USE OF LOW COPY NUMBER (LCN) DNA IN FORENSIC INFERENCE

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ABSTRACT

Development of a low copy number DNA profiling method has allowed the detection of very low levels of DNA. However, issues concerning the transfer and persistence of LCN DNA have required further research to be carried out; this has included studies of individuals' tendency to deposit and transfer DNA onto surfaces they have touched. Additionally, the effect on DNA of some of the enhancement treatments used on latent finger marks has been tested.
Key words: low copy number; shedder; secondary transfer; enhanced fingerprints

INTRODUCTION

Low copy number (LCN) DNA profiling using the SGM Plus™ multiplex (1) has been used in casework at the Forensic Science Service since January 1999. The sensitivity of the profiling process has been increased by raising the number of PCR cycles from 28 to 34. This allows DNA to be detected at much lower quantities, even down to the level of a single cell (2). Hence, strict guidelines are in place regarding the collection and processing of samples, and also the interpretation of results (3,4).

The LCN method can be used to analyse samples which have simply been touched; this follows findings reported by van Oorschot and Jones in 1997 (5), who used 28 PCR cycles to obtain genetic profiles from objects such as a telephone handset, pens and briefcase handles. The authors also reported that the amount of DNA recovered varied depending on the individual.

However, profiles obtained using LCN DNA analysis often originate from samples that show no visible source of biological material, consequently no presumptive test is available for contact traces. The question arises whether the profile is actually relevant to a case. Specific caveats are written into the court statements, pointing out that it is not possible to make conclusions about where the DNA originated, when it was deposited, or about the transfer and persistence of DNA. Further research into LCN DNA is driven by the need to answer these issues.

Questions regarding transfer have been previously investigated (again using 28 PCR cycles) by Ladd et al. (6) who tested whether secondary transfer of DNA could occur from individual A to individual B and then onto an object, or from individual A to an object and then onto individual B. They found some minor peaks attributable to secondary transfer but concluded that this was not likely to be an issue when presenting analysis results to a court. Van Oorschot and Jones reported that they had observed secondary transfer, as well as the persistence of DNA on experimental items for up to 84 days and on a casework item (a glove) for two years (5,7).

Finger marks found at a crime scene potentially offer two highly discriminating forms of evidence. Where marks are not smeared there will be the fingerprint ridge detail and the DNA of the blood or skin cells that have been transferred to a surface on deposition of the mark. Marks are usually enhanced using light sources and chemicals to allow greater visualisation, but a dilemma may be faced by investigators who also wish to obtain a DNA profile. It becomes necessary to be aware of any detrimental effects that enhancement methods may have on DNA profiling.

Previous research has been carried out in this field, predominantly into the effect of chemical treatments on marks made in blood. Several authors have reported that cyanoacrylate (CNA), when used to enhance bloody finger marks, has no detrimental effect on subsequent DNA analysis (8, 9, 10, 11). Zamir *et al*,

2000 have reported that CNA does not effect STR profiling of latent marks. The effect of dactyloscopic powders on DNA analysis has also been investigated (10, 11, 13). While powders such as White and Black powder were not found to inhibit DNA processing, metallic powders were found to limit the amount of DNA that could be recovered and profiled from latent marks (14). Two chemical treatments often applied to marks on paper are ninhydrin and 1, 8 diazafluoren-9-one (DFO). These enhancers have been observed to have little adverse effect on DNA profiling from marks in blood (11, 15), for ninhydrin this applies even when the mark has been left in its enhanced state for up to 56 days (16). Physical developer and iodine are also often applied to enhance marks on paper, both have been reported to degrade DNA in latent and bloody marks respectively (17, 8).

This paper examines further studies on shedder type (the tendency of an individual to deposit his/her DNA profile on a touched surface), transfer and persistence of LCN DNA, and discusses research that has been carried out to determine the effects of various enhancement methods on the DNA integrity of latent finger marks.

