



Persistence of DNA from laundered semen stains: Implications for child sex trafficking cases



Helen Brayley-Morris^{a,b}, Amber Sorrell^b, Andrew P. Revoir^c, Georgina E. Meakin^{a,b},
Denise Syndercombe Court^c, Ruth M. Morgan^{a,b,*}

^a UCL Centre for the Forensic Sciences, 35 Tavistock Square, London WC1H 9EZ, UK

^b UCL Department of Security and Crime Science, 35 Tavistock Square, London WC1H 9EZ, UK

^c Faculty of Life Sciences and Medicine, KCL, Franklin-Wilkins Building, London SE1 9NH, UK

ARTICLE INFO

Article history:

Received 13 January 2015

Received in revised form 22 June 2015

Accepted 14 July 2015

Keywords:

Child sexual exploitation

Internal child sex trafficking

Forensic science

Spermatozoa

DNA

Persistence

Transfer

ABSTRACT

In sexual assault cases, particularly those involving internal child sex trafficking (ICST), victims often hide their semen-stained clothing. This can result in a lag time of several months before the items are laundered and subsequently seized during a criminal investigation. Although it has been demonstrated previously that DNA can be recovered from clothing washed immediately after semen deposition, laundered items of clothing are not routinely examined in ICST cases, due to the assumption that the time delay and washing would result in no detectable DNA. The aim of this study was to examine whether viable DNA profiles could be recovered from laundered semen stains where there has been a significant lag time between semen deposition from one or more individuals and one or more washes of the stained clothing.

Items of UK school uniform (T-shirts, trousers, tights) were stained with fresh semen (either from a single donor or a 1:1 mixture from two donors) and stored in a wardrobe for eight months. Stained and unstained items (socks) were then washed at 30 °C or 60 °C and with non-biological or biological detergent. DNA samples extracted from the semen-stained sites and from the unstained socks were quantified and profiled.

High quantities of DNA, (6–18 µg) matching the DNA profiles of the semen donors, were recovered from all semen-stained clothing that had been laundered once, irrespective of wash conditions. This quantity, and profile quality, did not decline significantly with multiple washes. The two donor semen samples yielded ~10-fold more DNA from the T-shirts than from the trousers. This disparity resulted in the T-shirts yielding a ~1:1 mixture of DNA from the two donors, whereas the trousers yielded a major DNA profile matching only that of the second donor. The quantities of DNA recovered from the unstained socks were an order of magnitude lower, with most of the DNA being attributable to the donor of the semen on the stained clothing within the same wash, demonstrating the transfer of semen-derived DNA among items of clothing in the washing machine.

This study demonstrates that complete DNA profiles can be obtained from laundered semen stains on school uniform-type clothing, with an eight-month lag time between semen deposition and laundering, despite multiple washes and stains from two semen donors. These data emphasise the need to recover and examine the clothing of victims for semen and DNA evidence, even if the clothing has been stored for several months or washed multiple times since the sexual offence took place.

© 2015 Z. Published by Elsevier Ireland Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

1. Introduction

The sexual exploitation and internal trafficking of British children within the UK has received growing public and policy

attention in recent years. This has largely been in response to a number of high profile police investigations into cases involving teenage victims who had been sexually exploited by groups of adults across the UK [1]. Instances of sexual abuse in these cases involved multiple perpetrators committing child sexual offences in a variety of public and private locations to which victims were transported or 'trafficked' from close by or from another town or county.

* Corresponding author at: UCL Department of Security and Crime Science, 35 Tavistock Square, London, WC1H 9EZ, UK.

E-mail address: ruth.morgan@ucl.ac.uk (R.M. Morgan).

The children involved rarely acknowledged their own victimisation for a variety of reasons, including fear, shame and a normalisation of sexualised behaviour [2]. In many cases there was also a long lag time between the offences taking place and the subsequent police investigation, further complicating the prosecution of offenders [3]. For the purpose of this paper, the term internal child sex trafficking (ICST) shall be used in reference to the movement and sexual exploitation of children involving multiple offenders, as outlined in Brayley and Cockbain [4].

It has been identified that despite the sexual health risks and forensic potential of semen deposition, many sexual offenders do not use contraceptive protection during a sexual assault. In one study of multiple-perpetrator rapes, only 20% of offenders wore a condom during an attack [5]. Ejaculate left inside the victim, if still present, may be gathered during a routine forensic medical examination and is a well-known source of evidence for police. From a study of ICST cases, it was similarly found that offenders commonly did not use a condom and would often ejaculate directly onto the body or clothes of a victim [3].

