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ORIGINAL ARTICLE

Investigation of DNA transfer onto clothing during regular daily activities

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Abstract Low levels of DNA from an unidentified human source, often referred to as trace DNA, are ubiquitous, can be transferred onto objects by either direct or indirect methods and have an unknown longevity in situ. Clothing items from crime scenes are often submitted for trace DNA analysis, usually in attempt to identify a person of interest. This study examined the transfer of DNA onto three 10 × 10 cm areas located on the front, back and shoulder of an individual's external clothing ($n = 300$) during a regular day's activity. After wearing for a day, the DNA quantity on all three areas increased approximately 8-fold, which usually corresponded with an increase in the endogenous DNA from the wearer on the front area of the shirt. However, the back area of the shirt was more likely to demonstrate mixtures of endogenous and extraneous DNA. An additional study was also carried out to examine whether domestic laundering is a possible mechanism for the transfer of foreign DNA onto freshly laundered items and revealed that 74% of UV-treated cotton swatch samples produced DNA profiles after laundry with household garments. In summary, this study highlights the ease of DNA transfer onto an individual's external clothing during a regular day, and that extraneous DNA may be already on the clothing item prior to it being worn. The study provides empirical data to assist in the interpretation of trace DNA profiles and support a Bayesian approach to estimate statistical likelihoods for the transfer of foreign DNA.

Keywords DNA transfer · Trace · Clothing · Genotyping

Introduction

Trace DNA, also known as touch DNA, is defined as 'minute quantities of DNA transferred through skin contact' [1] and plays an important role in forensic investigations to obtain genetic information in the absence of discrete known biological samples (for example blood, semen or saliva). Various studies on trace DNA over the past two decades have revealed its dynamic and complex properties. Notably, individuals transfer inconsistent amounts of DNA over time [2] and this is influenced by various environmental factors [3–5]. Cellular materials can be transferred via primary (direct contact) or secondary (indirect contact) transfer, with both modes of transfer capable of producing reportable profiles [6]. In some instances, the DNA profile recovered is not from the last handler or the only person who had handled the item [7–10]. It has been shown trace DNA transfers more easily from a non-porous substrate than a porous substrate, and the amount deposited is generally lower on non-porous substrates than on porous substrates [11]. Improvements in the sensitivity of DNA analysis have also contributed to greater success in the recovery of trace DNA profiles from items of interest, including many commonly handled objects [12].

The transfer of trace DNA onto items touched by an individual provides an alternative way of obtaining a DNA profile in the absence of visible biological material. However, given

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its ubiquitous nature and ease of transfer, questions have arisen over the value of trace DNA as evidence. This is illustrated by recent criminal trials where the weight of inclusionary trace DNA evidence has been diminished by arguments relating to possible indirect or innocent transfer—for example, the recent Australian case *Fitzgerald v The Queen* [2014] HCA 28 [13]. It is now more common for the Defence in criminal trials to raise the possibility of innocent transfer of DNA as a benign explanation for inclusionary DNA evidence. This emphasises the fact that, although trace DNA can provide information regarding the genetic identity of the donor, it is frequently incapable of determining the mechanism by which the DNA was deposited.

This study was designed to examine the likelihood of DNA transfer onto external clothing during regular daily activities by determining the amount of endogenous (self) and extraneous (foreign) DNA deposited. A total of 50 participants each provided a freshly laundered shirt, with three areas (front, back and shoulder) tape-lift sampled before and after wearing for 1 day and the samples genotyped. In addition, several samples from clothing belonging to female donors underwent further testing using a Y-chromosome profiling kit to investigate for the presence of male DNA. Lastly, to investigate laundering as a possible mechanism for the transfer of DNA onto freshly laundered clothing, a subset of participants were given a UV-treated cotton swatch to be machine washed with their usual laundry, with both DNA quantity and profile examined after washing.

