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Australian Journal of Forensic Sciences

ISSN: (Print) (Online) Journal homepage: www.tandfonline.com/journals/tajf20

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To cite this article: Rachel H. Oefelein, Sarah Cresswell & Carney Matheson (2024) Establishing a baseline for background and purchaser DNA in new unwashed underwear for the purpose of evaluative reporting, Australian Journal of Forensic Sciences, 56:5, 483-492, DOI: 10.1080/00450618.2023.2231970

To link to this article: https://doi.org/10.1080/00450618.2023.2231970

Published online: 09 Jul 2023.

AUSTRALIAN JOURNAL OF FORENSIC SCIENCES
2024, VOL. 56, NO. 5, 483–492
https://doi.org/10.1080/00450618.2023.2231970

Establishing a baseline for background and purchaser DNA in new unwashed underwear for the purpose of evaluative reporting

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ABSTRACT

This study was designed to establish a baseline of both background deoxyribonucleic acid (DNA) from unknown sources and purchaser DNA that may be recovered from new unwashed children's and adult's underwear. Fourteen children's underwear and 10 adult's underwear items were tested. Despite no male donors being utilized for the study, male DNA was detected on 17 out of 24 of the samples, demonstrating that the presence of an unknown donor's DNA on new, unwashed, unworn underwear should be expected on most samples. Four samples (16.7%) were deemed suitable for comparison based on the laboratory's thresholds for comparison. Of the four samples deemed suitable, three resulted in inclusionary likelihood ratios (LR) for the donor that briefly handled the underwear, i.e. the purchaser. The percent portion of the assigned LR contributor position associated with the purchaser did not exceed 61.0%. This research suggests that very limited and transient contact with new underwear has resulted in DNA transfer sufficient for comparison, providing evidence that this level of contribution should be considered in analysis and interpretation when evaluating DNA profiles obtained from newly purchased, unwashed underwear.

ARTICLE HISTORY
Received 8 April 2023
Accepted 14 June 2023

KEYWORDS
Background DNA; underwear; DNA; activity level propositions; STRmix™; evaluative reporting

1. Introduction

Underwear is often considered a critical piece of evidence in alleged sexual assault cases. Forensic DNA profiling of the biological material present on underwear will form an important component in the investigation of an alleged sexual assault. However, if there is a chance for other contributors to be present in this biological material, it can adversely have an impact on the case. Evaluation of DNA profiles obtained from criminal evidence may require the consideration of persistence and transfer of DNA occurring from seemingly unlikely sources. One example of commonly encountered evidence posing this challenge is new clothing items. In this example of clothing, DNA transfer could come from the manufacturing staff, packing staff, shop staff who stock the items, prospective

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shoppers handling the item and even the purchaser of the item who is not necessarily the final owner of the clothing.

Evaluative reporting is described by the European Network of Forensic Science Institutes (ENFSI) as 'any forensic report containing an evaluative reporting section'[1]. The utilization of evaluative reporting has already been adopted in Europe and international and European organizations have released guidelines for evaluative reporting in forensic science[1–3]. However, this method of reporting, unlike investigative and technical reporting, has not yet become routinely incorporated into casework in the United States, and additional research is needed to provide the foundation for the incorporation of evaluative reporting in the United States. The most critical areas of research required for incorporation include, utilizing current DNA profiling systems and genetic analysers in real-world scenarios so that data can be effectively applied to Bayesian networks to evaluate activity level propositions[4]. One of the critical gaps in research where evaluative reporting is important is on evidence commonly encountered in alleged sexual assault scenarios. One of the most common items of evidence in an alleged sexual assault investigation is the examination of underwear.