In some ICST cases, it was identified that victims hid their semen-stained clothing from parents or carers to avoid having to discuss the assault [3]. Rather than discarding the clothes completely, it was observed that victims often stored the clothing for a period of time, ranging from several hours to over a year, before washing the items to remove the visible stains. It is therefore possible for DNA from the offender(s) to remain on these items of clothing. To date, such laundered items of clothing are not routinely examined in ICST cases, due to the assumption that the lag time, along with the laundering process, will have removed any detectable DNA from the deposited semen [3].

The internal trafficking of humans for the purpose of sexual exploitation was only formally acknowledged in UK law with the introduction of the Sexual Offences Act 2003. This piece of legislation relates to the exploitation of both adults and children but has to date rarely been used to prosecute ICST offenders. When ICST cases have gone to court the prosecution has relied heavily on victim accounts and testimony, and have rarely been supported by corroborating forensic science evidence [3]. It is therefore important to assess the viability of forensic analysis on items recovered during the investigation of these cases, as DNA profiles obtained from semen-stained laundered clothing may offer further evidence in future investigations and trials.

A small number of published empirical studies have demonstrated that spermatozoa cells can persist on items of clothing after they have been laundered in a washing machine, using various wash programmes, detergents and temperatures [6–11], and that DNA profiles can be obtained from laundered semen stains [6,8–10]. Importantly, none of these papers investigated whether

DNA profiles can be obtained from laundered semen stains where there has been a significant lag time between semen deposition and washing, along with semen deposited from more than one source, or, multiple washes of the stained clothing. All of these circumstances are more prevalent in ICST cases. It was therefore the aim of this study to examine whether viable DNA profiles could be recovered from laundered semen stains under conditions pertinent to the investigation of ICST and other sexual assault cases.

Experimental studies offer a means of developing an empirical basis for the development of forensic protocols in the investigations of specific crime types [12]. Such an evidence base for ICST cases will help enable the identification of situations when there is likely to be recoverable DNA from an item of clothing, and the extent to which a viable profile may be produced. Understanding the 'evidence dynamics' of this form of trace evidence within the context of ICST offences will, therefore, provide valuable insight into the best use of the often limited resources during the course of an investigation.

Studies into the UK ICST cases indicated that victims were often picked up while on their way to or from school [1,3] and as such, items of clothing that constitute elements of a school uniform in the UK (T-shirt, trousers, tights) were examined in this study. These items of clothing were stained with semen and then stored at the back of a wardrobe for eight months before being laundered. This process simulated how an ICST victim is known to have treated the clothing they were wearing when the sexual offence(s) took place. Given the more common incidence of multiple offenders and the potential for multiple washing of clothing to have occurred before a victim is identified in ICST cases, the persistence of DNA in semen stains on items laundered once, twice and three times, and in laundered semen stains from multiple donors were investigated.

2. Materials and methods

2.1. Samples and substrates used

To simulate typical evidential items recovered in ICST cases, fresh semen (<1 h old) taken from donors (who had no further involvement in the experiments) was deposited on items of children's clothing. Semen samples from two donors were used, as described below. Multiple ejaculates of each donor were required to set up the experimental samples listed in Table 1. Therefore, to ensure consistency, the ejaculates of each donor were initially combined to provide a stock solution before being deposited onto the items of clothing. The location on the clothing of the semen deposit was clearly marked with water-insoluble ink to allow its placement to be specifically targeted, in accordance with previous

Table 1
The combinations of items of clothing, washing conditions, and semen donors used in this study, alongside their summary DNA profiling results. Bio: biological detergent; non-bio: non-biological detergent; D1: semen donor 1.

Item of clothing	Temperature (°C)	Detergent	No. of washes	No. of semen donors	DNA profiles obtained
T-shirt	30	Bio	1	1	100% single-source matching D1 profile
Trousers	30	Bio	1	1	100% single-source matching D1 profile
Socks	30	Bio	1	–	See Table 3
T-shirt	30	Non-bio	1	1	100% single-source matching D1 profile
Trousers	30	Non-bio	1	1	100% single-source matching D1 profile
Tights	30	Non-bio	1	1	100% single-source matching D1 profile
T-shirt	60	Bio	1	1	100% single-source matching D1 profile
Socks	60	Bio	1	–	See Table 3
T-shirt	60	Non-bio	1	1	100% single-source matching D1 profile
Trousers	60	Non-bio	1	1	100% single-source matching D1 profile
T-shirt	30	Non-bio	2	1	100% single-source matching D1 profile
T-shirt	30	Non-bio	3	1	100% single-source matching D1 profile
T-shirt	30	Non-bio	1	2	See Table 2
Trousers	30	Non-bio	1	2	See Table 2

DNA persistence studies [13]. Items of clothing were new and unworn, and included T-shirts (cotton), trousers (polyester), and tights (nylon). The items had not been washed previously.

