before analyzing mean degradation levels, samples were analyzed to determine whether they had degraded DNA or not. First the CSF:TH01 ratios of the positive controls (swatches that were not exposed to fire) were analyzed. Then sample degradation ratios were compared to the positive control degradation ratios. Samples were considered degraded only if their ratios were higher than the positive control ratio plus one standard deviation for the relevant variable.

The t-test is a common statistical tool for analyzing differences between groups, and although it is a parametric tool, it is robust to departures from normality. T-tests were conducted to compare the mean DNA degradation levels based on biological fluids (blood versus semen), substrate (nylon carpet versus polyester carpet), and donor (Donor 1 samples versus Donor 2 samples). T-tests were also conducted for subsets of the data, such as blood versus semen for Donor 1 only or for nylon swatches only.

## Results

The semen samples turned dark orange in the presence of fire and so were easy to locate (Figure 4a). Similarly, the blood samples turned black and were also obvious (Figure 4b). Table 4 shows the replicate measurements of DNA concentrations for all samples. The table does not include measurements that crossed the amplification threshold more than 0.5 $C_T$ apart from the sample's other measurements. For every sample two averages were calculated: one for the replicate measurements of CSF concentrations (the shorter loci) and one for the replicate measurements of TH01 concentrations (the longer loci). Dividing the CSF average by the TH01 average gives a ratio of short-to-long DNA, which approximates the extent of degradation: the higher the ratio, the more degraded the sample. The range of DNA concentrations for all samples was large, from a minimum of 0.011 ng/µL to a maximum of 367.58 ng/µL. There was a wide range of DNA concentrations not only across all variables, but also within groups with

**Figure 4a:** Red arrows indicate post-fire semen samples, which appeared orange.

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**Figure 4b:** Red arrows indicate post-fire blood samples, which appeared black.

