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TECHNICAL NOTE

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The effectiveness of protective clothing in the reduction of potential DNA contamination of the scene of crime

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Abstract The use of ultra-sensitive low copy number (LCN) DNA typing allows the analysis of picogram amounts of DNA. Trace evidence accidentally left at a scene of crime (SOC) by the investigating team may be inadvertently collected and analysed, potentially leading to spurious evidence being introduced into the criminal investigation. A series of experiments were undertaken to determine the extent to which an investigator could contribute to any DNA contamination of a scene of crime under different simulated activities. Further, the degree to which any contamination was reduced by the use of commercially available protective clothing was demonstrated. Precautions that should routinely be taken at a scene of crime to reduce the risk of DNA contamination are recommended.

Keywords DNA · Contamination · Face mask · Scene suits · Scene of crime

Introduction

Recent advances in short tandem repeat (STR) analysis allow for the typing of sub-100 pg amounts of human DNA [1]. Indeed, recent studies have demonstrated that a DNA profile can be obtained from items that have been handled, even if only briefly [2] and from epithelial cells recovered from clothing or human skin in forensic casework [3, 4]. The ever-increasing sensitivity of DNA-based tests brings with it the increased possibility of the detection of accidental contamination of a crime scene with human DNA from those attending and working within the scene environment.

Previous work has publicised the potential problems of human DNA contamination within the mortuary, either on the cadaver [4], from the instruments [5, 6] or the work surfaces [7]. The concerns raised are equally applicable to all scenes of crime. It is possible that breathing or speaking may be sufficient to contaminate a crime scene although, to date, there is no published work to support or refute this hypothesis. Utmost care must be taken to prevent any such contamination of a scene by any evidence type, the consequence of which might mislead an investigation or potentially contribute to a miscarriage of justice.

This study reports the observations of a small number of experiments performed to investigate the potential for DNA contamination by a person attending a crime scene and considers the benefit of standard protective equipment that can be used to reduce contamination. Further, we propose a protocol to reduce the risk of DNA contamination by those working within a SOC.

Materials and methods

All experiments were performed by a single male subject, known to be of "good shedder" status as described by Lowe et al. [8]. In a laboratory used solely for the extraction of low levels of DNA, an assistant prepared the "test zone" and collected the samples by swabbing after each experiment as described below. The assistant was clothed in a disposable PrimeGuard "Howie" laboratory coat (Shield Medical, Farnham, UK), latex gloves (Johnson and Johnson Medical, Skipton, UK), paper mob cap (Fisher Scientific, Loughborough, UK) and disposable Barrier face mask (Johnson and Johnson Medical, Skipton, UK) at all times, in order to minimise the risk of contamination.

Experiments were categorised into four general groups, "No Movement", "Movement", "Talking" and "Coughing". Unless otherwise stated, the group of experiments that involved movement consisted of actions mimicking those that a crime scene examiner might make, including small arm movements, turning the head, and shifting weight from one leg to the other. Each experiment was performed in a standing and kneeling position, with and without protective clothing. The duration of each experiment was

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Table 1 Experimental conditions and DNA profiling results

