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Investigating Touch DNA on Porous and Non-Porous Surfaces

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Abstract

The aim of the experiment was to study the transfer of DNA in porous and non-porous surfaces. Using simple methods, obtain DNA profiles from a surface using time and the type of surface as variables.

Introduction

DNA fingerprinting is one of the widely used analytical techniques in association with other techniques for investigational purposes. It is very rarely used as a sole piece of evidence and thus has not displaced the importance of other techniques. It is quite robust material that survives under a variety of conditions. It is readily available from a variety of sources and with developing techniques relatively easy to extract and analyse. The research was based on Locard's Principle of Exchange, which states that "Every contact leaves a trace" (Hansson, 2009) as seen in fig 1.

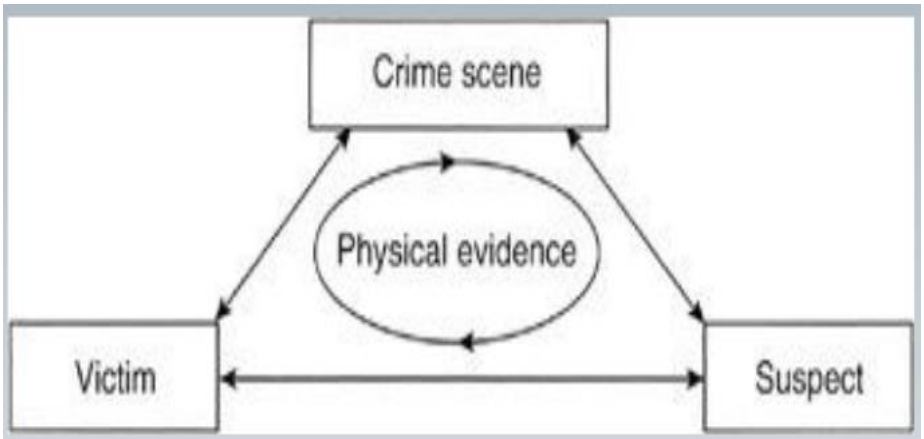

Figure 1: Locard's principle of exchange

It can be interpreted as, one person (A) held a pen and left his DNA on-to it. Another person (B) took the pen, now as the pen contains DNA of first person it can be used to link two people, this is also referred to as primary transfer. Now another person (C) borrows the same pen from person (B) and again exchange of DNA takes place and this is referred to as secondary transfer, as show in in fig 2.

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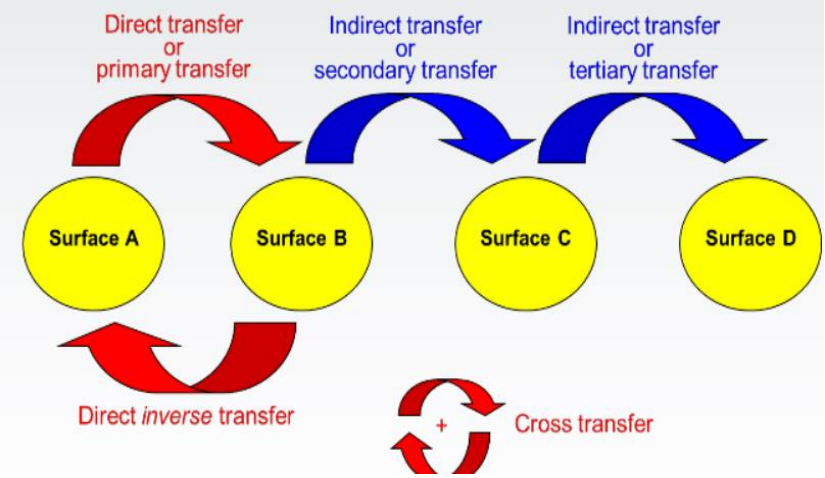

Figure 2: Principle of exchange between primary, secondary, and tertiary object

This study was focused on touch DNA. Tens and thousands of skin cells are shed every day and are transferred to every surface that we touch. When a crime is committed, if enough number of skin cells are left behind by the perpetrator on an item, and if that item is collected as a possible evidence, touch DNA analysis may be used to link the perpetrator to the crime scene (Milne, 2016).

