Investigation into DNA transfer during physical child abuse

Thesis submitted for the degree of Doctor of Philosophy at the University of Leicester

by

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Abstract

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The overall aim of this investigation was to determine whether DNA transfer can be used to identify perpetrators of physical child abuse. To this end two separate investigations were performed:

First, 12 areas of the head/neck of 32 children aged 0-5 years of age were swabbed in order to determine the 'normal' background levels of DNA present. The results indicated that person-to-person variation accounted for the differences in DNA profiles retrieved, while little non-subject DNA was observed.

The second part of the investigation was to determine if DNA is transferred during forceful contact, such as slaps and punches. This half of the study was divided into three phases: firstly 15 volunteers were asked to punch and slap a DNA free acetate sheet attached to a focus pad, 15 minutes after washing their hands. On a separate occasion they were asked to repeat the experiment but with an hour interval between hand washing and contact as well as with three punches/slaps rather than just one. Phase II was a preliminary test of person-to-person forceful contact involving two members of the Forensic Pathology Unit. Finally sixteen volunteers applied single punches/slaps to the upper arm of another volunteer. The results from all three phases indicated that DNA transfer does occur, onto DNA-free surfaces and between individuals, although the profiles retrieved varies between individuals. Slaps resulted in more transfer than punches, while no difference was observed between single and multiple (n = 3) contacts. Many of the volunteers exhibited non-subject alleles of unknown origin on their hands and arms which complicated interpretation.

Overall both studies indicated that perpetrators of physical child abuse may be determined by the DNA they deposit during forceful contact although the person-to-person variation observed means further research is necessary in this field.

Acknowledgements

I would like to say a huge thank you to Professor Guy Rutty for giving me the chance to continue this research project onto PhD level as well as for his support throughout the past five years. In addition thank you for all the additional opportunities, including the conferences and especially the trip to the Leeds Royal Armouries/LGC Firearms – that was awesome!

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Abbreviations

A	Adenine
ABI	Applied Biosystems
ALSPAC	Avon Longitudinal Study of Parents and Children
ANOVA	Analysis of variance
bp	Base pair
BSA	Bovine serum albumin
C	Cytosine
CCD	Charged coupled device
CODIS	Combined DNA index system
CPR	Child protection register
ct	Cycle threshold
df	Degrees of freedom
DNA	Deoxyribonucleic acid
EDTA	Ethylenediamine tetra-acetic acid
ESRC	Economic and Social Research Council
G	Guanine
GS	GeneScan
HVI	Hypervariable region I
HVII	Hypervariable region II
ID	Identification
IPC	Internal PCR control
LCN	Low copy number
MREC	Multi-centre research ethics committee
mtDNA	Mitochondrial DNA
ng	Nanogram
nm	Nanometer
NSPCC	National Society for the Prevention of Cruelty to Children
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction

pg	Picogram
pH	Potential of hydrogen
POP	Performance optimised polymer
qPCR	Quantitative PCR
RFLP	Restriction fragment length polymorphism
RFU	Relative fluorescent unit
Rpm	Revolutions per minute
RT PCR	Real-time PCR
Sig	Significance
SDS	Sodium dodecyl sulphate
SGM	Second generation multiplex
SLP	Single locus probe
SPSS	Statistical Package for the Social Sciences
STR	Short tandem repeat
T	Thymine
Taq	*Thermus aquaticus*
TBE	Tris borate EDTA
TE	Tris EDTA
TEMED	N, N, N', N – tetramethylethylenediamine
Tm	Melting temperature
Tris	2-amino-2 (hydroxymethyl)-1, 3-propandiol
µl	Microlitre
UK	United Kingdom
UP	Ultra pure
USA	United States of America
UV	Ultraviolet
VNTR	Variable number tandem repeat

1. Introduction

1.1 Forensic DNA

1.1.1 History

The road to forensic DNA profiling as it is performed today has been a long and eventful one. The first step on the path was made by Landsteiner in 1900 by identifying the ABO blood groups and how they differed between individuals (Landsteiner 1900). Four blood groups were identified as A, B, AB and O, depending on an individual's combination or lack of A and B antigens on the surface of their red blood cells. Blood groups can be determined quickly by the addition of antibodies to these A and B antigens and determination of the subsequent agglutination. However, in spite of the identification of the structure of DNA by Watson and Crick (1953) it wasn't until 1984 that a major breakthrough in forensic DNA science was made. This breakthrough was the discovery of Variant Number of Tandem Repeats or minisatellites by Professor Alec Jeffreys at the University of Leicester (Jeffreys et al. 1985b). He found that dispersed throughout the genome were numerous regions of tandemly repeated sequences of DNA, a combination of which could be found in no two unrelated individuals and formed a distinct 'DNA fingerprint'. To detect these minisatellites Jeffreys used a procedure involving Southern Blotting that was called Restriction Fragment Length Polymorphism. These techniques involved the use of restriction endonucleases to 'cut up' DNA into smaller fragments that are then separated by size on an agarose gel. The fragments are then transferred or blotted onto a nylon membrane to which probes of the specific sequence being looked at are then added and visualised either by autoradiography or chromogenic detection methods. This became the first protocol for human identity testing (Jeffreys et al. 1985c). The very same year as the technique was published it was employed in an immigration case involving a young boy of Ghanaian origin (Jeffreys et al. 1985a) however it was the following year that the first criminal case involving DNA fingerprinting occurred. In 1986 the technique was used in a mass screen of 4,000 adult men in three villages in Leicestershire to ultimately identify the double rapist and murderer Colin Pitchfork (Wong et al. 1987). Since then DNA fingerprinting has been used for a variety of purposes, from the identification of the remains of Nazi war criminal Josef Mengele (Jeffreys et al. 1992) and of the murdered Karen Price in 1989 (Johnson 1991) to the exoneration of death row prisoner Kirk Bloodsworth in 1993 (Donnelly 2007) and the

confirmation that Dolly the sheep was truly the first mammalian clone (Signer et al. 1998).

One year prior to the discovery of minisatellites by Professor Jeffreys, Kary Mullis and Cetus Corporation identified a method for the 'photocopying' of specific segments of DNA using a technique called the Polymerase Chain Reaction (Mullis et al. 1986). This development was the perfect partner for DNA fingerprinting enabling amplification and identification of individuals using small samples of DNA. Further advancements were made in the late 1980's including the identification of microsatellites, such as Short Tandem Repeats and the development) of the first capillary electrophoretic equipment and STR multiplex (Kimpton et al. 1993), all of which led to the creation of the UK DNA database in 1995 (Werrett 1997) and the American version, CODIS, in 1998 (Hoyle 1998). Both databases developed further with the creation of the more discriminatory STR multiplexes SGM (Sparkes et al. 1996) SGM plus® (Cotton et al. 2000) and the Powerplex 16 (Greenspoon et al. 2004).

Such techniques were soon employed by several researchers aiming to prove Locard's theory of 'every contact leaves a trace' (Locard 1930). In 1997, van Oorschot found that DNA could be retrieved and identified from the palm of the hand and that this could be transferred to other individuals during a brief handshake as well as to inanimate objects (van Oorschot and Jones 1997). Further evidence of this was observed by Lowe et al. (2002) who also found that mixed profiles from multiple individuals were generated from objects touched by a single person, indicating secondary transfer from one person to another who then deposited the DNA on the item. In 1997, Findlay et al. also found that it was possible to generate a DNA profile from a single cell using 34 cycles of PCR, leading to numerous other studies into DNA transfer from individuals to items such as cigarette butts (Hochmeister et al. 1991), drinks containers (Abaz et al. 2002) and insoles of shoes (Bright and Petricevic 2004). The use of 34 cycle protocols meant that DNA profiles could be obtained from minute quantities of DNA left behind by a suspect, although problems in profile interpretation were found when this technique was employed (Gill et al. 2000). Such problems include allele drop-in, allele drop-out and stutter, among others, that can lead to false profile generation. Therefore other markers, such as miniSTRs, Y-STRs

and mitochondrial DNA have been developed over recent years to ensure that forensic scientists can continue to retrieve identifiable DNA from even the smallest or most degraded of samples.

1.1.2 DNA extraction

Over the past few years the nature and number of samples submitted for DNA testing has increased and can now include cigarette butts (Hochmeister et al. 1991), hair (Higuchi et al. 1988), dandruff (Herber and Herold 1998) and even items that have been swabbed for cellular material such as fingerprints (van Oorschot and Jones 1997) in addition to the more usual samples; blood (Budowle et al. 1995), semen (Budowle et al. 1995), bones (Gill et al. 1994) and teeth (Alvarez-Garcia et al. 1996). Swabs are also routinely used to collect buccal (inner cheek) cells to provide reference profiles for criminals and live victims (Richards et al. 1993). As a result multiple techniques for extracting the maximum DNA possible out of each of these types of sample have been developed. Extraction techniques include the use of Chelex® 100 interchelating resin (Walsh et al. 1991), Organic/phenol – chloroform technique (Sambrook et al. 1989) and kits such as the Qiagen QIAamp® DNA mini and micro kits (Qiagen Ltd, Sussex UK).

1.1.2.1 Chelex® 100

The most common method for the use of Chelex® 100 was outlined by Walsh et al. (1991) and employs the affinity of the Chelex® resin for polyvalent ions, particularly magnesium which in turn inactivates the nucleases that destroy DNA. The sample solution is boiled in order to break open the cells, also denaturing the DNA, after addition of a 5% Chelex® solution. Once boiled the solution is centrifuged so the DNA can be removed, in the supernatant, from the Chelex® resin which is bound to the destructive nucleases, and used in subsequent PCR reactions. The authors (Walsh et al. 1991) found that this extraction technique enabled successful downstream processing from semen, bloodstains, semen stains, hair and oral swabs. It has also been determined that this technique is cheap and easy to use, reduces PCR inhibition, especially when extracting from blood and the use of a single tube for the entire protocol reduces the risk of contamination. Sweet et al. (1996) also found that the

recovery of DNA from swabs after Chelex® extraction was statistically more efficient than the traditional method of phenol-chloroform extraction, which was further increased if modifying the Chelex® technique by addition of proteinase K and a micro-concentration step (Sweet et al. 1996).

1.1.2.2 Organic

The Organic or phenol-chloroform technique involves the addition of multiple chemicals to a sample in order to produce pure high molecular weight DNA. SDS and proteinase K start the process by breaking open the cell walls releasing the DNA and proteins which are separated using the phenol-chloroform and a centrifugation step so the proteins can be discarded. Double-stranded DNA remains ready for analysis, but an ethanol precipitation step is often used to remove heme inhibitors which are a particular problem in blood samples or bloodstains.

As this method involves two overnight steps it automatically takes much longer than most other extraction techniques. Additionally the solution is transferred between several tubes which can increase the risk of contamination. While found to extract an abundant quantity of good quality DNA the organic technique was found to be more expensive and most hazardous in terms of chemicals involved, specifically the chloroform-phenol-isoamylalcohol (Hoff-Olsen et al. 1999). These chemicals are used instead of heating to break open cell walls and to destroy proteins in the sample hence the technique is often used for bloodstains or blood samples. The phenol-chloroform itself is used to separate DNA from proteins and other unwanted components ready for quantification and amplification.

1.1.2.3 Qiagen kits

An example of the pre-prepared extraction kits available is the QIAamp® DNA mini kit from Qiagen. It is more expensive than the phenol-chloroform and Chelex® but less hazardous and time consuming than the silica and organic methods. Greenspoon (Greenspoon et al. 1998) also found that the spin columns in the Qiagen QIAamp®

kits are more effective than the Chelex® extraction protocol. An added advantage is that the different kits ensure that all sample types can be extracted efficiently and all buffers and spin columns are included in the appropriate kit. The technique itself involves the addition of a sample to an eppendorf to which proteinase K and ethanol are added and heated in order to break open cell walls and release the DNA. The DNA in solution is then added to spin columns with a silica membrane to which buffers containing guanidine hydrochloride and alcohol are added to ensure the DNA binds to this membrane, while denatured proteins and cell debris are washed away. After these wash steps the DNA is released from the membranes using a buffer containing Tris-Cl and EDTA ready for quantification and PCR (Qiagen DNA Mini Kit Manual). An overview of the procedure is shown in Figure 1.1.

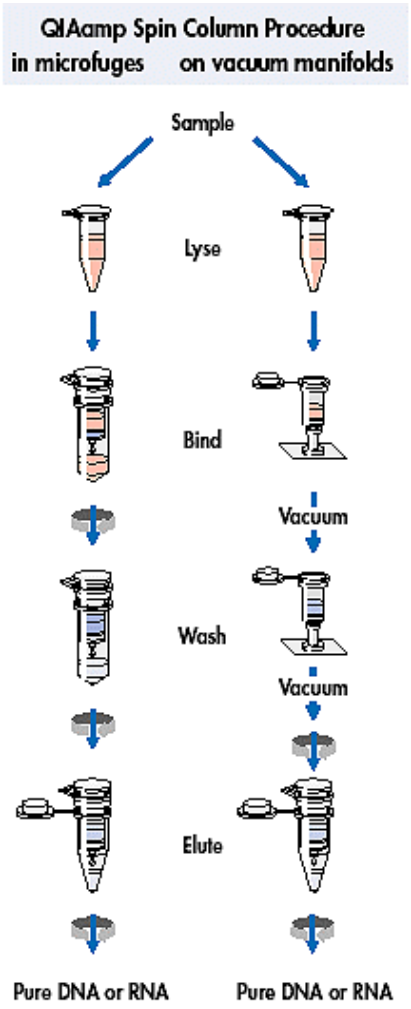


Figure 10-1: Overview of the Qiagen QIAamp® kits spin column protocol, from http://www1.qiagen.com/Products/GenomicDnaStabilisationPurification/QIAampSystem/default.aspx

1.1.3 DNA quantification

Developments in both PCR and DNA profiling over the years have made it possible to obtain full DNA profiles from minute quantities of DNA, even from single cells (Findlay et al. 1997). This sensitivity comes with associated problems as too much or too little template can produce artefacts that complicate analysis, as described later in the Chapter. As a result it is important that the amount of DNA added to the PCR reaction falls within the optimal range recommended by the manufacturer of that particular DNA amplification kit. Quantification is therefore an essential part of any forensic DNA protocol and as such, there are many different methods available. Those most commonly used today include the Picogreen® microtiter assay (Ahn et al. 1996), southern blots, spectrophotometry and real-time PCR methods.

1.1.3.1 Real-time PCR

Real-time PCR, otherwise known as quantitative PCR (qPCR), or kinetic analysis, is so called as the process involves instrumentation that measures DNA concentration during each cycle of PCR, as the template is amplified. The basis of this process is the polymerase chain reaction itself as qPCR incorporates fluorescent dyes into the reaction and measures the fluorescence that is produced as the quantity of DNA product increases cycle by cycle. The fluorescent signal is directly proportional to the product produced.

Real-time PCR can be divided into four separate phases; baseline, exponential, linear and the plateau, as shown in Figure 1-2. The point at which the exponential phase starts, is also known as the cycle threshold (CT); the number of cycles that have been completed at this point is inversely related to the template concentration at the beginning of the qPCR process. During the exponential phase of PCR all reagents are at their optimum levels, the dye, primers, magnesium and polymerase are present in sufficient quantities for amplification to continue efficiently. Hence the cycle threshold is the point at which the fluorescence versus cycle number is measured. A standard curve is generated using the cycle threshold values of a series of standards of known concentration. The CT values of the samples are then compared to this standard curve in order to determine the starting concentration (Kontanis and Reed 2006).

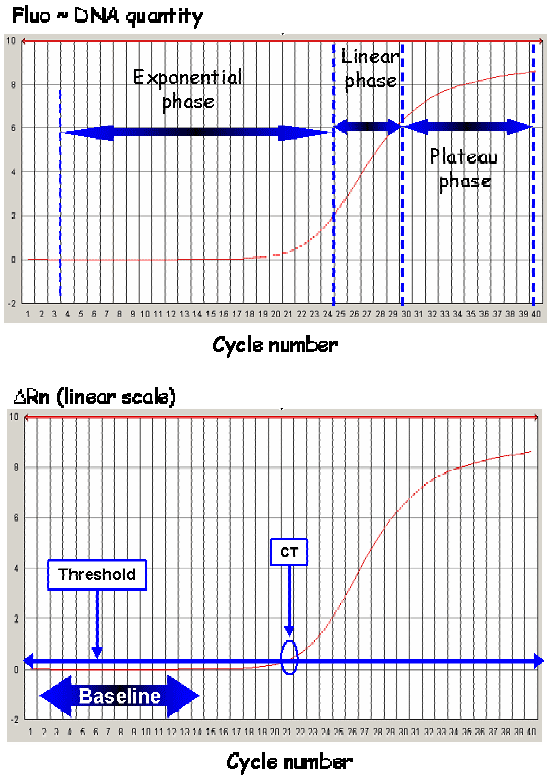


Figure 1-11: Phases of real-time PCR, from http://www.inra.fr/internet/Centres/toulouse/pharmacologie/pharmaco-moleculaire/technologie/PCR.html

At present there are two main approaches to real-time PCR; the use of interchelating dyes such as SYBR Green (Bowyer 2007) and the fluorogenic 5´ nuclease assays with Taqman probes, for example the Quantifiler® kit from Applied Biosystems.

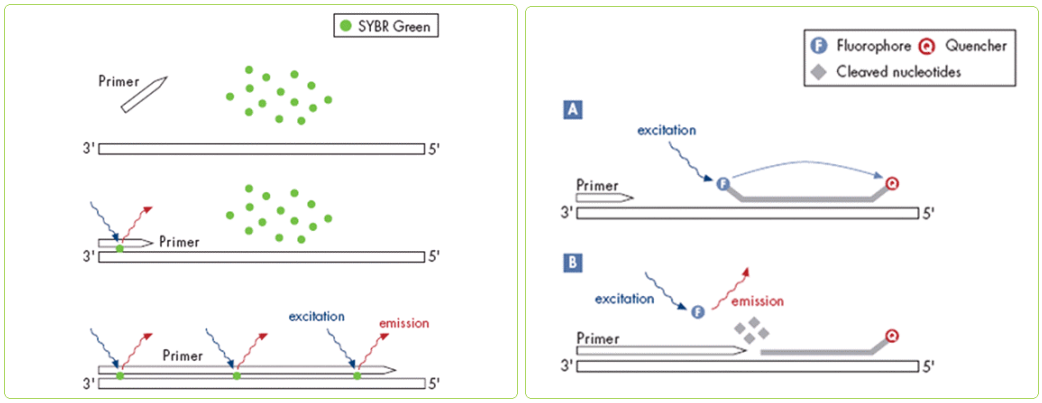


Figure 1-12: Schematics of SYBR green and Taqman approaches to real-time PCR from http://www.meduni-graz.at/zmf/mb/ab7900services.html, courtesy of Qiagen

The Quantifiler® kits involve the use of a fluorescent reporter dye and a quencher dye that are attached to the 5′ (reporter) and the 3′ (quencher) end of a probe (Taqman). The probe anneals to a specific target on the template DNA between the forward and reverse primers so that during PCR it is cleaved by the DNA polymerase, releasing the two dyes so that the reporter dye is able to fluoresce freely, as demonstrated in Figure 1-3. Hence, the fluorescent signal increases as more copies of the template are created and the probes are cleaved releasing more reporter dye (Richard et al. 2003). The main advantage of this system, in addition to the ease of use and fast throughput, is that the process can be species specific – the probe can be designed to target a sequence specific to the species of interest. For example the Alu repeat sequences in humans (Sifis et al. 2002). Quantifiler® human targets the human telomerase reverse transcriptase locus on chromosome 5, while Quantifiler® Y targets the sex reversal locus (SRY) on the Y chromosome. Another advantage of the Quantifiler® kits is the inclusion of an internal PCR control that labelled with a different reporter dye to the probe and hybridises with a synthetic template added to each reaction. This ensures that all aspects of the reaction are working correctly.

1.1.3.2 Picogreen® Microtiter assay

As with real-time PCR, the Picogreen® assay involves a set of standards of known concentration to which the unknown samples are compared. The Picogreen® dye itself is fluorescent and binds to AT-rich regions of the minor groove of double-stranded DNA which causes the dye to fluoresce to a greater extent. Oligreen® works in a similar manner for single-stranded DNA, as results after Chelex® extraction. The assay takes a similar length of time to perform as the RT-PCR but is not human-specific, potentially causing misleading results if bacterial or animal DNA is also present in the sample (Carrondo 1989).

1.1.3.3 Spectrophotometry

While easy to use and relatively cheap, after initial set-up, this method of quantification is unreliable for levels of DNA less than 1 ng/µl, as found by members of the Forensic Pathology Unit. In addition it is not human-specific as it measures total DNA in a sample and can be adversely affected by contaminating proteins, RNA,

or left over chemicals from extraction procedures. An example is the Nanodrop™ ND-1000 spectrophotometer (Nanodrop Technologies, USA) which involves the addition of only 1 µl of sample directly onto a pedestal on the machine. A light is pulsed through the sample and absorbance is measured at 260 nm. On the positive side, it is more accurate over 10 ng/µl and uses up only 1 µl per sample, taking only a couple of minutes per sample to perform.

1.1.3.4 Southern blot

Unlike the Picogreen® assay and spectrophotometry the southern blot method is human-specific and is sensitive, however it can take much longer to perform than either of them. The procedure involves the addition of genomic DNA to a nylon membrane to which a human-specific probe, such as the alpha satellite DNA sequence D17Z1, is then added and the chemiluminescent signal of the probe is compared to a set of standards of known concentration, as with real-time PCR and the Picogreen® microtiter assay. Initially the extracted DNA is digested and then the fragments are separated using agarose gel electrophoresis. The gel bands are blotted onto a special nylon membrane to which a primate specific oligonucleotide probe is added. This probe binds to the target sequence resulting in a radioactive, chemiluminescent or colorimetric signal depending on the probe used.

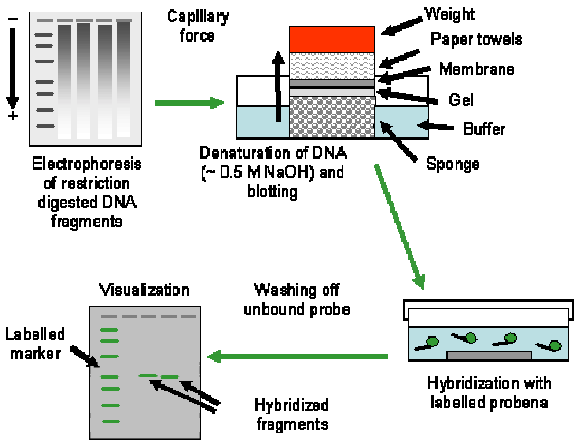


Figure 1-13: Principle of Southern blotting from http://www.campus.skelleftea.se/biomine/molecular/index_20.htm

1.1.4 PCR and STRs

1.1.4.1 PCR

In 1983 Kary Mullis working at Cetus Corporation identified a method for replicating DNA fragments that is now known as the Polymerase Chain Reaction (PCR) (Mullis et al. 1986). As PCR is such an effective tool for increasing the quantity of a specific DNA sequence it is now widely used in diagnostic and research laboratories across the world.