Experiment	Activity level	Protective clothing		Standing (S) or kneeling (K)	Talking	Coughing	Number of donor and non-donor alleles					Alleles in negative control
		Body	Face				Zone 1	Zone 2	Zone 3	Zone 4	Zone 5	
1	None	–	–	S	–	–	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0
2	None	–	–	K	–	–	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0
3	Normal	–		S	–	–	21 (0)	1 (0)	1 (0)	0 (0)	2 (0)	0
4	Normal	+	–	K	–	–	21 (0)	20 (0)	19 (0)	21 (0)	16 (0)	0
5	None	+	–	S	–	–	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0
6	None	+	–	K	–	–	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0
7	Vigorous	+	–	S	–	–	0 (0)	0 (0)	0 (0)	21 (0)	18 (0)	0
8	Vigorous	+	–	K	–	–	21 (0)	21 (0)	6 (2)	7 (1)	1 (0)	1
9	None	+	–	S	+	–	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0
10	None	+	–	K	+	–	19 (0)	20 (0)	9 (0)	11 (0)	0 (0)	0
11	None	+	–	S	–	+	2 (0)	7 (0)	0 (0)	1 (0)	21 (0)	0
12	None	+	–	K	–	+	0 (1)	17 (0)	10 (0)	4 (0)	1 (1)	0
13	None	+	+	S	+	–	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0
14	None	+	+	K	+	–	21 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2
15	None	+	+	S	–	+	0 (0)	0 (0)	0 (0)	0 (1)	1 (0)	0
16	None	+	+	K	–	+	0 (0)	1 (0)	0 (0)	0 (0)	0 (0)	0
17	None	+	+[a]	S	+	–	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0
18	None	+	+[a]	K	+	–	5 (4)	10 (12)	0 (0)	1 (0)	1 (1)	8

+[a] masked with visor

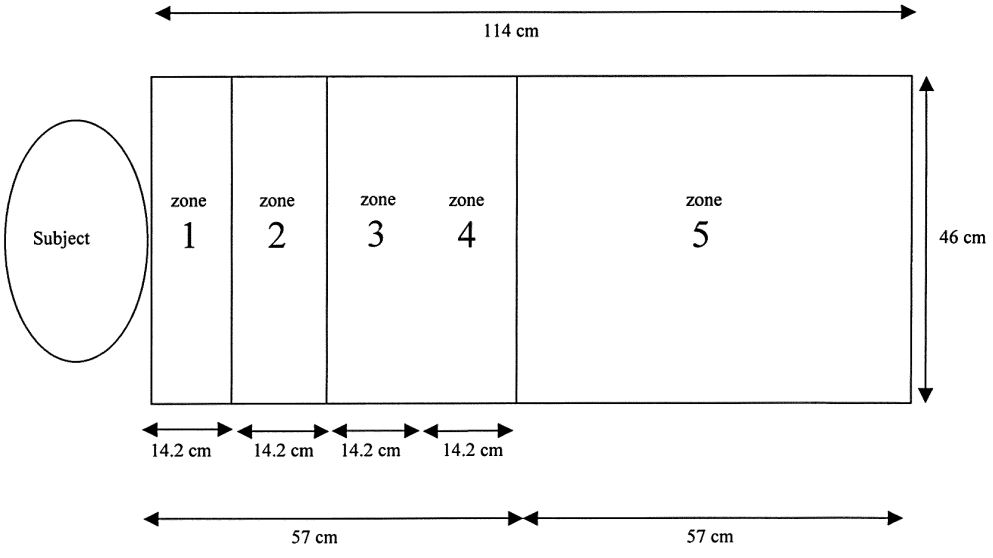

Fig 1 Diagrammatic representation of the test area

15 min except for the coughing experiments, which lasted for 10 seconds. A summary of the experiments is shown in Table 1. Where protective clothing was worn by the subject, this consisted of a full body suit with the hood up (Tyvek Pro-Tech chemical protective clothing, DuPont Nonwovens, Luxembourg), Sandra anti-static plastic overshoes (Henleys Medical, Welwyn Garden City, UK) and medical gloves (MarigoldIndustrial, Broxbourne, UK). Where a mask was worn, a disposable mask 9030V (ARCO, Hull, UK) was used.

For each experiment, a test area comprised of two new sheets of "Bench Kote" (Whatman, Maidstone, UK) measuring 46 cm× 57 cm were placed on the floor, polythene side up, to produce a non-absorbent area measuring 114 cm long by 47 cm wide. The paper represented the area immediately in front of an investigator at a scene. To prevent any contamination occurring from the action of the subject moving into position, the paper test area was placed in front of the pre-positioned volunteer. After each experiment the paper was removed and swabbed such that five zones (Fig. 1) were sampled by carefully swabbing the entire zone with a single cotton swab (Medical Wire and Equipment, Corsham, UK) moistened with sterile deionised water (a fresh vial for each experiment). A sixth swab was moistened with the same aliquot of water for use as a negative control. All swabs were immediately frozen without drying at –20°C prior to analysis. It was expected that any body fluids or cells deposited by the subject onto the test area would be retrieved using this protocol. Thus, any DNA shed by the subject could not only be collected but the distance of travel from the body would be known (Fig. 1).