| Sample # | BioFluid | Substrate | Donor | Locus | DNA concentration (ng/uL) | | | Mean | StDev | CSF:THO1 |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Repeat 1 | Repeat 2 | Repeat 3 | | | |
| 24 | Blood | Polyester | 1 | CSF | 0.91 | 0.76 | 0.77 | 0.81 | 0.06 | 0.575 |
| | | | | TH01 | 1.48 | 1.3 | 1.47 | 1.42 | 0.08 | |
| 25 | Blood | Polyester | 1 | CSF | 4.96 | 5.09 | 5.24 | 5.1 | 0.11 | 0.579 |
| | | | | TH01 | 9.07 | 8.7 | 8.64 | 8.8 | 0.19 | |
| 26 | Blood | Polyester | 1 | CSF | 1.38 | 1.38 | 1.34 | 1.37 | 0.02 | 0.537 |
| | | | | TH01 | 2.63 | 2.62 | 2.38 | 2.54 | 0.12 | |
| 27 | Semen | Polyester | 1 | CSF | 19.99 | 18.26 | 21.23 | 19.83 | 1.22 | 1.037 |
| | | | | TH01 | 19.53 | 18.15 | 19.68 | 19.12 | 0.69 | |
| 28 | Semen | Polyester | 1 | CSF | 15.26 | 16.14 | 15.75 | 15.72 | 0.36 | 1.128 |
| | | | | TH01 | 13.63 | 14.14 | 14.02 | 13.93 | 0.22 | |
| 29 | Semen | Polyester | 1 | CSF | 98.55 | 85.73 | 93.89 | 92.72 | 5.3 | 0.877 |
| | | | | TH01 | 111.21 | 101.36 | 104.66 | 105.74 | 4.09 | |
| 31 | Blood | Polyester | 1 | CSF | 7.99 | 7.47 | 7.56 | 7.67 | 0.23 | 0.63 |
| | | | | TH01 | 12.52 | 12.3 | 11.74 | 12.19 | 0.33 | |
| 32 | Blood | Polyester | 1 | CSF | 3.5 | 3.94 | 3.52 | 3.65 | 0.2 | 0.502 |
| | | | | TH01 | 7.27 | 7.63 | 6.94 | 7.28 | 0.28 | |
| 33 | Blood | Polyester | 1 | CSF | 1.3 | 2.05 | 1.81 | 1.72 | 0.31 | 0.512 |
| | | | | TH01 | 2.97 | 3.75 | 3.36 | 3.36 | 0.32 | |
| 34 | Semen | Polyester | 1 | CSF | 124.98 | 132.1 | 126.95 | 128.01 | 3 | 0.778 |
| | | | | TH01 | 163.25 | 166.36 | 163.72 | 164.44 | 1.37 | |
| 35 | Semen | Polyester | 1 | CSF | 19.89 | 10.51 | 21.1 | 17.17 | 4.73 | 1.168 |
| | | | | TH01 | 15.9 | 11.82 | 16.37 | 14.7 | 2.04 | |
| 36 | Semen | Polyester | 1 | CSF | 36.87 | 39.18 | 40.01 | 38.69 | 1.33 | 0.702 |
| | | | | TH01 | 54.72 | 55.13 | 55.42 | 55.09 | 0.29 | |
| 52 | Blood | Nylon | 1 | CSF | 33.26 | 30.79 | N/A | 32.03 | 1.24 | 0.68 |
| | | | | TH01 | 49.15 | 45.08 | N/A | 47.12 | 2.04 | |
| 53 | Blood | Nylon | 1 | CSF | 31.77 | 26.25 | N/A | 29.01 | 2.76 | 0.638 |
| | | | | TH01 | 48.77 | 42.19 | N/A | 45.48 | 3.29 | |
| 54 | Blood | Nylon | 1 | CSF | 35.22 | 29.37 | N/A | 32.3 | 2.93 | 0.665 |
| | | | | TH01 | 53.91 | 43.25 | N/A | 48.58 | 5.33 | |
| 55 | Semen | Nylon | 1 | CSF | 156.23 | 133.95 | N/A | 145.09 | 11.14 | 0.583 |
| | | | | TH01 | 270.76 | 227.3 | N/A | 249.03 | 21.73 | |
| 56 | Semen | Nylon | 1 | CSF | 105.38 | 97.95 | N/A | 101.67 | 3.72 | 0.555 |
| | | | | TH01 | 183.36 | 182.93 | N/A | 183.15 | 0.22 | |
| 57 | Semen | Nylon | 1 | CSF | 143.25 | 134.7 | N/A | 138.98 | 4.28 | 0.49 |
| | | | | TH01 | 288.57 | 279.23 | N/A | 283.9 | 4.67 | |
| 59 | Blood | Nylon | 1 | CSF | 15.58 | 12.23 | N/A | 13.91 | 1.67 | 1.188 |
| | | | | TH01 | 12.56 | 10.84 | N/A | 11.7 | 0.86 | |
| 60 | Blood | Nylon | 1 | CSF | 1.51 | 1.46 | N/A | 1.49 | 0.03 | 2.015 |
| | | | | TH01 | 0.73 | 0.74 | N/A | 0.74 | 0.01 | |

