The Effects of Green Dry-Cleaning on the Ability to Detect and Obtain DNA from Semen Stains on Three Different Types of Fabrics

By

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Abstract

The ability to detect and obtain DNA profiles from body fluid stains on clothing is important in solving crimes. However, many crimes are reported after a significant delay and stained clothing is sometimes exposed to water, detergents, and/or other cleaning agents before it is collected as evidence. Research on the effects of water immersion and aqueous-based cleaning methods (e.g. machine laundering, detergents, machine drying) indicates that a number of variables affect whether a stain can be detected post-exposure, including the body fluid examined, the fabric type, and the presence or absence of detergents and agitation. However, the effects of dry-cleaning on body fluid stains are not well understood, despite the fact that many fabrics are "dry-clean only." Additionally, most of the available information on dry-cleaning is based on the chemical perchloroethylene (Perc) and due to a 2007 ban on Perc, research was needed to examine the effects of available dry-cleaning alternatives. Three dry-clean-only fabrics were stained with semen and submitted for dry cleaning. Two green dry-cleaners were used, one using the petroleum-based DF2000™ and one using the silicone-based GreenEarth® process. After dry-cleaning, the stained fabrics were screened using a 5000 Å Crime-lite® and an acid phosphatase (AP) spot test. The sperm were then released from the fabric and detected using a Christmas Tree stain assay. Regardless of the results of the screening tests, the stains were removed and analyzed for DNA. The DNA was extracted using QIAamp® DNA Investigator kits, quantitated by qPCR using Quantifiler® Duo DNA Quantification kits, and genotyped using AmpFlSTR Identifiler® Plus kits. It was found that dry-cleaned semen stains were often difficult to detect with the Crime-lite® and the AP spot test but that sperm were present in abundance during the Christmas Tree stain assay. It was also found that enough DNA could be recovered to generate full Identifiler® Plus profiles from all samples. Therefore, it is important for analysts to exercise caution when screening dry-cleaned evidence as stains may be missed that carry probative genetic information.

Keywords: Forensic science, dry-cleaning, DF-2000, GreenEarth, DNA, semen, Crime-lite

1. Introduction

In 2007, the California Air Resources Board (ARB) approved requirements that will phase out the primary chemical used in dry-cleaning, perchloroethylene (Perc), by 2023[1]. Two of the dry-cleaning chemicals available as a replacement for Perc are DF-2000™, a petroleum-based product produced by ExxonMobil, and the liquid-silicone based GreenEarth® process. In the dry-cleaning community, DF-2000™ is often considered a close substitute for Perc while the GreenEarth® process is considered one of the more environmentally friendly options.

At the time of this project, much work has been done to examine the effects of washing and detergents on both blood and semen and on the ability to obtain a DNA profile from laundered fabrics. Cox[4] performed a study of 12 washable fabrics and determined that the retention of bloodstains depends on fiber composition, the screening test used, and if a detergent was used during laundering. No significant effect was found from stain drying time.

Alternative light sources (ALS) like the 5000 Å Crime-lite® were used to detect semen stains[5,6]. Kobus et al[5] found that several factors including the inherent fluorescence and absorbency of the fabric determine if semen stains will be readily detected using ALS. Vandenberg et al[6] found that residual laundry detergent can create false positives during the preliminary identification of semen stains. It is important to determine if dry-cleaning leaves residues similar to laundry detergents that could interfere with the identification of semen stains.

Kafarowski et al[7] examined the effect of machine laundering on semen stains and found that the amount of acid phosphatase in the stains was significantly decreased by washing but that enough spermatozoa usually remain on the stained fabric to conduct PCR and produce a profile. Therefore, in their study, the acid phosphatase test was not a good indicator of the presence of sperm (and DNA) on items that have been machine laundered. However, in a study by Joshi et al[8], both acid phosphatase and sperm were detected in semen stains immersed in water for up to 144 hours, indicating that water alone may not reduce the reliability of the acid phosphatase assay as an effective screening tool.