It doesn't require us to see anything but around seven to eight cells from outermost layer of our skin. It has been sampled successfully from numerous items such as mobile phones, gun grips, eating utensils, luggage handles etc. The key to getting effective Touch DNA results relies on perceiving things which may be suitable for Touch DNA analysis and utilizing the sampling technique that will recover the greatest number of skin cells. Despite the potential exchange of DNA, one may encounter difficulties trying to recover DNA, particularly if there is extremely low level of DNA or damage to the DNA due to degradation or contamination due to external factors (Van Oorschot, 2003).

Porous and Non-porous surface play different roles. DNA may get such between the lose spaces between porous surfaces, while non-porous surfaces keep them on the top and the DNA might get shed off or be cleared because of some other object or surface coming in contact with the porous/non-porous surfaces. It's an assumption that porous surfaces should give better results compared to the non-porous surfaces (Wickenheiser, 2010).

Methodology

Prior to conducting or starting with any work, all the equipment were sterilized. Beakers, Eppendorf tubes, and pipette tips were autoclaved. All the workstations were sanitized prior to when the experiment were started. The region to be used was marked on the surfaces before starting with sample collection. Different surfaces used were a pen (plastic), hammer (steel), and a pencil (wooden glazed) as non-porous and for the porous surfaces, objects used were a tissue, a woollen cloth and hammer (wood).

The objects were held for an interval of 1 minute followed by the immediate swabbing using a sterile Eppendorf tube. This was repeated for 5 times per sample and the tubes were labelled.as shown in table 1. After this, the same procedure was repeated but this time the objects were held for 5 minutes instead as shown in table 2.

Table 1: Different objects used for sampling and their labelling

Object	Type	Time held for	Sample Number
Pen	Non-porous	1 minute	1-5
Hammer(Wood)	Porous	1 minute	6-10
Hammer(steel)	Non-porous	1 minute	11-15
Pencil	Non-porous	1 minute	16-20
Paper Tissue	Porous	1 minute	41-45
Woollen cloth	Porous	1 minute	46-50

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Table 2: Various objects used for sampling, their labelling and time held for

Object	Type	Time held for	Sample Number
Pen	Non-porous	5 minutes	21-25
Hammer(Wood)	Porous	5 minutes	26-30
Hammer(steel)	Non-porous	5 minutes	31-35
Pencil	Non-porous	5 minutes	36-40
Paper Tissue	Porous	5 minutes	51-55
Woollen cloth	Porous	5 minutes	56-60

Apart from the samples, some references were taken, these were swabs that were taken from the surrounding area of the pre-marked zone. If any DNA was present, it could be detected and differentiated with the sample later. Negative controls were also taken which were treated the same way just contained no DNA. A positive (+ve) sample was also taken, which consisted of a buccal swab. The prepared samples were stored in a refrigerator until needed.

A Chelex solution was prepared, and the samples were then extracted using Chelex extraction method. The extracted products were then ana-lysed by Nanodrop Spectrophotometer, and the results were recorded, as shown in fig 3. The obtained results were then compared as show in fig 4; see results. After all the samples were analysed, 2 samples from each set of objects were selected with highest amount of DNA. These selected samples were then quantified using PCR technique.