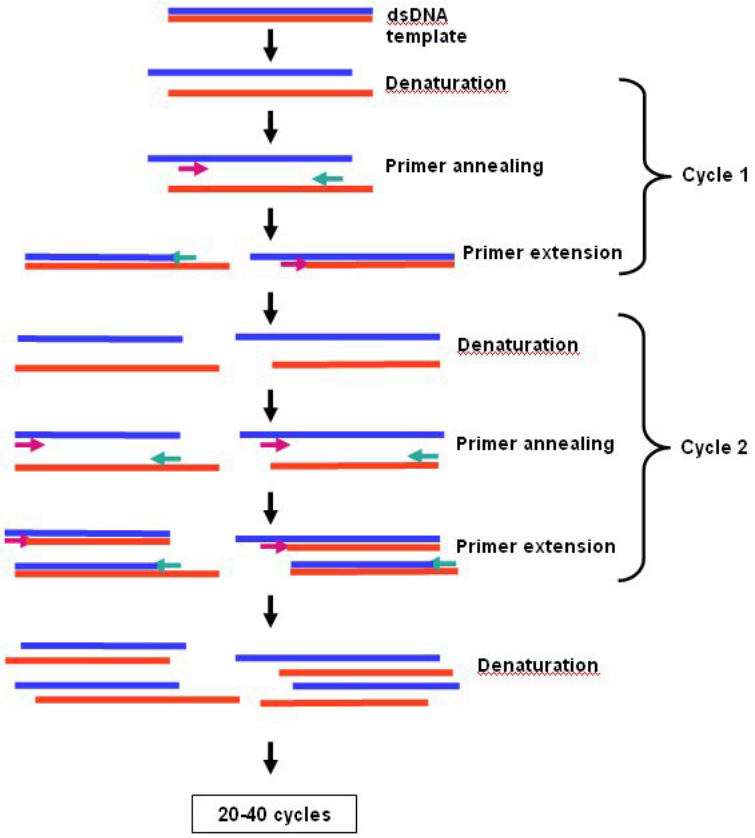


Figure 1-14: Principle of PCR from
http://www.apsnet.org/education/intraplantpath/PathogenGroups/plantViruses/text/fig28.htm

PCR involves multiple cycles of heating and cooling in order to copy a specific fragment of DNA, as demonstrated in Figure 1-5. For every molecule in the sample used, a copy of the target sequence is made every cycle. The target sequence is identified by a set of primers that bind to the sequence immediately before and after the desired region. This binding is promoted by a heating step specific to the primers

used. This annealing temperature is estimated using the following calculation Tm = 4(G + C) + 2(A + T) ºC which uses the combination of nucleotides in the primer sequences to determine the optimal temperature at which they will anneal to the template strand. However, before the primers are encouraged to anneal to the target two heating steps are performed: the first is an initial incubation step at 95ºC to prevent non-specific annealing of primers, in particular primer-to-primer and to activate the DNA polymerase. Secondly the reaction mixture is heated in order to denature the DNA fragment in order to allow the primers to anneal. After the primers have annealed the next stage involves the addition of free deoxynucleoside triphosphates (Adenine, Thymine, Cytosine and Guanine), included in the reaction mixture, to the primer in the complementary sequence to that of the target. This is performed by an enzyme known as DNA polymerase, commonly *Taq*, which is thermo-stable and generated from the bacterium *Thermus aquaticus* and able to withstand the high temperatures involved in PCR. Many DNA polymerases can start working before they have reached their optimal temperature resulting in non-specific annealing between primers, causing primer-dimers, or the template during PCR set-up. Such will then be preferentially amplified resulting reduced amplification efficiency of the template and primer-dimers may mask true alleles during visualisation. As a result 'hot start' PCR was introduced, adding the polymerase once the sample temperature reached optimum for polymerase activation, reducing the possibility of non-specific annealing. However, this procedure involves an additional step at which contamination can occur therefore a modified form of the *Taq* has been developed known as AmpliTaq Gold. This modified polymerase requires 10 minute incubation at 95ºC for activation, improving specificity.

A final step allows time for all products to be fully adenylated, i.e. the addition of an extra A nucleotide to the 3´ end, reducing the probability of split peaks being observed in the electropherogram. Table 1.1 shows the thermal cycling parameters for the AmpF*l*STR® SGM Plus ® PCR Amplification Kit (Applied Biosystems). This kit includes a reaction buffer containing buffer, salt, $MgCl_2$, the deoxynucleoside triphosphates dATP, dCTP, dTTP and dGTP, BSA and 0.05% sodium azide. The kit also includes AmpliTaq Gold® DNA polymerase, a control DNA sample of known DNA profile known a 007 plus an allelic ladder incorporating all common alleles and microvariants for the microsatellites included in the kit's primer set. The

AmpFlSTR® SGM Plus™ primer set includes fluorescently labelled primers, fluorophore incorporated at the 5´ end of the forward primer, for the following STRs plus the Amelogenin locus:

D3S1358, VWA, D16S539, D2S1338, D8S1179, D21S11, D18S51, D19S433, TH01 and FGA.

Step	Temperature	Time (minutes)	No. of cycles
Initial Incubation	95ºC	11	1
Denature	94 ºC	1	
Anneal	59 ºC	1	28
Extend	72 ºC	1	
Final Extension	60 ºC	45	1
Final Soak	25 ºC	Indefinitely	1

Table 1-1: Thermal cycling parameters for the AmpF*l*STR® SGM Plus ® PCR Amplification Kit from Applied Biosystems

1.1.4.2 STRs

In 1984 Professor Jeffreys found that dispersed throughout the human genome were simple tandemly repeated units of 10-100 basepairs (Jeffreys et al. 1985b). It was found that the number of times the core unit was repeated was highly variable, as differing between individuals. Minisatellites have midrange size repeats, often between 9 and 80 bp while microsatellite repeats range 2-6 bp. When multiple minisatellites were investigated at the same time the exact combination of repeat number for all markers was not observed for any two individuals, with the exception of monozygotic twins. Professor Jeffreys employed a method for identifying these repeat sequences, known as Restriction Fragment Length Polymorphism (RFLP) that

involved the hybridisation of probes to multiple minisatellites in restricted DNA and the creation of a DNA 'fingerprint' for the individual the sample was taken from (Jeffreys et al. 1985c). Soon after forensic scientists identified and employed single locus probes (SLP), one highly polymorphic RFLP to act as probe for the Southern blot. Multiple SLP's in conjunction ensured simple interpretation with a higher discriminatory power. From this point, groups such as the Forensic Science Service started the search for further sources of variation within the human genome that could be used for identity testing leading to the discovery of STRs or Short Tandem Repeats. These markers in conjunction with the Polymerase Chain Reaction now form the basis of modern day DNA profiling.

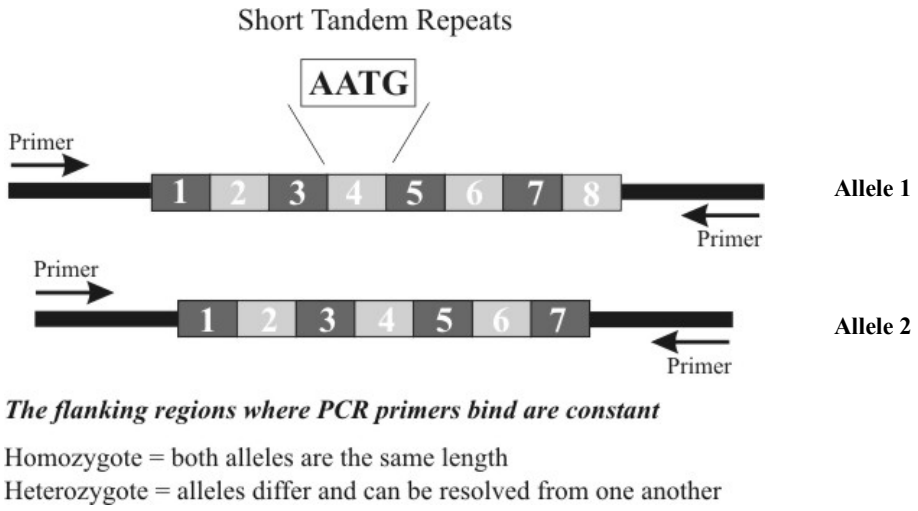


Figure 1-15: Illustration of a simple STR from http://nitro.biosci.arizona.edu/courses/EEB195-2007/Lecture05/Lecture05.html

The human genome comprises approximately three billion basepairs, 98% of which do not code for proteins and are therefore known as 'junk' DNA. It is in these regions that STRs are most commonly found (Schneider 1997). Short tandem repeats or microsatellites consist of a core sequence that is repeated in tandem multiple times, as shown in Figure 1-6, in the same way as minisatellites. However, STR core units are much smaller, being between 2 and 6 basepairs in length (Kimpton et al. 1993). There are several types of STR characterised by the number of basepairs in their core unit.

Dinucleotides have a two base repeat unit; trinucleotides have three and so on up to hexanucleotides with a six basepair core unit. As with minisatellites the polymorphism is due to the number of possible repeats of this core sequence which varies from person-to-person. Each individual has two copies, or alleles, of an STR, one inherited from each parent, consisting of a specific number of repeats of the core unit. Homozygous individuals will have exactly the same number of repeats for both alleles while heterozygosity indicates a different number of repeat units. In some cases individuals have an incomplete repeat unit as part of the sequence, these being known as microvariants. An example is allele 9.3 for the STR TH01, which has a core unit of four bases which is fully repeated nine times with an incomplete unit of three of the core bases. The repeat can also fall into one of three categories, namely simple, compound and complex. Simple indicates the repeat unit is consistent, while a compound can have multiple simple repeats in sequence, such as D3S1358 which has repeat units of AGAT and AGAC. Complex repeats are multiple core units of variable length in sequence (Urquhart et al. 1993; Gill et al. 1994b).

Name	Type	Repeat Unit	Location	Allele Range	Number of Alleles	Mutation Rate
TH01	Simple	TCAT	11p15.5	3-14	21	0.01%
D18S51	Simple	GAAA	18q21.33	7-39.2	55	0.22%
VWA	Compound	[TCTA] [TCTG] [TCCA]	12p13.31	10-25	29	0.17%
D3S1358	Compound	[TCTG] [TCTA]	3p21.31	8-20	27	0.12%
D21S11	Complex	[TCTA] [TCTG]	21q21.1	12-41.2	92	0.19%

Table 1-2: Five STR markers from the AmpF*l*STR® SGM plus™ kit and their characteristics. Data from www.cstl.nist.gov/div831/strbase

STRs are ideal for use in human identity testing due to their small size, usually between 100 and 400bp, as degraded samples are commonly encountered in forensic

samples. This small size range means that primers for several STRs can be combined in a multiplex reaction which in turn reduces the quantity of DNA necessary for analysis (Chamberlain et al. 1988). This combination of markers into one analysis, for both amplification and visualisation, increases the discriminatory power of the system (Kimpton et al. 1993). While several individuals may share the same combination of alleles at one locus the chances of two unrelated individuals having the same combination of alleles at multiple loci decreases as the number of STRs included in the multiplex increases. For each of the commonly used markers population studies have been performed to estimate the frequency of all known alleles within each ethnic group of that population. Once a profile has been generated the frequency of those alleles in that population can be multiplied together in order to determine the probability of two unrelated individuals having the exact same sequence, for example the match probability for the AmpF*l*STR® SGM™ plus kit is approximately 2.99 x 10^{-13} for US Caucasians and 7.91 x 10^{-14} for US African Americans (Applied Biosystems website). However diversity can be reduced within certain populations due to increased frequency of a few specific alleles at each marker. This can be as a result of a specific percentage of a population being killed or prevented from reproducing (population bottleneck); smaller population sizes resulting in inbreeding; or even if a small section of a population is separated from the main population (founder effect). However autosomal forensic profiles are highly variable between individuals due to independent assortment of chromosomes during meiosis, or random mixing of alleles from each gamete resulting in a unique combination reducing inter population variation (Jobling and Gill 2004). In spite of this independent assortment specific alleles for each marker will be found in the profiles of multiple members of the same family, but no two individuals however closely related, with the exception of identical twins, will have the exact same combination of alleles for multiple markers.

STRs with smaller repeat units, such as di and trinucleotides, seem better suited for DNA profiling than the larger hexanucleotides as they are easier to combine into such multiplexes. However, resolution of similarly sized heterozygote alleles when using di or trinuclotides is more difficult than for those with larger core repeats due to the smaller size range. A phenomenon known as stutter is also more commonly seen for di and trinucleotides than for larger microsatellites due to enzyme slippage during amplification. Slippage occurs when the primer and template separate during the

extension step resulting in the formation of a loop one repeat unit in length. This loop results in an amplicon, and therefore electropherogram peak, one repeat unit smaller, or occasionally larger than the true allele, complicating the identification of the true DNA profile of the sample. Tetranucleotides are thought to be less prone to this problem due to the larger size of their core repeat (Kimpton et al. 1993). These factors mean that tetranucleotides, being in the middle of the size range, and less prone to stuttering than di and trinucleotides are more commonly used for identity testing than the others (Primorac and Schanfield 2000).

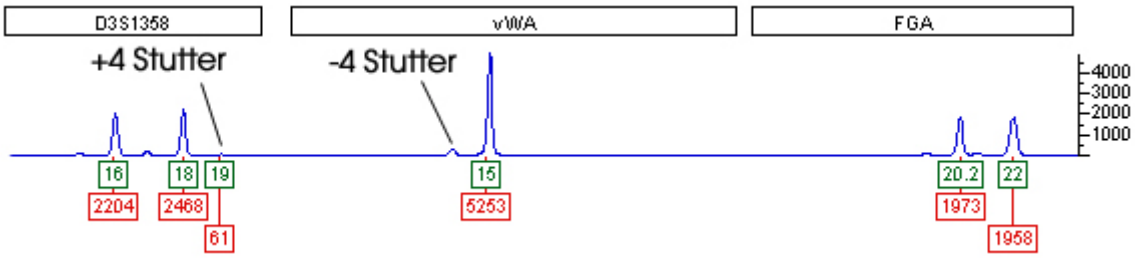


Figure 1-16: Example of an electropherogram exhibiting stutter peaks from http://www.bioforensics.com/genophiler/problems.html

In 1993 the Forensic Science Service produced the first multiplex of tetranucleotide STRs (Kimpton et al. 1993) consisting of TH01, VWA, FES/FPS and F13A1, this was followed by the SGM multiplex in 1996, TH01, VWA, FGA, D8S1179, D18S51 and D21S11 (Sparkes et al. 1996). The multiplex most frequently used in the UK today is the SGM plus® which was developed by Applied Biosystems in 1999 and includes the six markers of the SGM multiplex plus D3S1358, D16S539, D2S1338, D19S433 (Cotton et al. 2000) and the Amelogenin locus for sex typing (Sullivan et al. 1993). This is the multiplex now used for the UK National DNA Database and has a match probability of less than one in a billion (Gill 2002). CODIS, the American DNA database, utilises 13 STRs; CSF1PO, FGA, TH01, TPOX, VWA, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D21S11 and Amelogenin, all of which are included in the Powerplex 16 multiplex from Promega (Greenspoon et al. 2004). The Amelogenin gene codes for a protein in tooth enamel and has a 6bp deletion in intron 1 on the X chromosome. This deletion is not present on the Y resulting in a sequence 6bp longer than the X making this locus invaluable for sex

typing (Sullivan et al. 1993). The 5´ end of the forward primers for the STRs in these multiplex kits are labelled with fluorescent dyes so that the lasers in the different sequencers can detect the amplicons.

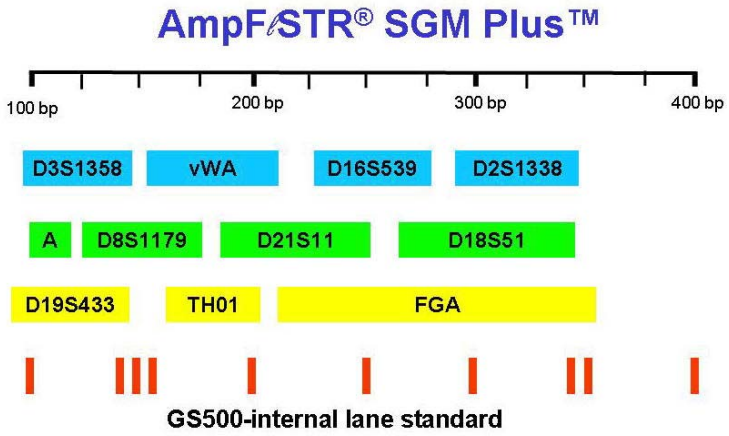


Figure 1-17: Schematic of the size ranges and fluorescent labels of the STR s in the AmpF/STR® SGM plus™ kit, from http://www.cstl.nist.gov/div831/strbase/kits/SGMPlus.htm

In many situations partial STR profiles are generated due to the highly degraded nature of the sample or due to PCR inhibition, in particular when the template has been subjected to fire or environmental contaminants (Coble and Butler 2005). Previous research has led to the resolution of these problems by increasing PCR cycle number. Gill et al. (2000) described this as Low Copy Number DNA profiling and it involves the amplification of less than 100pg of template using 34 rather than the standard 28 cycles of PCR. This technique enables the forensic scientist to generate a profile from much smaller quantities of DNA than was previously possible, widening the range of evidence types that can be submitted for DNA testing, including fingerprints (van Oorschot and Jones 1997), cigarette butts (Watanabe et al. 2003) and bedding (Petricevic et al. 2005). However, this technique has several problems associated with it that complicate profile interpretation, as described in Chapter 1.1.6.2. As a result researchers have aimed to solve this problem by reducing the size of the amplicons by moving the primers closer to the repeat sequence, creating 'miniSTRs'. The resulting smaller PCR products enables amplification from smaller segments of DNA increasing the probability of a DNA profile being generated from a

degraded sample. Another advantage is that existing technology does not need to be adapted as all protocols can be employed for miniSTRs as with the normal length STRs. As the miniSTRs are essentially shortened versions of existing STRs, compatibility with the DNA databases can be maintained and specific miniplexes of the 13 CODIS markers have been developed (Butler et al. 2003). Optimisation and concordance studies have shown that these miniplexes are effective for the amplification of degraded samples and maintaining the necessary compatibility with CODIS and the UK DNA database (Butler et al. 2003; Opel et al. 2006).

Mitochondrial DNA is present outside the cell nucleus in the mitochondria with thousands of copies per cell, compared to the two alleles of nuclear DNA. This makes it a very valuable tool for identification of ancient remains and those that are too degraded for nuclear analyses. A single spermatozoid has only a few hundred mitochondrial DNA molecules which are degraded after fertilisation of an oocyte, so the mitochondrial DNA sequence is passed only from mother to child. As recombination does not occur with the male sequence the same mitochondrial sequence can be passed down the maternal line through many generations (Bender et al. 2000). The mechanisms that ensure diversity within autosomal markers include independent chromosomal reassortment, recombination and mutation. In contrast mitochondrial DNA variability is only introduced through the mechanism of mutation thereby reducing diversity compared to autosomal markers. However, the main advantage of mitochondrial DNA is that hundreds to thousands of copies of the sequence are present per cell resulting in a greater chance of mtDNA profile when nuclear DNA is too degraded for use. In particular mtDNA is highly valuable for mummified/ancient remains and hair shafts, which have very low quantities of nuclear DNA (Higuchi et al. 1988). The reason behind this, in addition to the high copy number, is that mtDNA is circular which protects the DNA from the exonucleases that destroy nuclear DNA.

Similarly to autosomal DNA the regions of mtDNA used for forensic purposes are located within the non-coding or control region of the mtDNA sequence. As this region does not code for any gene products more mutations arise resulting in more polymorphisms than in the coding region (Bender et al. 2000). Two sections within the control region, HV1 and HV2, known as focus points of variation, are amplified,

using PCR and then sequenced before comparison to reference samples. In addition to the lack of diversity and each member of a matrilineage having the same haplotype there are two main problems with mitochondrial DNA. First different haplotypes tend to be associated with particular populations, for example it was found that 10.2% of the American Caucasian database could not be excluded if a sample were retrieved with one such haplotype. The second problem is heteroplasmy, when more than one haplotype is present in one individual. In some cases different haplotypes may be observed in different tissues of the same individual, which may lead to false inclusion or exclusion of a suspect depending on which tissue type is deposited and used as reference sample (Bender et al. 2000).

In addition to the autosomal STRs mentioned above, sex chromosome linked STRs are becoming increasingly popular for human identity testing. In some forensic cases, particularly rape, it is necessary to differentiate between male and female DNA in a mixed sample. While differential lysis can separate sperm from epithelial cells, it is not always successful as sperm DNA can be lost if the sample is very small or degraded and in some cases the rapist may be vasectomised resulting in a complete lack of sperm. In such cases the use of Y-chromosome STRs has become increasingly important as the very location of these markers indicates that a DNA profile can only originate from a male. In particular these markers can be used to determine how many individuals may have participated in gang rape as well as eliminating the need for complicated differential lysis to separate the male and female cells. In addition these markers can be used to determine the paternity of a male child, as the Y-chromosomes are directly passed from father to son, with a match probability of ~0.003 for 11 markers (Roewer et al. 2001). Y STRs can also enable paternity testing, of a son, when the potential father is not available for testing, as other male members of his family can be tested instead. While these factors are a useful tool in the forensic laboratory the only way variation can occur is by random mutations, so that a Y-STR profile match means that a male individual of that family is responsible, rather than being able to determine the specific perpetrator. However if a sample is found to differ from a suspect's profile then it is possible to exclude them from the investigation (Jobling et al. 1997). The lack of recombination and independent chromosomal reassortment, through most of the Y-chromosome does lower the diversity of Y-STRs when compared to autosomal markers, leaving only mutation to

cause changes, but the male specific nature does ensure their importance in forensic investigations. Because of their potential importance much research into Y-STRs has been performed resulting in the production of Y-STR multiplexes, in particular the AmpF*l*STR® Yfiler® from Applied Biosystems (Mulero et al. 2006).

1.1.5 Visualisation

After DNA has been amplified the markers can be visualised using gel electrophoresis. Visualisation of the DNA fragments separated by the gel matrix can be performed using either silver stain or fluorescent dyes, although the majority of forensic laboratories now use the automated and more efficient fluorescent technique. These systems are usually denaturing, involving chemicals such as formamide and urea, in order to maintain the DNA in a single-stranded state. This ensures better resolution of closely sized markers as single-stranded DNA is more flexible than double-stranded allowing more efficient separation through the gel or polymer. Formamide and urea form hydrogen bonds with DNA bases preventing them from bonding with their complementary sequence, initial separation is achieved by heating to 95ºC.

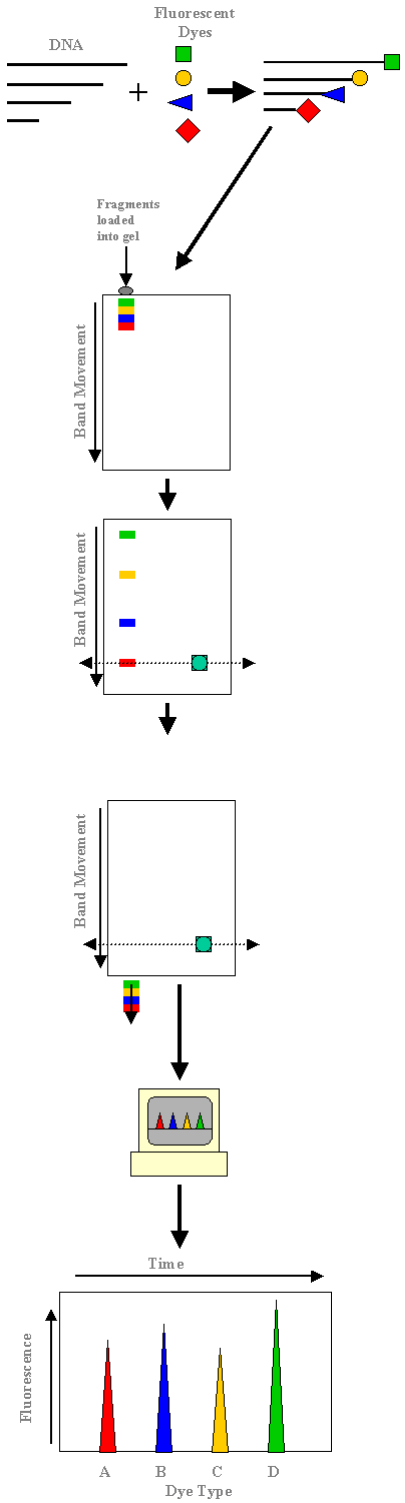
Recent history has seen the development of the capillary electrophoresis process that involves the use of liquid polymer instead of gels for electrophoresis (Buel et al. 1998; Moretti et al. 2001).

1.1.5.1 Gel-based electrophoresis

Gel based electrophoresis involves a solid gel matrix which the DNA fragments migrate through during the electrophoretic process. There are two types of gel that can be used, agarose and polyacrylamide, the type used depending on the size of the fragments to be separated. Polyacrylamide gels are better for separating smaller fragments as they have smaller pore sizes. Both involve the gel being placed in a buffer and electrodes attached to either end of the equipment. The samples are loaded into the gel at the opposite end to the positive electrode so the current draws the negatively charged DNA molecules through the gel. The size of the DNA fragments determines how fast they migrate through the gel, with smaller ones moving through the matrix faster than larger ones. Visualisation of the fragments can be performed in two ways, by silver staining of the fragments after electrophoresis or by fluorescent detection. Silver staining involves a number of washes of the gel including two soaks in a fixer (methylated spirits and glacial acetic acid) for 3 minutes, a 15 minute soak in 0.2% silver nitrate so that the silver deposits on the DNA molecules, two rinses in UP water, followed by soaks in developer (5M NaOH and formaldehyde) and neutraliser (Na_2CO_3) prior to the image being scanned straight from the gel into the computer. While this technique is efficient and relatively cheap, compared to fluorescent techniques, most laboratories now use the automated fluorescent techniques which are faster and less labour intensive.