Sample processing

After collection, all sample processing was carried out by a person other than the subject or the assistant, referred to hereafter as the

operator. The operator wore a mobcap, mask, gloves, and laboratory coat as described for the assistant. DNA was extracted from swabs using the QIAamp DNA mini kit (Qiagen, Crawley, UK) in accordance with the manufacturer's recommendations but no quantification of the DNA extracts was attempted. Each DNA extract was amplified in duplicate using AmpFlSTRSGM Plus (Applied Biosystems, Warrington, UK) at maximum volume following the procedure described by Gill et al. [1]. Amplification was carried out on a Perkin Elmer 9600 thermal cycler (Applied Biosystems, Warrington, UK) using the amplification parameters specified in Cotton et al. [9] except that 34 cycles of amplification were performed. Following electrophoresis and analysis as described previously [10], interpretation of STR results was performed blind with reference to Gill et al. [1].

Results and discussion

DNA profiling results are summarised as the number of donor and non-donor alleles in each test zone and the number of allelic peaks observed in each negative control (Table 1). In the case of the donor, 21 alleles constituted a full profile (Table 2). Alleles were called only if present in both duplicate amplifications [1]. As previously documented by Whitaker et al. [11], where partial profiles were observed, there was no bias towards drop-out of high molecular weight loci. In experiments where DNA was evident, those carried out in the kneeling position generally appeared to produce more alleles than those in the standing position. This is likely to be due to the closer proximity of the subject to the test zone possibly resulting in less chance of dispersal of shed material. All negative controls were negative, unless stated otherwise.

A total of 413 alleles were identified in this series of experiments, 34 of which were not attributable to the subject and are assumed to be a result of contamination (this figure includes any alleles seen in the negative controls). Of these non-subject alleles, 25 were found in a single experiment (experiment 18) and of these, 8 were in the negative control. Of the 25 alleles 13 were concordant with alleles of the operator and it is possible that these alleles were a result of contamination during the processing of the samples. The source of the remaining 12 alleles in experiment 18 was unknown and must be considered to be a result of random contamination. It is possible that all the non-subject alleles may have arisen from an individual or individuals having contact with the paper during manufacture, packing or storage at the laboratory, from a contaminated vial of water, or as a result of other contamination within the laboratory environment.

The remaining 9 non-subject alleles were seen in experiments 8, 14 and 15; 4 alleles were seen in experiment 8, including 1 in the negative control, the negative control in experiment 14 exhibited 2 alleles, 2 alleles were seen in experiment 12 and a single allele was in experiment 15.

Experiments carried out with no movement

No alleles were observed in samples taken from experiments 1 and 2 suggesting that an individual who is neither moving or talking is unlikely to shed detectable levels of contaminating DNA. Wearing protective clothing with no movement (experiments 5 and 6) provided similar negative results.

Experiments carried out with movement

If normal movement is introduced, such as might be carried out at a crime scene, a high rate of contamination is evident. Experiment 3 showed that a standing subject wearing no protective clothing and moving, shed sufficient contaminating DNA to generate a full SGM Plus profile from zone 1. When the subject was kneeling (experiment 4), gross contamination was detected in zones 1–4 with at least 19 alleles of the donors profile present. In zone 5, 16 alleles were recovered using the LCN technique suggesting that DNA contamination originating from a crime scene attendant may be expected at distances of 1 m or more. No non-subject alleles were evident in these experiments.

Clothing can contain detectable quantities of DNA in the form of shed epithelial cells [3] and it is possible that the high levels of contamination observed in the experiments without protective body wear could be due to the shedding of cells from the normal clothing of the subject due to movement as well as from the skin, head hair or respiratory system.