Dry-cleaning biological stains has not been frequently mentioned in the literature but the two direct references found are contradictory. One study found that "standard dry cleaning procedures that immerse fabric in an organic solvent are not effective for the removal of bloodstains[2]." Another found that "dry cleaning, however, pretty much eliminates the patterns that might be illuminated by the use of luminol[3]." Due to the 2007 ban on Perc and the shift to green dry-cleaning, even this contradictory knowledge of the effects of dry-cleaning will soon become outdated.

It is important to determine if the chemicals used in green dry-cleaning affect our ability to detect and obtain DNA profiles and what factors (e.g. presumptive tests, confirmatory tests, DNA quantitation) are affected. The current research examined three dry-clean only fabrics, 100% Wool, 65% Polyester/35% Cotton, and 65% Polyester/35% Rayon, and looked simultaneously at the effects of the dry-cleaning method and the fabric type on the ability to detect semen stains and recover DNA from them.

2. Methods

2.1 Sample Donation

A single donor was recruited to provide the entire semen sample for the study in order to allow generation of a single source autosomal STR profile. The donor ejaculated into a sterile collection vial over a 30 day period until the desired volume of 14 mL was obtained. The semen was stored at 4°C until use.

2.2 Staining

100 uL of semen was applied to the center of 30 swatches (12" x 12") of 65% Polyester/35% Cotton, 30 swatches (12" x 12") of 65% Polyester/35% Rayon, and 30 swatches (12" x 12") of 100% Wool for a total of 90 stained swatches. Each stain was encircled with a fabric safe pen in order to ensure

the entire stain could be collected post-cleaning, regardless of visibility or reaction to presumptive tests. All swatches were allowed to air-dry overnight (approximately 8 hours).

Ten swatches of each fabric type were used as positive controls, ten as experimental samples exposed to the dry-cleaning chemical DF-2000™, and ten as experimental samples exposed to the dry-cleaning GreenEarth® process. Two swatches of each fabric type were left unstained and served as negative controls.

2.3 Presumptive Tests

All swatches were examined using a 5000 Å Crime-lite® both before and after dry-cleaning. Fluorescence was scored as strongly positive (++), positive (+), or negative (-). An AP spot test was performed post-cleaning to detect the acid phosphatase found in human semen. Results were scored as strongly positive (++), positive (+), or negative (+).

A bright fluorescence upon exposure to 5000 Å Crime-lite® was scored as (++), a weak but visible fluorescence was scored as (+), and an absence of detectable fluorescence was scored as (-). For the AP spot test, a color change to deep purple within 30 seconds was scored as (++), a color change to deep or light purple within 3 minutes was scored as (+), and a color change after 3 minutes, or no color change, was scored as (-).

A Christmas Tree stain assay[9] was performed to see if spermatozoa were present, regardless of the results of the presumptive tests.

2.4 Sample Collection

The entire stained area of all experimental samples and positive controls, regardless of whether they tested positive in any of the presumptive tests, was taken for DNA analysis. The fabric was cut along the inside of the line drawn around the samples to ensure the entire sample was collected. A circle was cut from the center of the negative controls, approximately the same size and shape of those collected

from the positive controls and experimental samples. Each sample was cut into approximately six smaller pieces to ensure complete and even submersion during the extraction process.

2.5 DNA Extraction

DNA was extracted using QIAamp® DNA Investigator kits following the protocol, "Isolation of Total DNA from Bodily Fluid Stains[10]."

The following additional steps were added to the beginning of the protocol in order to perform the Christmas Tree stain assay[9]: 300 uL ATL buffer and 20 uL proteinase K were added to each sample. The samples were then vortexed and incubated at 56°C for 1 hour, with additional vortexing for 10 seconds every 10 minutes. After incubation, the fabric was placed in a spin basket and centrifuged (6000xg) for 1 minute. The fabric was then discarded and all but 30 uL of the supernatant was removed. The remaining solution was pipetted up and down to gently mix. 5 uL of the unlysed extract was reserved for use in the Christmas Tree stain assay[9].

The DNA was eluted from the columns in 100 uL of Qiagen ATE buffer.

2.6 DNA Quantification

All DNA extracts were quantitated by qPCR using Quantifiler® Duo DNA Quantification kits[11] on an ABI 7500 Real-Time PCR System.