STR primers in the multiplex amplification kits produced by companies such as Promega and Applied Biosystems are fluorescently tagged at the 5′ end of the forward primer so all fragments post-PCR are also fluorescently labelled. These single-stranded fragments are detected as they migrate through the gel, specifically when the pass the ‘read region’, approximately 4.5cm from the bottom of the glass plates, where a laser excites the dye and the data are collected by a CCD camera. This camera collects the data according to the wavelength of the light emitted by the dye so multiple fluorescent dyes can be detected in parallel. The use of multiple dyes enables the inclusion of several STR markers with overlapping size ranges in the one multiplex. These markers can then be separated by the dye they are tagged with, instead of having to analyse the same number of markers with multiple runs which would be necessary with silver staining (Frazier et al. 1996). Fragment size is determined by comparison to an internal size standard that is run in conjunction with each sample. An example of one of the sequencers available for gel electrophoresis

using fluorescent dyes is the 377 DNA Sequencer from Applied Biosystems; up to 96 samples, including ladders and controls, can be run at one time with this machine taking approximately three hours.



1. DNA fragments are labelled with fluorescent dyes via various labelling protocols.

2. For each sample:

1. Fluorescently labelled DNA fragments are loaded into a lane of a polyacrylamide gel.
2. Fragments are pulled through by an electric current (electrophoresis).

3. As the fragments move through the gel they separate into distinct bands according to size, due to:

1. The gelís matrix inhibiting movement.
2. Inhibition of movement being proportional to fragment size.

4. Near the base of the gel, bands fluoresce as they pass through the beam of a constantly scanning laser.

1. The laser excites the attached dyes within eachband.
2. The excited dyes then emit light at a wavelengh specific for the dye type.

5. The fluorescence is then detected by a camera and transferred to the computer.

6. The computer then identifies the band by the wavelength of emited light, and quantitates the fluorescent signal.

Figure 1-18: Overview of fluorescent gel based electrophoresis from http://www.bio.mq.edu.au/centres/sequencer/overviewpic.htm

The protocol for the 377 involves the preparation of a polyacrylamide gel using polyacrylamide, ammonium persulfate and TEMED (N, N, N', N – tetramethylethylenediamine). Prior to pouring the 36 cm gel plates are thoroughly

cleaned using deionised water and then mounted in a cassette that holds them in position. The gel is poured between the plates and is left to set for approximately two hours. A shark's tooth comb is then added to create wells for sample loading, any spilt polyacrylamide is removed and the cassette, with the plates, is loaded into the sequencer. A plate check is then performed to ensure the plates are clean and that the gel is not contaminated with undissolved urea crystals. If the scan window shows flat lines then the read region is clean and a pre-run can then be started in order to heat the gel up to the optimum temperature of 51ºC and laser power at 40.0mW, before loading the samples. Upper and lower buffer chambers are filled with 1 x TBE and a heat block is added to the front of the plates prior to the start of the pre-run. Once the optimal temperature is reached the samples, previously mixed with formamide to denature the sample, blue dextran loading dye and the appropriate internal line size standard, can be added to the wells along with an allelic ladder; the module number which includes the appropriate filter set, run time and read length, are set on the computer and electrophoresis can begin. When using samples amplified with the SGM plus® PCR kit the run module is GS Run 36F – 2400 which pre-programmes the sequencer to certain settings (377 DNA sequencer manual, Applied Biosystems) as described in Chapter 2. Software such as Genescan® (Applied Biosystems) is then employed to determine the size of each band detected as well as application of a matrix to separate the bands according to the fluorescent dye they were labelled with during amplification. A second piece of software called Genotyper® (Applied Biosystems) compares each fragment to an allelic ladder that consists of all alleles for each of the markers included in the multiplex used during PCR, in order to determine the DNA profile of the sample.

1.1.5.2 Capillary electrophoresis

Capillary electrophoresis is an updated more specialised version of gel electrophoresis. While still involving fragment migration dependent on size and fluorescent dye excitation and detection, a liquid polymer is used instead of the gel. Almost all of the technique is automated so smaller sample sizes are required and operator error is reduced by the autosampling, cross contamination between wells cannot occur and more samples can be run simultaneously than was possible with a gel. In addition the time taken is reduced as higher voltages can be used as the heat dissipates from the capillaries more readily (Moretti et al. 2001).

The most important difference to gel systems is the presence of individual capillaries for each sample rather than one gel with multiple wells. A liquid polymer, within the capillary, is the matrix through which the fragments migrate and is replaced by fresh polymer after the sample has completed its separation and the fluorescence signal detected by the CCD camera (Moretti et al. 2001). The samples are injected into the capillary by the machine rather than by operator loading, as with the gel based system reducing loading error and potential cross contamination between wells. One of the first capillary electrophoresis machines was the ABI Prism 310 Genetic Analyser (Applied Biosystems, Warrington, UK), with a single capillary, through which a single sample can be analysed every 30 minutes. Applied Biosystems (ABI 3700) and Molecular Dynamics/Amersham Pharmacia Biotech (MegaBACE) then introduced 96 capillary machines capable of analysing 1,000 samples every 24 hours. These are too high throughput and expensive for most laboratories so Applied Biosystems developed the 3100, with 16 capillaries, which can process 96 samples in approximately six hours.

The initial protocol for the capillary machines involves the installation of a capillary array onto the analyser, the number and length of capillaries depending on the equipment in use. The capillaries used for the SGM plus® amplified samples are usually 36 cm long. Then prior to electrophoresis the polymer must be added to the machine to fill the capillaries ready for sample injection. 1 x running buffer, including EDTA, is also added to ensure the current is properly conducted between the electrodes and so separation occurs across the capillary. Finally the samples are mixed with deionised formamide and the size standard in a 96 well plate that is added to the analyser. Each sample is injected into the capillary where separation occurs by size of DNA fragment. Once this is completed the polymer is flushed out with deionised water and replaced prior to injection of the next sample. A section of all the capillaries are grouped together so that the argon laser can be focused on that specific point so the fluorescent dyes can be excited and detected by the CCD. Prior to electrophoresis of samples spatial and spectral calibrations must be performed, the first enables the CCD to determine the exact position of the capillaries while the spectral acts as a matrix by determining the spectral overlap of the fluorescent dyes in use for that particular multiplex. As with the slab gels an argon ion, multi line laser

excites the fluorescent dyes incorporated into each fragment, emitting a light that is detected by the CCD. The CCD then transmits the length of time it took the fragment to migrate through the gel plus the wavelength of the light emitted to the computer software, such as Genemapper® ID (Applied Biosystems) which combines the functions of Genescan® and Genotyper® (Applied Biosystems).

1.1.6 Interpretation and Low Copy Number guidelines

1.1.6.1 Interpretation

Once the genotypes for each sample have been determined it is the responsibility of the operator to examine the data. There are a number of issues that may complicate the interpretation of a sample, especially if that sample is already a mixture. For example there are a number of artefacts that can appear in the electropherogram that are not actual alleles but at first glance may appear that way. Stutters are one such artefact that may arise when the *Taq* polymerase slips during amplification, resulting in a peak one repeat unit smaller than the actual allele, as in Figure 1-7. If the locus is heterozygous then stutters will usually be observed for both alleles at approximately 15% or less of the allele height (Gill 2002; Gill et al. 1998).

If a sample has been over-amplified or too much added to the gel/capillary a situation known as 'pull up' can occur. The matrix is unable to work properly by the excess of one off-scale or over amplified allele resulting in a smaller peak under it. If this smaller peak occurs at the same size as an actual marker then it may appear to be an allele (Clayton et al. 1998). If the operator suspects this has occurred the sample can be diluted, re-amplified and re-run.

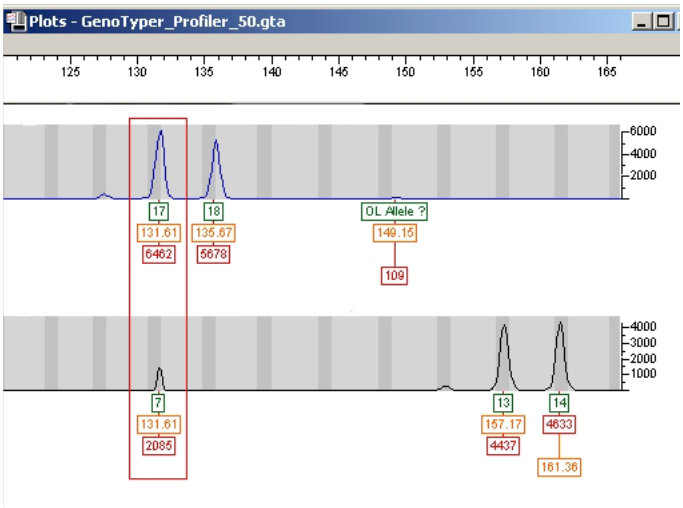


Figure 1-19: Example of an electropherogram with a pull-up peak as highlighted by the red box, from http://www.bioforensics.com/genophiler/problems.html

Under normal conditions heterozygous loci should be of approximately equal peak area. However if low levels of template are present two alleles for the same marker can be amplified to slightly different degrees. Heterozygote imbalance can also occur if the two alleles have very different molecular weights, as low molecular weight alleles tend to amplify more efficiently than those of a higher molecular weight (Clayton et al. 1998). To determine if the locus is heterozygous or if a mixture is present the peak area of the shorter allele should be divided by the peak area of the longer allele. A value over 0.6 (Hb_x) indicates a balanced heterozygous locus (Gill et al. 1998).

While these problems can be observed after the conventional 28 cycles of PCR and visualisation they are more commonly observed when the Low Copy Number technique (34 cycles) is employed (Gill et al. 2000).

1.1.6.2 Low Copy Number guidelines

For most of the multiplex systems involving STRs, the optimum amount of DNA template to be added is 1ng, although as little as 250pg can be amplified using 28 cycles of PCR. However in 1997 van Oorschot and Jones (1997) found that items only briefly touched could be swabbed for identifiable DNA. Findlay et al. (1997) found that in 91% of cases they were able to amplify DNA from a single cell, with full profiles from 50%. Similarly, Gill (2001) found that less than 100pg of template could be amplified using 34 cycles of PCR, more than this did not increase sensitivity but did increase artefact. He also found that the artefacts seen occasionally after 28 cycles were more commonly seen after 34, as well as stutters and heterozygote imbalance.

As a result of these observations a number of studies have concluded that the following guidelines should be employed when using LCN, but preferably avoid the technique if possible.

As laboratory contamination is difficult to completely eliminate due to the sensitivity resulting from the increase in PCR cycle number to 34, a consensus profile must be generated (Taberlet et al. 1996). If an allele is observed when the sample has been

amplified and visualised in two separate rounds of PCR and electrophoresis then it can be called as an allele. Any peaks that only appear in one set of results can be counted as either contamination or artefact such as stutter. Gill found that only 4 out of 1225 comparisons showed a duplicated spurious allele (p=0.003) (Gill et al. 2000; Whitaker et al. 2001). Alleles that are observed in a sample must be excluded if observed in duplicated negative controls (Gill et al. 2000).

Stutters in normal PCR can be observed up to 15% of the peak area of the actual allele. Low copy numbers stutters can often be greater in size (Gill et al. 2000). If the peak is 9,999 RFU or less in area then stutters can be up 40% of the allele, but this value is 20% if the peak is over 10,000 RFU in area (Whitaker et al. 2001).

As cycle number increases and sample size decreases stochastic variation causes certain alleles, often the low molecular weight ones to be preferentially amplified resulting in increased heterozygote imbalance or in the extreme, allelic dropout (Gill et al. 2000). Whitaker et al. (2001) found that when calculating heterozygote imbalance for peaks generated using LCN the Hb_x can be as low as 0.2. Values less than this were indistinguishable from the background and these peaks counted as allelic dropout.

Allelic dropout can occur when LCN work is performed due to the preferential amplification of one allele of a heterozygote, to the extent that one allele cannot be observed, when low levels of template are added to the PCR (Gill 2001). Whitaker et al. (2001) found that when using LCN for known heterozygotes allelic dropout can occur at a rate of approximately 10% per locus. This is not normally seen using the standard 28 cycles. If a single peak is observed using LCN then the peak area must be considered. Whitaker et al. (2001) noted that when a single peak was observed and it was over 10,000 RFU no allelic dropout occurred. But allelic dropout could occur and leave an allele up to 9,400 RFU. Therefore if a single peak over 10,000 RFU is observed then it can cautiously be called as a homozygote genotype, although it is safer to say allele failed. 9,999 or under it must be assumed that dropout has occurred (Whitaker et al. 2001).

While every precaution should be taken to avoid contamination when analysing samples, extra care is needed for LCN samples. Gill (2001) and Rutty et al. (2003) recommend:

1. A separate laboratory is used for extraction and PCR set-up, well away from post-PCR preparation areas and the thermocyclers should also be kept separate.
2. Gloves, masks and lab coats, all disposable, should be worn at all times, with regular changes of gloves.
3. UV light and bleach should be used to clean equipment and surfaces before and after use.
4. Negative controls should be used to check for contamination at every step.
5. LCN PCR should be performed in duplicate.
6. A staff database, comprising DNA profiles of all members of staff working in the area, should be compiled to try and detect the source of any contamination.

Due to the problems outlined above LCN PCR has been treated with caution by researchers. These issues complicate the interpretation of DNA profiles and unless a rigorous computer program designed to identify each of the artefacts described is employed profiling could be open to operator error. The potential evidentiary value of retrieving a perpetrator's DNA from a single cell is amazing, however the associated problems may outweigh the benefits. The ability to generate a profile from a single cell does not necessarily mean that that cell is related to the crime in question as secondary and even tertiary DNA transfer has been shown to occur (Lowe et al. 2002). When the cell was deposited or even what type of cell it is can be impossible to determine, leaving only a DNA profile surrounded by questions. In addition the possibility of contamination from consumables, Scene of Crime Officers and laboratory personnel is greatly increased.

Several alternatives to LCN do exist, including increasing PCR product concentration by reducing volume; nested PCR; whole genome amplification; filtration of PCR product to remove ions that interfere with electrophoresis and adding more PCR product to the analyser (Budowle et al. 1995). More alternatives and guidelines are

being developed in the wake of the Caddy Report produced after criticism of LCN during the Omagh trial in 2007. The report concluded that the theory was sound but that national guidelines needed to be employed for interpretation and in particular scrupulous anti-contamination protocols in the laboratory and at the point of sample collection need to be performed including the use of 'DNA-free' consumables; in addition dilution of the PCR product could reduce stochastic variation (Section 3.3 Caddy Report, 2008). Alternatives included the 'clean-up' of PCR products amplified using 28 cycles prior to electrophoresis, resulting in sensitivity equivalent to the 34 cycle protocol. This method, known as DNA SenCE, uses a staged approach including three more steps; 1) increased volume of sample added to the analyser, 2) increased injection time and 3) increased injection voltage. A combination of all four resulted in an increase in peak number and peak height as well as a reduction in stochastic products. The conclusion of this study was that if these alterations to standard 28 cycles of PCR were performed in stages an equivalent or better alternative to LCN could potentially be provided. The first stage over standard 28 cycle protocols was to clean-up the PCR product and double the volume of sample loaded. Stage two was to increase injection time from 10 seconds to 30 seconds and injection voltage from 3 kV to 4 kV (Forster et al. 2008).

Overall the report concluded that the technique could prove valuable after strict national and international consensus and validation studies have been performed provided the laboratories involved can show anti-contamination and results are of highest standards.

1.1.6.3 Mixture analysis

A mixed sample can often be identified by the presence of more than two bands at one or more loci. While these bands may be PCR artefacts the guidelines outlined in Chapters 1.1.6.1 and 1.1.6.2 should exclude these leaving behind true allele peaks. Clayton et al. (1998) describes four steps for determining the individuals whose DNA is present in a particular sample.

The first step is to determine whether the profile that was generated is a mixture of two or more individuals. A complex mixture of more than two individuals is likely if

more than four alleles are noted at one or more loci after stutters etc have been excluded. Secondly, the ratio of the alleles at each locus should be calculated, which can indicate whether a particular allele is shared by more than one contributor as well as the original proportion of each donor's DNA in the mixture. Once it has been determined whether any alleles are common to multiple donors then all possible combinations of alleles at each locus can be transcribed, prior to comparison with the reference profiles of potential suspects. These steps can also help identify the major and minor contributors to a mixture. Noting of all possible combinations of alleles at each locus should be done without knowledge of the profiles of potential contributors to ensure objectivity (Clayton et al. 1998). To maintain such objectivity the majority of forensic laboratories employ computer systems to perform mixture analysis especially for LCN samples, for example the *LoComatioN* software application (Gill et al. 2006). As mixtures are expected with LCN samples and due to the complications resulting from stochastic variation, systems like *LoComatioN* are recommended for such samples rather than manual calculations like the Likelihood Ratio Calculations that are too complex to perform manually (Gill et al. 2006).

1.2 DNA transfer

Locard's principle states that every contact leaves a trace (Locard 1930). Modern forensic science is based on this theory as crimes scenes are now examined for fingerprints, hairs, footprints, semen and saliva. Locard's principle has also been applied to DNA. An average human being sheds approximately 400,000 cells every day (Wickenheiser 2002), with the excretion of sweat bearing possible free DNA sources. Contact between the skin and objects or other people should therefore follow Locard's principle and transfer traces of DNA with the potential for identification (Wickenheiser 2002).

A study by van Oorschot and Jones (1997) has been influential in instigating a number of other studies into the propensity of individuals to transfer DNA as well the routine sampling of trace DNA from crime scenes. The study showed that when swabbing the palm of the hand DNA yields varied significantly between individuals but full genetic profiles could be generated. Further to this, when the subject gripped polypropylene tubes for varied lengths of time the authors found that sufficient DNA was deposited onto the tubes on initial contact to generate full profiles although this did vary after hand washing (van Oorschot and Jones 1997). One-minute handshakes were also investigated and shown to reveal transfer of DNA from one individual to another. Secondary transfer was also observed, as alleles of individuals who did not touch the object involved in the study but shook the hand of the individual who did were picked up on some of the objects. Lowe et al. (2002) also found that mixed profiles could be obtained from objects that only one individual had touched indicating that secondary transfer and therefore DNA transfer from person-to-person can occur. Although transfer is known to occur results are difficult to obtain due to the small quantities of DNA that are involved. However van Oorschot and Jones (1997) found that objects handled regularly yielded between 1 and 75ng of DNA and complete profiles were obtained. These results have since been disputed by Ladd et al. (1999) who claimed that the quantity of DNA recovered from inanimate objects was actually only equivalent to background levels of DNA. Such quantities of DNA are at the lower end of sensitivity recommended by manufacturers of the STR systems necessary for analysis. Ladd et al. (1999) also disputed the claims of van Oorschot and Jones (1997)

to generate DNA profiles indicating secondary transfer. However Ladd et al. did not test the ability of their subjects to deposit DNA as Lowe et al. (2002) did, as an extension to the work of van Oorschot and Jones (1997), which shows that individuals do vary significantly in their propensity to shed cells, especially immediately after hand washing.

In 2006 Phipps and Petricevic published their work on transfer from individuals to handled items (Phipps and Petricevic 2006). When repeating the shedder test as described by Lowe et al. (2002) with five individuals on four different days they found that none of the volunteers produced the same number of alleles on each occasion. Like Lowe et al. (2002) they did find more DNA was deposited the longer the period since hand washing and that more alleles were observed from the non-dominant hand than the dominant hand in a large scale experiment involving 60 volunteers. In addition they found little evidence of secondary transfer. Overall the results of the Phipps and Petricevic (2006) study indicate that to classify individuals as good or bad shedders is to oversimplify the issue and further research is required before a definitive answer can be provided as to the usefulness of testing for shedder status.

Outside of these studies into shedder status there have been several others investigating the propensity and means of individuals to transfer DNA to one another and onto inanimate objects. In 2004 Bright and Petricevic (2004) demonstrated that DNA could be obtained from the soles and top of the feet, with higher yields from the top. Additionally they found that DNA profiles could be obtained from the insoles of shoes, with profiling success being reduced if the insoles were made of leather. Other items from which DNA profiles have been generated after contact include toothbrushes (Tanaka et al. 2000), lip cosmetics (Webb et al. 2001), drinks containers (Abaz et al. 2002) and bedding after just one night's sleep (Petricevic et al. 2005).

Research in the Forensic Pathology Unit of the University of Leicester has included the spread of DNA during speaking which found that individuals can deposit their DNA up to a metre away during a single, short sentence. Prior to this experiment Rutty et al. (2003) had investigated the effectiveness of protective clothing in the prevention of DNA transfer. Lack of movement with and without protective clothing

did not result in DNA deposition. Movement with and without protective clothing resulted in varying degrees of transfer, although the protective suits did reduce this. DNA deposition during talking was only observed when the subject knelt over the test area. Additionally, Graham and Rutty (2007) investigated manual strangulation, including the mapping of 'normal' DNA profiles from the adult human neck. They found that DNA was transferred and that spurious alleles of unknown origin were frequently found. This investigation followed on from previous research by Rutty (2002) and Wiegand and Kleiber (1997) into the transfer of DNA during simulated manual strangulation. Both found that DNA could be transferred from offender to victim and could survive on the neck for up to ten days. Several alleles of unknown origin could be detected during the same time period from test and control sites of the neck, as found by Graham and Rutty (2007).

1.3 Child abuse

1.3.1 Types of abuse

There are five main categories of child abuse, namely physical, sexual, emotional, neglect and Munchausen's by Proxy (Morris et al. 1997). Physical abuse has been classified as "hitting, shaking, throwing, poisoning, burning or scalding, drowning, suffocating or otherwise causing physical harm to a child." (NSPCC Inform, Childline Casenotes, 2006). Munchausen's by Proxy is similar, in that an individual, usually the parent or carer of the child, fabricates the symptoms of an illness or even goes so far as to induce an illness in the child (NSPCC Inform, Childline Casenotes, 2006). Sexual abuse can include activities from flashing to indecent imagery and unwanted touching to rape and buggery. Emotional abuse is a little harder to define but is set as "the persistent ill treatment of a child which affects their emotional development" (NSPCC Inform, Understanding the Links, 2005) and can include shouting, verbal abuse and generally making the child feel unloved and worthless. Neglect is defined as "persistent failure to meet the physical and/or psychological needs of a child; for example failing to provide adequate food, warmth, shelter, clothing, emotional care or medical treatment" (NSPCC Inform, Understanding the Links, 2005). While each of these types of abuse can be seen in isolation, it is more common to see combinations of different types of maltreatment. For example, physical abuse can be used as a means to intimidate children into keeping quiet about neglect or sexual abuse and in addition can be used as a means to sexual abuse (NSPCC Inform, Childline Casenotes, 2006).

While physical abuse would seem the easiest of these to identify, many visible effects such as bruising can be passed off as accidents by 'carers'. Signs of sexual abuse and neglect are more difficult to explain once detected. Therefore the aim of this project is to determine if DNA can be used to differentiate between accident and physical abuse.

1.3.2 Incidence

The annual number of child homicides in England and Wales has remained constant at one or two a week for the past thirty years with the most at risk age group being those under one year old (Home Office Statistical Bulletin, 2005/2006). Childline is a UK helpline for children, aiming to help them cope with anything from exam stress and safe internet use to physical and sexual abuse. Of the children who call Childline, a third talked about physical abuse, averaging two calls every hour of the year, approximately 12,513 children in 2004/2005. In the same time period (2004/2005) 2,588 children called Childline about physical abuse combined with emotional abuse or neglect, while 1,513 complained of sexual abuse facilitated by physical force (NSPCC Inform, Childline Casenotes, 2006). In the period 2005/2006 the number of children calling Childline about sexual abuse alone was more than 9,000, two thirds of who had allegedly been raped (NSPCC Inform, Childline Casenotes, 2006).