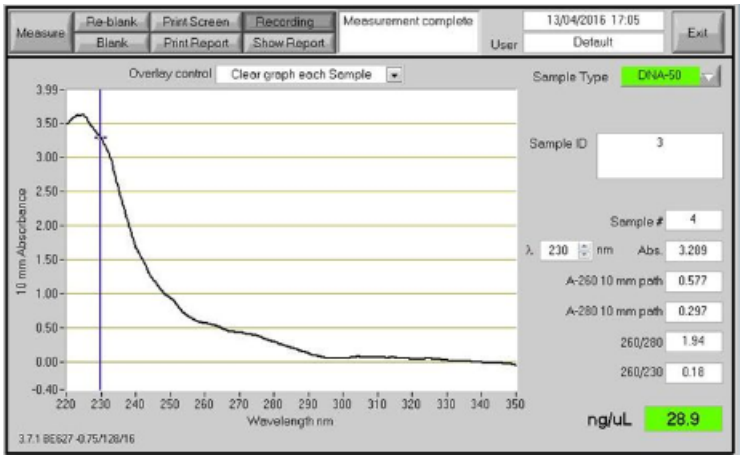

Figure 3: A sample result from Nanodrop photo spectrometer

After all the samples were prepare, TBE buffer was prepared followed by the production of Agar gel. Once the gel was made and all set up, the samples were treated to make them suitable for running on gels. They were then poured into the wells followed by gel electrophoresis. After an hour of running, the gels were then taken out and viewed un-der a UV transilluminator as shown in fig 5.

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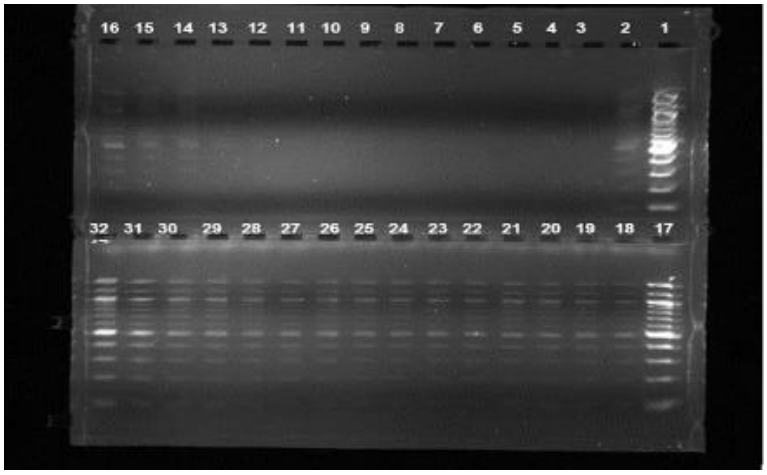

Figure 4: View of gel under UV light under UV transilluminator

Results

Table 3: DNA Purity in samples, Results from Nanodrop and the samples used, shown in highlight

Sample Number	DNA Purity $\frac{OD\ 260}{OD\ 280}$
1	1.67
2	1.47
3	1.94
4	2.11
5	1.74
6	1.36
7	1.54
8	1.42
9	1.46
10	2.34
11	-1.41
12	1.48
13	1.52
14	1.62
15	1.42
16	1.45
17	1.53
18	1.53
19	1.38
20	1.39

Sample Number	DNA Purity $\frac{OD\ 260}{OD\ 280}$
21	1.26
22	0.91
23	0.94
24	0.77
25	0.85
26	0.96
27	1.11
28	1.08
29	1.09
30	0.96
31	1
32	0.98
33	0.97
34	1.84
35	1.09
36	3.2
37	0.51
38	-1.44
39	0.02
40	0.67

Sample Number	DNA Purity $\frac{OD\ 260}{OD\ 280}$
41	1.04
42	1.1
43	2.69
44	1.11
45	0.9
46	1.03
47	0.74
48	0.73
49	1.7
50	0.75
51	0.54
52	0.55
53	0.71
54	0.82
55	0.72
56	1.37
57	1.22
58	1.32
59	1.28
60	1.26

Sample Number	DNA Purity $\frac{OD\ 260}{OD\ 280}$
R_1	1.4
R_2	0.85
R_3	0.93
R_4	0.91
R_5	1.37
R_6	1.37
1^-	1.57
2^-	1.46
3^-	1.59
4^-	1.54
Positive (+ve)	1.11
Chelex negative (C^-)	1.76