On some items, 1 ml of a single source of semen was deposited and on others, 2 ml of semen as a 1:1 mixture of semen from two donors was deposited (Table 1). For these latter items, the semen from the first donor was deposited on the item and allowed to dry, then the semen from the second donor was applied directly to the first stain to simulate separate deposits of semen from two different sources. After the deposition of semen, the items of clothing were allowed to dry at room temperature. Once dry, the semen-stained items of clothing were stored individually in paper bags at the back of a wardrobe for 8 months; such a delay in evidence recovery is commonly encountered in ICST cases [3].

The stained items of clothing were then washed at different temperatures and with different detergents (Table 1) to investigate whether DNA could be recovered from semen stains after the clothing had been laundered. Items of clothing not stained with semen (socks) were also washed alongside some of the semen-stained clothes in order to examine the possibility of DNA transfer from semen-stained items to other items within a washing machine. To verify the source of any DNA recovered, buccal swabs were taken from both semen donors (D1 and D2), one of the regular users of the washing machine (W) and from the laboratory analyst (L) processing the items of clothes.

2.2. Laundering protocol

All semen-stained items of clothing were laundered in a domestic washing machine (Hotpoint BHWM129) using a programme with a duration of 90 min and a spin cycle of 1200 rpm. As indicated in Table 1, items of clothing were washed at 30 °C with non-biological detergent (Non-Bio Persil tablets), 30 °C with biological detergent (Persil Colour Care water capsules), 60 °C with the same non-biological detergent, or, 60 °C with the same biological detergent. Those clothes laundered using the same programme were washed together. No fabric conditioner was used in any of the washes.

Some items of clothing were washed once, some twice and others three times to test the impact of multiple laundering on the recovery of DNA from semen-stained clothing (Table 1). For the washes with biological detergent, unstained socks were also included in both the 30 °C and 60 °C programmes. All combinations were run in triplicate. After washing, the items of clothing were dried on a clotheshorse at room temperature overnight, before

being washed again, if applicable. The laundered items were stored individually in paper bags, until being processed on the following day.

2.3. Sample processing and analysis

An area of 0.5 cm² was cut out from the semen-stained site on each item of clothing and from the unstained socks. DNA was extracted from these samples using the EZ1 DNA Investigator Kit (Qiagen) with the EZ1 BioRobot (Qiagen), as per the manufacturer's instructions for extracting DNA from body fluid stains. In particular, during the cell lysis stage prior to use of the EZ1 BioRobot, 1M DTT was added to all the samples (including those from the socks) to promote release of the DNA from any sperm cells present. Extracted DNA was eluted into 50 µl sterile deionised water. DNA was also extracted from the buccal swabs using the EZ1 DNA Investigator Kit and BioRobot, as per the manufacturer's instructions for extracting DNA from buccal swabs, with the extracted DNA being eluted into 100 µl sterile deionised water.

All DNA samples were quantified using the Quantifiler[®] Human DNA Quantification Kit (Applied Biosystems) with the ABI PRISM[®] 7000 Sequence Detection System (Applied Biosystems), as per the manufacturer's instructions. As described below, the quantification results revealed very high concentrations of DNA in the extracts from the semen-stained items, such that considerable dilution of the samples was required prior to further processing. DNA profiles of the diluted samples were then obtained using the PowerPlex[®] ESI 16 System (Promega) with the 3130xL Genetic Analyzer (Applied Biosystems) and analysed with GeneMapper[®] ID version 3.2 software (Applied Biosystems), all as per the manufacturer's instructions. For profile interpretation, a peak height threshold of 50 relative fluorescence units (rfu) was employed, along with a 150 rfu threshold for homozygotes.

3. Results

3.1. Recovery of DNA from laundered semen stains on clothing

Given the eight-month delay between the semen deposition and washing of the items of clothing, it was anticipated that only low levels of DNA would be recovered from the stains. In order to increase the concentration of DNA obtained during the extraction process and thereby improve the chance of obtaining good quality DNA profiles, the DNA samples were eluted into 50 µl rather than the routinely