While children calling Childline regarding maltreatment is one way of estimating the incidence of abuse, it cannot be used in isolation as many children are too afraid to talk to anyone about what they are going through; in addition younger children are unable to call the helpline. If they do they fear reprisals or getting the abuser, who may often be a carer, into trouble. Another way is by examining the numbers of children placed on Child Protection Registers. In 2006 there were 11,800 children on the register as a result of neglect, 3,600 after physical abuse, 2,300 sexual abuse and 6,000 from emotional abuse (NSPCC Inform, Child Protection Register Statistics – England, 2007). However it is thought that these figures are a gross underestimate of the actual incidence of each type of maltreatment, with possibly as few as 5% of cases being reported (Morris et al. 1997). More accurate figures are those of child homicides, with 55 incidents in 2005/2006, 7% of all homicides in this period, approximately half resulting from a loss of temper (Home Office Statistical Bulletin, 2005/2006). The most at risk age group, as in previous years, was found to be the under ones.

1.3.3 Physical abuse and bruising

Although the number of children placed on Child Protection Registers in England as a result of emotional abuse is greater than those suffering from physical abuse, the reverse is true for those children calling Childline. While emotional abuse is traumatic for the children and can cause behavioural problems in later life, physical abuse has the direct effect of injury whether mild (e.g. bruises and abrasions) or severe (e.g. head injury) and can sometimes result in death. If the abuse is not fatal the children in turn can often become aggressive, with problems interacting with others and may in turn abuse their own children. The majority of those children who called Childline regarding physical abuse stated it was ongoing rather than a one-off incident. One child informed them that his "parents had been beating me since I was eight (was 16 at the time). It used to be just slaps but now dad will knock me down and kick me in the ribs" (NSPCC Inform, Childline Casenotes, 2006). A number of studies have also indicated that those most at risk of physical abuse are too young, less than five years of age, to inform anyone what is going on and often do not know what is happening to them is wrong (Hornor 2005; Morris et al. 1997) (Childline Casenotes, 2006). Ellimann and Lynch (2000) estimated that approximately 8% of child abuse and neglect could be prevented if someone, whether authorities, social workers, friends or neighbours could intervene at an early stage.

In many cases physical abuse can begin as corporal punishment before escalating (Elliman and Lynch 2000). Corporal punishment has been defined by Professor Straus as the "use of physical force with the intention of causing a child to experience pain but not injury for the purposes of correction or control of the child's behaviour" (Elliman and Lynch 2000). The effect of corporal punishment in decreasing misbehaviour can only be maintained if the threat of punishment is maintained and often the intensity has to increase to sustain effectiveness, hence possible escalation into physical child abuse. Physical abuse can also result from parents losing control as they punish their children. Studies have shown that 90% (USA), 75% (Canada), and 90% (UK) parents had used corporal punishment on their children at some point (Ateah and Durrant 2005; Ateah et al. 2003; Youssef et al. 1998). Only 2% believed it was acceptable to use implements such as belts or slippers, to punish their children (NSPCC Inform, ESRC, 2007), a decade ago this was 23%. In 2004 the UK

government outlawed the hitting of children that resulted in permanent marks. However, in spite of campaigns by such groups as the NSPCC parents in the UK are still allowed to physically punish their children. While education may help reduce cases of physical child abuse by teaching parents how to look after their children safely and maintain their tempers when the child misbehaves, this will not be successful for all. If early signs of physical child abuse can be identified and help provided for the families, escalation may be prevented.

Bruises are an important early sign of physical child abuse. A bruise is defined as the "escape of blood into the skin or subcutaneous tissue or both, following the rupture of blood vessels, usually capillaries, by the application of blunt force" (Stephenson 1995). Although common in childhood, a number of factors can indicate a non-accidental origin. Studies by Carpenter (1999), Sugar et al. (1999), Warrington et al. (2001), Dunstan et al. (2002) and Sibert (2004) all investigated the occurrence of bruising in non-abused children. All agree that the skin is a common site of accidental and non-accidental injuries, with bruising being seen most frequently in both cases. While accidental injury and abuse are difficult to distinguish there are certain signs that can indicate an accidental origin. For example, age, infants less than nine months old are not independently mobile and therefore have few bruises. Those learning to stand occasionally have bruises on the forehead if they pull to stand using a table or chair. When learning to walk or toddling the incidence of bruising increases but the majority are on bony areas on the front of the body such as the shins and knees. Fleshy areas such as cheeks, buttocks, arms and especially the ears were not bruised accidentally.

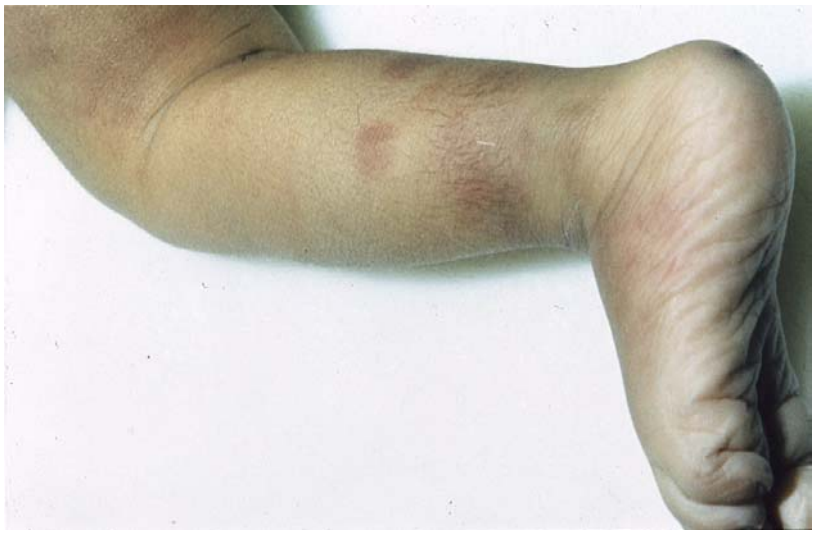


Figure 1-11: Photograph of an infant with fingertip bruising on the lower leg, indicating a forceful grip. Picture courtesy of Professor Guy Rutty

When non-accidental injures were investigated the bruising tended to be over 5cm in diameter (Sibert 2004), situated predominantly on fleshier areas such as the ears, buttocks, limbs and abdomen, but were linear in shape, when the hand or weapons were used. Face, head and neck were the most common sites of abusive bruising in head injured children (Atwal et al. 1998).

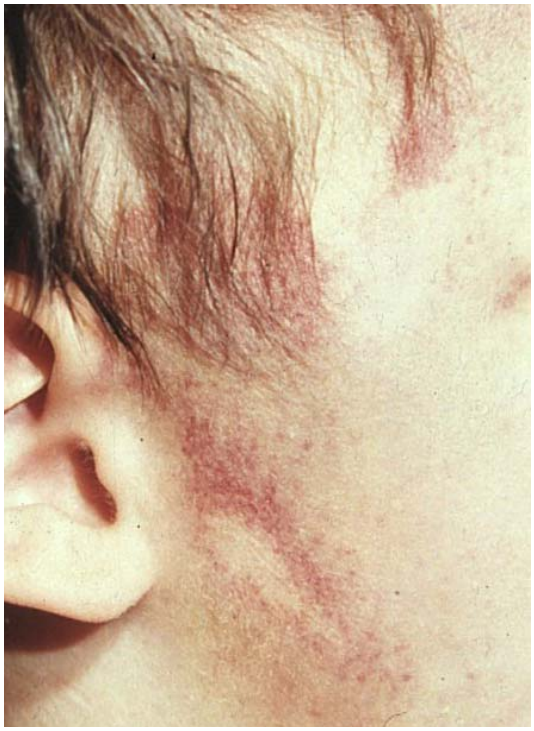


Figure 1-12: Photograph of an infant with linear bruises on the right side of their face, forming a distinct handprint. Picture courtesy of Professor Guy Rutty

The studies also noted that the bruises often formed a pattern indicative of the object causing the bruise, for example clusters of bruises 1cm in diameter, indicating a forceful grip, as seen in Figure 1-11, or two parallel lines left by the outer edges of a looped cord, shown in Figure 1-13.

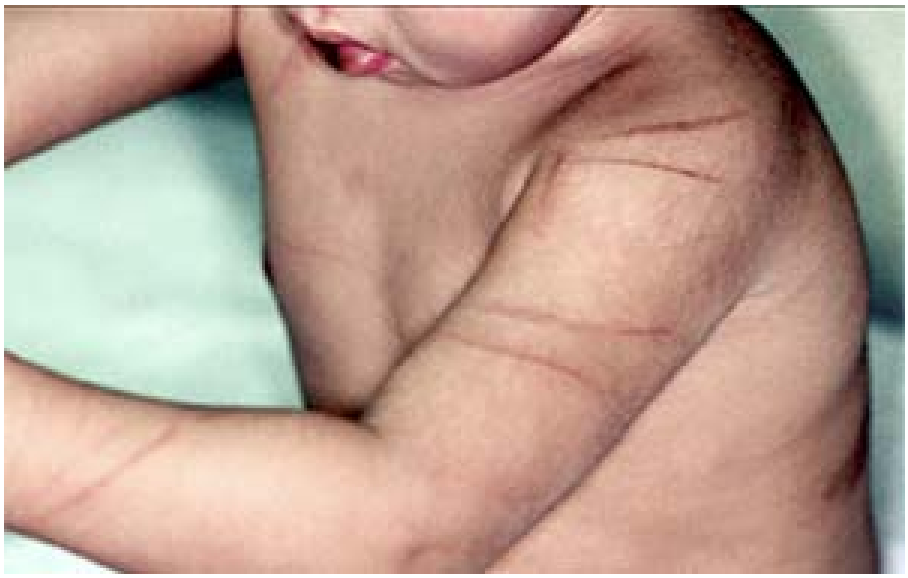


Figure 1-13: Photograph of an infant with bruising on the arm and torso displaying the pattern of the looped cord that was used. Picture taken from the New York Mandated Reporter (2006) http://www.wildirismedicaleducation.com/courses/199/index_mand.html (January 2008)

Multiple bruises of different ages, indicated by varying colours, over different sites may support abuse as do repeat occurrences. Infants younger than nine months with any bruising should be cautiously regarded as potentially abused and require careful investigation.

However the interpretation of bruises should be undertaken with caution. Definitive opinion of aging of bruises by colour observation especially should be limited as several studies have indicated no two bruises change colour in the same manner over the same time period (Maguire et al. 2005). Site of bruising while indicative of cause should also be used cautiously as accidental bruises can be seen across all areas of the body although some are more common than others (Labbe and Caouette 2001). A number of factors can also affect the tendency of individuals to bleed making them more prone to easy bruising for instance steroid inhaler use, non-steroidal anti-inflammatory drug use, anticonvulsants, haemostatic disorders, e.g. haemophilia, von Willebrand's disease and collagen disorders such as Ehler-Danlos and Marfan's Syndromes (Vora and Makris 2001). Additionally there are conditions that can mimic bruising, for example natural skin conditions such as Mongolian spots,

neuroblastoma, idiopathic thrombocytopenic purpura, vasculitis, hemorrhagic edema of infancy, cultural remedies, e.g. coining and cupping, both non-abusive, and even pen marks can all result in false interpretation of abuse if not properly diagnosed or identified (Kos and Shwayder 2006). An example of a natural skin condition that can be mistaken for abusive bruising is shown in Figure 1-14.

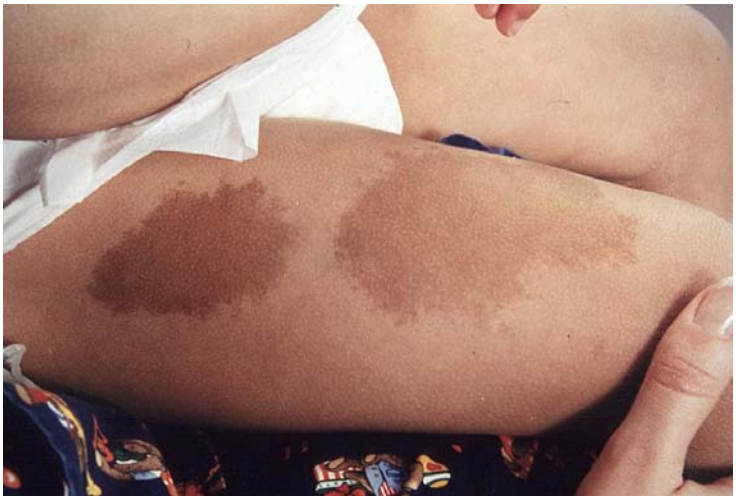


Figure 1-14: Natural skin naevi may be mistaken for abusive bruising. Picture courtesy of Professor Guy Rutty

1.3.4 Perpetrators

A big problem with preventing and investigating physical child abuse, after diagnosis, is the determination of the perpetrator. Often the child is too afraid to tell who abused them for fear of reprisal or they don't want to tell as they don't want to get the abuser in trouble as they are a parent or carer (NSPCC Inform, Childline Casenotes, 2006). Many cases involve children too young to understand what is going on. When investigating 185 physically abused 0-8 year olds 15.1% were abused by the father, 14.6% both parents, 13.5% mother, 6.5% babysitter/educational, 3.2% sibling, 2.7% mother's boyfriend, 1.6% other adult member of family, 0% strangers and 42.7% unknown perpetrator (Martrille et al. 2006). Martin found two cases of physical child abuse by babysitters, while Green (1984) described four cases of sibling abuse, all of whom had been abused by one or other of their parents. As shown by the statistics

strangers are rarely responsible for physical abuse of children in contrast to the amount of publicity these cases receive. Multiple carers may look after the child and may have been present within the possible time-frame of abuse. As most cases (53.3%) occur within the child's home (Cairns et al. 2005) there are no witnesses outside the caring environment, thus physical evidence may be the only means of determining the person responsible. However as those responsible are frequently the carers, physical evidence such as DNA may be explained by normal day-to-day activities.

1.4 Aims and objectives

1.4.1 Aims

The overall hypothesis of this thesis is that the perpetrators of physical child abuse will deposit sufficient DNA during forceful contact for identification purposes. Research has shown that simulated manual strangulation results in transfer of DNA between the victim and offender however no data have been generated on DNA transfer by other forms of forceful contact. Therefore my thesis aims to fill this gap and determine if current techniques can be employed to this end.

1.4.2 Objectives

The research will be divided into two separate investigations. The first stage is to determine the normal, background DNA present on the faces of children aged 0-5 years. This age group was chosen as they are most at risk of physical abuse and the face is the most common location of abusive bruising, an early indicator of physical child abuse. It is hypothesised that mixed profiles will be generated as this age group require a lot of care and therefore frequent contact with parents or carers. Reference samples will be taken from the children and their carers in order to determine the origin of any non-subject DNA sampled from the faces. The head and neck will be divided into twelve sections to ease sampling as well as to determine any differences between the sites. Questionnaires will also be provided for the carers to detail the techniques employed in the care of their child in order to ascertain any effect on the DNA profiles retrieved from the children's faces.

The second stage will investigate whether DNA is transferred by forceful contact such as punching and slapping. In order to do this, three phases will be performed, beginning with the application of force to a DNA-free surface. Both punches and slaps will be applied under three conditions; contact 15 minutes after hand washing, contact one hour after hand washing and multiple (n = 3) contacts applied one hour after hand washing.

The objective of phase II is to determine the feasibility of person-to-person application of force with two subjects prior to recruitment of further volunteers. Both subjects will apply a single punch and slap to the upper arm of the other subject on three separate occasions. The final phase will expand on phase II with multiple pairs of volunteers in order to establish the DNA profiles retrieved and determine if the offenders DNA can be distinguished from that of the victim and vice-versa.

2. Materials and methods

2.1 Materials

AmpF*l*STR ® SGM Plus ® PCR Amplification Kit	Applied Biosystems
Blue dextran loading dye	Applied Biosystems
Chelex® 100 Resin	Bio-Rad Laboratories
Dryswab™ woodstick shaft in labelled tube	Medical Wire and Equipment
Footprint lifting sheets	WA Products
Formamide, for molecular biology (≥99.5% GC)	Sigma
GeneScan® 500 ROX™ Size Standard	Applied Biosystems
Hi-Di™ Formamide	Applied Biosystems
Long Ranger® Singel® Polyacrylamide Gel Packs	Cambrex Bioscience
MicroAmp™ Optical 96 well reaction plates	Applied Biosystems
MicroAmp™ Fast Optical 96 well reaction plates	Applied Biosystems
MicroAmp™ Optical adhesive film	Applied Biosystems
Nanodrop™ ND-1000 Spectrophotometer	Nanodrop Technologies
QIAamp® DNA Mini Kit	Qiagen Ltd
Quantifiler® Human DNA Quantification Kit	Applied Biosystems
Sodium Hypochlorite Solution	Sigma
Tamper evident police bags	WA Products
Water, molecular biology grade	Sigma
Water, fluorescence grade	Fisher

2.2 Contamination prevention

All laboratory work apart from PCR and pre-electrophoresis set-up was carried out in a dedicated room into which no amplified DNA was taken. Benches were cleaned with 10 % (w/v) sodium hypochlorite and treated with UV light ~ 254nm (Astec Microflow Hood). UV irradiation causes cross-linking of adjacent Thymine nucleotides resulting in inhibition of polymerase activity and therefore amplification of any contaminating DNA molecules. All equipment and reagents that were suitable for de-contamination by UV light were treated in a UV crosslinker (Syngene Crosslinker, wavelength 254 nm) for a minimum of ten minutes (Tamariz et al. 2006). Plasticware was guaranteed DNase and RNase free and all pipette tips were changed between samples. Lab coats, masks (3M 9913 particulate respirators) and sterile, disposable gloves were worn throughout the experimental work and gloves were changed regularly. A database of profiles of all individuals working in the room was created for exclusion purposes in case of any contamination issues (Gill 2001).

2.3 DNA extraction

2.3.1 Chelex® 100 resin

The head of each cotton swab was snapped off from its shaft then incubated, at room temperature, in 500 µl of ultra-pure (UP) water for 30 min. The swabs were then centrifuged at 14,000 rpm for two min before the swab was removed from the liquid and placed into a 0.5 ml eppendorf, with a small hole in the bottom that was inside a 2 ml screw top tube. This 'piggyback' was then centrifuged for two min at full speed. The swab and 0.5 ml tube were then discarded and the liquid transferred to the original eppendorf for a further two min of centrifugation. All but 20-30 µl was discarded and 200 µl of 5 % (w/v) Chelex® solution was then added to each. This was followed by incubation of the DNA solution at 56 ° C for twenty min. The tubes were then vortexed for five to ten seconds, before another incubation of eight min at 100 ° C. All of the supernatant, containing the DNA, was then transferred to a correspondingly labelled eppendorf tube for storage. Protocol from Walsh et al. (1991) and slightly modified as by Sweet et al. (1996).

2.3.2 Qiagen QIAamp® DNA mini kit

The extraction was performed as per manufacturer's instructions for buccal swabs but with reduced volumes of PBS and Buffer AL.

Each swab was snapped off into 1.5 ml eppendorf tubes into which 200 µl of PBS had been added. 20 µl of Proteinase K and 200 µl of buffer AL were also added before fifteen seconds of vortexing. This was followed by incubation at 56 ° C for ten minutes and a brief centrifugation. At this point the piggybacking step from the Chelex® method was performed: the swab was transferred to a 2 ml screw top tube that contained a smaller (0.5 ml) eppendorf with a hole at its base. The swab was then centrifuged for one minute at full speed, the swab was discarded and the liquid added to that in the original tube before a brief centrifugation. 400 µl of 95 % ethanol was added to the sample, which was then vortexed and centrifuged briefly. 700 µl of this mixture was transferred to a QIAamp® spin column for centrifugation at 8000 rpm for one minute. The spin column was then removed from the collection tube, and placed into a new collection tube. These last two steps were then repeated before adding 500 µl of buffer AW1 and another centrifugation at 8000 rpm for one minute. The collection tube was again discarded and replaced by a fresh one and 500 µl of buffer AW2 was added to the spin column. The samples were then centrifuged at full speed for three minutes and the spin column was placed into a clean eppendorf and the collection tube was discarded. 60 µl of buffer AE was added, incubated for one minute at room temperature and centrifuged for one minute at 8000 rpm. Final sample volume was approximately 60 µl.

2.4 DNA quantification

2.4.1 Nanodrop™ ND-1000 spectrophotometer

The Nanodrop™ ND-1000 spectrophotometer (Nanodrop Technologies, USA) protocol requires the addition of 1.2 µl of a sample to a pedestal that holds a fibre optic cable, while a second fibre optic cable is held in the lid so that the sample acts as a bridge between the two cables. A xenon lamp acts as light source and is analysed after passing through the sample by a CCD array.

After switching on the Nanodrop™ ND-1000 the system was initialised by cleaning the measurement pedestals with a tissue then adding 1.2 µl of UP water. The lid was then closed to allow the machine to initialise. The pedestals were then cleaned again with a tissue. A blank was created by adding 1.2 µl of buffer AE, closing the lid and selecting the blank option. All samples were measured by adding 1.2 µl of the extract to the measurement pedestal and selecting measure. Both pedestals were cleaned with a tissue in between samples. Results were given in ng/µl based on absorbance at 260 nm.

2.4.2 Real-time PCR, Quantifiler® Human DNA quantification kit

The assay contains two 5´ nuclease assays, one specific for the human telomerase reverse transcriptase gene and an internal PCR control assay (IPC). Included are primers for the human specific sequence as well as for the IPC, plus a FAM-labelled Taqman MGB probe to detect the amplified sequence, while a similar probe labelled with VIC dye detects the amplified IPC.

A series of DNA standards were set-up as shown in Table 2-1.

Standard	Concentration (ng/µl)	Amounts	Dilution Factor
1	50.000	10 µl (200 ng/µl stock) + 30 µl TE	4x
2	16.700	10 µl (Standard 1) + 20 µl TE	3x
3	5.560	10 µl (Standard 2) + 20 µl TE	3x
4	1.850	10 µl (Standard 3) + 20 µl TE	3x
5	0.620	10 µl (Standard 4) + 20 µl TE	3x
6	0.210	10 µl (Standard 5) + 20 µl TE	3x
7	0.068	10 µl (Standard 6) + 20 µl TE	3x
8	0.023	10 µl (Standard 7) + 20 µl TE	3x

Table 2-1: DNA quantification standards dilution series as described in the Quantifiler® Kits User Manual

Once the standards were prepared a master mix was created using 10.5 µl Quantifiler® Human Primer Mix and 12.5 µl Quantifiler® PCR Reaction Mix per sample. The master mix was then vortexed before 23 µl was added to each reaction well of the MicroAmp™ fast optical 96-well reaction plate as needed. 2 µl of sample or standard was then added to the appropriate wells, with the standards being added in duplicate. A sheet of MicroAmp™ optical adhesive film was used to seal the plate before running the plate in the 7500 Fast Real-Time PCR system (Applied

Biosystems, Warrington, UK). The cycling conditions were set as Absolute Quantification, 9600 emulation with two stages:

Stage 1 – 95 º C for ten minutes (Once)
Stage 2 – 95 º C for fifteen seconds then 60 º C for one minute (Forty cycles)

2.5 Polymerase Chain Reaction

2.5.1 AmpF*l*STR® SGM Plus® PCR amplification kit

PCR was set-up using the AmpF*l*STR® SGM Plus® PCR amplification kit from Applied Biosystems. The primers included within the kit target the following ten STRs plus the Amelogenin locus (Sullivan et al. 1993):

D3S2358	Li et al. (1993)
vWA	Kimpton et al. (1992)
D16S539	GenBank G07925
D2S1338	Watson et al. (1998)
D8S1179	Oldroyd et al. (1995)
D21S11	Sharma and Litt (1992)
D18S51	Urquhart et al. (1995)
D19S433	Lareu et al. (1998)
TH01	Edwards et al. (1992)
FGA	Mills et al. (1992)

Table 2-2: The ten STRs included in the AmpF*l*STR® SGM Plus® PCR amplification kit

For the background levels study, the PCR was set-up as per manufacturer's instructions: 21 µl reaction mixture, 11 µl primer set and 1 µl Ampli Taq Gold DNA polymerase per sample. Sample DNA was added up to a maximum of 20 µl, dependent on concentration, with the maximum being added to the majority of LCN PCR reactions. These volumes were quartered for the forceful contact samples (5.25 µl reaction mixture, 2.75 µl primer set and 0.25 µl Ampli Taq Gold) (Fregeau et al.