There are several studies relating to the transfer, persistence, prevalence, and recovery (TPPR) of DNA in underwear, but these studies have been conducted utilizing DNA profiling systems not routinely used in the United States such as the Applied Biosystems AmpFlSTR® SGM Plus® PCR Amplification Kit a 10-marker system, and AmpFLSTR™ NGMSElect™ PCR Amplification Kit a 17-marker system[5–7]. This study seeks to expand on this previous research using a DNA profiling kit routinely used in the United States and internationally, the Applied Biosystems GlobalFiler™ PCR Amplification Kit a 24-marker system. This system is commonly used in the United States because it encompasses the core loci required by the Combined DNA Index System (CODIS)[8,9]. It has been estimated that as many as 300,000 to 400,000 sexual assault kits remain untested in the United States[10], and many of these will contain underwear, a routine component of sexual assault kits. As such, this study has focused on female adult's and children's underwear, of two commonly encountered fabric types.

2. Methods

This study consisted of measuring the level of background DNA and purchaser DNA recovered on new unwashed underwear. The underwear items were in both children's and adult's sizes. Fourteen children's sized underwear from the brand Fruit of the Loom were used. The children's underwear was 100% cotton and categorized as tag-free, full-seated, size 4, girl's underwear. Ten adult's sized underwear from the brand QOVOQ were used in this study. These were all 90% nylon and 10% spandex, categorized as tag-free, thong-style, extra-small, women's style underwear. All underwear was purchased online through Amazon. There were two female donors employed in this research. The DNA profiles of the donors were obtained by means of buccal swab collection. Donor 1 briefly handled each of the children's underwear for approximately 30 seconds, touching with their hands throughout the underwear, and then bagging the item. The amount of time was selected in an effort to be comparable to the amount of time an individual may handle the underwear in the act of purchasing the item. Donor 2 completed the same

process with all the adult's underwear. The donors did not wash their hands within 1 hour prior to handling the underwear.

The sampling method utilized was a single wet swab wetted with water. Samples were collected by cutting the swab material from the swab stick and adding the swab cutting into sterile extraction tubes. Samples were processed using the PrepFiler™ DNA Extraction Kit, the Promega PowerQuant® system, and the Applied Biosystems GlobalFiler DNA profiling system. Quantitation is an essential part of the DNA testing process for determining if DNA is present, if male DNA is present then what is the male-to-female ratio, and to calculate the normalization of the sample. For the purposes of this study, male and female refer to the chromosomal type of XX for female and XY for male. Quantitation is also valuable to assess the total amount of DNA, suitability for testing, and the presence of male DNA, when no known male has handled the item in question. The final elution for the PrepFiler™ DNA Extraction Kit, automated on a TECAN Freedom EVO 150, is approximately 45 µL after using 2 µL for quantitation. The total nanograms (ng) obtained was calculated by taking the value in ng/µL obtained from the Promega PowerQuant® system and multiplying that amount by 45.

All processing steps were automated on a TECAN Freedom EVO 150 system. Normalization followed the laboratory's internal protocol of optimal input of 1.00 nanogram (ng) based on the internal validation for this DNA profiling system. Amplification using the manufacturer's guidelines was followed by capillary electrophoresis on an Applied Biosystems™ 3500xL Genetic Analyser. The data were collected with 3500xL Series Data Collection software v.3 and analysed using GeneMapper® ID-X software v1.4. The STRmix™ v2.6.02 software was used for mixture deconvolutions and likelihood ratio (LR) generation. The allele frequencies utilized for calculations were the Expanded Federal Bureau of Investigation (FBI) DNA population database[8,9,11–13], and a theta value of 3.00% was applied. The quantitation values obtained, whether the values met the routine cut-off for polymerase chain reaction (PCR) amplification for casework, the autosomal DNA to male DNA ratio, and whether the developed profile was suitable for comparison were evaluated. The interpretation of these data followed the operational guidelines, thresholds, and cut-offs of a standard accredited forensic DNA laboratory in the United States. Human Ethics approval was granted by Griffith University Research Ethics for this research (GU ref no: 2021/790).