| 61 | Blood | Nylon | 1 | CSF | 0.02 | 0.02 | N/A | 0.02 | 0 | 1.017 |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | TH01 | 0.01 | 0.02 | N/A | 0.02 | 0.01 | |
| 62 | Semen | Nylon | 1 | CSF | 45.36 | 42.02 | N/A | 43.69 | 1.67 | 2.575 |
| | | | | TH01 | 17.66 | 16.28 | N/A | 16.97 | 0.69 | |
| 63 | Semen | Nylon | 1 | CSF | 0.83 | 0.83 | N/A | 0.83 | 0 | 4.527 |
| | | | | TH01 | 0.14 | 0.22 | N/A | 0.18 | 0.04 | |
| 64 | Semen | Nylon | 1 | CSF | 159.19 | 185.44 | N/A | 172.32 | 13.13 | 1.269 |
| | | | | TH01 | 139.59 | 131.89 | N/A | 135.74 | 3.85 | |
| 66 | Blood | Nylon | 1 | CSF | 0.01 | 0.02 | N/A | 0.02 | 0.01 | 0.83 |
| | | | | TH01 | 0.02 | 0.03 | N/A | 0.02 | 0.01 | |
| 67 | Blood | Nylon | 1 | CSF | 0.34 | 0.3 | N/A | 0.32 | 0.02 | 5.906 |
| | | | | TH01 | 0.06 | 0.05 | N/A | 0.05 | 0.01 | |
| 68 | Semen | Nylon | 1 | CSF | 90.79 | 78.47 | N/A | 84.63 | 6.16 | 0.934 |
| | | | | TH01 | 102.02 | 79.23 | N/A | 90.63 | 11.4 | |
| 74 | Blood | Nylon | 2 | CSF | 0.06 | 0.12 | 0.1 | 0.09 | 0.02 | 2.428 |
| | | | | TH01 | 0.04 | 0.04 | N/A | 0.04 | 0 | |
| 75 | Blood | Nylon | 2 | CSF | 30.87 | 28.44 | 29.04 | 29.45 | 1.03 | 0.736 |
| | | | | TH01 | 41.1 | 41.03 | 37.92 | 40.02 | 1.48 | |
| 76 | Blood | Nylon | 2 | CSF | 2.27 | 2.29 | 2.22 | 2.26 | 0.03 | 1.943 |
| | | | | TH01 | 1.08 | 1.19 | 1.22 | 1.16 | 0.06 | |
| 79 | Semen | Nylon | 2 | CSF | 21.19 | 19.12 | 20.41 | 20.24 | 0.85 | 6.345 |
| | | | | TH01 | 3.36 | 3.15 | 3.06 | 3.19 | 0.13 | |
| 81 | Blood | Nylon | 2 | CSF | 29.14 | 26.21 | 26.65 | 27.33 | 1.29 | 0.835 |
| | | | | TH01 | 35.37 | 30.88 | 31.93 | 32.73 | 1.92 | |
| 82 | Blood | Nylon | 2 | CSF | 34.57 | 36.38 | 39.03 | 36.66 | 1.83 | 0.747 |
| | | | | TH01 | 50.15 | 48.31 | 48.76 | 49.07 | 0.78 | |
| 83 | Blood | Nylon | 2 | CSF | 4.98 | 5.7 | 4.56 | 5.08 | 0.47 | 1.503 |
| | | | | TH01 | 3.35 | 3.21 | 3.58 | 3.38 | 0.15 | |
| 84 | Semen | Nylon | 2 | CSF | 182.16 | 151.9 | 183.43 | 172.5 | 14.57 | 0.61 |
| | | | | TH01 | 309.7 | 262.03 | 276.01 | 282.58 | 20.01 | |
| 85 | Semen | Nylon | 2 | CSF | 174.14 | 165.07 | 156.37 | 165.19 | 7.26 | 0.456 |
| | | | | TH01 | 367.58 | 362.98 | 355.12 | 361.89 | 5.14 | |
| 86 | Semen | Nylon | 2 | CSF | 77.37 | 78.98 | 78.15 | 78.17 | 0.66 | 1.303 |
| | | | | TH01 | 61.95 | 59.86 | 58.19 | 60 | 1.54 | |
| 117 | Blood | Nylon | 2 | CSF | 0.21 | 0.22 | 0.19 | 0.2 | 0.01 | 6.718 |
| | | | | TH01 | 0.04 | 0.02 | 0.03 | 0.03 | 0.01 | |
| 118 | Blood | Nylon | 2 | CSF | 0.62 | 0.62 | 0.75 | 0.66 | 0.06 | 5.289 |
| | | | | TH01 | 0.14 | 0.14 | 0.1 | 0.12 | 0.02 | |
| 120 | Semen | Nylon | 2 | CSF | 19.15 | 24.66 | 19.17 | 20.99 | 2.59 | 3.526 |
| | | | | TH01 | 5.64 | 6.42 | 5.8 | 5.95 | 0.34 | |
| 121 | Semen | Nylon | 2 | CSF | 7.38 | 8.41 | 6.41 | 7.4 | 0.82 | 9.391 |
| | | | | TH01 | 0.69 | 0.99 | 0.69 | 0.79 | 0.14 | |
| 133 | Blood | Polyester | 2 | CSF | 6.05 | 6.26 | 6.68 | 6.33 | 0.26 | 0.734 |
| | | | | TH01 | 8.43 | 8.86 | 8.58 | 8.62 | 0.18 | |
| 134 | Blood | Polyester | 2 | CSF | 19.75 | 18.87 | 23.57 | 20.73 | 2.04 | 0.855 |
| | | | | TH01 | 23.59 | 23.43 | 25.75 | 24.26 | 1.06 | |
| 135 | Blood | Polyester | 2 | CSF | 20.73 | 20.85 | 21.21 | 20.93 | 0.2 | 0.609 |
| | | | | TH01 | 33.6 | 35.19 | 34.25 | 34.35 | 0.65 | |
| 136 | Semen | Polyester | 2 | CSF | 182.93 | 175.06 | 170.59 | 176.19 | 5.1 | 1.381 |
| | | | | TH01 | 128.84 | 130.62 | 123.36 | 127.61 | 3.09 | |
| 137 | Semen | Polyester | 2 | CSF | 0.25 | 0.28 | 0.22 | 0.25 | 0.02 | 0.992 |
| | | | | TH01 | 0.24 | 0.2 | 0.31 | 0.25 | 0.05 | |
| 138 | Semen | Polyester | 2 | CSF | 160.36 | 173.57 | 161.57 | 165.17 | 5.96 | 0.826 |
| | | | | TH01 | 180.22 | 212.58 | 207.19 | 200 | 14.16 | |
| 140 | Blood | Polyester | 2 | CSF | 14.94 | 16.78 | 17.57 | 16.43 | 1.1 | 0.668 |
| | | | | TH01 | 23.89 | 24.77 | 25.13 | 24.6 | 0.52 | |
| 141 | Blood | Polyester | 2 | CSF | 14.16 | 16.85 | 12.23 | 14.41 | 1.89 | 0.682 |
| | | | | TH01 | 21.23 | 22.86 | 19.33 | 21.14 | 1.44 | |
| 142 | Blood | Polyester | 2 | CSF | 8.54 | 9.89 | 9.34 | 9.26 | 0.55 | 0.593 |
| | | | | TH01 | 15.44 | 16 | 15.41 | 15.62 | 0.27 | |
| 143 | Semen | Polyester | 2 | CSF | 99.93 | 103.94 | 98.88 | 100.92 | 2.18 | 0.723 |