2.7 DNA Genotyping

A representative group of the DNA extracts were genotyped using AmpFlSTR Identifiler™ Plus kits[12] on an ABI 310 Genetic Analyzer using GeneMapper® ID v3.2. The representative group included a positive control, DF-2000™ cleaned sample and GreenEarth® cleaned sample for each of the fabric types for a total of 9 extracts.

3. Results

3.1 Presumptive Tests (Table 1)

All positive controls and experimental samples on the 65% Polyester/35% Rayon fabric exhibited no fluorescence either before or after dry-cleaning. Fluorescence, ranging from weak to intense, was observed from the positive controls and experimental samples on the other two fabrics. All negative controls exhibited no fluorescence when observed with the Crime-lite®. Background fluorescence was not observed with any of the three fabrics used.

All stains cleaned with DF-2000™ tested strongly positive for acid phosphatase. All stains cleaned using the GreenEarth® process tested negative for acid phosphatase.

All samples yielded abundant spermatozoa during the Christmas Tree stain assay (data not shown).

Table 1. Presumptive test results. Bright fluorescence upon exposure to the 5000 Å Crime-Lite® was scored as strongly positive, weak but visible fluorescence was scored as positive, and an absence of detectable fluorescence was scored as negative. In the AP spot test, a color change to deep purple within 30 seconds was scored as strongly positive, a color change to deep or light purple within 3 minutes was scored as positive, and a color change after 3 minutes, or no color change, was scored as negative.

	65% Polyester/35% Rayon			100% Wool			65% Polyester/35% Cotton		
Sample	Pre-cleaning Fluorescence	Post-cleaning Fluorescence	AP Spot Test	Pre-cleaning Fluorescence	Post-cleaning Fluorescence	AP Spot Test	Pre-cleaning Fluorescence	Post-cleaning Fluorescence	AP Spot Test
PC1	-	-	++	++	++	++	+	+	++
PC2	-	-	++	++	++	++	+	+	++
PC3	-	-	++	++	++	++	+	+	++
PC4	-	-	++	++	++	++	+	+	++
PC5	-	-	++	++	++	++	+	+	++
PC6	-	-	++	++	++	++	+	+	++
PC7	-	-	++	++	++	++	+	+	++
PC8	-	-	++	++	++	++	+	+	++
PC9	-	-	++	++	++	++	+	+	++
PC10	-	-	++	++	++	++	+	+	++
DF1	-	-	++	++	++	++	+	+	++
DF2	-	-	++	++	++	++	+	+	++
DF3	-	-	+	++	++	++	+	+	++
DF4	-	-	++	++	++	++	+	+	++
DF5	-	-	++	++	++	++	+	+	++
DF6	-	-	++	++	++	++	+	+	++
DF7	-	-	++	++	++	++	+	+	++
DF8	-	-	+	++	++	++	+	+	++
DF9	-	-	+	++	++	++	+	+	++
DF10	-	-	++	++	++	++	+	+	++
DFNC	-	-	-	-	-	-	-	-	-
GE1	-	-	-	++	+	-	+	+	-
GE2	-	-	-	++	+	-	+	+	-
GE3	-	-	-	++	+	-	+	+	-
GE4	-	-	-	++	+	-	+	+	-
GE5	-	-	-	++	++	-	+	+	-
GE6	-	-	-	++	+	-	+	+	-
GE7	-	-	-	++	+	-	+	+	-
GE8	-	-	-	++	+	-	+	+	-
GE9	-	-	-	++	+	-	+	+	-
GE10	-	-	-	++	+	-	+	+	-
GENC	-	-	-	-	-	-	-	-	-

PC = Positive control, DF = cleaned with DF-2000™, GE = cleaned with GreenEarth®, NC = negative control
- = negative result, + = positive result, ++ = strong positive result

3.2 Cleaning Method (Table 2, Figures 1 - 4)

Ample DNA was recovered from the samples to allow for STR genotyping. The average DNA yield from the 65% Polyester/35% Rayon was 7.4E+02 ± 1.9E+02 ng (positive control), 3.1E+02 ± 8.7E+01 ng (DF-2000™), 6.9E+01 ± 6.0E+01 ng (GreenEarth®). The average DNA yield from the 100% Wool was 9.0E+02 ± 3.6E+02 ng (positive control), 3.6E+02 ± 3.0E+02 ng (DF-2000™), 1.9E+01 ±

1.0E+01 ng (GreenEarth®). The average DNA yield from the 65% Polyester/35% Cotton was 1.5E+03 ± 5.9E+02 ng (positive control), 1.2E+03 ± 5.6E+02 ng (DF-2000™), 3.4E+02 ± 2.2E+02 ng (GreenEarth®). Figure 1 illustrates the abundance of DNA available as compared to the 1 ng required to perform STR genotyping.