Materials and methods

Experimental design

A total of 50 participants were recruited for the study. Participants were asked to place a freshly laundered shirt of their own, to be worn as external clothing, into a paper evidence bag provided (routinely used by New South Wales Police Force for evidence storage). The wearing history of the shirts provided by the test subjects ranged from worn a few times to worn frequently. Three 10 × 10 cm areas, located on the front, back and shoulder of the shirt were tape-lift sampled prior to wearing (Fig. 1) and labelled 'before wearing' (BeW).

The shirt was then returned to the participant in the evidence bag for it to be worn the following day. Participants engaged in their regular daily activities (for example work, study, socialising) and then placed their shirt into the same evidence bag at the end of their work day or after they arrived home. Repeat tape-lift samples were then collected from the same three areas and labelled as 'after wearing' (AfW) samples. Participants were also given a survey to briefly describe their activities during the day (data not shown). The majority

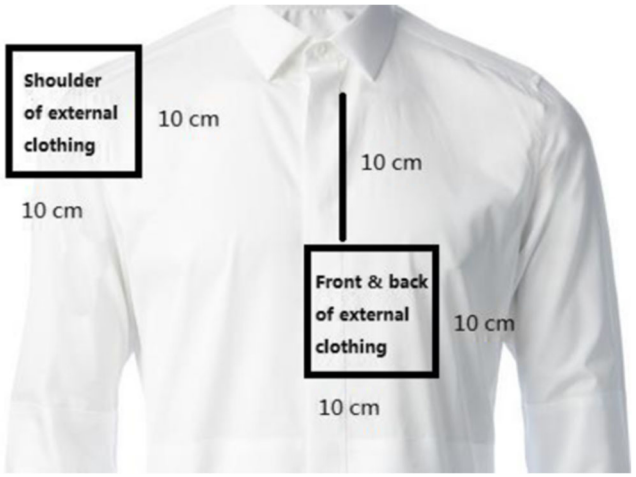

Fig. 1 The three areas on the shirt tape-lifted. Each area is 10 × 10 cm in size

of participants wore their shirt for approximately 9 h (for example 8.30 a.m.–5.30 p.m), with a minimum wearing time of 7 h and a maximum of 12 h. A total of 300 tape-lift samples (150 BeW + 150 AfW) were collected.

For the laundry study, 38 participants were each given a piece of cotton swatch approximately 10 × 10 cm in size (obtained from a pristine, white 100% cotton T-shirt) to be machine washed and dried with their laundry using their own household washing machine and usual detergent. The cotton swatches were UV-treated for 30 min at 253.7 nm wavelength in an Eco-Tech Fume Cupboard fitted with UV C lamps for microbial and germicidal decontamination (Mechatronic Environmental System, Australia) prior to distribution. Two cotton swatches were randomly chosen as negative control.

Sample processing and quality control

Participant reference profiles were obtained using buccal swabs (Tubed Sterile DrySwab™, MWE Medical Wire, UK) and applying the wet swab directly to a Whatman® Non-Indicating® FTA Mini Card (GE Healthcare Life Sciences, UK). The Luminex BSD® 600PLUS Semiautomatic Punch System (Thermo Fisher Scientific, USA) was used to isolate a small sample (1.2 mm in diameter) from the centre of the FTA Mini Card, which was directly processed to obtain a DNA profile.

For experimental samples, one DNA tape-lift kit (Lovell Surgical Supplies, Australia) was used per sample. The tape was 2.5 × 2 cm in size and was pressed using normal pressure against the target area four times across and repeated five times down to ensure the entire 10 × 10 cm target area was covered. The tape was placed inside an AutoLys tube (Hamilton Company, USA), and DNA was extracted using PrepFiler® Automated Forensic DNA Extraction Kit (Thermo Fisher Scientific, USA) using the Hamilton Microlab® AutoLys

STAR liquid handling workstation (Hamilton Company, USA). The final elution volume was 50 μL.