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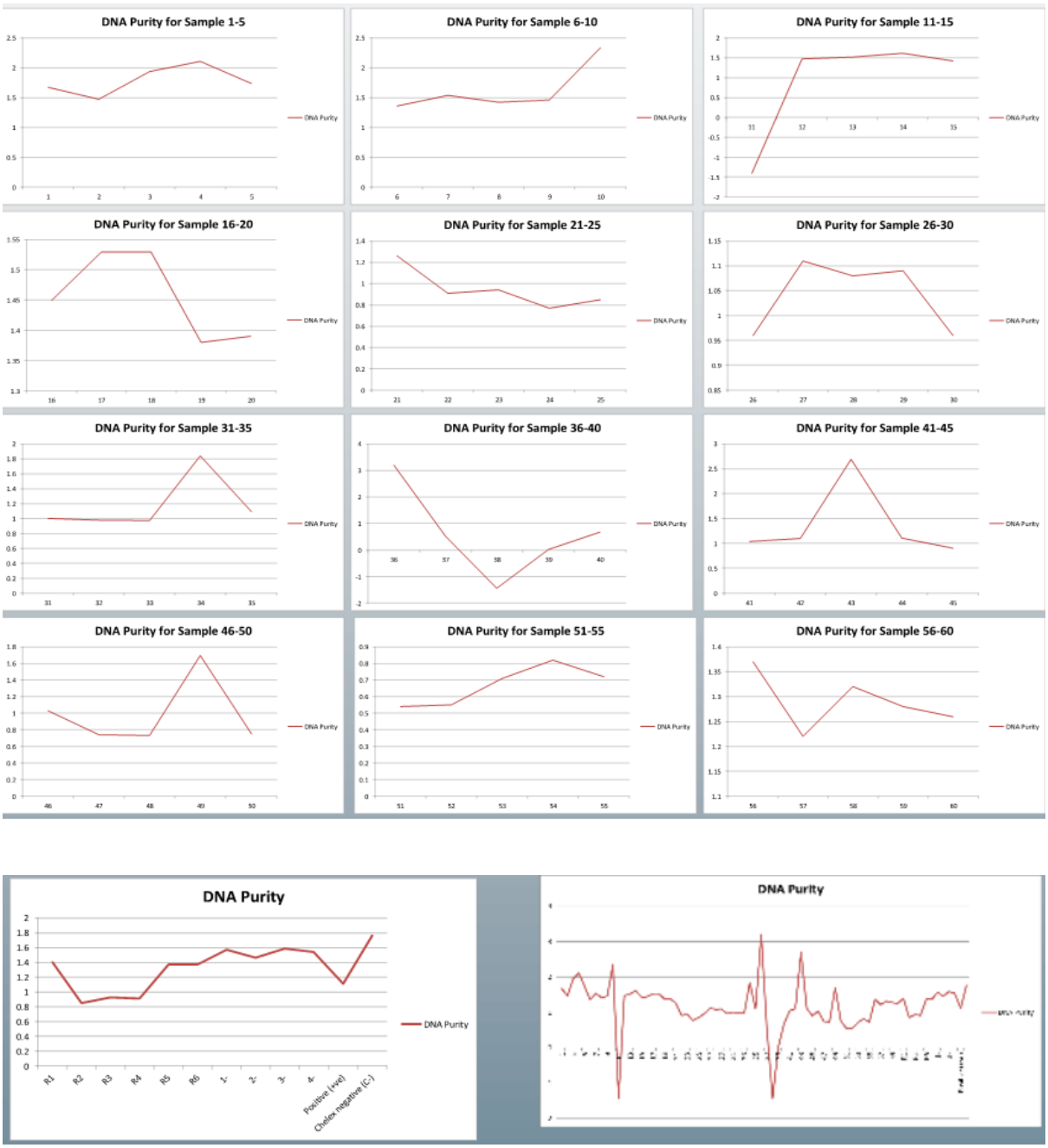

Figure 5: Graphical representation of Nanodrop results

Discussion

Trace DNA analysis has become an important part of forensic laboratory workload and an important tool for investigation. Recently, there has been seemingly high amount of research being conducted to investigate the fundamentals of trace DNA and to review the procedures and skills used to collect and explain even the smallest samples encountered in terms of forensic biology. Before collection, the first stage is to identify the area that needs targeting. As trace samples cannot be identified with a naked eye, sometimes fingerprinting agents are used to identify the touched area. Alternatively, several exhibits are swabbed/taped on the assumption as to where DNA might be pre-sent. Some of these fingerprinting methods affect the quality of DNA. In this research, the touched area was pre-demarcated, so it was easier to locate the area that needed to be swabbed.