3. Results and discussion

3.1 Quantitation

The total autosomal DNA and the total Y-DNA quantities were assessed. The DNA quantities obtained from the children and adult's samples are shown in Figures 1 and 2 respectively. The average amount of total DNA in nanograms for the children's underwear was 0.08 ng for autosomal DNA and 0.02 ng for the male DNA present (Figure 1). The average amount of total DNA in nanograms for the adult's underwear was 0.17 ng for the autosomal DNA and 0.07 ng for any male contribution present. The calculated averages for the DNA recovered from the adult's underwear were strongly influenced by replicate 8. The removal of replicate 8 would have changed the averages for the adult's samples to 0.09 ng for the autosomal DNA and 0.01 ng for the male DNA present, considerably more comparable to the children's samples (Figure 3). There are many reasons why replicate 8

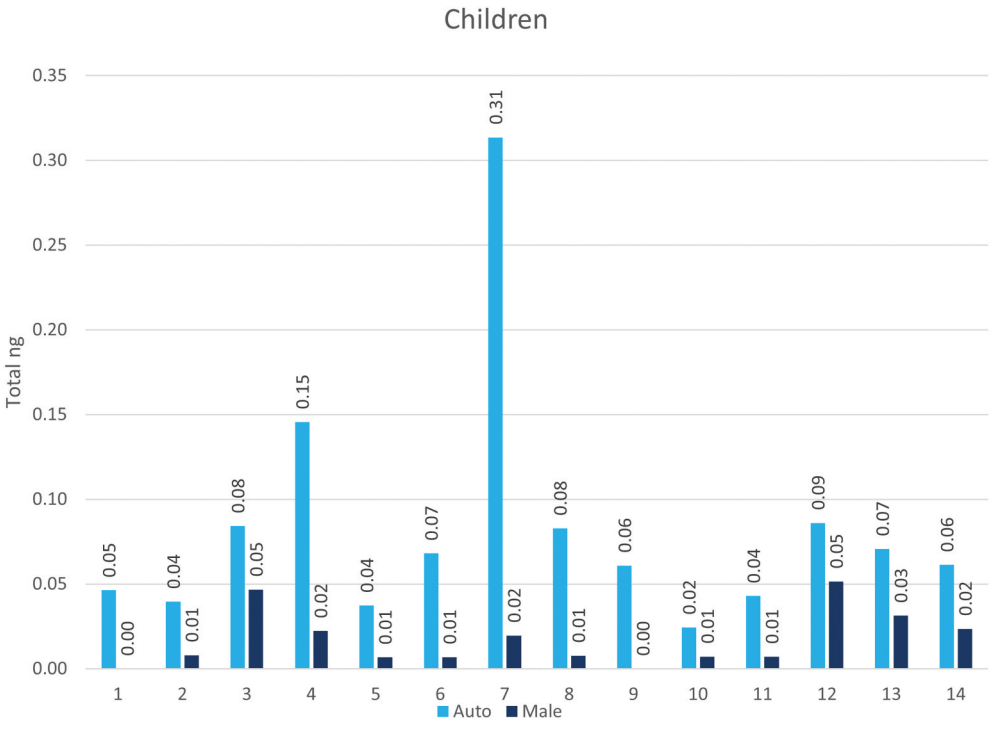

Figure 1. Total quantity of DNA recovered from children's samples.