| | | | | TH01 | 135.1 | 145.43 | 138.26 | 139.6 | 4.32 | |
|---|---|---|---|---|---|---|---|---|---|---|
| 144 | Semen | Polyester | 2 | CSF | 109 | 100.24 | 114.88 | 108.04 | 6.02 | 0.725 |
| | | | | TH01 | 150.36 | 147.27 | 149.35 | 148.99 | 1.29 | |
| 145 | Semen | Polyester | 2 | CSF | 159.09 | 203.03 | 187.9 | 183.34 | 18.23 | 0.924 |
| | | | | TH01 | 169.43 | 216.86 | 209.07 | 198.45 | 20.77 | |
| 147 | Blood | Polyester | 2 | CSF | 21.31 | 19.8 | 20.61 | 20.57 | 0.62 | 0.591 |
| | | | | TH01 | 34.26 | 34.71 | 35.48 | 34.82 | 0.5 | |
| 148 | Blood | Polyester | 2 | CSF | 0.03 | 0.03 | 0.02 | 0.03 | 0.01 | 0.592 |
| | | | | TH01 | 0.03 | 0.06 | 0.05 | 0.05 | 0.01 | |
| 149 | Blood | Polyester | 2 | CSF | 17.74 | 15.98 | 16.98 | 16.9 | 0.72 | 0.564 |
| | | | | TH01 | 31.54 | 28.62 | 29.71 | 29.96 | 1.2 | |
| 150 | Semen | Polyester | 2 | CSF | 17.1 | 16.52 | 15.94 | 16.52 | 0.47 | 0.966 |
| | | | | TH01 | 16.9 | 17.62 | 16.76 | 17.09 | 0.38 | |
| 151 | Semen | Polyester | 2 | CSF | 28.4 | 25.72 | 24.66 | 26.26 | 1.57 | 0.858 |
| | | | | TH01 | 31.66 | 31.95 | 28.26 | 30.62 | 1.68 | |
| 152 | Semen | Polyester | 2 | CSF | 42.18 | 43.58 | 46.83 | 44.2 | 1.95 | 1.545 |
| | | | | TH01 | 28.89 | 28.76 | 28.19 | 28.61 | 0.3 | |