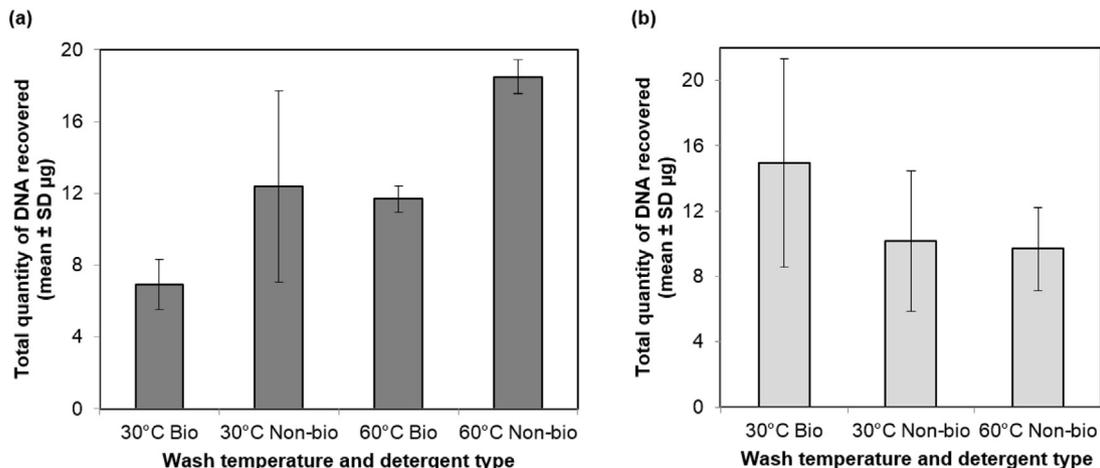


Fig. 1. The recovery of DNA from laundered semen stains on cotton T-shirts (a) and polyester trousers (b). Quantities of DNA are presented as means of three replicates ± one standard deviation (SD); bio, biological detergent; non-bio, non-biological detergent.

used 100 μ l. Contrary to this expectation, considerable amounts of DNA, ranging from 6 to 18 μ g, were recovered from the single-donor semen stains on the T-shirt and trousers that had been laundered once (Fig. 1). All these stains resulted in complete single-source DNA profiles matching that of the donor of the semen (Table 1).

Variable quantities of DNA were recovered within each group of samples of the same material, wash temperature and detergent (Fig. 1). For the semen stains on cotton T-shirts, there appeared to be a general trend of reduced DNA recovery when washed with biological detergent compared with non-biological detergent, with mean DNA quantities reducing from 12.4 to 6.9 μ g for 30 °C washes and from 18.5 to 11.7 μ g for 60 °C washes (Fig. 1(a)). In contrast, the opposite trend was suggested by the mean DNA quantities recovered from the polyester trousers, which increased from 10.2 and 9.7 μ g with non-biological detergent at 30 °C and 60 °C to 15.0 μ g with biological detergent at 30 °C (Fig. 1(b)).

With respect to the nylon tights, there was difficulty in their preparation, due to the semen congealing on the material, that resulted in semen-stained tights only being washed under one condition (30 °C with non-biological detergent), and tested in triplicate. As with the T-shirts and trousers, a high level of DNA was recovered from the tights (10.4 \pm 2.3 μ g), from which complete single-source DNA profiles matching that of the donor of the semen were obtained (Table 1).

3.2. Recovery of DNA from semen stains on cotton T-shirts after multiple washes

Having demonstrated the recovery of complete DNA profiles from semen stains on clothing that had been laundered once, the effect of further washing was investigated. This was to establish whether there is value in casework to seize clothing purported to have been worn at the time of the incident, which may have been washed multiple times in the intervening period. Cotton T-shirts stained with semen from one donor were washed once, twice or three times at 30 °C with non-biological detergent and DNA was recovered from the stains. High quantities of DNA were recovered from the semen stains, irrespective of the number of times the T-shirts were washed (Fig. 2). Full single-source DNA profiles matching that of the donor were obtained from the stains (Table 1), and the stains were still visible even after three washes. An overall decline in the mean quantity of DNA recovered from the semen stains was observed with each additional wash, with a reduction

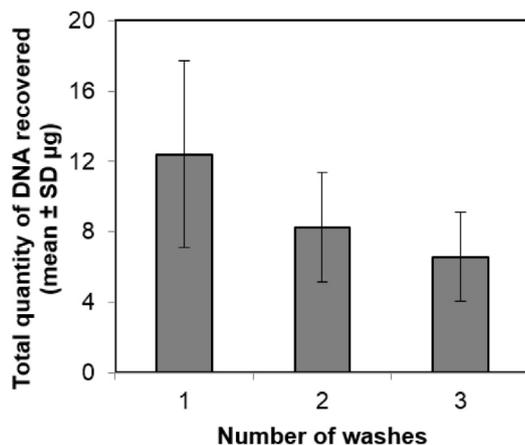


Fig. 2. The effect of multiple washings at 30 °C with non-biological detergent on the quantity of DNA recovered from semen stains deposited on cotton T-shirts. Quantities of DNA are presented as means of three replicates \pm one standard deviation (SD).

from 12.4 to 8.3 to 6.6 μ g for one, two and three washes, respectively (Fig. 2).