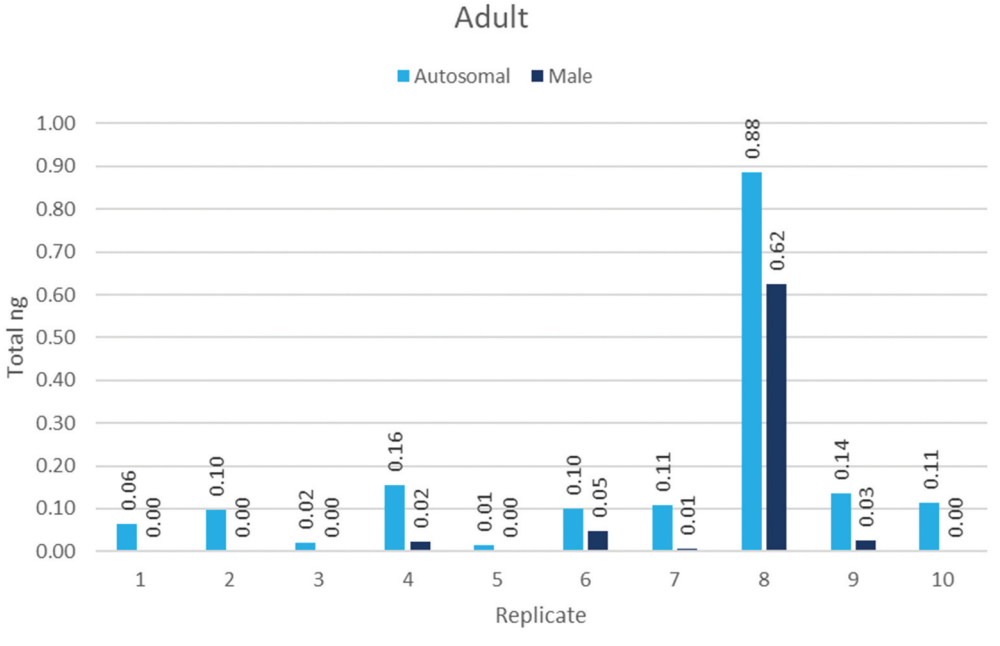

Figure 2. Total quantity of DNA recovered from adult's samples.

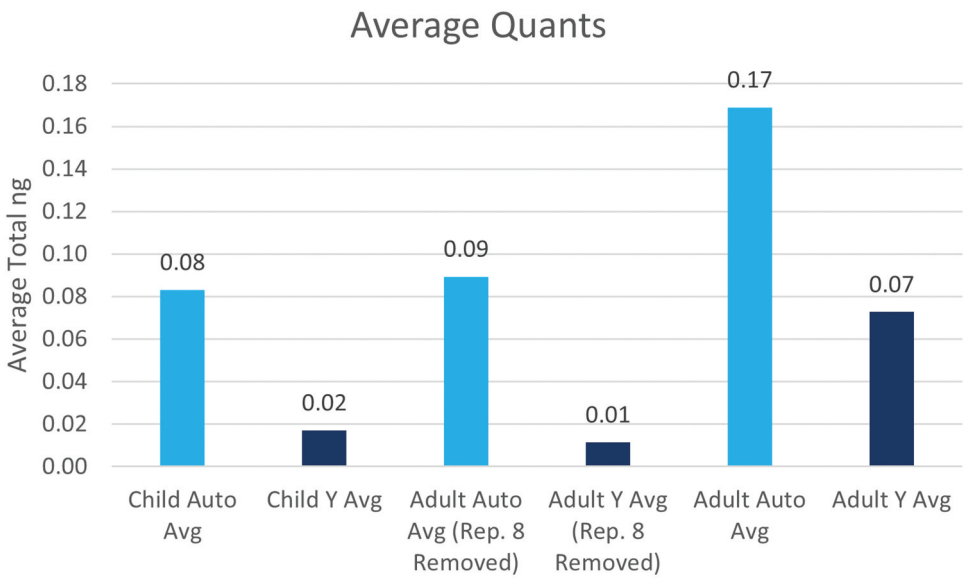

Figure 3. Average total quantity of DNA recovered from all samples.

may have had an increased level of DNA, including but not limited to a sneeze from the donor, dislodgement of cellular material under a nail while handling, an opened abrasion on their hand, etc. When taking out the consideration of the replicate 8 outlier from the adult's underwear the overall averages were quite similar between adult's and children's samples which also translates to the difference in textiles, cotton versus nylon/spandex blend (Figure 3). This similarity is supported by a p-test, in which the total autosomal DNA using the first 10 replicates of the children's samples in comparison to all of the adult's samples total autosomal DNA results in a P-value of 0.03. However, when replicate 8 for the adult's samples is removed and the remaining replicates are compared to the first nine replicates of the children's samples a P-value of 0.51 was obtained demonstrating no statistical significance. The P-test was repeated twice with different sets of nine replicates from the children's samples against the adult's sample with P-values of 0.31 and 0.54 being obtained. The replicate tests were conducted to ensure the P-value remained greater than 0.05 when different replicates were selected.