All post-lysis procedures were performed on the Tecan Freedom EVO® 150 Extraction robotic workstation (Tecan Group Ltd., Switzerland) with appropriate anti-contamination procedures as specified by the New South Wales Forensic and Analytical Science Service (NSW FASS) Forensic Biology Laboratory protocols. Samples were quantified with Quantifiler® Human DNA Quantification Kit (Thermo Fisher Scientific, USA) on the Applied Biosystems 7500 Real-Time PCR Systems (Thermo Fisher Scientific, USA). Amplification was performed on the Applied Biosystems GeneAmp® PCR System 9700 Thermal Cycler (Thermo Fisher Scientific, USA) using the PowerPlex® 21 System (Promega, USA). Ten samples from female subjects were additionally amplified with AmpFLSTR® Yfiler® (Thermo Fisher Scientific, USA). Capillary electrophoresis was performed on the Applied Biosystems 3500xL Genetic Analyzer (Thermo Fisher Scientific, USA) following manufacturer's recommendations.

Data analysis and statistical interpretation

GeneMapper® ID-X Software v1.4 (Thermo Fisher Scientific, USA) was used for the analysis of STR profiles, with an allele calling threshold of 175 relative fluorescence unit (rfu) and stutter determined following the analysis guidelines of NSW FASS. For AmpFLSTR® Yfiler® analysis, the threshold for calling alleles was 100 rfu. Statistical analysis was performed in Minitab® 17 (Minitab Inc., USA).

Mixture analysis

Mixture analysis was carried out by an experienced reporting forensic biologist using the STRmix® software v2.3.08 (Institute of Enviromental Science & Research Ltd ("ESR"), NZ). The analyst determined whether the sample was suitable for further interpretation and the number of contributors in the mixture using the NSW FASS Forensic Biology Laboratory guidelines. Greater than four person mixtures were not analysed. All STRmix® deconvolutions were performed assuming the wearer as a contributor to the mixture for the clothing samples or, for the laundry study, the test subject as a contributor. The number of uploadable alleles from additional contributors (i.e. other than the test subject) was determined from the STRmix® results.

Results

DNA quantity increased significantly on all three areas after wearing

On average, DNA quantity on the three areas of the shirt increased approximately 8-fold after it had been worn for a day (Table 1 and Fig. 2). The average DNA quantities across the entire 10 × 10 cm area for BeW samples were 0.96 ng (front), 0.48 ng (back) and 0.81 ng (shoulder). For AfW samples, the average DNA quantity across the entire 10 × 10 cm area were 9.51 ng (front), 3.96 ng (back) and 5.64 ng (shoulder). There were 12 BeW samples that had no detectable amount of DNA, while all AfW samples produced detectable amounts of DNA. Seven AfW samples (1× front, 3× back and 3× shoulder from six shirts) showed a decrease in DNA quantity compared to their respective BeW sample. Paired *t*-test shows the difference in the mean of DNA quantity between BeW and AfW samples were significant for all three areas ($p < 0.05$).

DNA profiles recovered were mostly mixtures

Mixed DNA profiles were recovered in the majority of the samples tested regardless of area or time sampled (i.e. before or after wearing), with two to three person mixtures being the most common (Table 2). STRmix® analysis of the mixed DNA profiles produced profiles suitable for uploading onto a database (greater than 14 alleles from additional contributors as determined by STRmix®, with the wearer as an assumed contributor) in 22–38% of all of the BeW samples tested compared to 20–26% from the AfW samples. The amount of two to four person mixtures in the AfW samples increased by 6% for front, 30% for back and 10% for shoulder samples. The back samples also produced the most complex mixture samples in the study, approximately 6% of greater than four contributors (not analysed with STRmix®). However, there was a 20 and 16% increase in the number of single source wearer profiles recovered from the front and shoulder samples respectively compared to a 4% increase in the back AfW samples. Therefore, samples from the front and shoulder areas produced more single source profiles attributable to the wearer compared to the back samples.

For two of the three couples who participated in this study, the uploadable alleles determined by STRmix® could be attributed to the wearer's partner. The remaining couple for whom the foreign alleles did not match their partner do not cohabit. DNA recovered from 22 to 32% of the BeW samples were evaluated as too weak for interpretation, while virtually all of the AfW samples provided interpretable profiles, either single source or mixtures.