3.3. Recovery of DNA from laundered semen stains deposited by two donors on clothing

Assault of victims by more than one perpetrator is often seen in ICST cases [14]. To investigate the potential for DNA recovery in such cases, cotton T-shirts and polyester trousers stained with semen from two donors were washed once at 30 °C with non-biological detergent, and DNA was recovered from the stains. Surprisingly, and in contrast to Fig. 1, considerably less DNA was recovered from the semen-stains on the trousers (1.5 \pm 0.8 μ g) than from those on the T-shirts (12.2 \pm 0.6 μ g). This difference was reflected in the DNA profiles obtained (Table 2), in which an approximately equal 1:1 mixture of DNA from the two donors was obtained from the T-shirt stains, whereas a major DNA profile matching only that of donor 2 was obtained from the trouser stains. One allele that could have come from donor 1 was also observed in the DNA profiles obtained from the trousers (Table 2). However, that allele could also have come from the laboratory analyst.

3.4. Potential for DNA transfer between items of clothing in a washing machine

It has previously been shown that sperm cells from a semen stain on women's underwear, deposited by drainage from the vagina, were transferred to unstained underwear when machine washed together [9]. Kafarowski et al. [9] hypothesised that it may be possible to obtain DNA profiles from the transferred sperm cells, although they thought it would be unlikely due to the small number of sperm transferred in their study. As a preliminary study into the potential for semen-derived DNA transfer between items of clothing within the washing machine, unstained socks were washed with clothing stained by semen from one donor (D1) using biological detergent at both 30 °C and 60 °C.

The quantities of DNA recovered from the socks in washes of both temperatures were an order of magnitude lower than those recovered from the laundered semen stains, being 8.6 \pm 7.9 ng for 30 °C and 2.6 \pm 4.6 ng for 60 °C. In the 30 °C washes, a complete major DNA profile matching that of donor 1 was obtained from the socks (Table 3). These profiles also showed one or two minor alleles that could have come from the laboratory analyst (Table 3). In the 60 °C washes, a complete single-source DNA profile matching that of donor 1 was obtained from the first sock (Table 3).

The DNA profiles obtained from the two other replicates at the 60 °C temperature were, however, more complex (Table 3), in which two-person mixtures of DNA were obtained that could not be reliably separated into major and minor components. Given the experimental set-up in this study, it is possible to attribute these DNA profiles to a mixture of DNA from donor 1 (D1) and the regular user of the washing machine (W). An example of an epg observed for one of these samples is shown in Fig. 3(a) with the profiles of

Table 2

DNA profiling results from three replicates of depositing semen from two donors on cotton T-shirts and polyester trousers and washing them at 30 °C with non-biological detergent. D1: semen donor 1, D2: semen donor 2, () indicates number of PowerPlex[®] ESI 16 STR loci at which alleles were detected (out of 15).

DNA profile correspondence (number of STR loci)	
T-shirt	Trousers
1:1 mixture D1 (15):D2 (15)	Major D2 (15) and minor (1)
1:1 mixture D1 (15):D2 (15)	Major D2 (15) and minor (1)
1:1 mixture D1 (15):D2 (15)	Major D2 (15) and minor (1)

Table 3

DNA profiling results from three replicates of unstained socks that had been washed using biological detergent in the same load as items of clothing stained with semen from donor 1. D1: semen donor 1, L: lab analyst, W: regular user of washing machine, () indicates number of PowerPlex® ESI 16 STR loci at which alleles were detected (out of 15).

DNA profile correspondence (number of STR loci)	
30 °C	60 °C
Major D1 (15) and minor L (2)	D1 (15)
Major D1 (15) and minor L (1)	D1 (13) and W (3)
Major D1 (15) and minor L (1)	D1 (15) and W (15)

D1 and W (Fig. 3(b)). If these results were the product of casework DNA profiles, in which the contributors of the DNA were unknown, it would be difficult to separate the mixtures into individual contributors based on the profiles obtained. However, with a DNA profile from a suspect, a probabilistic analysis could be conducted to evaluate the likelihood that DNA from the suspect could have contributed to the mixtures.

4. Discussion

The results from this study demonstrate for the first time that profilable DNA can be recovered from laundered semen stains after an eight-month lag time between semen deposition and laundering, as is often seen in ICST cases. The results also demonstrate that profilable DNA can be obtained from semen-stained clothing that has been laundered multiple times, and confirm the findings of previous research [6,8–10] that DNA profiles can be obtained from semen-stained clothing laundered just once.