If DNA concentration techniques prior to amplification is being utilized with a cut-off of 0.001 ng/µL, 18 of the samples (75.0%) would have been suitable for amplification based on the laboratory's casework quantitation cut-off for the DNA profiling system. Without concentration, utilizing a cut-off of 0.005 ng/µL 2 samples (8.33%) would have been suitable for amplification.

Donor 1 and Donor 2 were females, enabling the ability to determine the presence of male DNA recovered from the samples as exclusively background DNA, assuming no secondary, tertiary, quaternary transfer from Donor 1 and Donor 2. In total, 17 samples (70.8%) had male DNA detected (Figures 1 and 2). The male-to-female ratio was calculated by using the formula: (Auto-Y)/Y that ranged from 0.42 to 16.1 with no particular trend (Figure 4). The female-to-male ratio cut-off for autosomal STR testing for this DNA profiling system in the laboratory is 99:1 for alleged sexual assault casework with

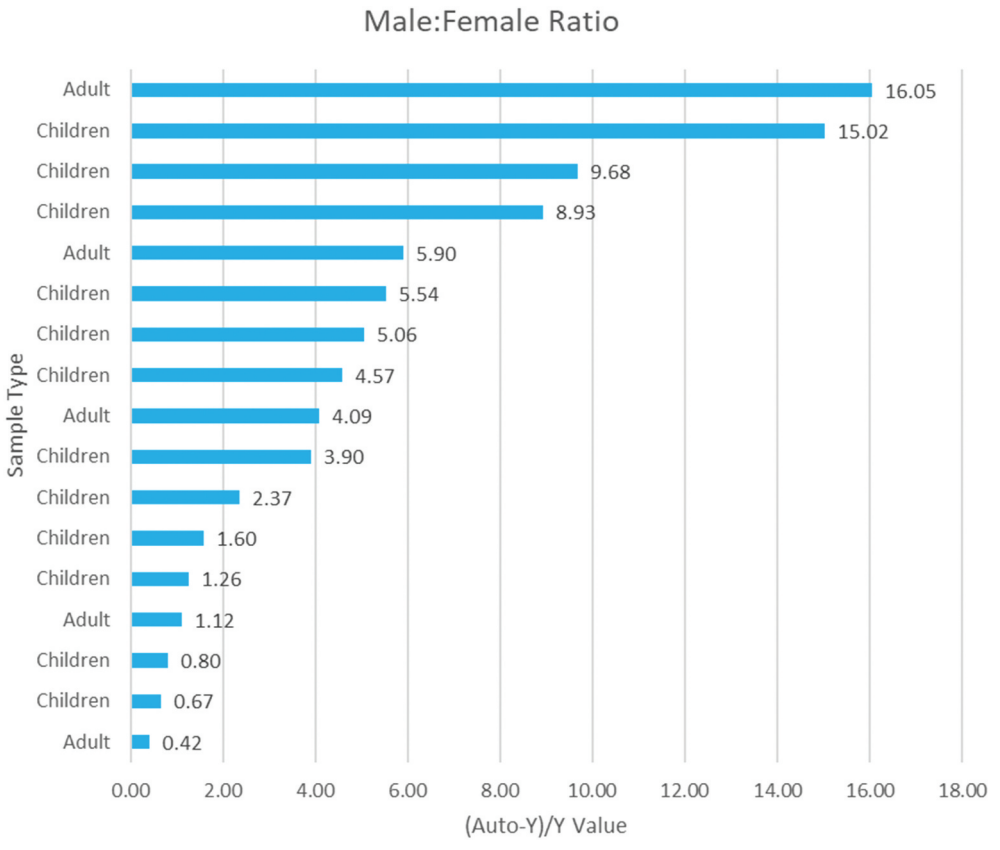

Figure 4. Female:Male ratios obtained from all samples with male DNA detected.

a male assailant and a female victim. This value was selected based upon the internal validation studies, where it was observed that when the quantity of female DNA exceeded 99 times the male DNA, the male DNA profile was typically not observed in the presence of the excess female DNA. All samples (n = 24) were within the cut-off for the male-to-female ratio. Note that this data is summarized in Figure 4; however, seven replicates were not included due to no male DNA being observed at quantitation. The sample type is shown along the Y-axis to demonstrate that regardless of sample type (adult's versus children's), and as such regardless of fabric type, there is no trend in the male to female ratio.