Y-specific allele was recovered from both before and after wearing samples from female participants

BeW ($n = 69$) and AfW ($n = 69$) samples from 23 female participants were also examined for the presence of the Y-allele at the amelogenin locus in PowerPlex® 21 System. The Y-allele was detected in 37/69 (54%) of BeW samples and 49/69 (71%) of AfW samples.

Table 1 The lowest, highest and average DNA quantity recovered from the 10 × 10 cm target areas of the shirt before and after wearing

DNA quantity	Before wearing samples			After wearing samples			Average change
	Lowest	Highest (ng)	Average (ng)	Lowest (ng)	Highest (ng)	Average (ng)	
Front	Undetected	7.95	0.96	0.33	42.30	9.51	×9.9
Back	Undetected	3.51	0.48	0.18	60.90	3.96	×8.3
Shoulder	Undetected	8.43	0.81	0.18	31.80	5.64	×7.0

Additionally, AmpFLSTR® Yfiler® kit was used on five BeW female samples and their respective AfW samples to determine whether there was an increase in the number of Y-specific alleles recovered. All Y-specific alleles that were present on BeW sample were present on the AfW sample. Moreover, all of the AfW samples for this subsample of participants demonstrated additional Y-specific alleles (see Supplementary Fig. 1).

Extraneous DNA were transferred onto cotton swatches during laundering

The quantity of DNA recovered from the laundered cotton swatches ranged from undetected to 4.98 ng with the average being 1.00 ng. The majority of cotton swatch samples (76%) showed either clear single source DNA profiles (21%) or mixed DNA profiles (55%) (Table 3). STRmix® analysis carried out on suitable mixed DNA profiles, and assuming the test subject as a contributor, provided results with greater than 14 uploadable alleles from a second proportionally highest contributor in 37% of all of the samples. Of the mixtures analysed, the majority were two to three person with only one being a four person mixture. One of the three person mixtures provided greater than 14 alleles for upload from the 3rd contributor. DNA profiles recovered from 24% of the swatch samples were determined as too weak for further analysis. DNA recovered from one of the samples was a single source profile which did not match the test subject. For cotton swatches given to female participants, 14/17 (82.4%) showed the presence of the amelogenin Y-allele. No DNA was detected on the two negative control cotton swatches. The participants used 14 different brands of automatic washing machine and nine different commercial washing detergents.

Discussion

The primary focus of this study was to assess the dynamics of DNA transfer onto an item of external clothing during a regular day's activities, in a cohort of individuals with a variety of daily work, transport and social schedules. This approach aimed at closely mimicking 'real life' situations, which may occur in various crime scenarios. In other words, the results give an indication of the amount of background DNA on an individual's external clothing, which could be expected to be present at the time a crime was perpetrated. The study also indicates that the presence of DNA on the external surfaces of an individual's clothing is commonplace even on freshly laundered garments. In addition, although a proportion of the DNA recovered was attributable to the wearer alone, a