SHEDDER INDEX

A group of 29 people were tested for their ability to deposit their DNA profile onto touched objects. It was found that a typical good shedder leaves a complete profile on the surface of a plastic tube after contact of only 10 seconds, whereas at the other end of the scale a poor shedder will leave only a few alleles, possibly with several loci dropping out completely. Figure 1 shows the results from this group, which suggested that with the collection of data from more individuals a continuous distribution would result.

PRIMARY TRANSFER

Work was carried out to determine whether DNA profiles could be obtained from clothing; specifically, plain white T-shirts. After 8 hours wear, more of the wearer's DNA was recovered from the front of the T-shirt than the back. Targeting the neck area maximised the chance of obtaining a useful result. In a series of simulated assaults, where one person grabbed the shoulder of another for a period of 30 seconds, mixed profiles were obtained from the grabbed area of the T-shirts. The "assailant" always contributed the major component to this mixture, regardless of his/her shedder type.

SECONDARY TRANSFER

Experiments were carried out to determine whether it was possible for individual A to transfer his DNA to individual B through contact, who could in turn transfer A's DNA onto an object. We began with a scenario which was most likely to yield a result: a good DNA shedder (A) shook hands with a poor shedder (B), who then gripped a plastic tube for 10 seconds. The results from swabs of the tubes showed that on five separate occasions all of the good shedder's profile was recovered, with none of the poor shedder's alleles appearing.

The experiment was then repeated, but with the introduction of a delay of 30 minutes between the time of the handshake and the tube-holding. The results (Figure 2) indicated that although the poor shedder deposited some alleles, secondary transfer of the good shedder's DNA still occurred.

Further experiments are underway to determine the length of time between contact and tube-holding where no secondary transfer occurs, and to examine other types of transfer situations.

PERSISTENCE

Many factors may affect the persistence of low level DNA; time, temperature, humidity, etc. While it is unreasonable to test every combination of variables, some generic experiments have been undertaken and certain scenarios addressed.

A time-study of the persistence of DNA is currently underway, where touched items have been stored at room temperature and tested to find out how much DNA can be recovered after certain periods of time.

Figure 3 shows the results; full profiles were still recovered from surfaces touched by a good shedder even after 4 months, whereas a marked decrease in the recovery of the poor shedder's DNA was observed.

An exchange of identical wrist-watches between certain shedder types was carried out to ascertain the period of time needed for the original wearer's DNA profile to be replaced by that of the new wearer. Generally we found that a good shedder completely replaced the original wearer's profile in 2-3 weeks, and after only a few days had become the major component of a mixture. An example of this is shown in Figure 4. In contrast, a poor shedder typically took around 2 weeks just to comprise the major component.

ENHANCED LATENT FINGER MARKS

The effect of various treatments used to enhance latent marks on either porous or non-porous surfaces was investigated. The finger marks used for these analyses were all deposited by the same individual on either acetate or paper for non-porous and porous surfaces respectively. The chemical treatments tested were CNA (in and out of a vacuum), aluminium powder, metal deposition, DFO, ninhydrin and physical developer. The effect of these chemicals on STR profiling was observed on freshly enhanced marks and marks that had been left for 100 days before DNA analysis was carried out. The mean results of replicate analyses carried out on single finger marks can be seen in Table 1.

It was observed that overall, better recovery of DNA was possible from marks deposited on the non-porous surface. Marks enhanced with CNA, aluminium powder and metal deposition yielded full DNA profiles when DNA processing was carried within a week of treatment. However, recovery of DNA decreased when marks had been left in the enhanced state for 100 days. While some of the drop in DNA recovery can perhaps be accounted for by general degradation, the inability to recover any alleles from the marks treated with metal deposition suggests that some chemicals do radically effect DNA over time. A similar result was obtained from ninhydrin treated marks. Further observation of the results appears to indicate that the recovery of DNA from vacuum CNA treated marks increases over time post enhancement. However, this is unlikely to be the case. The reason for improved recovery may be due to two different pieces of vacuum CNA equipment being used for each sample set. Slight modifications to enhancement methods have been shown previously to improve recovery of DNA from marks in blood.