**Table 4:** Sample Data, con't.

isolated variables. For example, even when the researchers examined only the TH01 locus from Donor 2's semen DNA on polyester swatches, the DNA concentration varied by 216.67 ng/μL.

The Shapiro-Wilks tests indicated that neither the blood nor semen ratios were normally distributed. Recall that normally distributed data will have a Shapiro-Wilk Score (W) greater than Wc. For the blood samples, W is 0.553 and Wc is 0.902 at p=0.01; since W is less than Wc, the blood ratios are not normally distributed. Likewise, the semen ratios have a W of 0.587 and Wc of 0. 896 at p=0.01, meaning the semen ratios are not normally distributed.

Additionally, histograms of both sets of ratios show the non-normal distributions (Figures 5 and 6). For both blood and semen, the degradation ratios primarily fell into bins 1 and 2 (ratios between 0 and 1, and between 1 and 2, respectively). The set of blood DNA ratios included 22 ratios in bin 1; semen DNA had 16 ratios in bin 1. In contrast, both datasets had fewer ratios in the larger bins; for example, each dataset had only one ratio in bin 7.

The positive controls (the swatches with either blood or semen that were not exposed to fire) had an average ratio of 0.616 (SD-0.064). Nylon positive controls had the lowest positive control ratio at 0.570 (SD-0.035). Polyester positive controls had the highest positive control ratio at 0.652 (SD-0.071). These results meant that, for example, polyester samples were only considered degraded if they had ratios higher than 0.723 (0.652+0.071). A summary of the positive control ratios can be found in Table 5.

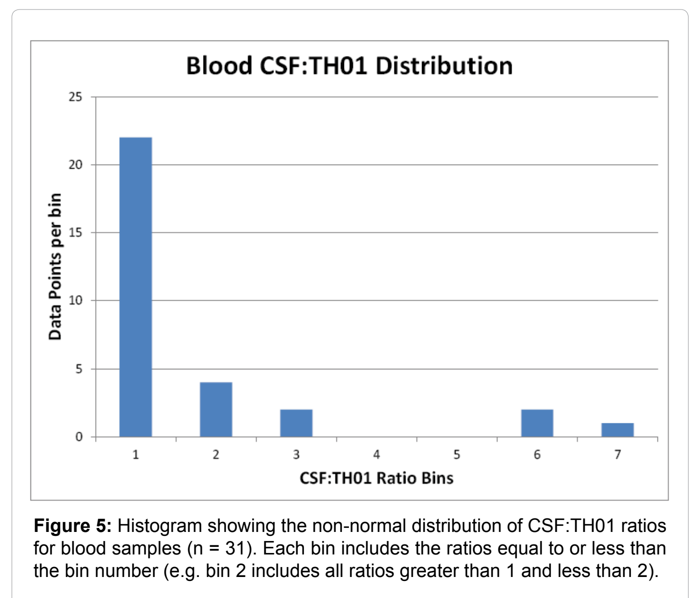


**Figure 5:** Histogram showing the non-normal distribution of CSF:TH01 ratios for blood samples (n = 31). Each bin includes the ratios equal to or less than the bin number (e.g. bin 2 includes all ratios greater than 1 and less than 2).