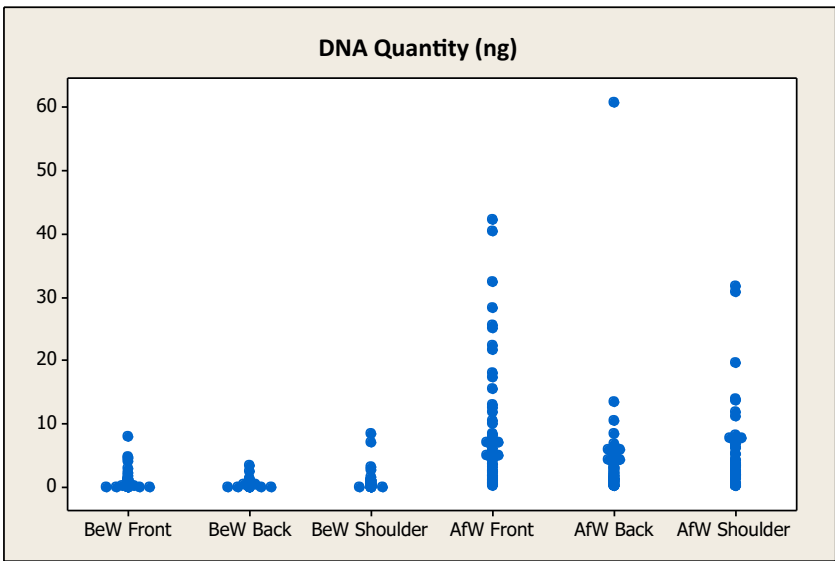

Fig. 2 BeW and AfW DNA quantity on three 10 × 10 cm target areas of the shirt ($n = 50$ for each category)

Table 2 Summary of the DNA analysis results from the shirt samples. The 2nd contributor refers to the next proportionally highest contributor to a DNA mixture as determined by STRmix®, assuming the wearer as the first contributor

Number of samples with uploadable profiles						
Description	Front BeW	Front AfW	Back BeW	Back AfW	Shoulder BeW	Shoulder AfW
Too weak for interpretation	11 (22%)	0 (0%)	16 (32%)	1 (2%)	13 (26%)	0 (0%)
Single source wearer samples	11 (22%)	21 (42%)	10 (20%)	12 (24%)	10 (20%)	18 (36)%
Single source non-wearer samples	2 (4%)	0 (0%)	2 (4%)	0 (0%)	0 (0%)	0 (0%)
2–3 person mixture samples	26 (52%)	25 (50%)	22 (44%)	30 (60%)	27 (54%)	27 (54%)
≥ 4 person mixture samples	0 (0%)	4 (8%)	0 (0%)	7 (14%)	0 (0%)	5 (10%)
Total	50 (100%)	50 (100%)	50 (100%)	50 (100%)	50 (100%)	50 (100%)
Mixture samples with > 14 uploadable alleles attributable to the 2nd contributor	16 (32%)	10 (20%)	11 (22%)	12 (24%)	19 (38%)	13 (26)%
2–3 and 4 person mixture samples—uploadable alleles from 2nd contributor						
Description	Front BeW	Front AfW	Back BeW	Back AfW	Shoulder BeW	Shoulder AfW
0 no uploadable allele From 2nd contributor	3	12	1	15	2	3
1–13 uploadable alleles from 2nd contributor	7	7	10	10	6	16
14–29 uploadable alleles from 2nd contributor	8	7	8	3	13	4
30–40 uploadable alleles from 2nd contributor	8	3	3	9	6	9
Total	26	29	22	37	27	32

significant amount of mixed DNA profiles were also recovered from both BeW and AfW samples which yielded interpretable profiles and sufficient foreign alleles for uploading onto a DNA database. It should be noted that although the NSW FASS threshold for uploading profiles onto the evidence database is 14 or more alleles, profiles of less than 14 alleles may still be used for intelligence purposes in casework involving more serious crimes against persons. The three target areas of each individual's shirt demonstrated an increase in the DNA recovered after wearing for a day in the majority of samples (only 7/150 samples showing a decrease). The highest average increase in DNA recovery was evident in the samples taken from the front of the shirt, while the lowest recovery came from the samples from the back of the shirt. The difference in the average DNA quantitation between the front and back of the shirt was 0.48 ng in BeW samples and this difference was increased over 11-fold in AfW samples (5.55 ng difference). As would be expected, the increase in DNA quantity after wearing corresponded to an increase in the peak heights and number of reportable alleles (both self and foreign) recovered, and this was evident in the majority of samples.