Different techniques can be incorporated for the collection of the touch DNA such as taping method, swabbing and new wet vacuum touch DNA recovery system (M-Vac). swabbing was used. Furthermore, there is either wet swabbing or a dry swabbing. To enhance the collection process, swabbing the area multiple times with a dry swab is recommended, as was used in this case. Hence, double swabbing was used. Several inhibitory factors

were found to be present in the swab that inhibited the interpretation of the obtained profiles. Chelex 100 have is commonly chosen by forensic practitioners to be used for extracting DNA for their samples. Nowadays, commercially available kits and methods, customized for specific samples are commonly used. In recent years, there has been quite some developments which us-es magnetic beads with silica-coating to separate DNA from the remaining lysed cell such as Promega's DNA IQ and Invitrogen's Charge switch and optimization of the same to make them compatible with the automated apparatus. Chelex solution was washed 3 times as told in the method, this was done to get rid of any impurities present within the Chelex.

Amplification was carried out using PCR. The temperatures used for PCR are mostly primer dependant. There are different types of primer such as Amelogenin, THO1 and others. Primer used was Amelogenin. To increase the number of DNA, number of PCR cycles can be increased, this pro-cess was developed in the UK by the Forensic Science Services is commonly known as an LCN analysis. Most of the older kits recommend 28 cycles of PCR, contrary to the new one that takes advantage of LCN analysis and yield results depending upon the increased number of cycles such as the Applied Biosystems Minifiler (29 cycles), Yfiler (30), and NGM (29) and Promega's ESX and ESI kits (30 cycles). All this process makes the sample more sensitive which increases the risk of contamination. Taq polymerase was added right in the end so that its reaction with other chemicals can be prevented to form monomers, dimers, and polymers. Alternatively, chemicals like Bouvine serum albumin can be used to prevent inhibitory chemicals, reducing the activity of Taq polymerase by sequestering of phenolic compounds which would have otherwise reacted with the polymerase. For the preparation of Agarose gel, a conical flask must be used specifically rather than a beaker or any other type of container as during heating the solution up in microwave the pointed side of the beaker prevents it from coming out, thus reducing a risk of hazard.

"In 2007, a high-profile case in Northern Ireland raised questions regarding the appropriate interpretation methods of low template DNA and the subsequent UK Forensic Regulator's report recommended the development and validation of methods specific to trace DNA amounts." Reference samples were taken from the surroundings of the surface near to the marked zone, so if any DNA present already can be made aware of. Positives were taken to help us identify if we have any sort of DNA present in the solution or not. Negative samples were taken so that when we run them, they will let us know about any contamination. Contamination is one of the most common issues in the analysis and the interpretation of trace DNA analysis.

Looking at the gel, we can clearly see multiple bands for most of the samples. Interestingly, the ladder would have been incorporated into all the other wells by some sort of contamination that needs to be found out. All negatives gave bands which further shows that there was something wrong. Primer Amelogenin was used which is supposed to attach to DNA and form one band at around 106 bp or/ and 112 bp. A ladder of 100 bp was used, which forms bands at 100bp, 200bp, 300bp and so on. On the contrary it showed multiple bands which showed that ladder might have leaked into the wells.

Conclusion

The results obtained were quite inconclusive as there were ladder bands that could be seen clearly in the gel. This could be due to a variety of reasons like addition of ladder into the wells, broken wells, or contamination of samples. Troubleshooting needs to be done. Experiment needs to be repeated to determine the root cause of contamination. Looking at the Nanodrop results, they are quite inaccurate as well. Negative samples are not supposed to have any DNA, but the analysis showed otherwise

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