## Blood versus semen

There were 31 blood samples, and 19 had degradation ratios above the blood positive control threshold. Out of 28 semen samples, 23 had degradation ratios above the semen positive control threshold. T-tests showed no significant difference in degradation levels for blood DNA (M=1.87, SD=1.91) compared to semen DNA (M=1.93, SD=2.14) across all samples; t(40) 0.110, p=0.05. T-tests also showed no significant difference in DNA degradataion between blood and semen when examining only samples from Donor 1, Donor 2, nylon swatches, or polyester swatches.

## Donor 1 versus donor 2

Out of 27 samples from Donor 1, 17 had degradation ratios above the positive control threshold; there were 32 samples from Donor 2, and 23 had degradation ratios above the positive control threshold. T-tests showed no significant difference in degradation levels for Donor 1's samples (M=1.61,SD=1.47) compared to Donor 2's samples (M=2.23,SD=2.39); t(37)=1.02,p=0.05. This result held when comparing Donor 1 and Donor 2 DNA degradation for only samples from blood, semen, nylon swatches, or polyester swatches.

## Nylon versus polyester

Out of 29 samples on nylon swatches, 25 had degradation ratios above the nylon positive control threshold. There were 30 samples on polyester swatches, and 15 of them had degradation ratios above the polyester positive control threshold. T-tests showed significant differences in DNA degradation levels for samples on nylon swatches (M=2.54, SD=2.42) compared to samples on polyester swatches (M=0.98, SD=0.06); t(25)=3.19,p=0.05. This result held when comparing only blood samples on nylon (M=2.07, SD=2.03) to only blood samples on polyester (M=0.79, SD=0.01); t(15)=2.50,p=0.05. Nylon samples also had significantly more DNA degradation when comparing only semen samples on nylon (M=3.39, SD=2.94) to only

**Citation:** Snyder ML, Aldredge RC (2016) Trial by Fire: Comparing DNA Degradation in Blood versus Semen after Fire Exposure. J Forensic Res 7: 352. doi: 10.4172/2157-7145.1000352



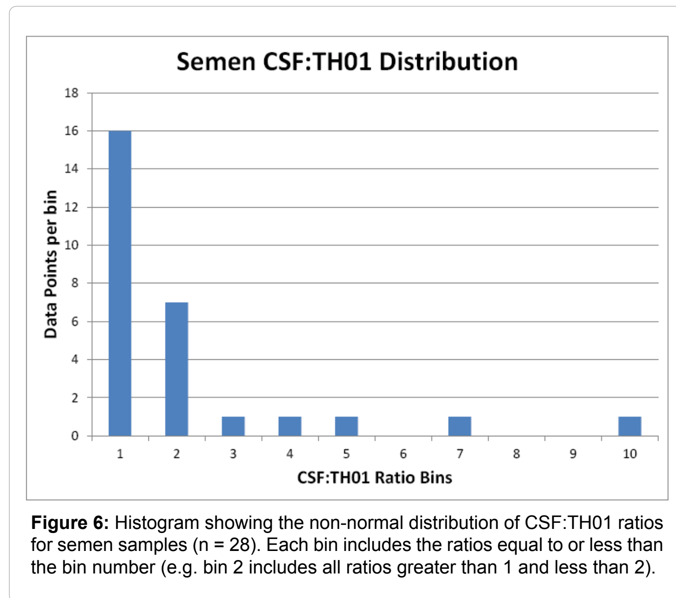


**Figure 6:** Histogram showing the non-normal distribution of CSF:TH01 ratios for semen samples (n = 28). Each bin includes the ratios equal to or less than the bin number (e.g. bin 2 includes all ratios greater than 1 and less than 2).