2003; Leclair et al. 2003). Although validation studies for this reduction in reaction volume were not performed tests were carried out within the unit to determine the effectiveness of the technique. The results indicated that not only was the reduction cost effective but more sensitive than the full volume. All samples underwent the following PCR cycles in a GeneAmp 9700 thermocycler:

Temperature	Time	Cycles
95 ° C	11 min	1
94 ° C	1 min	
59 ° C	1 min	28 or 34
72 ° C	1 min	
60 ° C	45 min	1
4 ° C	Indefinitely	

Table 2-3: The cycle protocol for the AmpF/STR® SGM Plus® PCR amplification kit. The steps in red are repeat cycles of either 28 or 34

When 34 cycles of PCR were employed each sample, including all controls, were amplified and visualised in duplicate so that a consensus profile could be generated.

2.6 Electrophoresis/visualisation

2.6.1 ABI 377 DNA sequencer

Electrophoresis Voltage	3,000 V
Electrophoresis Current	60.0 mA
Electrophoresis Power	200 W
CCD Offset	250
Collection Time	2.25 hrs
Gel Temperature	51 º C
Laser Power	40.0 mW
CCD Gain	2

Table 2-4: 377 DNA sequencer settings for samples amplified using the AmpF*l*STR® SGM Plus® PCR amplification kit (Applied Biosystems)

All products from the background levels study that were amplified using the ABI AmpF*l*STR® SGM Plus® PCR kit, underwent electrophoresis using a 377 DNA sequencer (Applied Biosystems, Warrington, UK). A polyacrylamide gel was created using Long Ranger® Singel® packs (Cambrex Bioscience) containing 6 M Urea, 1 x TBE, 0.05 % (w/v) Ammonium Thiosulphate, and 0.07 % (w/v) TEMED which are mixed together, in the pack, on an orbital shaker at medium speed. The 377 gel plates were assembled using 0.2 mm spacers, to allow room for the gel between the 36 cm plates, and a cassette that holds the plates in place for mounting into the sequencer. Prior to assembly the plates, spacers and cassette were thoroughly cleaned using 0.1 M nitric acid and hot deionised water, three times per plate. Once the gel was poured into the plates the straight edge of a sharks-tooth comb was inserted between the plates and bracers were clamped on in order to hold the comb in place and prevent leakage. The gel was then left to polymerise for two hours at room temperature.

After polymerisation the sharks-tooth comb was inverted to create the loading wells and the cassette holding the plates was then mounted into the 377 DNA sequencer before addition of 1 x TBE to the buffer chambers. A plate check was then run to

ensure the plates were completely clean, followed by a pre-run until the temperature reaches 51 º C.

Before electrophoresis the samples were prepared by mixing 500 µl formamide, to denature the sample, with 100 µl of blue dextran loading dye. For each sample 5 µl of the formamide/dye mixture was mixed with 0.55 µl of the internal size standard GeneScan™ 500 ROX ™. 5 µl of this was then mixed with 4 µl of the sample. During the machine set-up the samples were denatured for two minutes at 95 ° C then placed immediately on ice. 1.5 µl of sample was loaded into the gel along with two lanes of the supplied allelic ladder (Applied Biosystems). The sequencer was then run for two and a half hours at 51 ° C. GeneScan® software v3.1 was employed to determine the dye colour and molecular weight of each peak in each sample by comparison with the size standard. Allele determination was then carried out using the Genotyper® software v3.7, which compared each peak to the allelic ladder run on the same gel as the sample, using the KaZam macro specific for the AmpF*l*STR® SGM Plus® kit alleles. (Applied Biosystems, Warrington, UK). Peaks that were identified using the software that were less than 50 RFU in height were excluded as background (Lygo et al. 1994).

2.6.2 ABI 3130 Genetic Analyser

All products from the forceful contact study, amplified using the ABI AmpF*l*STR® SGM Plus® PCR kit, underwent electrophoresis in the 3130 Genetic Analyser (Applied Biosystems). The 3130 was set-up prior to electrophoresis by installation of an array and by the addition of a fresh bottle of POP 4 polymer. 1 x running buffer was added to the anode and cathode buffers as well as deionised water in the water reservoir. Before electrophoresis the samples were prepared by mixing 8.5 µl Hi-Di™ formamide with 0.5 µl of the internal size standard GeneScan™ 500 ROX™. 9 µl of the Hi-Di™/ROX™ master mix was then added to 1 µl of the sample in MicroAmp™ optical 96 well reaction plates. During the machine set-up the samples were denatured for three minutes at 95 ° C then placed immediately on ice. The plates were then placed in the analyser ready for electrophoresis. A plate record was then created

describing the samples being analysed, the polymer and size standard in use as well as the AmpF*l*STR® kit used during amplification. Electrophoresis was then performed. After electrophoresis the data were analysed using the GeneMapper® ID software v3.2, which combines the functions of both GeneScan® and Genotyper®. Peaks under 50 RFU were excluded as background. Full profiles were determined as those consisting of the 20 alleles for the autosomal markers, i.e. minus the Amelogenin locus.

2.7 Interpretation guidelines (Low Copy Number)

1. A consensus profile must be generated. If an allele is observed when the sample has been amplified and visualised in two separate rounds of PCR and electrophoresis then it can be considered as an allele.

2. If the peak is 9,999 RFU or less in area then stutters can be up 40 % of the area, but this value is 20 % if the peak is over 10,000 RFU (Whitaker et al. 2001).

3. Alleles that are observed in duplicated negative controls must be excluded if seen in sample results (Gill et al. 2000).

4. Allelic dropout can occur when LCN work is performed. If a single peak over 10,000 RFU is observed then it can safely be called as a homozygote genotype. 9,999 or under it must be assumed that dropout has occurred (Whitaker et al. 2001).

5. Peaks under 50 RFU can be excluded as background.

2.8 Mixture analysis

1. Identify potential stutters, 'pull-up peaks', imbalanced heterozygous loci and N-bands (Clayton et al. 1998).
2. If more than two peaks present at one or more loci after exclusion of stutters etc then mixture analysis should be performed (Clayton et al. 1998).
3. Determine the numbers of individuals whose alleles are present in the profile (Clayton et al. 1998).
4. Determine the major and minor contributors to the profile by calculating the admixture ratio (Clayton et al. 1998).
5. Identify the possible pairings of alleles at each locus (Clayton et al. 1998).
6. Compare the generated DNA profiles to known reference samples and determine whether the suspect can be excluded from contributing to the sample (Clayton et al. 1998).

2.9 Statistical analysis

Prior to the actual statistical testing, the descriptive statistics function of SPSS was used in order to determine if the data from both studies was normally distributed and therefore whether parametric or non-parametric tests should be used. Normally distributed data forms a symmetrical shape when plotted on a histogram or has a significance value of 0.05 or over using the Kolmogorov-Smirnov test. Both datasets were produced using the explore function within the descriptive statistics. All the data generated as part of the thesis was found not to be normally distributed and therefore non-parametric statistical testing was employed. Although non-parametric tests are less sensitive than parametric they were deemed appropriate for these results due to the small sample size rather than transforming the data so that parametric tests could be utilised. Normal distribution test data can be seen in the Appendix (6.1.5 and 6.2.4).

2.9.1 Children's faces

Using SPSS 14.0 for Windows it was found that the data retrieved from this study was not normally distributed. The explore function of the descriptive statistics was used to analyse the data and the resulting histograms and Kolmogorov-Smirnov statistics indicated that the data from this study was not normally distributed. Therefore non-parametric statistical tests were employed, including the Friedman test, Spearman's Rank Order Correlation, Mann-Whitney and Kruskal-Wallis, as described by the software.

The Friedman test is a non-parametric version of ANOVA was used to determine the significance of any variations in allele number observed between the 12 areas of the head and neck as well as the comparison of left versus right side of the head and neck. The test was chosen as each subject was measured repeatedly and the data were found not to be normally distributed. The confidence interval was 95 %.

Spearman's Rank Order of Correlation is used to determine the strength as well as direction of any correlation between two groups of data which are not normally distributed. The correlation can indicate that one of the variables affects the other in either a positive or negative manner in addition to providing a value of the strength of the relationship. This statistical test was used to compare the effects of wash number, time since washing and age on the total number of alleles observed across the entire head and neck. The confidence interval was 95 %.

Sex and the time of day that sampling was performed were tested, statistically, using the Mann-Whitney U Test, which is the non-parametric version of the independent samples t-test. This test compares the differences between two groups using a continuous measure, such as total allele number. The confidence interval was 95 %.

The final test used was the Kruskal-Wallis which is also a non-parametric equivalent of ANOVA. Unlike the Friedman this test does not compare results for the same individual who has been tested repeatedly, but compares three or more groups of individuals, similar to the Mann-Whitney. Therefore Kruskal-Wallis was used for the

data when divided into groups of cloth and cleanser type used for washing the faces of the subjects. The confidence interval applied was 95 %.

2.9.2 Forceful contact

As with the Background Levels study statistical testing was performed using SPSS 14.0 for Windows. Some of the data was found to be non-normally distributed and so the Kruskal-Wallis, Friedman and Wilcoxon Signed-Rank tests were used. Although other data was found to be normally distributed using the Kolmogorov-Smirnov test it was determined that non-parametric testing should be used for all data sets. The Kruskal-Wallis and Friedman tests were both employed for the results generated from the Background Levels study.

The Wilcoxon Signed-Rank test is a non-parametric version of the repeated measures t-test and was used for samples that were measured on a number of occasions, in particular when the samples were amplified using both 28 and 34 cycles of PCR.

3. Determination of the ‘normal’ distribution of DNA across the faces of children aged 0-5 years

3.1 Introduction

Over the past ten years there have been several studies investigating the propensity of individuals to shed and transfer DNA. These include transfer between individuals (van Oorschot and Jones 1997; Rutty 2002; Banaschak et al. 1998), as well as several studies determining the DNA transfer from individuals to inanimate objects (Table 3-1):

Tanaka et al. (2000)	Toothbrush
Webb et al. (2001)	Lip Cosmetics
Abaz et al. (2002)	Drinks Containers
Watanabe et al. (2003)	Cigarette Butts
Bright and Petricevic (2004)	Shoes
Esslinger et al. (2004)	Exploded Pipe Bombs
Petricevic et al. (2005)	Bedding
Graham et al. (2007)	Earprints

Table 3-1: Published studies investigating DNA transfer onto inanimate objects

These studies and others have shown that transfer of DNA, both primary and secondary, can occur between individuals as well as between individuals and objects. Rutty (2002) investigated transference by simulated manual strangulation and took control samples from the non-contact side of the neck. One out of the 29 control samples had a partial profile matching the individual applying the force to the test area. Several of the control samples also exhibited alleles of one or more unknown individuals. These results indicate that DNA can be transferred without direct contact between an individual and a particular surface and therefore DNA on an individuals skin retrieved after an assault may not necessarily be that of the offender. Bright and Petricevic (2004) also directly swabbed the skin of the upper and sole of the feet as

well as the hands but while they gathered different quantities of DNA depending on the individual, no non-subject DNA was amplified. With the exception of the mapping of DNA profiles on adult necks by Graham and Rutty (2007), no other research has determined what DNA is normally present on the skin through daily activities and contact with others, either in adults or children.

3.1.1 Aims and objectives

While the signs of physical abuse may be easy to identify, proving the individual responsible is often a much more difficult task. Children under the age of five are one of the most at risk age groups with regard to physical abuse, with the head and neck being common targets (Atwal et al. 1998). DNA profiling is now one of the most widely employed forensic techniques, but in order for it to be used in the identification of the perpetrators of physical child abuse data needs to be generated on what DNA profiles can be generated from a child's skin under 'normal' conditions. Therefore the aim of this Chapter is to determine the normal levels of DNA that can be retrieved from the head and neck of children aged 0 – 5 years. In addition details on care techniques such as washing habits will be recorded to determine their effects on the DNA retrieved.

3.2 Methods

3.2.1 Sampling

Prior to the beginning of the investigation a protocol was designed for the sampling and downstream processing of swab samples taken from the children's head/neck. This included obtaining ethical permission from the Multi Centre Ethics Committee, Cardiff, 18th March 2003 (MREC number 03/9/29). A face map, as seen in Figure 3-1, was then devised, using Poser V5 (Curious Labs, CA, USA) in order to divide the children's head and neck into easy to swab areas that also corresponded with the most common sites of abusive bruising (Atwal et al. 1998). The sampling protocol was laid out to ensure a consistent approach towards the sampling of each child and can be seen in Appendix 6.1.1.

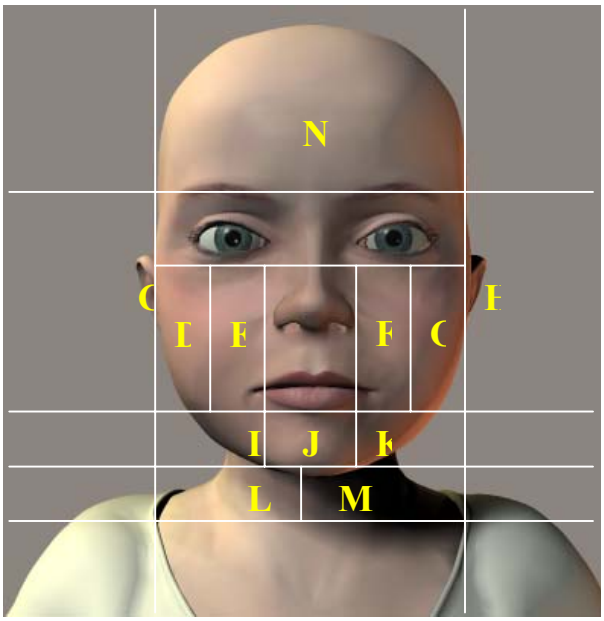


Figure 3-20: The twelve areas of the head and neck used for sampling

Before sampling all swabs were labelled with a code in order to anonymise the samples; this was performed in Leicester prior to transportation to Cardiff. Questionnaires were provided (see Appendix 6.1.2) to log the childcare details of each volunteer, on the same day as sampling only and consent forms were signed by the child's carer. For each subject sampling was performed by the same child protection

nurse to remove sampler variation. The sampler was requested to swab the skin firmly but without causing reddening of the skin. Several adult practice cases were performed to ensure the sampler knew the pressure to apply. Each area of Figure 3-1 was swabbed with a sterile swab, dipped in sterile water while wearing sterile gloves, individually packaged, but no masks or lab coats. This protocol was identified as being acceptable during work performed prior to the initiation of this particular research (Maguire et al. 2007). The sampler also refrained from talking within one metre of the subject (Rutty et al. 2003). A sterile swab was left unopened to act as a control for the swab batch, and to act as an extraction blank, while another was dipped in the water to act as a water control. This control identifies any contamination in the water that is used to moisten the cotton swabs prior to sampling. A number of swabs were also included for buccal samples and were collected from the subject whose face was being swabbed, the sampler, as a positive extraction and sampling control, and from the relatives/carers who had contact with the subject in the hours immediately prior to sampling. The samples were sealed in police tamper-proof evidence bags (WA products, UK) and couriered to Leicester on dry ice. Thirty-two children were sampled in Cardiff in this manner. They were recruited by a paediatric nurse who contacted friends and relatives with children aged five years and under. All parents/guardians were fully informed of what participation would involve and were given at least 48 hours to think about their decision. Sampling was then performed in the home of the subject, at a time convenient to them, mostly with the subject being held on the carer's knee to hold them still. No repeat sampling was performed once the twelve surface swabs and buccal samples had been obtained.

3.2.2 Sample processing

After the samples were delivered to Leicester they were stored in a -80ºC freezer until processing. DNA was extracted using the Chelex® method and quantified using the Nanodrop™ spectrophotometer, as the only quantification method available to us at the time. The Nanodrop™ results chart included a ratio of the sample absorbance at 260 and 280nm. A ratio of approximately 1.8 indicated the DNA was pure, lower meant contamination with proteins while higher meant RNA was the primary component. In all samples for this study the ratio was approximately 1.8.

Amplification was performed in the GeneAmp 9700 thermocycler using the SGM plus® PCR amplification kit. Both 28, as per manufacturer's instructions and 34 cycles (LCN protocol), of PCR were used. The resulting PCR products were visualised using a 377 DNA Sequencer and analysed using Genescan® and Genotyper® software. Statistical testing was performed using SPSS 14.0 for windows non-parametric tests. All protocols and LCN interpretation guidelines are detailed in Chapter 2.

3.3 Results

All facial and buccal samples from all 32 subjects were amplified using 28 and 34 cycles of PCR. But only four subjects exhibited any alleles under the standard, 28 cycles, conditions (Appendix 6.1.6), therefore all charts were generated using the LCN data. Tables of quantification results, and profiling results, both 28 and 34 cycles, can be seen in the Appendix 6.1.4 to 6.1.7.

After 28 cycles of PCR all controls were free from contamination. When amplified using 34 cycles two of the swab controls exhibit DNA profiles that do not fully match the sampler, subject or the individual processing the samples. Both the swabs were from the same batch and the profiles are not consistent with each other and are not seen as contamination through the other swabs in the batch either. It is possible that they are sporadic contamination from the general environment and/or operators, as seen when performing LCN PCR (Gill et al. 2000). The same pattern is seen for the six water controls with contaminating alleles.

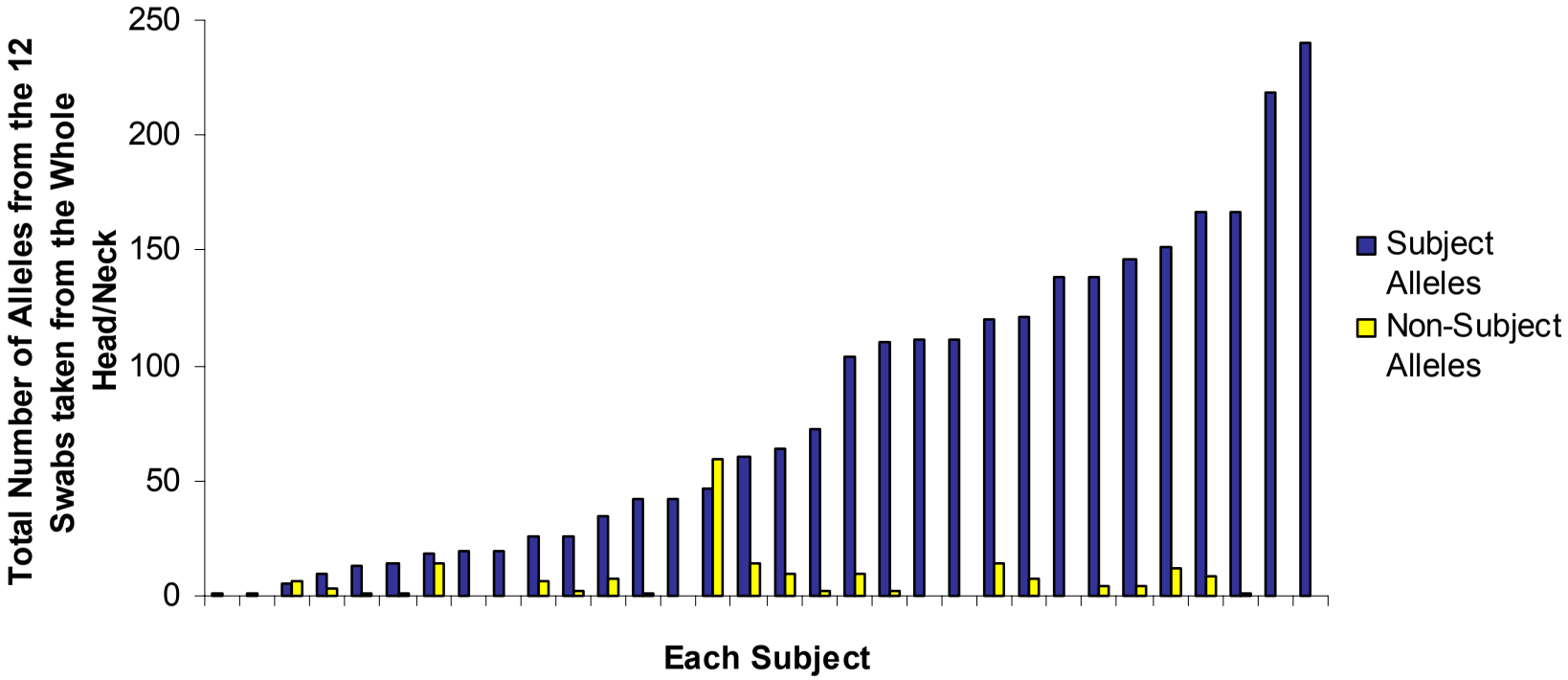


Figure 3-21: Total number of alleles amplified (max = 240) across the whole face for each of the 32 subjects

Using SPSS the results in Figure 3-2 were found not to be normally distributed with a significant result using the Kolmogorov-Smirnov test (sig – 0.029 for the subject alleles and 0.00 for non-subject alleles). A non-significant result of 0.05 or more indicates the data is normally distributed (SPSS manual). The total number of alleles sampled from all areas of the head and neck for each of the 32 subjects can be seen in Figure 3-2. The profiles generated were split into two categories, those that matched the reference profile of the subject and those that did not. A full profile at all of the sites sampled would produce a total of 240 alleles. Homozygous loci were still counted as two alleles. As the chart shows, the number of subject alleles ranges from only one to the full complement of 240, with a mean value of 80. The number of non-subject alleles ranges from zero for 10 of the 32 subjects, to 59 with a mean of six.

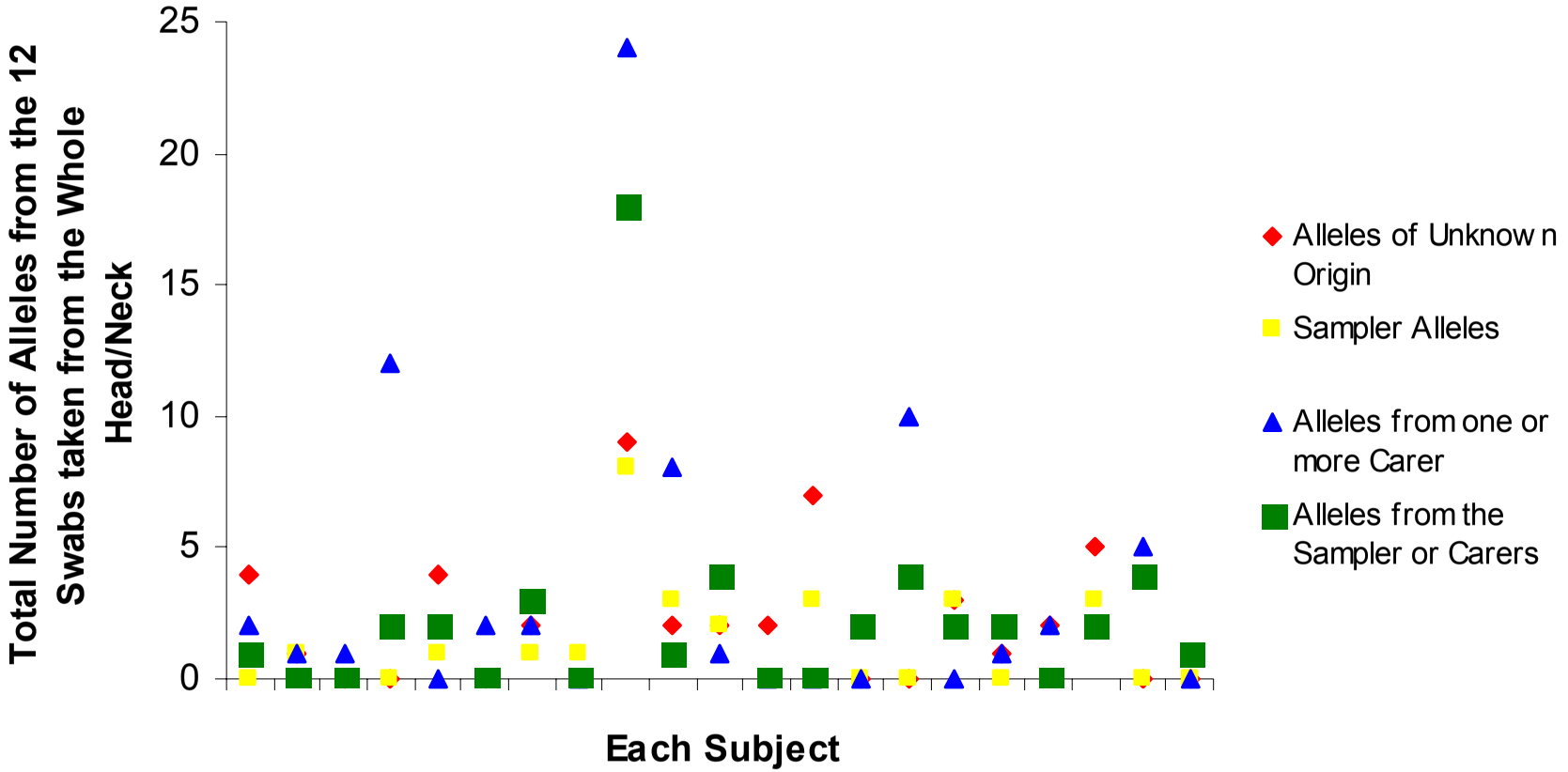


Figure 3-22: Total number of non-subject alleles and their possible origin from the whole face for each of the 21 subjects with non-subject DNA

The non-subject data from Figure 3-2 are shown in Figure 3-3 in more detail to determine the possible origin of these alleles. As part of the questionnaire data the subject's carer was asked to detail the individuals who had been in contact with the child in the twelve hours prior to sampling. Buccal samples were obtained from these individuals, such as mother, father, grandparents and babysitters, as well as from the sampler. As the samples were taken directly from the skin of a particular subject alleles matching their reference profile were first extracted from the DNA profile generated once artefacts were removed. The remaining alleles were compared to the reference profiles of the relatives and the sampler. Alleles were then categorised into the three categories listed in Figure 3-3. Any alleles not matching any of the reference profiles of individuals involved in the study were described as unknown. The number of alleles retrieved from the faces that do not match the profile of these 21 subjects range from one to 59. 76% display ten or fewer non-subject alleles across the whole head and neck. The remaining 24% exhibit between 12 and 59 non-subject alleles spread over at least four of the 12 areas. In all cases these alleles appear to be from more than one individual, supporting results by Rutty (2002) and Graham and Rutty (2007) who both found mixed DNA profiles during their investigations of simulated manual strangulation. However, relatives would be expected to share multiple alleles with the subject. Under standard PCR conditions these common alleles would be distinguished by determining peak height/area ratios, a procedure that is not possible under LCN due to stochastic variation inherent with 34 cycles of PCR as described earlier.