Vigorous activity, even when wearing protective garments can still cause contamination of a crime scene (experiments 7 and 8). It is possible that the friction created between the subject and the protective clothing, particularly at the cuffs and around the face, may cause sloughing of cells from the skin. These are likely to be deposited on the floor or other surfaces around the donor, some potentially being dispersed over a wide area owing to the air currents generated by the physical movement. This level of activity, which included scratching, waving of arms and contact of gloved hands with the face should not be considered normal activity at a scene of crime by an investigator. However, if an offender had been moving around the scene, particularly if there was evidence of vigorous activity, it should be considered possible that the offenders DNA may be present at the scene.

It is noteworthy that there was less contamination when wearing body protection whilst performing vigorous activity (experiment 8), compared to wearing no protective clothing and simulating normal activity (experiment 4). This finding suggests that the use of protective

Table 2 AmpF/STR SGM Plus DNA profile of the donor

Locus	D16	D2	D3	VWA	Amel	D18	D21	D8	D19	FGA	TH01
Designation	11, 12	19, 20	14, 15	14, 18	X, Y	14, 15	29, 30	12, 13	13, 13	21, 25	9, 9.3

clothing reduces the risk of contamination of the crime scene by the investigator. A single allele matching both the assistant and the operator was exhibited in the negative control of experiment 8.

Talking

No DNA was detected in any test zone whilst the subject was standing and talking, whether a mask was worn or not (experiments 9, 13 and 17). When the experiments were repeated whilst kneeling, gross contamination of zones 1–4, was observed if no facemask was worn (experiment 10). This contamination was reduced to a large extent when a mask was worn (experiment 14). It is interesting to note that a full profile of the subject was seen in zone 1 when talking whilst wearing a mask. The action of talking causes the mask to rub against the face, which is likely to cause sloughing of cells which may fall directly to the floor. This is also likely to occur if a person agitates their mask, so called "mask wiggling" [12] and this could be further exacerbated if the choice and use of the mask was inappropriate or sub-optimal. Although this area was not specifically investigated it has been reported previously that the type of mask worn, its duration of use and the degree of fit, especially if the person using it has a beard or moustache, will influence its ability to inhibit respiratory particle fallout [13, 14, 15]. Furthermore, within the DNA laboratory environment it has been observed that wearing masks (in addition to laboratory coats and mobcaps) is not 100% effective in preventing contamination. However, wearing masks and enforcing a no-talk rule within the lab substantially reduces the contamination rate (unpublished observation).

The dispersal of contaminating material was found to be greater whilst wearing a visor (experiment 18), compared with a mask (experiment 14). However, only a partial profile resulted from experiments with the visor, which may be explained by the reduced contact of the visor with the face, resulting in a decreased likelihood of cell sloughing from the face as hypothesised for the mask. Despite this result, the experiments demonstrate that the visor does not function as a suitable barrier between the subject and the test area.

Coughing

Experiments 11 and 12 show that coughing caused contamination across the test area in both standing and kneeling postures but the pattern of contamination varied according to subject stance. Whilst standing, the greatest proportion of subject alleles were recovered from zone 5, whereas the kneeling experiment (12) demonstrated the greatest contamination in zone 2. This observation may be explained in part by the distance of the source of contamination, i.e. the mouth and respiratory tract, from the ground whilst coughing (an object of similar size, trajectory and momentum will travel further if launched from a greater height). Consider also the following: the greater the distance from the ground, the more likely the smaller saliva droplets will be affected by environmental air currents as they fall to the ground. A combination of these two effects might explain the results: larger droplets falling quickly to the floor are likely to follow the expected trajectory (contaminating zone 5 when standing, and zone 2 when kneeling), whilst finer droplets may be affected by air currents within the laboratory and, depending on the direction of the draught, land closer or further afield from the subject. Alternatively, the observed effects could be due to random spatter.