Table 3 Summary of the DNA analysis of the laundry samples. The 2nd contributor refers to the next proportionally highest contributor to the mixture as determined by STRmix®, assuming the test subject as the first contributor

Description	Cotton swatch samples
Too weak for interpretation	9 (24%)
Single source wearer samples	8 (21%)
Mixture samples	21 (55%)
Total	38 (100%)
Mixture samples—uploadable alleles from 2nd contributor	
0 no uploadable allele from 2nd contributor	3
1–13 uploadable alleles from 2nd contributor	4
14–29 uploadable alleles from 2nd contributor	7
30–40 uploadable alleles from 2nd contributor	7
Total	21
Number of 2 person mixture samples	10
Number of 3 person mixture samples	10
Number of 4 person mixture samples	1

The detection of foreign alleles on the wearers' shirts both before and after wearing is clear evidence of transfer of DNA onto these items, although the determination of the mechanism of transfer i.e. direct or indirect, active or passive etc. was outside the scope of this study [14]. In addition, the results indicate that the increase in the amount of foreign DNA acquired during a regular day was particularly evident on the back area of the shirt.

In reviewing the samples from the three areas of the shirt, there was an increase in single source DNA profiles matching the wearer recovered from AfW samples from the front and shoulder of the shirt, compared to AfW samples from the back of the shirt. It could be speculated that these areas may be more susceptible to exposure to the wearer's DNA from, for example, saliva produced by talking and breathing, as well as the wearer directly touching the area. However, it is acknowledged that further investigation would be required to support

this proposal, such as identifying the possible cellular source of the wearer's DNA on the front and shoulder of the shirt, e.g. amylase screening for saliva. The increase in wearer's DNA would also have the effect of masking lower levels of foreign DNA which may have been acquired during the day. It is also evident from the DNA profiling results that the back area, which is not so directly exposed to possible high yield sources of wearer's DNA (such as saliva) demonstrated an increased recovery of mixed DNA profiles in the AfW samples. In consideration of this result, it could be argued that the back area of the shirt is more likely to contact possible DNA transfer 'vectors', such as chair backs in office areas, in cafes and restaurants, on public transport or even in private cars driven by more than one individual. However, additional studies on the likelihood of this transfer would be required to confirm this proposal.

Acquired foreign DNA from multiple donors was demonstrated in both BeW and AfW samples by DNA profiling, but the greatest increase was seen with the AfW samples as would be expected. Although some of these samples produced complex DNA mixtures which were not suitable for most routine mixture interpretation protocols (greater than four possible contributors), many others were suitable for the determination of uploadable profiles using the STRmix® software and assuming the wearer as a contributor.

Three sets of couples were included in the study and, for these individuals, the majority of foreign alleles found on their clothing could be matched to their partner, except the one couple who did not cohabit (data not shown), indicating the ease of DNA transfer in close relationships and domestic settings.

A somewhat surprising finding was the amount of DNA in the BeW samples, which were collected from freshly laundered clothing, representing the background levels of DNA which can be expected to be detected on the surface of clean clothing provided by the participants. Apart from the donor's own profile, many of these samples produced interpretable mixtures from which uploadable foreign DNA profiles could be determined. In some cases the donor of the clothing was not even the predominant DNA profile in the sample. This finding has serious implications for forensic DNA casework when elimination samples from family members or cohabiting individuals are often not provided.

It was very common for shirts from female subjects to contain the Y amelogenin allele in the PowerPlex® 21 System DNA profiles recovered, particularly in AfW samples (49/69, 71%). This was less prevalent in the samples from the front of the shirt. However, as previously mentioned, the predominance of the wearer's DNA on this area probably masked the presence of any male DNA. Further investigation on a small subsample of the female samples using the AmpFLSTR® Yfiler® profiling kit demonstrated a Y profile which could have originated from at least seven males in one of these samples (Supplementary Fig. 1). This particular individual worked in hospitality and was therefore exposed to multiple males during her day. Although Y-STR profiles are not currently used for database search comparisons with a reference sample database, they still may provide important intelligence information to investigating officers indicating that DNA from an unknown male is present. This information can become the focus of an investigation of crimes perpetrated on females by male offenders. However, as indicated by the study without suitable elimination samples from, for example, cohabiting males, Y-profiles recovered from clothing may have no probative value in an investigation.