Tables 3-1 to 3-3 are examples of the DNA profiling results for three subjects, further emphasising the differences observed between each subject. But the results do indicate that of the subjects with non-subject DNA on their faces the alleles do appear to be from multiple origins, each too few for definite conclusions as to their potential origin.

	D3	VWA	D16	D2	D8	D21	D18	D19	TH01	FGA
Swab Control	/	/	/	/	/	/	/	/	/	/
Water Control	/	/	/	/	/	/	/	/	/	/
82C	14,16	16,18	12,13	24,25	10,14	28,29	12,13	14,15	7,8	21,25
82D	14,16	16,18	12,13	24,25	10,14	28,29	12,13	14,15	7,8	21,25
82E	14,16	16,18	12,13	24,25	10,14	28,29	12,13	14,15	7,8	21,25
82F	14,16	16,18	12,13	24,25	10,14	28,29	12,13	14,15	7,8	21,25
82G	14,16	16,18	12,13	24,25	10,14	28,29	12,13	14,15	7,8	21,25
82H	14,16	16,18	12,13	24,25	10,14	28,29	12,13	14,15	7,8	21,25
82I	14,16	16,18	12,13	24,25	10,14	28,29	12,13	14,15	7,8	21,25
82J	14,16	16,18	12,13	24,25	10,14	28,29	12,13	14,15	7,8	21,25
82K	14,16	16,18	12,13	24,25	10,14	28,29	12,13	14,15	7,8	21,25
82L	14,16	16,18	12,13	24,25	10,14	28,29	12,13	14,15	7,8	21,25
82M	14,16	16,18	12,13	24,25	10,14	28,29	12,13	14,15	7,8	21,25
82N	14,16	16,18	12,13	24,25	10,14	28,29	12,13	14,15	7,8	21,25
Subject	14,16	16,18	12,13	24,25	10,14	28,29	12,13	14,15	7,8	21,25
Sampler	15,16	15,17	9,13	18,19	10,13	29,29	13,16	12,14	9.3,9.3	19,24

Table 3-2: DNA profiling results for subject T0082 after 34 cycles of PCR

The results in Table 3-2 show that the samples taken from subject T0082 resulted in full profiles of the subject from all areas of the face and neck. This is the only subject in Figure 3-2 with the full 240 alleles. Both control samples are free from contamination. No alleles were observed that did not match the reference profile of this subject.

	D3	VWA	D16	D2	D8	D21	D18	D19	TH01	FGA
Swab Control										
Water Control										
21C					12					
21D								12		
21E	14,15							12,14		
21F	14,15	18,19			8,13			12,14		
21G	14,15							12,14		
21H										
21I		16	9,10		12,13	28				
21J	14							12,14		
21K	14,15							12,14		
21L										
21M	14							14		
21N										
Subject	14,15	18,19			8,8	28,29	14,14	12,14	9.3,9.3	22,25
Sampler	15,16	15,17	9,13	18,19	10,13	29,29	13,16	12,14	9.3,9.3	19,24
Relative	14,17	18,19	9,11	18	8,14	30		14		22
Relative	14,15	18,19			13,14	30,30		12,14		22,25

Table 3-3: DNA profiling results for subject T0021 after 34 cycles of PCR. Alleles highlighted in red are of unknown origin, green match either the sampler or one of the subject's relatives

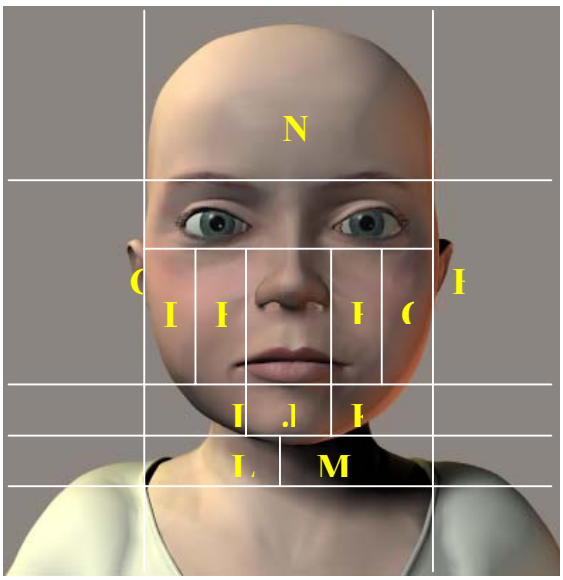
In contrast to subject T0082 in Table 3-2, only partial profiles have been generated from eight of the twelve areas for subject T0021 as shown in Table 3-3. Several non-subject alleles are also highlighted. Four of these are red and have no known origin, either from family members of the subject or lab staff. However two of these, alleles 9 and 10 at locus D16, may match the profile of the subject, but problems with the reference

sample from the subject failing to fully amplify in spite of multiple attempts, means that the origin of these alleles is unknown. The three remaining alleles all match the sampler as well as one or other of the two relatives.

	D3	VWA	D16	D2	D8	D21	D18	D19	TH01	FGA
Swab Control	16,17		10		12			14		
Water Control		18			13	29,30		14		
57C								14	7	
57D	15,17	14,18						16		
57E		18	9		13	28,30		14	7,9.3	
57F	16,17	15,16,17	9	17	12,13	28,29,30		14	7	
57G	17									
57H	16,17	15,16,17,18	10		10,11,12,13	28,29,30		12,14	7,9.3	
57I	15,16,17	16			12			14	9.3	
57J	16	15								
57K								14,15		
57L	15,16,17	16,17,18			12,13	28,29,30		12,14,17		
57M	17	16,17,18		20	12,13	30	12	14	7	
57N	15,16,17	14,16,18	9,10,11,12,14		13	28,29,30		12,13,14,16	7,9.3	
Subject	15,17	18,19	9,12	16,20	13,14	27,29	12,17	16,16	7,9.3	23,23
Sampler	15,16	15,17	9,13	18,19	10,13	29,29	13,16	12,14	9.3,9.3	19,24
Relative	14,15	16,19	11	20,24	12,13	27,30	17	14,16	7,9.3	20,23
Relative	17,17	16,18	9,10	16,25	11,13	29,31	12,16	15,16	6,9.3	18,23
Relative	14,17	16,18	12,13	16,20	13,14	27,29	16,17	14,15	6,9.3	23,23
Relative	15,17	16,18	12,13		12,13	27,29	12	14,15	7,9.3	18,20
Relative	15,16	14,15	12,12	22,25	14,15	30,31.2	15,15	14,15	9,9.3	22,24

Table 3-4: DNA profiling results for subject T0057 after 34 cycles of PCR. Alleles highlighted in red are of unknown origin, yellow indicates they match the sampler. Blue alleles match the profiles of one or more of the subject's carers, while green means the allele could be blue or yellow in origin.

Subject T0057 in Table 3-4 exhibits similar levels of subject DNA to that shown in Table 3-3. However there are many more non-subject alleles than seen previously, with a total of 59 across the whole head and neck, as noted in Figures 2 and 3. Eleven of the 12 sites exhibit alleles from a source other than the subject, five showing an unknown donor. Seven (E, F, H, I, L, M and N) indicate multiple origins of the non-subject alleles, even when excluding those of definite unknown origin. No single relative or the sampler matches all of the non-subject alleles in any of these seven sites. Some contamination is observed in the controls, but it is not the same for both and is not seen consistently through all of the samples. As the swab control was unopened until DNA extraction was performed contamination of this swab could only have occurred during manufacture or sample processing in the Forensic Pathology Unit. When the reference profiles of the Unit members are compared to the contaminating alleles no single match is found. All the swabs used for the samples in Table 3-4 were from the same batch, so contamination of the actual swab would be expected through all 21 samples. However unpublished data in the Forensic Pathology Unit has noted that contamination of individual swabs can be observed. Gill (2001) also noted that due to the sensitivity of the LCN protocol only a single molecule is necessary to contaminate a sample and therefore may be observed in only one out of a batch processed at the same time with no contamination in the control samples.



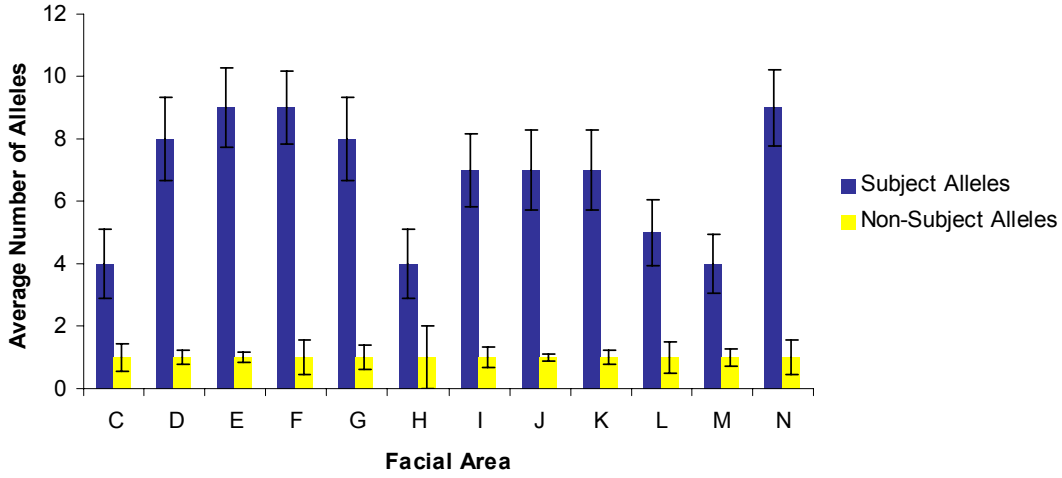


Figure 3-23: Average number of alleles from all 32 subjects for each facial area sampled

The average number of alleles from each of the areas of the head and neck for all 32 subjects are displayed in Figure 3-4. When examining the subject data, there is a pattern of slightly higher numbers of alleles on the inner cheeks of the children (E and F) than the outer (D and G) with equal averages for the left versus the right side. All three areas of the chin (I, J and K) exhibit the same average number of subject alleles, while the ears (C and H) are also even. Figure 3-4 appears to show a symmetrical pattern between the left and right sides of the face, with similar numbers of subject alleles being generated from the same areas of the left versus right side. This observation is confirmed in Figure 3-5. The average number of non-subject alleles is consistent across all areas of the head and neck. Non-parametric statistical testing

using the Friedman test (66.979, 11df) indicates a significant difference between the number of subject alleles observed across the 12 areas of the head and neck ($p < 0.005$). A significant difference (27.623, 11df) was also observed for the non-subject alleles ($p < 0.004$). This difference may be due to different degrees of moisture for each of the facial areas. More sweat may be secreted from areas such as the chin, forehead and cheeks than the ears resulting in more subjects DNA. When non-subject DNA was observed it was commonly on the chin and forehead, potentially areas that carers may have most contact with during cleaning and kissing. Overall the results do indicate that the location a sample was taken from will affect the DNA profile generated.

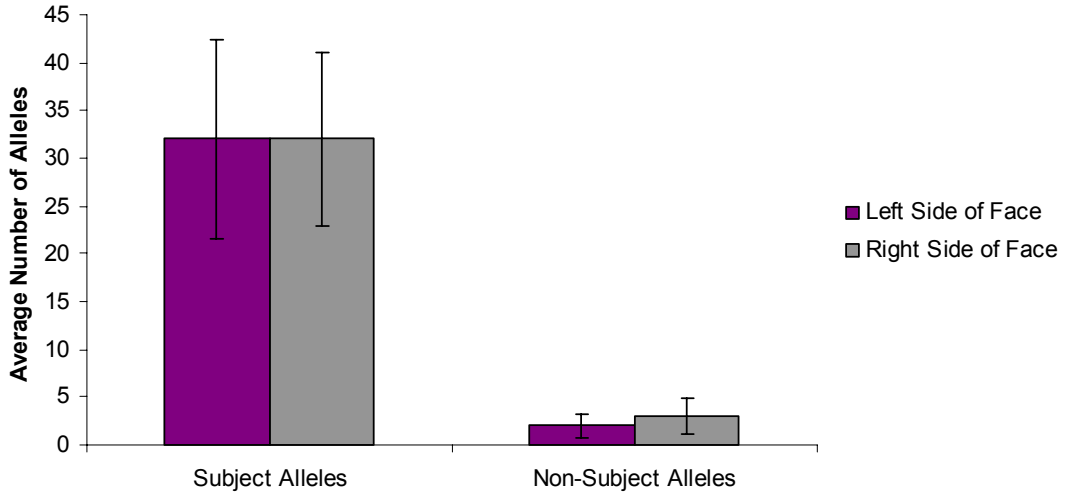


Figure 3-24: Average number of alleles from all 32 subjects from the left versus the right side of the face

When the sampled sites are grouped by left versus right side of the child, excluding sites J and N which are central, no difference is observed for subject alleles, with a marginally higher average for non-subject alleles sampled from the right side. Non-parametric statistical testing also indicated no significant difference between the two sides of the head/neck with regard to both subject and non-subject alleles.

3.3.1 Comparison of care techniques

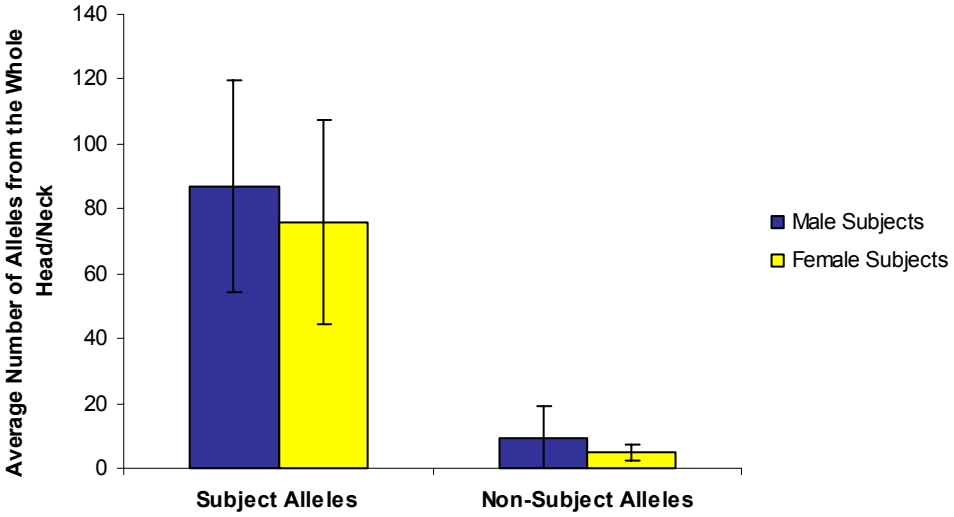


Figure 3-25: Average number of alleles from the whole face for male (n = 11) versus female (n = 21) subjects

For Figure 3-6 the 32 subjects were divided by sex to give 21 females and 11 males. The results for both subject and non-subject alleles indicate a slightly higher average number of alleles from the whole head and neck for male subjects than for female subjects. However no significant difference (Mann-Whitney test) was found between males and females with regard to subject or non-subject alleles.

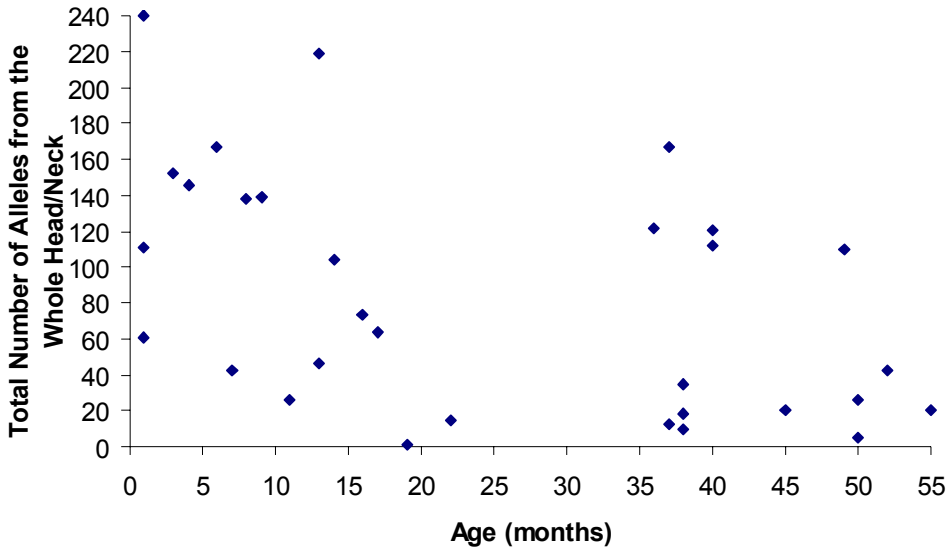


Figure 3-26: Total number of subject alleles (max = 240) from the faces of 31 subjects arranged by age

Questionnaire data regarding age were unavailable for one of the subjects. The scatter chart in Figure 3-7 shows the total number of subject alleles from the whole face for each of the 31 subjects, plotted by age. While there is a spread of alleles consistent with the expected person-to-person variation, as demonstrated by the three subjects aged one month (61 alleles, 111 alleles and 240 alleles), there is a general downward trend with age. This is confirmed by the Spearman's Rank Correlation Coefficient (-0.522) that indicates a strong negative correlation between total allele number and age ($p < 0.01$). This may be due to a decrease in care levels as the child ages and requires less constant attention in terms of wiping of food from the face, or application of baby lotion. A higher yield of DNA may be a result of friction between the face and bedding as the child sleeps, so as the infants age and sleep less sleep this may result in the general decrease in allele number observed in Figure 3-7. However it is likely that this apparent trend is purely due to the sample size and may not be a true reflection of change in shedder status with age. A study involving the sampling of individuals from birth throughout the years would provide a more accurate picture of how or even if shedder status alters with age.

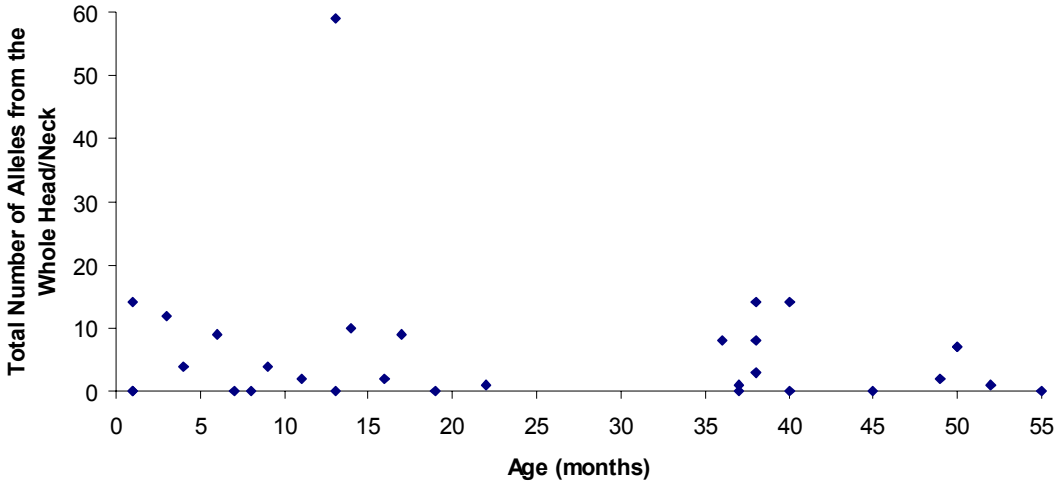


Figure 3-27: Total number of non-subject alleles (max = 240) from the faces of 31 subjects arranged by age

As with Figure 3-7, the chart in Figure 3-8 represents all 32 subjects displayed by age and total number of non-subject alleles observed across the whole head and neck. With the exception of one subject, aged 13 months, all have fewer than 15 non-subject alleles across all the sampled areas. Unlike the subject data there does not appear to be a downward trend in non-subject allele number with age. Equally an upward trend is not shown which may be expected as the number of individuals the child has contact with increases by going to nursery or play groups. The Spearman's Rank Correlation Coefficient indicates no significant trend for non-subject alleles with age.

Although the data shown in figures 3-7 and 3-8 demonstrates significant variation between individuals the results were averaged for the following figures in order to try and determine if any of the variables investigated were responsible for the variation already mentioned.

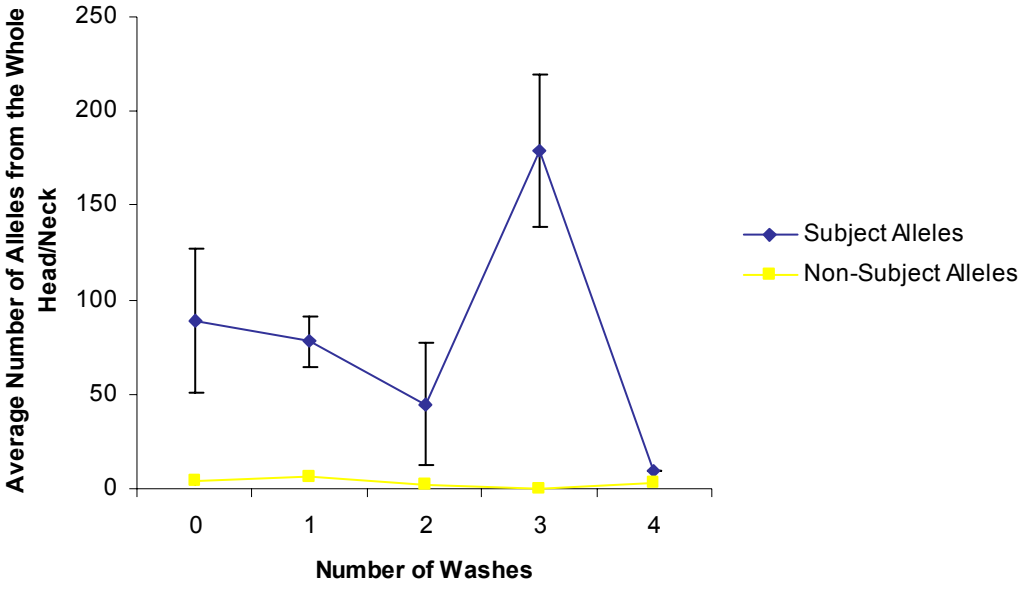


Figure 3-28: Average number of alleles from the faces of 30 subjects, grouped by the number of times their faces were washed. 0 washes, n = 6; 1 wash, n = 17; 2 washes, n = 4; 3 washes, n = 2; 4 washes, n = 1.