Repeating these experiments with the use of a mask reduced the contamination in the entire test area noticeably in each case. This observation supports the previous observation that wearing a mask reduces the risk of contamination from the mouth and respiratory system.

The experiments in which the subject was kneeling more closely simulate the actions of an investigating team at a SOC. This study demonstrates that under these circumstances the risk of contamination appears to be highest, and as such, the above recommendations should be observed more strictly.

Some individuals can naturally and consistently deposit more DNA than others on contact with an inert object. Those leaving sufficient DNA to produce a full DNA profile are termed "good shedders"; those leaving insufficient DNA to gain a full profile are "poor shedders" [8]. The subject in this series of experiments was a good shedder and the results of these experiments might therefore represent a worst case scenario, i.e. a good shedder may be more likely to contaminate a SOC than a poor shedder without contact. In our experiments, normal activities of a good shedder left enough evidence on the test area to gain a full DNA profile. An estimated 45% of the population can be categorised as good shedders and 70% of the population are likely to deposit sufficient DNA to produce 70% or more of their DNA profile (A.L. Lowe, personal communication). It might be expected that similar proportions of SOC attendees are likely to deposit DNA at the SOC.

It should be noted that the test area sampled in this series of experiments was essentially clean (with the exception of experiment 18) and therefore, we consider it more likely to be able to detect relatively small numbers of DNA molecules. It is likely that small amounts of DNA deposited by an individual onto a surface where DNA from another individual is present in a ratio of 1:10 would not yield an amplification products since this is the limit of detection of SGM Plus (E.A. Cotton, personal communication). It is likely that DNA from persons with legitimate reasons for being present at a scene will be present at a scene, along with any DNA from the perpetrator of a crime. However, if samples are collected in a targeted manner, e.g. from items known to have been recently cleaned, items that have been touched, or spoken over by the perpetrator of a crime, and if contamination of the scene by the investigator is minimised, it may be possible to profile the offender's DNA from trace evidence at the

SOC. Comparison of DNA evidence recovered from a SOC to DNA profiles from suspects, or searching a database of known offender profiles, can provide the investigation team with vital intelligence as to the possible identification of the suspect.

Recommendations

The duration of each test carried out in the course of this work was limited. In a casework scenario, a scientist may spend many hours examining a scene and it is likely that with an increased duration at a particular scene or exhibit there is an increased risk of contamination of the scene. The limited number of experiments performed in this study show that an individual visiting a crime scene can potentially deposit enough DNA evidence to allow their full STR profile to be determined. Wearing appropriate protective clothing (including body suits, head protection, and facemasks) can reduce, but is unlikely to eliminate this accidental DNA contamination of a SOC. Additional precautions could further reduce the contamination risk as follows:

- With regard to facemasks, it is recommended that the wearer refrains from talking or otherwise manipulating the facemask
- Similarly, wearers of scene suits should not interfere with their protective clothing in such a way as to risk compromise of the scene
- Movement within the SOC should be kept to the minimum possible for the task in hand
- Regular changing of gloves and barrier masks outside the SOC will not only add to the comfort of the attendant but may also help to reduce contamination and cross-contamination of the crime scene with DNA
- Strictly limiting access to the scene to those persons that need it
- Minimising verbal communication whilst within the SOC will help to reduce DNA contamination by the investigating team.

Authorities with the responsibility of overseeing work undertaken at a SOC should also consider taking DNA samples from each member of the investigating team (and indeed anyone else who has had access to the scene) as a routine precaution. This allows for cross-reference against any evidence recovered and may prevent misleading information (i.e. any contaminating DNA originating from the investigators) being introduced into the investigation. Such samples are currently taken from the police and scenes of crime officers within the police forces of England and Wales, and from staff at the Forensic Science Service. This allows the identification of DNA profiles in criminal casework that might be a result of DNA contamination originating from the investigating team and therefore, along with other relevant control samples, help to establish the relevance of the DNA profiles in a case.

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