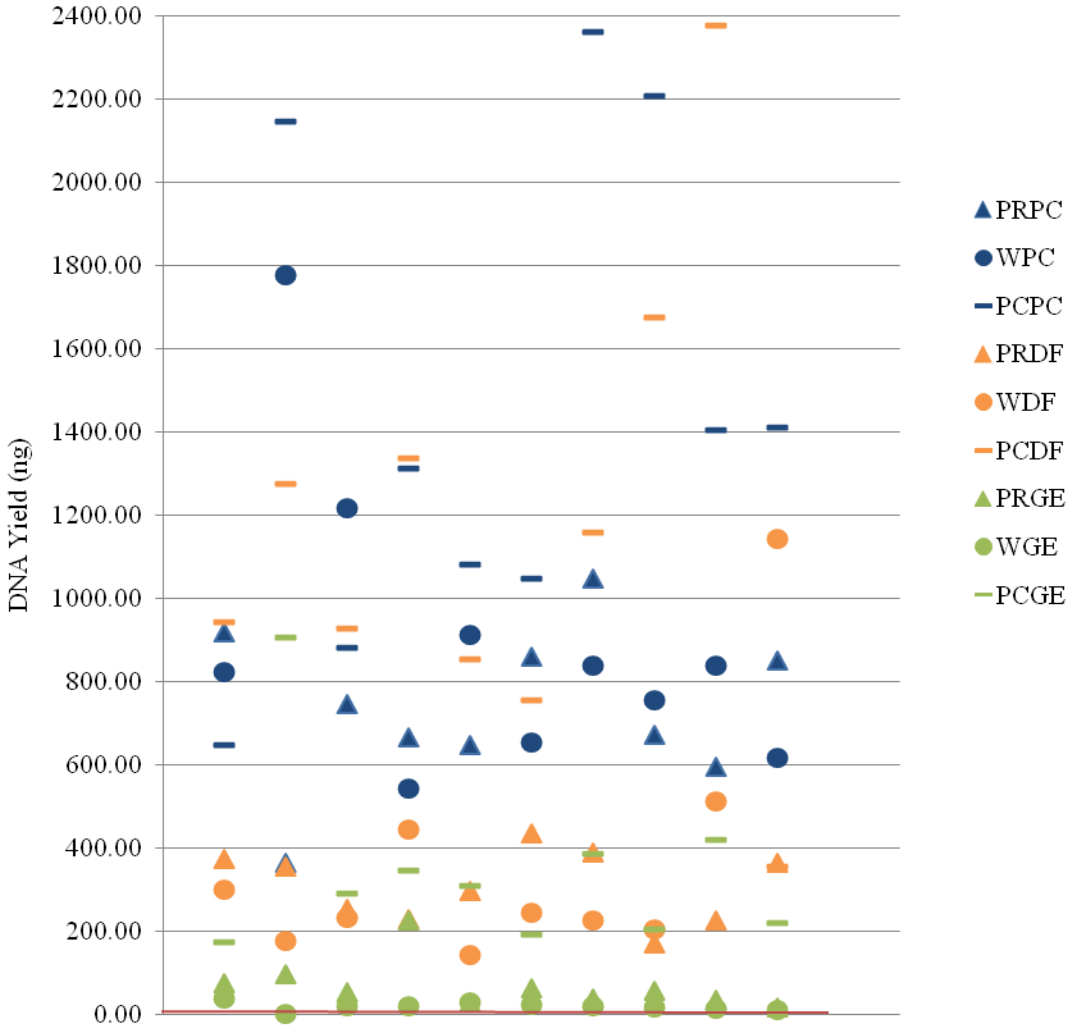

Figure 1. DNA yield of all positive control and dry-cleaned samples. The horizontal red line (almost indistinguishable from the baseline) indicates the 1 ng of DNA required for genotyping. Positive controls (PC) shown in blue, samples cleaned using DF-2000™ (DF) shown in orange, and samples cleaned using GreenEarth® (GE) in green. Samples on the 65% Polyester/35% Rayon (PR) are indicated by a triangle, 100% Wool (W) by a circle, and 65% Polyester/35% Cotton (PC) by a line.