3.2 Amplification and DNA typing results

Regardless of the quantity of DNA obtained, all samples were amplified, and sample concentration was not utilized. The DNA profiles obtained were assessed for suitability for comparison with approximately four samples (16.7%) being suitable for comparison. The suitability for comparison is based on the operational guidelines, where the minimum loci required for suitability for comparison are six non-sex-determining loci. To be clear, all four of these samples required probabilistic genotyping for comparison and none would

have been deemed suitable for upload to a database due to no profile, known or unknown contributors, being resolved using STRmix™. In order for a profile to be considered resolved, based on the criteria of the standard operating procedure at the operation laboratory where the research was conducted, the component interpretation summary in STRmix requires a weighting greater than 99% at a locus for a genotype and a minimum of six loci would need to be characterized.

Eight samples (33.0%) resulted in mixtures of at least two individuals and one sample (4.1%) resulted in a mixture of at least three individuals. The remaining 15 samples were categorized as at least one contributor. It should be noted that laboratory policy is that there must be two instances of evidence for an additional contributor to outright increase the number of contributors. This policy is in place to account for elevated stutters and/or tri-alleles. Peak height ratios, allele counts, and out of range diagnostics can be considered as individual reasons at each locus or overall to increase the number of contributors (NOC). For example, allele counts at locus SE33 in conjunction with elevated peak height ratio at CSF1PO could satisfy the two required reasons to increase the NOC for an individual profile. The diagnostic features evaluated include forward stutter variance, back stutter variance, −2BP stutter variance, Gelman Rubin (GR) diagnostic, average log likelihood, and effective sample size[14].

3.3 Donor results

The presence of alleles recovered from the donor, i.e. the purchaser, was evaluated in two ways. First, the expected versus the observed alleles for the handling donor, for all samples were evaluated (see Figures 5 and 6). The highest representation observed was 30.0% of the expected alleles.

The presence of a single allele or even several alleles consistent with a donor does not necessarily reflect the presence of that donor, rather it may be due to allele sharing with another individual. As such, the profiles deemed suitable for comparison were further evaluated utilizing probabilistic genotyping. The two hypotheses considered were the DNA profile originated from the purchaser and N-1 unknown individuals (where N is the estimated number of contributors in the mixed DNA profile), or the DNA profile originated from N individuals.

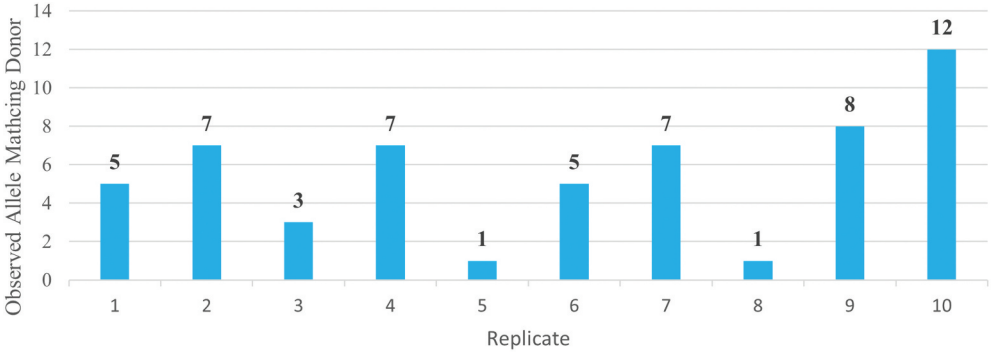

Figure 5. Count of observed alleles corresponding to Donor 1 (children samples).