High quantities of DNA, in the microgram rather than nanogram range, were recovered from the laundered semen stains. These

were significantly higher than the quantities of DNA of approximately 0.6–7.5 ng previously recovered from semen-stained underwear (calculated from the concentrations and elution volume reported by Farmen et al. [7]). This difference in the quantities of DNA between the present study and the study of Farmen et al. [7] could be due to differences in the wash programmes used (e.g. the temperature setting and type of detergent used), given that similar initial semen deposits and materials were used (0.5 cm² samples of 1 ml semen stains versus 1 cm² samples of 0.5 ml semen stains). It is also hypothesised that the long lag period in the present study, as opposed to just 24 h between semen deposition and laundering in the study by Farmen et al. [7], could have made the stains more resistant to the washing process. It is also possible that, as different donors were used in these two studies (one donor in this study, and 5 donor mixture in the Farmen et al. [7] study), a variation in sperm count may account for a difference in the amount of recoverable DNA.

Similarly high quantities of DNA were recovered from the laundered semen stains, regardless of wash temperature, detergent used, material type, or number of washes, although very varied results were obtained (Figs. 1 and 2). Wash conditions and type of material have previously been found to have varying degrees of influence on DNA recovered from laundered semen stains. For example, Nussbaumer et al. [10] reported that the highest amounts of DNA from laundered semen stains were found after washing at 60 °C, whereas Farmen et al. [7] concluded that twice the amount of DNA was recovered from semen stains laundered at 40 °C than at 60 °C. Transfer of DNA among items of clothing within the washing machine may have also affected the amounts of DNA recovered.

The potential impact of multiple washings on DNA retention in semen stains had not previously been addressed. In the present study, multiple washes of semen-stained cotton cloth produced