Two patterns begin to emerge in Figure 1. First, the groupings of colors show that the positive controls (shown in blue) tend to have the most DNA and the samples cleaned with GreenEarth® (shown in green) tend to have the least DNA. Also, within each color group (cleaning method), the samples on the 65% Polyester/35% Cotton (represented by a line) tend to have the most DNA. The implications of these patterns will be discussed later on.

Table 2. Data from the one-way ANOVA performed on each fabric type to determine if the cleaning method affected the DNA yield. H_0 [1,2]: The cleaning method does not affect the mean DNA yield.

	Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
65% Polyester/35% Rayon	Between Groups	2286318.6	2	1143159.3	71.17	1.718E-11
	Within Groups	433666.9	27	16061.7		
100% Wool	Between Groups	3913805.2	2	1956902.6	26.75	3.941E-07
	Within Groups	1975440.7	27	73164.5		
65% Polyester/35% Cotton	Between Groups	6585024.3	2	3292512.1	13.91	7.033E-05
	Within Groups	6389703.6	27	236655.7		

[1] : Reject H_0 when $p < 0.05$

[2] : Reject H_0 when $F > F_{crit}$; $F_{crit} = 3.354$

A one-way analysis of variance (ANOVA) was performed on the data from each of the fabric types to determine if the cleaning method affected the average DNA recovered (Table 2). On the 65% Polyester/35% Rayon and 100% Wool fabrics, both cleaning methods removed a statistically significant amount of DNA as compared to the positive control and the GreenEarth® method removed statistically more DNA than the DF-2000™ (Figs. 2 and 3).

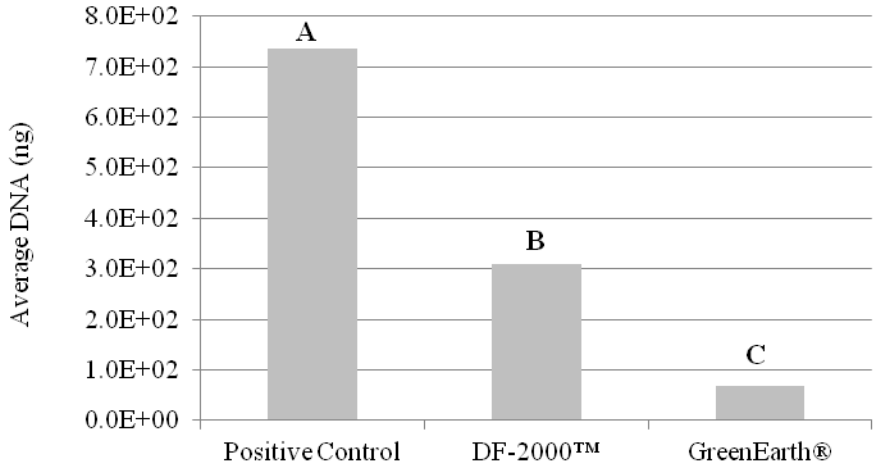

Figure 2. Tukey HSD post-hoc analysis of the one-way ANOVA ($p < 0.05$) performed on the 65% Polyester/35% Rayon data. Means with the same letter are not significantly different from each other.