An additional experiment using aluminium powder was also carried out. The relative levels of profile recovery from powdered marks in situ and from tape lifts of the marks were investigated. It was found that equal amounts of DNA could be recovered from the mark in situ and the lifted mark, both yielding approximately 70% of the fingerprint donors profile. This finding was important as it suggested that a mark could be lifted and preserved on tape for ridge detail analysis while the original deposit could be swabbed for DNA.

The views expressed in this paper are not necessarily the policy of the FSS.

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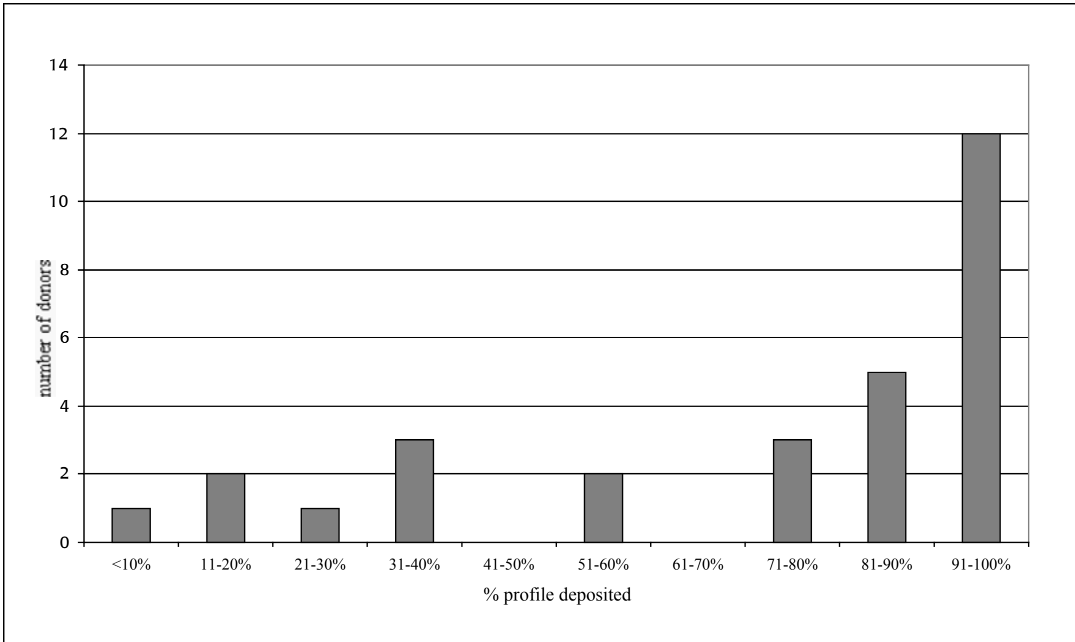

Figure 1: Distribution of shedder type of 29 individuals – percentage of DNA profile deposited on a touched surface.