The investigation of the DNA recovered from cotton swatches washed with the subject's regular laundry demonstrated an acquisition of endogenous and foreign DNA during this process, which has also been shown by others [15–21]. However, in the current study the laundry was performed in the subjects own washing machines and the mixtures obtained were STRmix® analysed assuming the subject as a contributor. Results are presented that demonstrate that DNA transfer events have occurred during the laundry process. The recent publication by Voskoboinik et al. (2017) [21] demonstrated that 22% (7/32) of samples from new unworn socks with no traceable DNA prior to experiment produced DNA profiles post-laundry. In our study, we observed DNA profiles in 74% (28/38) of the cotton swatches post-laundry. The explanation for the increased detection of DNA mixtures in the current study may be due to the sensitivity of the DNA profiling kit utilised i.e. PowerPlex21® System used in this study versus AmpFISTR® SGM Plus™ Kit used by Voskoboinik et al. In addition, although it may be assumed that the actual machine washing process was the major mechanism for transfer, it is also possible that DNA transfer may have occurred during other steps in the washing and drying of clothing. For example, the mixing of dry/wet clothing in the laundry basket or the mode of clothes drying utilised may also have contributed to DNA transfer onto BeW clothing. This part of the study was designed to demonstrate the propensity of household laundering to impact on the recovery of incidental DNA profiles; as such it was not intended to provide a reductionist analysis of laundering activities.

The results of this study further reaffirms that any DNA profiles obtained from casework garments should be treated with extreme caution with regards to their case relevance. It could be hypothesized that in real life scenarios, the biological material found on a worn garment could originate from at least three sources: the wearer him/herself (endogenous), donor who had social interaction with the wearer during the day (extraneous) and donor who cohabit with the wearer (extraneous). All of these factors, both individually and combined, would contribute to the prevalence of the recovery of mixed DNA profiles from a garment. Moreover, continuous improvement in the sensitivity of DNA typing technologies

would further increase the chances of obtaining a DNA profile from trace evidence.

Conclusion

The adventitious transfer of trace DNA means that the DNA recovered in forensic casework may not always have evidentiary relevance. In addition, the efficiency of DNA profiling of low yield DNA samples has been greatly facilitated by the increased sensitivity of profiling kits and the availability of software programs such as STRmix® for the interpretation of mixed DNA profiles. In some cases, mixture interpretation is not possible due to the large number of foreign alleles present, but in others interpretable mixtures are recovered which can provide unknown DNA profiles suitable for uploading onto DNA evidence databases. The results of this study demonstrate that the transfer of foreign DNA onto an individual's external clothing during a regular day is commonplace and that extraneous DNA may be already present on the clothing item prior to it being worn [21]. This study also demonstrated the apparent ease with which DNA was transferred to an item during the laundering of clothing, as a possible mechanism for the deposition of this 'pre-wear' DNA. This information presents an important cautionary note for criminal investigations, involving the recovery of trace DNA from external clothing, which may prevent the expensive pursuit of false leads (for example familial searching, Interpol searches, obtaining covert samples for comparison etc.) and the uploading of non-evidentiary DNA profiles onto already overburdened databases. In particular, the evidentiary relevance of trace DNA recovered from clothing in offences committed within domestic settings will certainly be limited. Therefore, obtaining appropriate elimination samples from individuals who cohabit with the victim may save investigators the time and expense of attempting to identify the source of unknown DNA profiles and this applies to both autosomal and Y chromosome STR profiling. Elimination reference samples would also assist with the interpretation of complex DNA mixtures and the identification of possible probative DNA profiles, by supplying more information on known contributors to input into forensic mixture analysis software such as STRmix®. Moreover, certain trends which are indicated by this study, such as the increased tendency for the wearer's DNA to predominate the front area of the shirt after a day's wear, can be further investigated to add to our current knowledge of the probability of direct and indirect transfer of DNA onto external clothing. Information such as this may support a Bayesian approach to estimate statistical likelihoods for the transfer of foreign DNA [22]. Further studies of this kind, which examine 'background' DNA acquisition, are recommended to gain a better understanding of the mechanisms which lead to the transfer of trace DNA.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Research involving human participants All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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