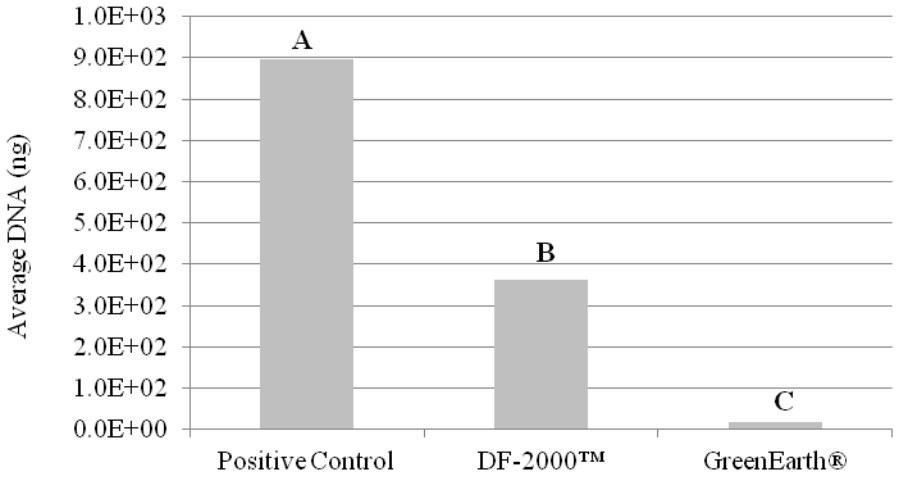

Figure 3. Tukey HSD post-hoc analysis of the one-way ANOVA ($p < 0.05$) performed on the 100% Wool data. Means with the same letter are not significantly different from each other.

On the 65% Polyester/35% Cotton fabric, only the GreenEarth® method removed a statistically significant amount of DNA, as compared to both the positive control and DF-2000™ (Fig. 4).

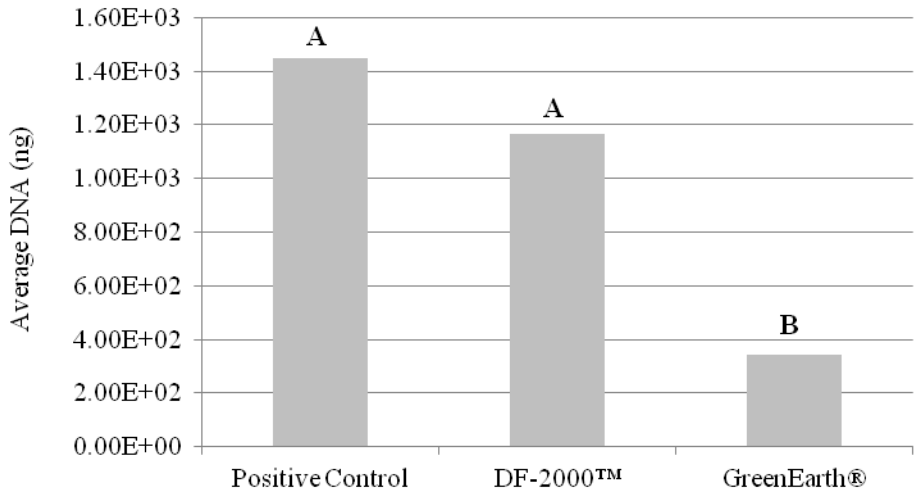

Figure 4. Tukey HSD post-hoc analysis of the one-way ANOVA ($p < 0.05$) performed on the 65% Polyester/35% Cotton data. Means with the same letter are not significantly different from each other.

3.3 Fabric Type (Tables 3 - 4, Figures 5 - 6)

A one-way ANOVA was performed on the data from each cleaning method to determine if fabric type affected the average DNA recovered (Table 3). With both DF-2000™ and GreenEarth®, the 65% Polyester/35% Cotton retained statistically more DNA than the 100% Wool or 65% Polyester/35% Rayon (Figs. 5 and 6).

Table 3. Data from the one-way ANOVA performed on each cleaning method to determine if the fabric type affected the DNA yield. H_0 [1,2]: The fabric type does not affect the mean DNA yield.

	Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
DF-2000™	Between Groups	4600501.3	2	2300250.6	16.94	1.708E-05
	Within Groups	3665715.4	27	135767.24		
GreenEarth®	Between Groups	614277.55	2	307138.77	18.45	8.907E-06
	Within Groups	449545.65	27	16649.839		