As with the data on age, the information on washing habits was detailed in the questionnaire that the subjects carers were asked to fill in prior to sampling. These data was only available for 30 of the subjects, due to time constraints during sampling, with six experiencing zero washes, 17 one wash and four were washed twice. Of the remaining three, two were washed three times and one four times, in the 12 hours prior to sampling. The allele numbers from the subjects with the same wash number were averaged, resulting in a slight downward trend as wash number increased. Little change is displayed for the non-subject data. However, statistically no significant difference (Spearman Rank Correlation Coefficient) was found in the number of subject or non-subject alleles as a result of different numbers of washes.

The data produced in the questionnaires that were filled in by the subjects' carers indicated a wide range of time intervals between last face wash and sampling. These intervals ranged from 15 minutes to in excess of 24 hours. Several of the subjects had the same interval period and therefore their allele numbers were averaged. Non-subject alleles remain reasonably consistent across the interval. A slight downward trend was indicated but no significant correlation between subject and non-subject allele number and time since washing was observed using Spearman's Rank Correlation Coefficient.

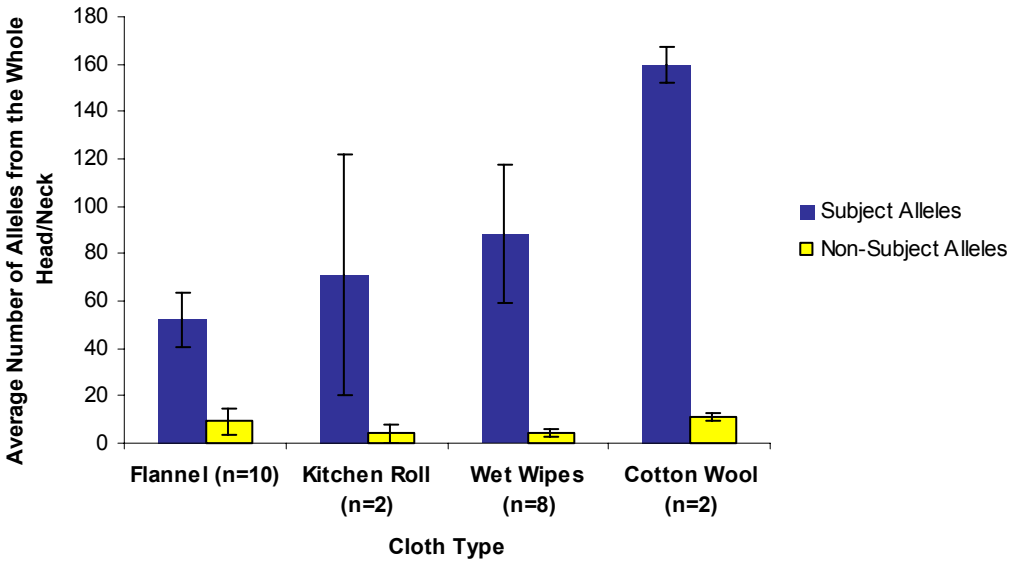


Figure 3-29: Average number of alleles on the faces of 22 subjects by the type of cloth used to clean their faces

Further details on wash habits that were recorded on the questionnaires included the items used for washing which have been broken down into cloth and cleanser, although wet wipes fall into both categories. Figure 3-10 represents the four cloth types used by the carers. Only two subjects had been cleansed using kitchen roll, and another two with cotton wool. Eight were washed with wet wipes and the remaining ten with a flannel. The error bars, generated for subject alleles after wash with kitchen roll show a big difference between the two subjects, one of whom had only 20 subject alleles across their entire face, while the other had 121. The large variation shown by the error bars may be due to the small sample size as well as the degree of variation between individuals previously noted. Those who had been washed using cotton wool exhibit on average over triple the number of subject own alleles compared to those who were washed with a flannel. However, non-parametric statistical testing (Kruskal-Wallis) shows no significant difference. Little difference is also observed in the number of non-subject alleles for any of the cloth wash technique, confirmed by the Kruskal-Wallis test.

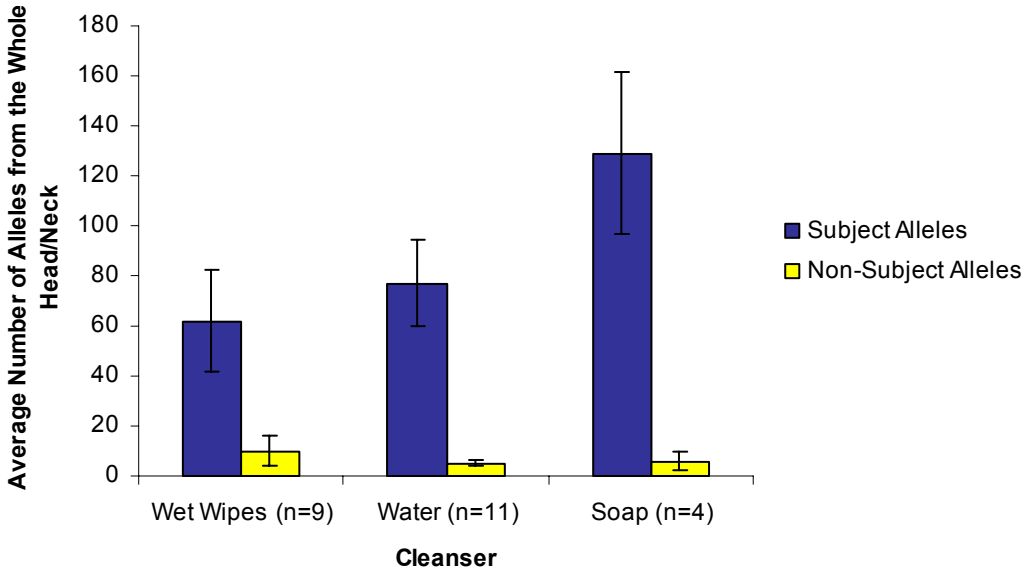


Figure 3-30: Average number of alleles on the faces of 24 subjects by the type of cleanser used to clean their faces

Four of the subjects were washed using soap, nine with wet wipes and eleven just with water. The non-subject data when segregated by cleanser type shows a similar pattern to Figure 3-10, in that little difference is observed between the three types. Both water and wet wipes show an average of less than 80 subject alleles from the entire head and neck, which is less than a third of the possible total. As was observed for the cloth type results no significant difference was found for subject and non-subject alleles (Kruskal-Wallis).

3.3.2 Comparison of sampling protocol

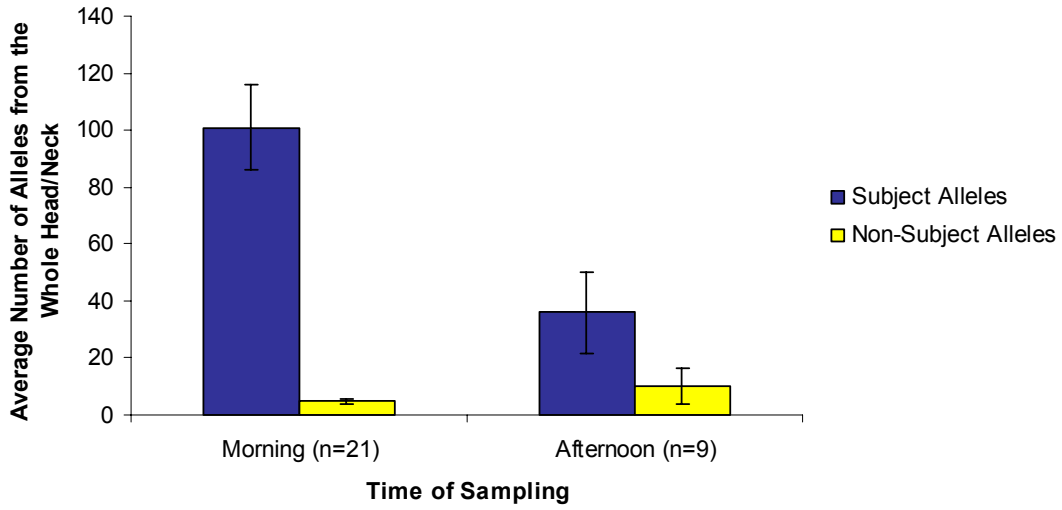


Figure 3-31: Average number of alleles for 30 subjects, grouped by the time of day they were sampled

In order to determine if the time of day that the samples were taken affected the subjects obtained, Figure 3-12 was plotted using the recorded time of sampling. Twenty-one of the subjects were sampled in the morning, while nine were sampled in the afternoon/evening. The chart indicates a definite decrease in subject alleles between morning and afternoon sampling, while a slight increase is observed for the non-subject alleles, but this increase is not significant (Mann-Whitney). The decrease observed between morning and afternoon sampling for subject alleles was found to be significant using the Mann-Whitney test (-2.671, $p < 0.008$), possibly due to those who were sampled later in the day having more contact with other people and objects depositing their free DNA prior to sampling, which may also account for the slight increase in non-subject alleles on those sampled in the afternoon.

3.3.3 Comparison of subjects with the same care techniques

Each of the tables for the subjects show differences between individuals with regard to the number of subject and non-subject alleles obtained from the head and neck. No single care technique significantly affected the subject or non-subject alleles sampled from the head and neck of the subjects. As there were several different combinations of care protocol described in the questionnaires, it was decided to compare the DNA retrieved from subjects with the same care profile.

	D3	VWA	D16	D2	D8	D21	D18	D19	TH01	FGA
Swab Control										
Water Control										
22C	15,16	14,17	9,12	18	10,12	29	14	14	7	19,24
22D	15,16				12			14		
22E										
22F	15,16				12			12,14		
22G	15,16	17						12,14		
22H	15,16							12,14		
22I	15,16					29		12,14		
22J						29		12		
22K										
22L		17				29				
22M								14		
22N										
Subject	15,16	17,18			12,12	28,29		12,14	9,9	24,27
Sampler	15,16	15,17	9,13	18,19	10,13	29,29	13,16	12,14	9.3,9.3	19,24
Relative	14,15	16,17	9		12,13			12		
Relative	15,16	17						14		
Relative	15,15	14,14	12,13	18,23	13,14	29,30		14,16	6,9.3	19,24

Table 3-5: DNA profiling results for subject T0022 after 34 cycles of PCR

	D3	VWA	D16	D2	D8	D21	D18	D19	TH01	FGA
Swab Control										
Water Control										
51C										
51D										
51E	14							15		
51F								15		
51G										
51H								12,15		
51I										
51J	15	14,19				28		15		
51K										
51L								15		
51M										
51N								14,15		
Subject	14,15	15,19	11,11		14,14	30,31.2		14,15	9.3,9.3	20,24
Sampler	15,16	15,17	9,13	18,19	10,13	29,29	13,16	12,14	9.3,9.3	19,24
Relative	15,17	15,17			13,14	31.2		15		20,21
Relative	14,17	18,19	11,11	18,23	12,14	28,31.2	14,16	14,15	7,7	22,24

Table 3-6: DNA profiling results for subject T0051 after 34 cycles of PCR

Subjects T0022 and T0051 both had the same care background details listed in their questionnaires. Both were female, 38 months old and had been washed once using flannel and water two hours prior to sampling. T0022 had a total of 35 subject alleles from all areas of the head and neck compared to only ten for T0051. Non-subject alleles numbered eight for T0022 and only three for T0051. Of the non-subject alleles the eight for T0022 are all located on site C (ear) and appear to be from multiple sources. The non-subject alleles for T0051 are also from multiple sources, and in addition are spread over two sites, H (ear) and J (chin). While the total numbers of subject and non-subject alleles observed for these two subjects are similar the location of the non-subject DNA does differ. It may therefore be possible that the combination of variables does influence the level of DNA retrieved but has no effect on the location of any transfer.

	D3	VWA	D16	D2	D8	D21	D18	D19	TH01	FGA
Swab Control										
Water Control										
32C	14,15	16,17						13.2,14		
32D	14,15	16,17	9,13	19,20	11,15	28,32.2	14,16	13.2,14	6	21,23
32E	14,15	16,17	9,13	19,20	11,15	28,32.2	14,16	13.2,14	6	21,23
32F	14,15	16,17			11,15	32.2		13.2,14		
32G	14,15	16,17								
32H	15									
32I	14,15	16,17			11,15	28,32.2		13.2,14		
32J	14,15	16,17	9		11,15	28,32.2		13.2,14	6	21,23
32K	14,15	16,17	9,13	19,20	11,15	28,32.2	14,16	13.2,14	6	21,23
32L	15									
32M										
32N	14,15	16,17			11,15	28,32.2		13.2,14		
Subject	14,15	16,17	9,13	19,20	11,15	28,32.2	14,16	13.2,14	6,6	21,23
Sampler	15,16	15,17	9,13	18,19	10,13	29,29	13,16	12,14	9.3,9.3	19,24
Relative	14,16	14,17	13,13	17,19	11,11	28,30	13,16	14,14	6,7	20,21
Relative	14,15	16,18	9,12	20,21	10,15	28,32.2	13,14	13.2,14	6,6	22,23
Relative	14,16	16,17	9,13	17,20	10,11	28,28	13,16	14,14	7,9.3	21,22

Table 3-7: DNA profiling results for subject T0032 after 34 cycles of PCR

	D3	VWA	D16	D2	D8	D21	D18	D19	TH01	FGA
Swab Control										
Water Control	13									
33C										
33D	14,15,16	16,17	9		10,11,15	28		14	6	21,23
33E	14,16	16,17	9,13		10,11	28		14	7,9	21,22
33F	14,16	16,17	9,13	17	10,11	28	13	14	7,9	21,22
33G	14,15,16	16,17	9		10,11,15	28,32.2		14	6,9	21
33H										
33I	14,16	16,17			10,11	28		14	7,9	21
33J	14,16	16,17	9,13	20	10,11	28	13,16	14	7,9	21,22
33K	14,16	16,17	9,13		10,11,15	28		14	7,9	21,22
33L	14,15,16	16,17	13		10,11	28		14	7	
33M		16			10,11			14		
33N	14,15,16	16,17	9		10,11,15	28,32.2		14	6,7,9	21,22
Subject	14,16	16,17	9,13	17,20	10,11	28,28	13,16	14,14	7,9	21,22
Sampler	15,16	15,17	9,13	18,19	10,13	29,29	13,16	12,14	9.3,9.3	19,24
Relative	14,16	14,17	13,13	17,17	11,11	28,30	13,16	14,14	6,7	20,21
Relative	14,15	16,18	9,12	21,21	10,15	28,32.2	13,14	13.2,14	6,9	22,23
Relative	14,15	16,17	9,13		11,15	28,32.2	14,16	13.2,14	6,6	21,23

Table 3-8: DNA profiling results for subject T0033 after 34 cycles of PCR

The two subjects in Tables 3-7 and 3-8 also represent two subjects with the same care profiles. Both T0032 and T0033 are females, aged 40 months who were washed once

with soap approximately two hours prior to sampling. No non-subject alleles can be seen in any of the samples from T0032 while T0033 has 14 spread over five of the twelve areas of the head and neck. All 14 potentially match one of two relatives who had spent time with the subject in the previous four hours. One hundred and twelve subject alleles were retrieved from the head and neck of T0032, while 120 were amplified for T0033. As with the previous comparison similar numbers of subject alleles were retrieved from both T0032 and T0033. However, there is a difference in the non-subject DNA profiles. Therefore it is more likely that the combination of care techniques employed have a consistent effect on the subject's own DNA that is retrieved but not on the non-subject. Transfer may result more from hugs and kisses and general contact than through actual childcare. A single contaminating allele is shown in the water sample from subject T0033, but is not present in the profiles generated from any of the other samples. This may be due to random LCN artefact or contamination as this allele does not match the profiles of the subject, sampler, subject's relatives or any of the laboratory users.

3.4 Discussion

The aim of this investigation was to determine the 'normal' distribution of DNA across the faces of children aged five years and under. Thirty-two children were recruited and sampled in a manner that caused as little distress as possible, before the

samples were processed as previously described in Chapter 2 and by Maguire et al. (2007).

While DNA profiles could be obtained from some of the children's faces using 28 cycles of PCR, the majority, 28 out of the 32, failed. Therefore LCN became a necessary part of the investigation, increasing risk and probability of contamination, as well as artefacts such as drop-in, drop-out and stutter. When the faces were sampled and samples were amplified using 34 cycles the resulting DNA profiles varied across the different facial areas as well as between individuals. This variation is consistent with results from most investigations into transfer especially those by van Oorschot and Jones (1997), Ladd et al. (1999), Abaz et al. (2002), Lowe et al. (2002), Bright and Petricevic (2004) and Phipps and Petricevic (2006) who all found that the propensity of individuals to transfer DNA varied from person-to-person, with the reason being as yet unknown. These results have been generally attributed to an individual's shedder status, identifiable by the DNA profile they deposit 15 minutes after hand washing. While it is not known what determines shedder status it is thought that the issue is not straightforward with several influencing factors such as time since washing and hand used. It has also been shown that an individual's shedder status is not always consistent on different days even when the time since hand washing was maintained (Phipps and Petricevic 2006). However shedder status has not been tested in children and no research has been performed on shedder differences between the face and hands. The variability between individuals is shown in the present study both in the subject and non-subject DNA profiles retrieved from the children's faces and it is probable that if they were sampled on multiple occasions the differences noted by Phipps and Petricevic (2006) would be observed. However, with the exception of one subject, the non-subject DNA has been shown to be minimal and consisting of mixtures of individuals, including relatives, the sampler and unknown individuals. The presence of alleles of unknown origin may be due to secondary transfer(van Oorschot and Jones 1997; Lowe et al. 2002) an occurrence observed by other studies but concluded to be of a limited nature (Wickenheiser 2002; Phipps and Petricevic 2006). Although few unknown alleles were observed they may have been present as a result of contamination, although any alleles present in the negative controls were excluded from the sample profiles and only alleles appearing in both repeat amplifications were included in the consensus. The probable origin of these alleles is either sporadic contamination during processing that can be seen in LCN profiles or

via secondary transfer from objects such as telephones or door handles via the subject's carers (Rutty 2002). This presence of non-subject DNA on the skin's surface was also found by Graham and Rutty (2007) when swabbing the necks of adult volunteers, although some of this was concluded to be transfer from partners or spouses as fewer non-subject alleles were observed on the necks of subjects who were single at the time of the study.

Prior to sampling, the head and neck was divided into 12 areas representing the most common sites of abusive bruising (Atwal et al. 1998). When the number of alleles detected for the 32 subjects was averaged for each area there were three groups of higher numbers of subject alleles – the cheeks, chin and forehead. The chin was lower than the cheeks or forehead, unexpectedly as potentially full profiles may have been generated from saliva on the chin (Graham, work in progress). However it is possible that these groups may shed more due to higher secretions, carrying cells with them, in these areas compared to lower secretions from the neck and ears (Wickenheiser 2002). When the non-subject alleles were averaged, no difference was observed between the facial areas indicating person-to-person variation, however there was found to be significantly more non-subject DNA on the forehead and inner cheek (F) than anywhere else. This may be due to location of kisses by carers, although the only study into transfer by kissing was by Banaschak et al. (1998) and investigates adult intra-oral kissing, with transfer between the subjects being observed.

In 2006, Phipps and Petricevic hypothesised that shedder status varied depending on washing habits and sampling occasions. Data were collected in questionnaires about the care techniques used for each of the 32 subjects of this study in order to determine their effect, if any, on the DNA retrieved from the head and neck. Statistical testing indicated no difference in subject or non-subject DNA between males and females, or any of the wash techniques employed, including number of times the face was washed, the time since last wash and the cloths and cleansers used. These results correspond with those found by Graham and Rutty (2007) when investigating the normal levels of DNA on the adult neck, as wash time and sex had no significant effect on the DNA recovered. Although no difference was observed when the faces were washed on several occasions to just once, significantly more subject alleles were retrieved when the sampling was performed in the afternoon compared to the morning. Those sampled in the afternoon tended to have had their faces washed more

often than those sampled in the morning but due to the previous finding this is unlikely to be the cause for the higher frequency of alleles. It is possible that more activity throughout the day may have resulted in friction and more epithelial cells and secretions being present on the skin's surface of those sampled in the afternoon leading to the amplification of more subject alleles (Wickeheiser 2002). While this may be the case, no difference was noted in non-subject alleles for the two groups. The final variable tested was age of subject; no difference was observed for non-subject alleles but a strong negative correlation was found between age and number of subject alleles. This may be due to friction with bedding or carers clothes. Such contact may decrease as the child ages resulting in less friction and therefore sloughing of epithelial cells.

Although, individually, each of the care factors discussed have little or no effect on the DNA profiles observed it is possible that a combination of these may be responsible (Phipps and Petricevic 2006). When comparing pairs of subjects with the same care profile, including age and sex, a similar number of subject alleles were observed. However this pattern did not follow with the non-subject alleles sampled from the head and neck.

While the mapping of DNA on children's faces has not been performed previously and the results provide the basis of new data for DNA transfer research, some limitations to the protocols were noted. Firstly, the sampling was performed in Cardiff and processing in Leicester. This could not be avoided as our colleagues in Wales had the access to the children and child protection nurses and therefore appropriate training for dealing with children. In addition they had obtained ethical approval for the project and limited funding, but they did not have facilities for DNA profiling. As a result all samples had to be transferred between the two areas after sampling. While studies have shown that this can be carried out with successful profiling at the conclusion (Walker et al. 1999) for buccal samples, the facial samples have far less DNA to begin with. Additionally on a number of occasions the samples had also been delayed in delivery. These were not included in the study as they were too degraded to get any results. But a number of the others showed evidence of degradation, even in the buccal samples (subjects T0021 and T0022). At present alternatives to shipping of swabs as described in this study are being investigated, including buffer systems such

as the Oragene DNA Self Collection kits from DNA Genotek which enable long-term storage of DNA at room temperature.

Additionally, some contamination was observed from the sampler. This was probably due to the lack of lab coats and masks however it was determined prior to this study that wearing of masks caused unacceptable distress to the children. During this study shedder status was not tested as the nurse informed us that to get the children to perform the shedder test as set out by Lowe et al. (2002) was not feasible and was also cause further distress to the child, due to the extra time involved. The shedder test as set out by Lowe et al. (2002) involved the gripping of a sterile tube for 10 seconds 15 minutes after hand washing. Our child protection nurse concluded from experience that to get many of the children in this age group to hold the tube as required without putting it in their mouths would be very difficult and potentially distressing to the child.

Due to the issues mentioned it would be advisable to repeat the research preferably with sampling performed in the same location as processing and with greater numbers of children involved. This would reduce the travelling time of the samples as well as potential freeze-thaw cycles that can result in DNA degradation. In addition the increase in subject number would improve the statistical significance of the data produced. With these data, information on who had kissed the child would also be useful for analysis, in case of transfer to the kiss site. An investigation into the testing of oral shedder status is already in progress in our lab which may be acceptable to children. Recently the Medical Research Council has awarded a grant to the Leicester/Cardiff research group enabling expansion of the investigation, including a greater number of subjects and the use of DNA extraction kits, Real-Time PCR equipment and capillary electrophoresis such as the 3130 Genetic Analyser from Applied Biosystems.

Further work could also be performed to explain how DNA may be transferred to children's faces other than through forceful contact. Information from the child protection nurse who performed the sampling indicated that abusive parents have explained away ominous bruising on their children as being caused by falling objects, the accidental hitting of a child with an object by a sibling, or a fall against a surface such as a corner of a table. It would be useful to determine if DNA can be deposited

over time onto a number of different objects under 'normal' conditions and see how long it can remain there before complete degradation occurs. These objects will also be swabbed and hit against other surfaces/objects over different time periods to see if DNA can be transferred from the object onto an individual and identified. The results can then, possibly, be used to eliminate these excuses. Similarly, if DNA is found to be useful as a means of determining the perpetrator of physical abuse then it is anticipated that the perpetrator(s) will explain away the presence of their DNA on areas of bruising by saying they had kissed the child after they had banged their head. The determination of possible transfer through kissing would be an important investigation. Banaschak et al. (1998) did investigate DNA transfer through kissing, but this involved adult intra-oral kissing with saliva exchange and is not relevant to this study. These studies could explain the presence of the non-subject DNA that was observed on the faces of the children in this study and potentially be used in conjunction with the data generated from the investigation into DNA transfer through forceful contact.