| Samples | n | Avg CSF:TH01 | StDev |
|---|---|---|---|
| Blood | 5 | 0.593 | 0.065 |
| Semen | 5 | 0.639 | 0.053 |
| Nylon | 4 | 0.57 | 0.035 |
| Polyester | 4 | 0.652 | 0.071 |
| Donor 1 | 6 | 0.61 | 0.047 |
| Donor 2 | 4 | 0.624 | 0.082 |
| All | 10 | 0.616 | 0.064 |

**Table 5:** Positive control thresholds.

semen samples on polyester (M=1.01, SD=0.06); t(8)=2.41,p=0.05. There was no significant difference in degradation levels for Donor 1 samples on nylon (M=1.85, SD=1.70) to Donor 1 samples on polyester (M=1.00, SD=0.03); t(11)=1.73,p=0.05. However the t-test indicated a significant difference in degradation levels for Donor 2 samples on nylon (M=3.18, SD=2.86) compared to Donor 2 samples on polyester (M=0.98, SD=0.27); t(12)=2.76,p=0.05) (Table 6)

## Discussion

This experimental design controlled for as many variables as possible, such as swatch size, biological fluid volume, storage conditions, and sample positions during each fire trial. Despite this consistency, the resulting DNA concentrations across all trials were highly variable. This variation is most likely due to the inherent inconsistency of both DNA sampling and the fire itself. Even though the biological fluid volume was constant, one aliquot of sperm or blood may have had a different number of cells (and thus different amount of DNA) than another aliquot. Additionally, variables between fire trials (and sometimes within the same fire trial) included flame height, presence of smoke or ash, peak temperature of the fire, peak temperature of the trash can, and weather conditions. This variability mimics the fluctuating nature of fires in real crime scenes, but it most likely contributed to the highly variable DNA concentrations.

The t-test results indicate that semen DNA was not significantly more degraded than blood DNA. Crime scene investigators and DNA analysts may be surprised by this result because traditionally sperm are thought of as particularly hardy cells. Sperm nuclear membranes, which protect the nucleus and thus DNA, contain protein disulfide bridges; these bridges make the membranes more resistant to destruction compared to the nuclear membranes of other types of cells. In contrast, leukocytes (white blood cells), which contain the DNA found in blood, have no disulfide bridges. And yet the results indicate that semen DNA was no more resistant to degradation than blood DNA. In fact a higher proportion of semen samples than blood samples were considered degraded; 82% (23 out of 28) of semen samples were above the semen positive control threshold compared to 61% (19 out of 31) of blood samples that were above the blood positive control threshold.

| Comparison | t | $t_{crit}$ | Sig. Dif. | More Degraded |
|---|---|---|---|---|
| | Blood vs. Semen | | | |
| Blood vs. Semen | 0.11 | 2.021 | No | N/A |
| Donor 1 Blood vs. Semen | 0.347 | 2.131 | No | N/A |
| Donor 2 Blood vs. Semen | 0.359 | 2.069 | No | N/A |
| Nylon Blood vs. Semen | 1.497 | 2.08 | No | N/A |
| Polyester Blood vs. Semen | 1.899 | 2.11 | No | N/A |
| | Nylon vs. Polyester | | | |
| Nylon vs. Polyester | 3.191 | 2.06 | Yes | Nylon |
| Blood Nylon vs. Polyester | 2.503 | 2.131 | Yes | Nylon |
| Semen Nylon vs. Polyester | 2.411 | 2.306 | Yes | Nylon |
| Donor 1 Nylon vs. Polyester | 1.725 | 2.201 | No | N/A |
| Donor 2 Nylon vs. Polyester | 2.761 | 2.179 | Yes | Nylon |
| | Donor 1 vs. Donor 2 | | | |
| Donor 1 vs. Donor 2 | 1.02 | 2.026 | No | N/A |
| Blood Donor 1 vs. Donor 2 | 0.421 | 2.131 | No | N/A |
| Semen Donor 1 vs. Donor 2 | 0.712 | 2.08 | No | N/A |
| Nylon Donor 1 vs. Donor 2 | 1.429 | 2.08 | No | N/A |
| Polyester Donor 1 vs. Donor 2 | 0.069 | 2.131 | No | N/A |