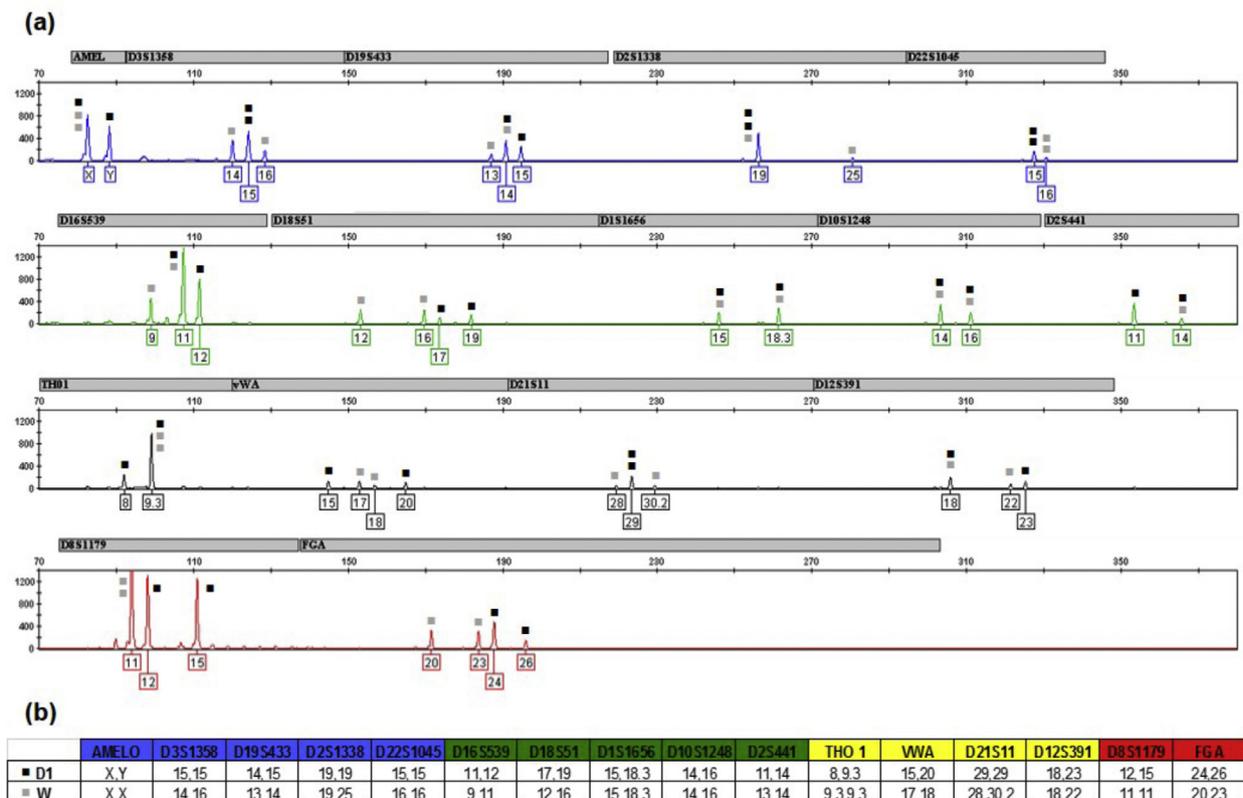


Fig. 3. An example epg of the DNA samples taken from the unstained socks washed at 60 °C (a) with the DNA profiles of the two potential contributors (b): the semen donor (D1) and the regular user of the washing machine (W).

only a minimal loss of recovered DNA suggesting that multiple washes may not materially affect DNA recovery (Fig. 2). These results suggest that, in casework with items of clothing on which semen is believed to have been deposited prior to laundering of the clothing, it would be appropriate for items to be analysed for DNA, possibly irrespective of the number of times the clothing has been washed. However, these are preliminary data, and further work is required to establish whether multiple washings would reduce DNA recovery under other conditions, such as with varying initial semen deposits (either with respect to volume or sperm count) or on different types of material. This clearly has implications for casework where the original volume of semen or number of sperm cells deposited will be unknown.

In casework, there are essentially three ways to screen an item for the possible presence of semen prior to potentially preparing samples for microscopic analysis: a visual examination for appropriate-looking stains, an examination with an alternate light source, and presumptive testing, such as Acid Phosphatase (AP) testing. Under the specific conditions of this study, the semen staining was still visible even after multiple washes, suggesting that visible screening might assist scientists to identify possible semen staining on washed clothing. However, further research is required to see whether the staining would still be visible under different conditions. With respect to AP testing, previous research has consistently shown that semen-stained items of clothing laundered with detergent does not give a positive AP reaction [6–9], so AP testing would not be appropriate when examining clothing washed after the deposition of semen. The use of an alternate light source to identify semen stains on washed clothing has yet to be considered and research into its use under such conditions would be useful to help inform the best way to screen such items for semen.

When using single-donor semen deposits, little difference in the quantities of DNA recovered was observed between the three material types: cotton (T-shirts), polyester (trousers), and nylon (tights). However, when semen was deposited from two donors, considerably less DNA was recovered from the polyester material than the cotton material, which was also reflected in the nature of the DNA profiles obtained. Even though equal volumes of semen from both donors were deposited on both types of material, only the cotton T-shirts gave 1:1 mixtures of complete DNA profiles from both donors. The polyester trousers gave a complete DNA profile of donor 2, but only one allele from a second source which matched DNA from both donor 1 and the lab analyst. In both cases, semen from donor 2 was placed on the item after semen from donor 1 had dried, suggesting that semen from donor 2 would be more readily removed during the washing process. This is a limited experiment and as such further work is required to investigate whether the difference in material type could explain why a complete DNA profile from only one semen donor was found on the trousers, in contrast to a complete profile being derived from both donors on the T-shirts.

The inclusion of unstained socks in washes with semen-stained clothing provides preliminary data of secondary transfer of DNA from the stained items of clothing to unstained items. Complete DNA profiles matching that of the semen donor were found on the majority of the socks (Table 3). This finding supports the recent presentation by Noël et al. [15] who found that interpretable male DNA profiles could be obtained from pristine panties that had been washed with a bed-sheet stained with semen. However, further work is required to investigate whether the sperm cells themselves are being transferred or whether just the DNA from the sperm cells is transferred. The additional finding of alleles that could have come from the regular user of the washing machine on two of the socks washed at 60 °C suggests that DNA has also been transferred to the socks from the washing machine. This supports the concept

of transfer of 'wearer DNA' between items of clothing in a washing machine, which had previously been proposed by Stouder et al. [16], but has yet to be demonstrated empirically. Further research is required to establish whether the nature of the semen staining (for example, whether the stain is visible or whether sperm cells are identified, and if so, the number of those cells) or the quantity of DNA obtained from the stain post-washing could be used to suggest whether the staining is as a result of primary deposition or secondary transfer within the washing machine. Depending on the case circumstances, such research, including this initial study, will assist forensic scientists in evaluating the findings of DNA from semen-stained clothing.