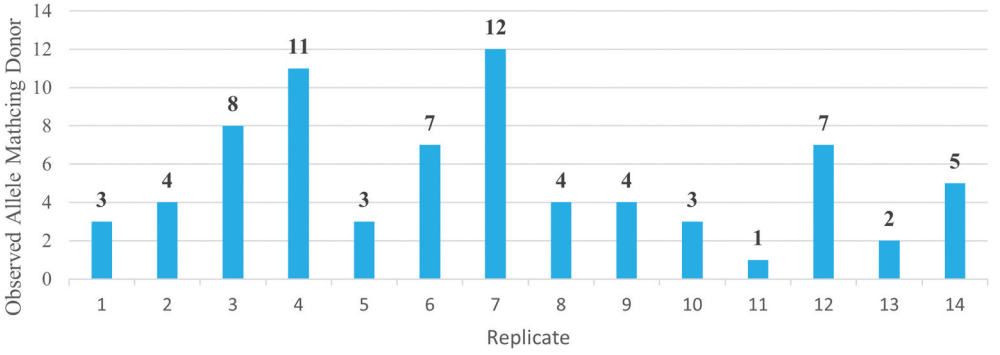

Figure 6. Count of observed alleles corresponding to Donor 2 (adult samples).

Hypothesis 1 (H1): Purchaser Donor & N Individuals-1

Hypothesis 2 (H2): N Individuals

The first sample suitable for comparison was a two-person mixture obtained from the seventh replicate from the children's samples. When evaluated as a two-person mixture based on apparent observations, an LR of 1.40E13 was obtained, very strong support, based on the Scientific Working Group on DNA Analysis Methods (SWGDAM) recommendations for LR reporting[15]. The remaining three samples were two 2-person mixtures from the adult's underwear with LRs of 1.07E9 and 2.08E4 (very strong and strong support based on SWGDAM recommendations), and a three-person mixture with an LR of 4.23E-1 (limited support based on SWGDAM recommendations when reporting 1/LR for H2). For the three inclusionary LRs, the percentage proportion of DNA from the assigned contributor position was 63.0% for the children's underwear sample, 60.0% and 61.0% for the adult's samples. Previously a study was published examining the presence of male cohabitants DNA profiles detected in the underwear of the female when no sexual activity occurred. That study found that out of 103 samples only five samples resulted in complete PowerPlex Y23 profiles, demonstrating that in the absence of sexual activity it is still possible to obtain DNA profiles of cohabitants; however, this data is often limited and will not be detected in the majority of samples[7]. This research is consistent with the findings of this study.

4. Conclusions

This study has shown that DNA may be obtained from the purchaser or handlers of new, unwashed, unworn underwear due to deposition of cellular material from direct DNA transfer. Most samples may be too limited in DNA quantity to proceed for amplification when laboratory cut-offs are employed. However, as cut-offs get lowered and typing systems become more sensitive, this poses a point of consideration. For example, examination of profiles utilizing probabilistic genotyping with reduced analytical thresholds beyond what was internally validated at the laboratory. Based upon the

observations in this study, samples collected under similar conditions and processed using similar parameters are expected to be primarily inconclusive for comparison purposes. It is possible to obtain inclusionary LRs for individuals that briefly handle the underwear and as such this should be taken into consideration when examining DNA profiles obtained from newly purchased unwashed underwear for the purposes of evaluative reporting. Additionally, when evaluating the DNA profile and LRs obtained from underwear in casework, the quantities of DNA obtained, suitability for comparison, and the associated LRs should also be taken into consideration. DNA profiles consistent with individuals not associated with the case may be obtained due to background DNA on the underwear from unknown sources, i.e. packers, stockers, other shoppers, and manufacturers. The importance of this research is that the presence of unaccounted for DNA profiles may not be attributable to the activities of the owner of the underwear when the item is new and unwashed.

5. Further research

Evaluation of the persistence of unsourced background DNA and purchaser DNA in worn underwear may also be beneficial for evaluative reporting data. Additional research will be conducted using a wide variety of current methodologies for DNA processing of evidence routinely encountered in alleged sexual assault case scenarios. The goal of the additional research will be to share the research data in the public realm so that forensic scientists may access the data for use in evaluating reporting for evidence.

Acknowledgments

The authors would like to thank the contributions of the forensic and legal communities in these ever-evolving times as well as the support of DNA Labs International, Griffith University, and Jayson Burkhardt.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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