**Table 6:** Summary of t-test results (All differences measured at p=0.05)

T-test results indicate no significant degradation difference between Donor 1's DNA and Donor 2's DNA for all samples or for different subsets of samples. However, Donor 2's DNA was significantly more degraded on nylon samples than on polyester samples, while Donor 1's DNA showed no significant difference in degradation based on substrate. It's possible the DNA from different individuals will degrade differently in similar circumstances, but this question would be best explored in a study with a greater number of donors and a greater number of samples from each donor.

Finally, the t-test results indicate that samples on nylon swatches were significantly more degraded than those on polyester swatches. This result is underscored by the proportion of degraded samples in each subset: only 50% of samples on polyester (15 out of 30) were above the polyester positive control threshold compared to a full 86% of samples on nylon (25 out of 29) above the nylon positive control threshold. This disparity is likely due to the different absorbencies of the swatches. The nylon swatches did not readily absorb the biological fluids; the fluids rested at the top of the carpet fibers, and so a greater portion of each fluid was directly exposed to fire with no protection from the carpet. In contrast, the polyester swatches easily absorbed the biological fluids, such that most of the fluids had soaked into the fibers and then spread along the base of the carpet before fire exposure. The polyester carpet itself offered some protection during the fire trials.

The different outcomes for nylon versus polyester confirm that substrates can affect the quality of post-fire evidence. Nylon and polyester are common types of carpet, but the crime scenes for fire can include a wide range of substrates. It is possible that other non-absorbent substrates will also result, as the nylon swatches did, in higher DNA degradation levels.

Whatever the crime scene conditions, it is important that arson investigators are made aware of potential degradation in their samples. Degradation may affect forensic processes from sample collection to analysis. Investigators who suspect degradation may interpret screening results differently and may collect different types or numbers of samples. DNA analysts may likewise use different screening methods and may try to extract DNA from more locations or different locations on the evidence. Analysts might also interpret DNA typing results differently, as degraded DNA can affect DNA profile results.

It may be useful to conduct future research on a wider variety of substrates using a greater number of donors to further explore the effects of substrate and donor on DNA degradation. Similarly, it may be useful to analyze the effects of different types of fuel (other than wood) and of various fire extinguishing methods; previous research suggests both blood and semen stains can persist after exposure to water [19-21]. It might also be useful to compare DNA results from post-fire blood and semen samples to DNA results from post-fire hair samples; DNA from hair has been shown to be more resistant to degradation than DNA from either blood or semen when exposed to warm temperatures over a long time period (90 days or more) [22], but this robustness may be negated if hair samples burn away more easily than blood and semen samples.

## Conclusion

Overall, fire exposure did not degrade blood DNA any more than semen DNA. However, samples on nylon were significantly more degraded than samples on polyester. Though investigators and analysts might expect blood DNA to generally be more degraded than semen DNA, this study suggests that is not the case.

### Acknowledgement

Thanks to the nurses at the UC Davis Clinical and Translational Science Center and the firefighters at the Sacramento Metro Fire Station for helping ensure the experiment was conducted safely. Thank you to Dr. John DeHaan for lending his expertise regarding fires and fire investigations. The project described was supported in part by the National Center for Advancing Translational Sciences (NCATS), National Institutes of Health (NIH), though grant #UL1 TR000002, which paid for phlebotomy services.

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