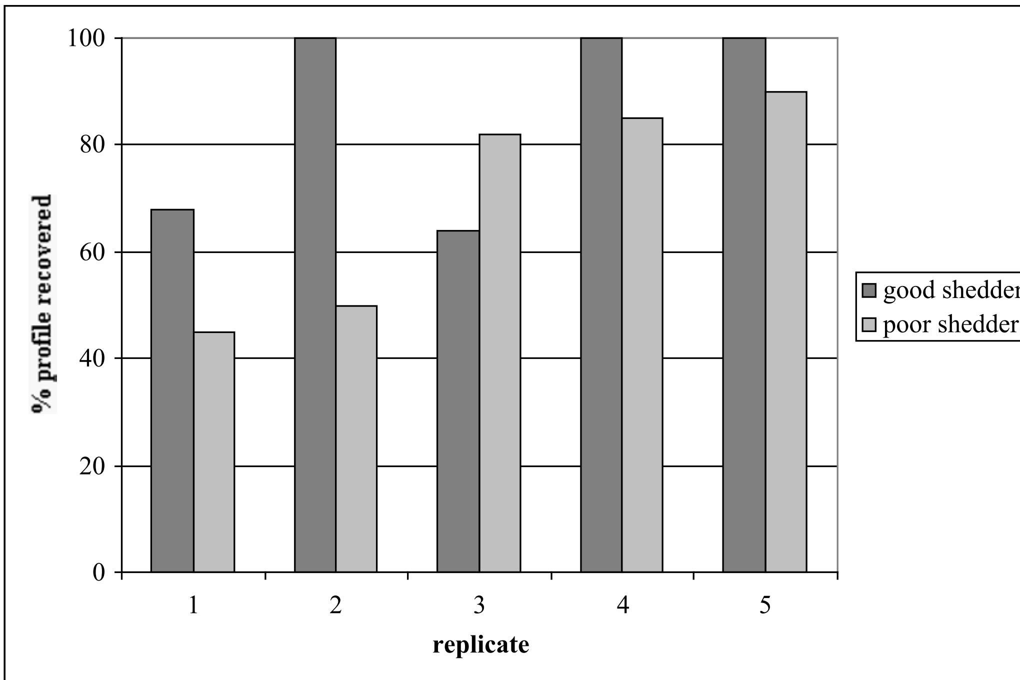

Figure 2: Percentages of good shedder's and poor shedder's DNA profiles deposited on touched surface 30 minutes after handshake, in 5 replicate experiments.

Figure 3: Amount of DNA profile recovered from touched surfaces at various time intervals post-contact, for two shedder types.

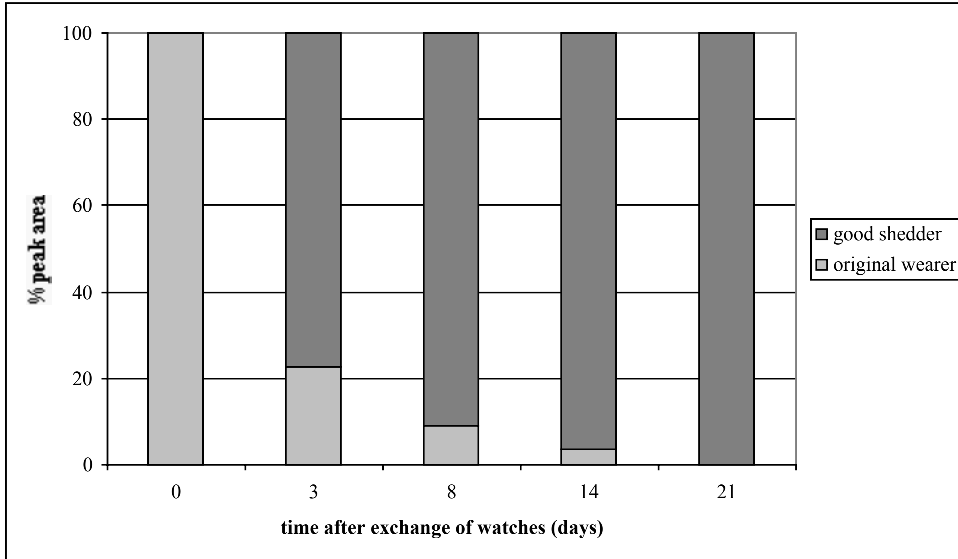

Figure 4: Proportion of recovered DNA from wristwatch attributable to original wearer and new wearer (good shedder) at various time intervals post-exchange.

	% Donor Profile Recovered	
Treatment	**Freshly enhanced**	**100d post enhancement**
Untreated NP	100	77
CNA NP	100	68
CNA (vacuum) NP	49	86
Aluminium powder NP	96	77
Metal deposition NP	100	0
Untreated P	74	76
Ninhydrin P	44	35
DFO P	79	29
Physical developer P	33	2

Table 1: Recovery of DNA profile from single latent marks after chemical enhancement. (P = porous surface; NP = non-porous surface)