[1] : Reject H_0 when $p < 0.05$

[2] : Reject H_0 when $F > F_{crit}$; $F_{crit} = 3.354$

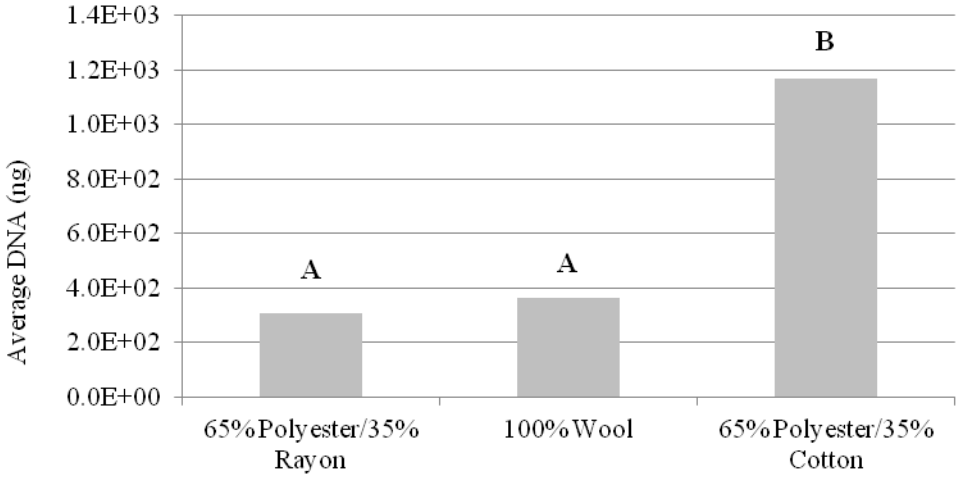

Figure 5. Tukey HSD post-hoc analysis of the one-way ANOVA ($p < 0.05$) performed on the DF-2000™ data. Means with the same letter are not significantly different from each other.

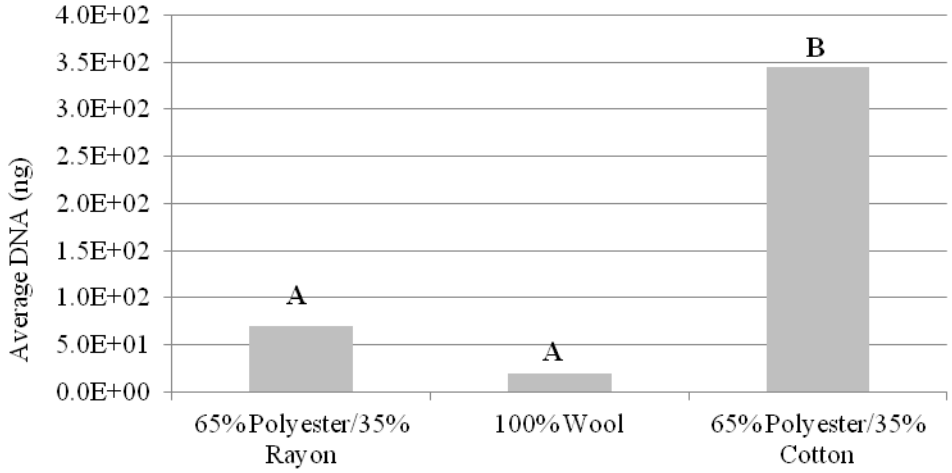

Figure 6. Tukey HSD post-hoc analysis of the one-way ANOVA ($p < 0.05$) performed on the GreenEarth® data. Means with the same letter are not significantly different from each other.

A two-way ANOVA was performed on the entire data set and the results in Table 4 indicate that there is interaction between the cleaning method and the fabric type. These results, as discussed in the

next section, may have been exacerbated by factors including the time the semen sample spent at 4°C prior to use.

Table 4. Data from the two-way ANOVA performed to determine any interaction between the effects of the cleaning method and the effects of the fabric type. H_0 [1,2]: There is no interaction between cleaning method and fabric type.

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	F_{crit}
Cleaning Method	3287506.1	1	3287506.1	43.14	2.069E-08	4.020
Fabric Type	4255081	2	2127540.5	27.92	4.728E-09	3.168
Interaction	959697.8	2	479848.9	6.30	3.484E-03	3.168

[1] : Reject H_0 when $p < 0.05$

[2] : Reject H_0 when $F > F_{crit}$

3.4 Genotyping

Full genetic profiles were obtained from the samples submitted for STR genotyping.