5. Conclusion

In ICST cases, it is common for offenders to ejaculate directly onto their victim's clothing, for victims to be wearing school uniform at the time of the assault, for multiple offenders to be involved, and for victims to hide their stained clothing for lengthy periods of time before laundering them. This study demonstrates that complete DNA profiles can be obtained from laundered semen stains on school uniform-type clothing, even with an eight-month lag time between semen deposition and laundering. On cotton T-shirts, complete DNA profiles can also be obtained after multiple washes of semen stains and from laundered stains of two semen donors. These data emphasise the need to recover and examine the clothing of victims for semen and DNA evidence, even if the clothing has been stored for several months or washed multiple times. Potential ICST victims' clothing may be examined to locate possible semen stains using an enzyme presumptive test: this enzyme activity will be lost with washing which may be problematic for the investigation. Nevertheless, in this study, staining was visible even after washing and differentially stained areas could be targeted for DNA tests in forensic analysis. The recovery of such evidence may be crucial to ICST cases, given that the majority of these cases rely heavily on victim accounts and testimony with forensic evidence being rare [3]. These findings are also applicable to other sexual assault cases and indicate that evidence recovery should be included in investigations even if a lag time between the offence and its investigation has taken place.

Acknowledgements

This research was approved by the National Research Ethics Service (REC: 11/LO/0928) to enable the use of human tissue. The authors acknowledge that this research was funded by the Engineering and Physical Sciences Research Council of the UK through the Security Science Doctoral Research Training Centre (UCL SECRéT) based at University College London (EP/G037264/1).

References

- [1] H. Brayley, E. Cockbain, G. Laycock, The value of crime scripting: deconstructing internal child sex trafficking, *Policing* 5 (2011) 132–143.
- [2] E. Cockbain, H. Brayley, G. Laycock, Exploring internal child sex trafficking networks using social network analysis, *Policing* 5 (2011) 144–157.
- [3] H. Brayley, Designing Police Investigation for Child Sex Offending Groups (PhD), University College London, UK, 2013.
- [4] H. Brayley, E. Cockbain, British children can be trafficked too: towards an inclusive definition of internal child sex trafficking, *Child Abuse Rev.* (2014).
- [5] J.C. Chambers, M.A.H. Horvath, L. Kelly, A Typology of multiple-perpetrator rape, *Crim. Justice Behav.* 37 (2010) 1114–1139.
- [6] G. Crowe, D. Moss, D. Elliot, The effect of laundering on the detection of acid phosphatase and spermatozoa on cotton T-shirts, *Can. Soc. Forensic Sci. J.* 33 (2000) 1–5.
- [7] R.K. Farnen, P. Cortez, E.S. Frøyland, Spermatozoa recovered on laundered clothing, *Forensic Sci. Int.: Genet. Suppl. Ser.* 1 (2008) 418–420.

- [8] R.M. Jobin, M. De Gouffe, The persistence of seminal constituents on panties after laundering. Significance to investigations of sexual assault, *J. Can. Soc. Forensic Sci.* 36 (2003) 1–10.
- [9] E. Kafarowski, A.M. Lyon, M.M. Sloan, The retention and transfer of spermatozoa in clothing by machine washing, *J. Can. Soc. Forensic Sci.* 29 (1996) 7–11.
- [10] C. Nussbaumer, T. Halama, W. Ostermann, DNA-typing of sperm collected from skin and clothes after washing in Proceedings of the Third European Academy of Forensic Science Meeting, *Forensic Sci. Int.* 136 (Suppl. 1) (2003) 47.
- [11] J. Spector, D. Von Gemmingen, The effect of washing on the detection of blood and seminal stains, *Can. Soc. Forensic Sci. J.* 4 (1971) 3–9.
- [12] R.M. Morgan, J. Flynn, V. Sena, P.A. Bull, Experimental forensic studies of the preservation of pollen in vehicle fires, *Sci. Justice* 54 (2014) 141–145.
- [13] J.J. Raymond, R.A.H. van Oorschot, P.R. Gunn, S.J. Walsh, C. Roux, Trace evidence characteristics of DNA: A preliminary investigation of the persistence of DNA at crime scenes, *Forensic Sci. Int.: Genet.* 4 (2009) 26–33.
- [14] E. Cockbain, Trafficking of British Children with the UK for Sexual Exploitation: A Situational Analysis (PhD), University College London, UK, 2013.
- [15] S. Noël, K. Lagacé, D. Granger, C. Jolicoeur, Intrafamilial sexual assaults on children: have you considered the possibility of DNA transfer in the laundry? 25th International Symposium on Human Identification (2014) .
- [16] S.L. Stouder, K.J. Reubush, D.L. Hobson, J.L. Smith. Trace evidence scrapings: A valuable source of DNA? *Forensic Science Communications*. 2001, 4 Online; Available: <http://www.fbi.gov/about-us/lab/forensic-science-communications/fsc/oct2001/stouder.htm>.