4. Discussion

Due to the 2007 ban on Perc and a general lack of research on the effects of dry-cleaning, this study aimed to determine the differences between two available green dry-cleaning chemicals and their effects on our ability to detect and obtain DNA from semen stains. This study also examined the effect of fabric type on DNA detection and retention.

Initial screening using a 5000 Å Crime-Lite® was not consistently effective at predicting the location of semen stains. Kobus et al[5] showed that fabrics can fluoresce at the same wavelength as semen, overwhelming any fluorescence that may have been observed from the stain itself. This is known as background fluorescence. In the current research, the fabrics were screened using the Crime-lite® prior

to deposition of a semen stain to determine background fluorescence. Fluorescence could be observed from nearby objects but no fluorescence was observed from the fabrics themselves. After deposition of the semen stains, the fabrics were again screened using the Crime-lite® to determine pre-cleaning fluorescence. The stains on the 100% Wool and 65% Polyester/35% Cotton fabrics were clearly visible but the stains on the 65% Polyester/35% Rayon fabric could not be seen. Kobus et al[5] stated that fabrics may possess characteristics which may cause them to absorb the fluorescence that would otherwise be seen from the stain.

The AP spot test was also unreliable as a method of determining the location of semen stains. Samples cleaned using the GreenEarth® process yielded negative AP spot test results, though further analysis showed that sperm and DNA were present. The quality of the DNA in the semen stain was unaffected by the GreenEarth® process, indicating that something in the GreenEarth® process interferes with either the enzymatic activity of acid phosphatase or with the creation of the colorimetric response.

It was observed that samples on the 65% Polyester/35% Cotton retained more DNA than the other fabrics when compared to their respective cleaning type groups (Fig. 1). This effect may also be seen in the results of the post-hoc analysis of the cleaning method on the 65% Polyester/35% Cotton (Fig. 4) where, unlike the 100% Wool (Fig. 3) and 65% Polyester/35% Rayon (Fig. 2) fabrics, the DF-2000™ did not remove a statistically significant amount of DNA as compared to the positive control. It should be noted that the 65% Polyester/35% Cotton was the last fabric tested and due to the research method (using a single pooled semen donation throughout the research) the semen sample was required to be kept at 4°C for the duration of the experiment. By the time the 65% Polyester/35% Cotton was tested, the sample had been in and out of refrigeration multiple times. The viscosity had increased and small crystal formations were observed throughout the sample. This could have led to a lack of homogeneity in the sample and irregular deposition onto the fabric being tested. This may also have affected the results of the two-way ANOVA which indicated there is interaction between the cleaning method and fabric type.

5. Conclusions

This study found that dry-cleaning using either DF-2000™ or the GreenEarth® process was ineffective at removing DNA from semen stains. This would indicate that items that have been dry-cleaned using the DF-2000™ or GreenEarth® processes should not be eliminated as evidence, even though they may have tested negative during screening assays. It was also found that while the Crime-lite® is an effective tool for screening multiple items quickly, it may yield negative results even when semen is present, depending on the absorbency of the fabric. Items believed to contain semen stains that do not fluoresce under the ALS should be subjected to additional presumptive tests. Finally, the AP spot test will yield negative results if the item is cleaned using the GreenEarth® process, even though sufficient DNA remains for DNA profiling. Items believed to contain semen stains that were cleaned using the GreenEarth® process should be subjected to confirmatory testing (i.e. visual confirmation of the presence/absence of spermatozoa) before being eliminated as potential evidence. Preventing the unnecessary loss of evidence may aid investigators in their efforts to solve crimes quickly. It is therefore recommended that analysts exercise caution when interpreting screening results in order to prevent the unnecessary loss of probative evidence.

6. Future Research

Although DF-2000 and GreenEarth are becoming more popular with commercial dry-cleaners, there are still several options available that may increase in popularity as Perc is phased-out. Additional research is needed on the dozens of other dry-cleaning chemicals available, including a comparison of the effectiveness of these chemicals to Perc. It would also aid this researcher, and future researchers, to study the effects of refrigeration on semen samples and the reproducibility of the DNA yield when pipetting semen from a refrigerated sample.

Conflict of Interest

None declared.

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Ethical Approval

Institutional Review Board protocol #265362-2, expiring October 18, 2014.

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