Finally expansion of the study to determine if the DNA profiles obtained from the child's skin changes as they age and care techniques alter could provide valuable information with regard to shedder status and DNA transfer. Sampling could be performed on individuals from birth through puberty and onwards. While multiple individuals of different age groups could be tested the inherent variations in shedder status observed in current research papers indicate that the ideal scenario would be to sample the same individuals at multiple points throughout their life time.

4. DNA transfer through forceful contact

4.1 Introduction

There have been several studies investigating the propensity of individuals to transfer DNA between each other as well as to and from objects. However there are only three that tackle the issue of DNA transfer through forceful contact, all involving simulated manual strangulation. In 1997 Wiegand and Kleiber simulated manual strangulation on the upper arm with sixteen suspect and victim pairs. Of the three STRs used to profile the epithelial cells retrieved from the victim after the simulation, successful typing of the suspect was achieved in 73% cases for VWA, 80% for FGA and 85% for CD4. Rutty (2002) performed a similar study involving a single male offender and female victim pair. The fingers of the offender were rubbed against the victim's neck for one minute, following the protocol set by Wiegand and Kleiber (1997). The situation was repeated ten times on multiple occasions allowing longer periods between contact and sampling. Of the 29 post-contact samples 19 yielded DNA profiles, 12 of which only matched the victim. Seven were mixtures of the victim and offender. Seventeen swabs were further amplified using LCN resulting in full victim and partial offender profiles in all cases, retrievable up to ten days after contact. However several of the control swabs, taken from the neck where no contact had been made, also yielded DNA matching the offender and one or more third parties. Similar results were found by Graham and Rutty (2007) when they simulated manual strangulation on the necks of ten adult volunteers. As with the previous two papers transfer to and from the victim was noted. In addition secondary transfer of the victim's partner's DNA from the neck was noted on the fingers of the offender. 24% of these samples also generated alleles of unknown origin, i.e. did not match laboratory staff or partners/spouses of the volunteers, a finding also noted by Rutty (2002). In addition to the manual strangulation simulation Graham and Rutty (2007) also determined the normal distribution of DNA across five areas of the neck of 24 adult volunteers. Person-to-person variation was found, as with van Oorschot and Jones (1997), Lowe et al. (2002) and Phipps and Petricevic (2006), but 14 of the 24 volunteers exhibited DNA of unknown origin, with greater numbers of alleles being noted for those with partners or spouses. No difference was observed between the five areas sampled and between male and females. Time since washing had no significant effect, in contrast to the results that have been obtained when samples are taken from

the hands (Lowe et al. 2002; Phipps and Petricevic 2006) although this may be due to all neck wash intervals being greater than one hour (Graham and Rutty 2007).

4.1.1 Aims and objectives

The studies outlined previously indicate DNA transfer through forceful contact can occur, however the only type of blow investigated was a rubbing motion. Punching and slapping are commonly seen in child and spousal physical abuse (Morris et al. 1997) but no research has been performed to determine if this mode of transfer has any effect on the DNA transferred between the individuals involved.

The overall hypothesis for this study is that DNA transfer will occur between individuals during a punch or slap. Therefore the first aim is to determine if DNA is transferred to a clean surface during forceful contact. Secondly the study will also aim to determine if offender DNA can be distinguished from the victim's after forceful contact and vice versa. In addition the study will investigate the effects of time between hand washing and contact as well as multiple blows on any DNA transferred.

4.2 Methods

4.2.1 Sampling

4.2.1.1 Focus pad investigation

Recruitment for this investigation was performed by poster advertisement and announcement in the departmental lab meeting. Ethical approval was requested but the South Leicester Committee informed us that it was unnecessary. However the subjects were provided with an information sheet and asked to sign a consent form prior to commencement. Subjects were asked to wash their hands fifteen minutes prior to sampling; additionally they were asked to fill in a questionnaire detailing their washing habits. Each was then asked to slap a sterile acetate sheet that was attached to a focus pad that is used for boxing and martial arts training. The sheet had been sampled with a sterile moistened swab prior to contact, as a control, and this was repeated after the slap was applied with only the area of contact being swabbed. This was then repeated with a single punch on a new sheet, using the same hand but on a separate occasion. All sampling was performed by the same individual.

All fifteen subjects were asked to return, provided they were happy to do so, on two more separate occasions to repeat the experiment under slightly different conditions. Both of these involved allowing one hour between hand washing and sampling, but the first involved only a single punch/slap, while the second involved punching and slapping the sheet three successive times on approximately the same spot.

Although acetate sheets are not an accurate representation of human skin, inexpensive alternatives to actual human contact for this preliminary investigation were difficult to come by. Pig skin was one such alternative but after consultation with potential volunteers it was deemed unsuitable as few people felt comfortable with its use.

4.2.1.2 Preliminary person-to-person investigation

Two subjects were recruited for the preliminary investigation after giving informed consent. Each was asked to refrain washing their hands for at least one hour prior to contact in order to minimise the effects of shedder status (Lowe et al. 2002). All

swabs were labelled prior to sampling and a universal tube of water was treated with UV light. The non-dominant hand and arm of each subject were swabbed with a moistened sterile swab prior to contact, to act as a control. Both subjects were then asked to firmly punch the upper right arm of the other, with sufficient force to redden the skin slightly. The areas of contact on the hands and arms of both subjects were then swabbed with sterile moistened cotton swabs. An unopened swab from the same batch was placed in an evidence bag with the test swabs before being placed in the -20ºC freezer. The procedure was repeated twice more on separate occasions. The whole experiment was then repeated with a short, sharp slap to the upper arm instead of the punch. Assailant A applied the blow to victim B and assailant B applied the blow to victim A for each of the repeats for each contact type.

4.2.1.3 Person-to-person contact investigation

As with the focus pad study ethical approval was requested but deemed unnecessary by the South Leicester Ethics Committee. Subjects were then recruited using posters and lab meeting announcements. All were requested to allow one hour between hand washing and. Prior to sampling all subjects were asked to fill in questionnaires and consent forms. The forearm of each subject as well as the palm and knuckles of their non-dominant hands were sampled with sterile moistened swabs prior to application of the blow. Each subject then slapped the upper arm of their partner, with their dominant hand, who then did the same. This was then repeated with the subjects punching the other arm of their partner. Areas of contact, as visualised by slight reddening of the skin, were sampled with sterile moistened swabs.

4.2.2 Sample processing

DNA was extracted from the swabs using the Qiagen QIAamp® DNA mini kit and quantified using the Quantifiler® Real-Time PCR kit from Applied Biosystems, with the exception of the preliminary study samples which were quantified using the Nanodrop™ ND-1000. Amplification was performed in the GeneAmp 9700 thermocycler using the SGM plus® amplification kit and 28 and 34 cycles of PCR. The resulting PCR products were visualised using a 3130 Genetic Analyser and analysed with Genemapper® ID software. All methods are described in Chapter 2. Mixture analysis was not performed on the samples taken from the acetate sheet as the primary objective was to determine the transfer from one individual and analysis was

performed with prior knowledge of the subject's reference profile. In addition mixture analysis was not carried out on the LCN samples due to the complications outlined in Chapter 1.

4.3 Results

4.3.1 Focus pad investigation

4.3.1.1 Example raw data after 28 cycles

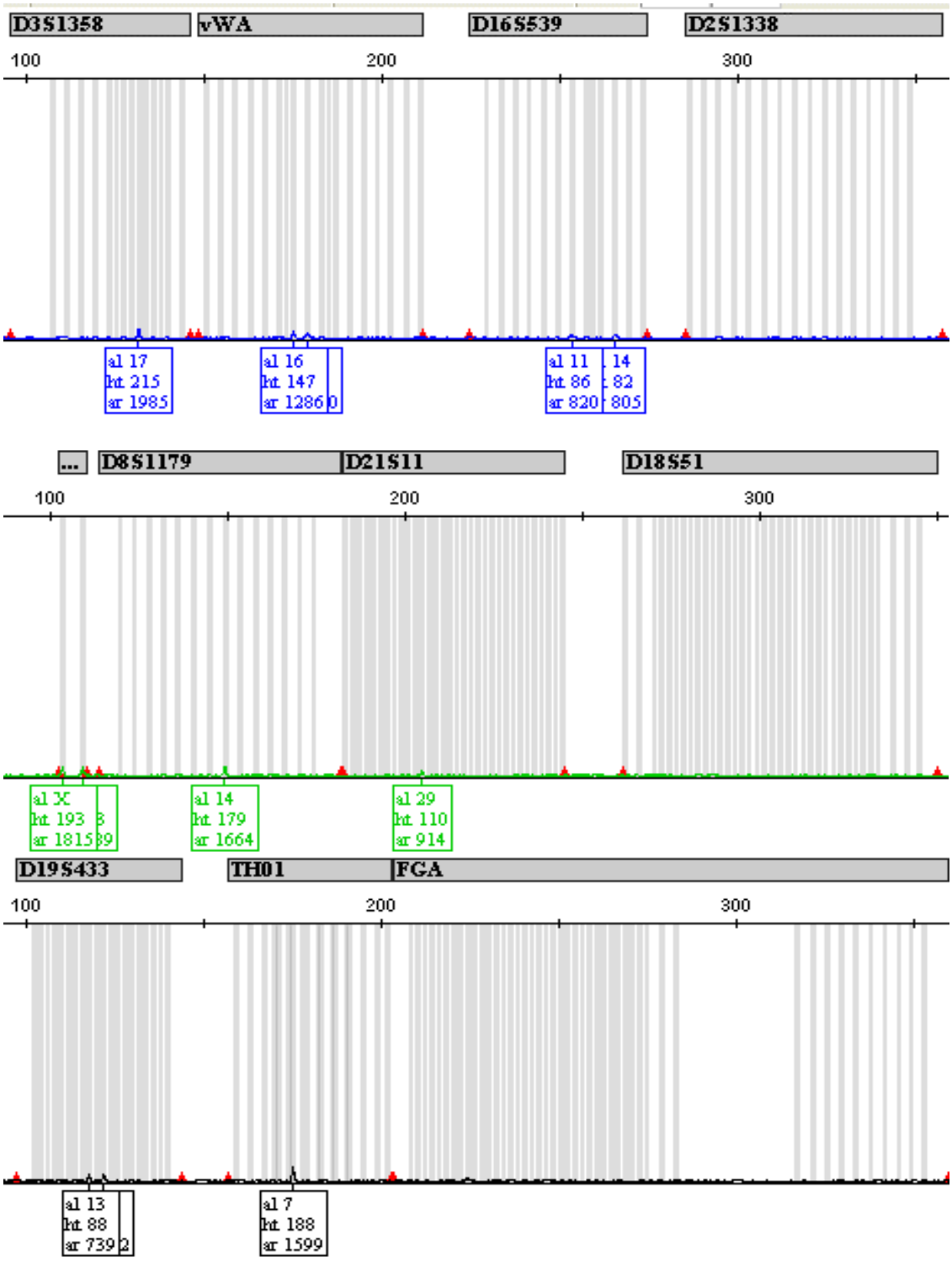


Figure 4-1: Electropherogram of the DNA transferred from one subject to the focus pad after a single slap, one hour after hand washing

Figure 4-1 is an example of the electropherograms generated from the samples when amplified using 28 cycles of PCR. Peaks can be seen at eight of the eleven loci included in the SGM plus® kit. The loci that are missing are D2, D18 and FGA due to the small quantity of amplifiable DNA present shown in the Appendix. Quantification using the Quantifiler® kit indicated only 15.7pg/µl of human DNA was present in this sample, a lot less than the optimum 1 ng.

The peak height and area data from the samples taken prior to contact can be seen in the Appendix 6.2.5.

		Loci									
Variable	Blow	**D3**	**VWA**	**D16**	**D2**	**D8**	**D21**	**D18**	**D19**	**TH01**	**FGA**
15 mins	Punch										
1 hour	Punch					59					
Multiple	Punch										
15 mins	Slap	75,115	162,202			52,186			173	137	
1 hour	Slap	215	147,127	86,82		179	110		88,95	188	
Multiple	Slap	79	56			61			52	113	

Table 4-3: Peak height data (RFU) for one subject after application of the blow, using 28 cycles of PCR

Despite the low concentration of DNA in the sample taken after a single slap, one hour after this subject washed their hands peaks can be seen in eight of the eleven loci on the electropherogram and the peak heights noted in Table 4-1 are all greater than the baseline threshold of 50 RFU.

Variable	Blow	Loci									
		D3	**VWA**	**D16**	**D2**	**D8**	**D21**	**D18**	**D19**	**TH01**	**FGA**
15 mins	Punch										
1 hour	Punch					537					
Multiple	Punch										
15 mins	Slap	812,1251 (0.65)	1575,1936 (0.81)			514,1827 (0.28)			1426	1244	
1 hour	Slap	1985	1286,1150 (0.89)	820,805 (0.98)		1664	914		739,772 (0.96)	1599	
Multiple	Slap	668	532			573			436	991	

Table 4-4: Peak area (RFU) data for one subject after application of the blow, using 28 cycles of PCR. The values in brackets show the heterozygote balance (Hb_x) of the two peaks

The peak areas displayed in Table 4-2 indicate a general trend of heterozygote balance, as expected at 28 cycles of PCR and shown by the Hb_x values all being over 0.6. The exception being the D8 locus for the sample taken after a single slap 15 minutes post-hand wash with Hb_x value of 0.28 indicating an imbalanced locus. This is because these two peaks represent a mixture of two individuals – the subject is homozygous for this marker and the smaller peak represents the secondary profile. Some allele dropout can be seen from the multiple slaps sample at vWA, amelogenin and D19. The single slap sample 15 minutes after hand washing also exhibits dropout at the D19 locus.

Variable	Blow	Loci									
		D3	**VWA**	**D16**	**D2**	**D8**	**D21**	**D18**	**D19**	**TH01**	**FGA**

15 mins	Punch							
1 hour	Punch				14			
Multiple	Punch							
15 mins	Slap	14, 17	16,17		13,14		13	7,7
1 hour	Slap	17,17	16,17	11,14	14,14	29,29	13,14	7,7
Multiple	Slap	17,17	17		14,14		13	7,7

Table 4-3: DNA profiles for one subject after application of the blow, using 28 cycles of PCR

As noted with the peak area data two alleles that do not match the reference profile of the subject are highlighted in Table 4-3. The control samples taken from the acetate sheet prior to contact were DNA free indicating that these alleles probably were transferred onto the sheet along with the subject's own DNA. Both the single punch 15 minutes after hand washing and the multiple punch samples show no transfer, while the single punch, one hour after hand washing only transferred a single allele as shown at the D8 locus. In contrast transfer can be seen after all three slap conditions. The transfer appears to increase when the interval between hand washing and contact doubles, from seven to 14 subject alleles. This corresponds with the data from Lowe et al. (2002) and Phipps and Petricevic (2006) that indicated shedding increases with time since washing. When multiple slaps are applied the transfer appears to decrease compared to the single contact, from fourteen to eight subject alleles. The decrease may indicate that most transfer occurs upon initial contact (van Oorschot and Jones 1997).

4.3.1.2 Example raw data after 34 cycles

The results in this section are shown prior to the generation of a consensus sequence and comparison to the reference profile of the subject.

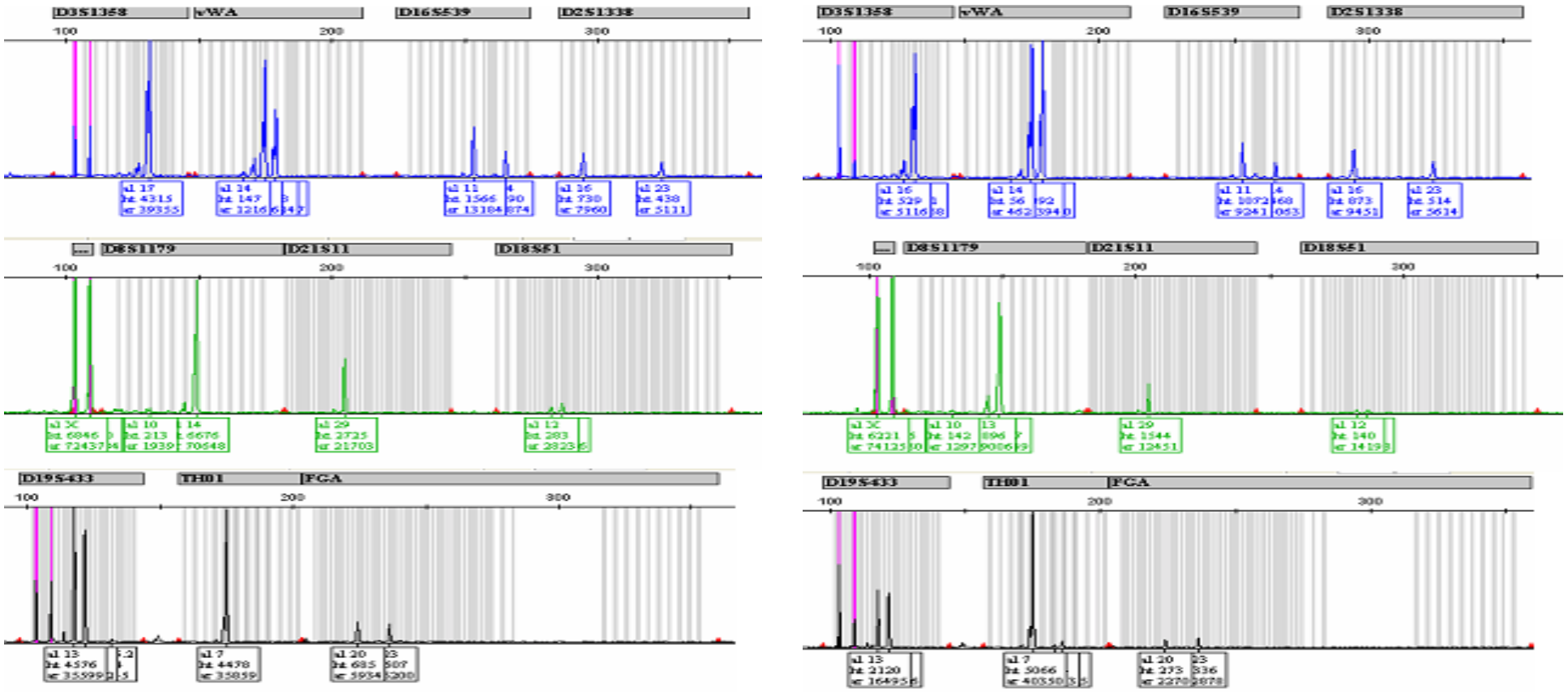


Figure 4-2: Electropherograms of the DNA transferred from one subject to the focus pad after a single slap, one hour after hand washing

Figure 4-2 represents the repeat DNA profiles obtained from one subject after a single slap that was applied one hour after hand washing, when amplified using 34 cycles of PCR. Both show full profiles that fully match the reference profile of the subject. In addition both show allele 10 at locus D8, which does not match the subject but its presence in both profiles indicating it is an allele rather than drop-in. Stutter can also be seen at the vWA locus both profiles as well as D3 and D8 in the second repeat. There is also evidence of drop-in at TH01 in the second profile that is not demonstrated in the first.

The peak height and peak area data for the samples taken prior to the application of the blow can be seen in the Appendix 6.2.5, along with the DNA profiles retrieved before contact. Alleles observed on the acetate sheet prior to contact were excluded from the post-contact profiles.

As expected when samples are amplified using 34 cycles of PCR each repeat shows different profiles, indicating the presence of spurious alleles as described by Gill et al. (2000), shown in Tables 4-4 and 4-5. Known heterozygous loci show imbalanced peaks, for example the first repeat of the single slap, one hour after hand washing at locus D16 has two peaks of 13184 and 6874 (highlighted in blue), as indicated by the Hb_x values under 0.6. Similar patterns are seen throughout the alleles listed, with some exceptions of more balanced heterozygotes, for example the peaks highlighted in red. Table 4-5 presents the corresponding DNA profiles for this subject, prior to the generation of the consensus profiles and comparison to the reference profile. At this point all samples show transfer of at least one allele.

Variable	Blow	Loci									
		D3	**VWA**	**D16**	**D2**	**D8**	**D21**	**D18**	**D19**	**TH01**	**FGA**
15 mins	Punch	16560	15901,28704 (0.55)	14961,7589 (0.51)	1598		11209		18399,2654 (0.14)	5391,4817 (0.89)	5093,1009 (0.20)
15 mins	Punch	2300	15993, 23557 (0.68)		12342	11322,14962, 26553,4663	7746,6374 (0.82)			3191,9611, 1781	2709,3683 (0.74)
1 hour	Punch	17742	8259,7299 (0.88)	2671	6817,1460 (0.21)	684,2970 (0.23)	7194,2390 (0.33)	1815,3020, 734	4614,10928,8555	2306,14853 (0.16)	1789,1972, 1701
1 hour	Punch	14231, 9214 (0.65)	8814,14808, 8068,7631	9084,6076 (0.67)	4149,7417 (0.56)	739,531,31890	14155	3824	5266	30646	6199,2425 (0.39)
Multiple	Punch	10237, 28417 (0.36)	7854,13674 (0.57)	1658,2811, 4075	1621	10009,1469,8136	3155	681	252941	17882,8102 (0.45)	3235
Multiple	Punch	12918	6213,1124 (0.18)		2545,696, 3025	719,2925,7658	2342,10227, 1457		1743,5295,3157	687,7689, 1055	497
15 mins	Slap		10093			13940,17324 (0.80)			15059		
15 mins	Slap					22222,99834 (0.22)					
1 hour	Slap	39355	1216,4926,3624, 18167	13184,6874 (0.52)	7960,5111 (0.64)	1939,70648 (0.03)	21703	2823,4486 (0.63)	35599,29182,745	35839	5934,6200 (0.96)
1 hour	Slap	34768	462,34394,5350	9241,4063 (0.44)	9451,5614 (0.59)	1292,61049 (0.02)	12451	1419,1193 (0.84)	16495,15976 (0.97)	40350,633, 1975	2270,2878 (0.79)
Multiple	Slap	33389	14912,40066,19125	12483,5395 (0.43)	1248,997 (0.80)	35358,3035 (0.09)	17602	803	24468,21535 (0.88)	40015,1872 (0.05)	1480,933 (0.63)
Multiple	Slap	9801	3672,8990,13008	2778,2424 (0.87)	719	2180,22334 (0.10)	8606		4680,20332,22060, 1179	26249,635 (0.03)	452,440 (0.97)

Table 4-4: Peak area data for one subject after application of the blow, using 34 cycles of PCR. The values in brackets show the heterozygote balance (Hb_x) of the two peaks

Variable	Blow	Loci									
		D3	**VWA**	**D16**	**D2**	**D8**	**D21**	**D18**	**D19**	**TH01**	**FGA**
15 minutes	Punch	15	17,18	9,11	17		27		12,13	7,9	23,24
15 minutes	Punch	15	17,18		17	9,12,13,14	27,30			6,8,9	23,24
1 hour	Punch	17	16,17	11	16,23	10,14	29,32.2	12,13,18	12,13,14	6,7	20,23,24
1 hour	Punch	15,17	14,16,17,19	11,14	20,21	8,10,14	29	12	13	7	23,26
Multiple	Punch	14,17	14,17	11,12,14	23	8,10,14	29	12	13	7,9.3	23
Multiple	Punch	17	16,17		16,22,23	10,13,14	28,29,31.2		12.2,13,14	6,7,9	20
15 minutes	Slap		14			10,13			13		
15 minutes	Slap					10,13					
1 hour	Slap	17	14,15,16,17	11,14	16,23	10,14	29	12,13	13,14,16.2	7	20,23
1 hour	Slap	17	14,16,17	11,14	16,23	10,14	29	12,13	13,14	7,9,9.3	20,23
Multiple	Slap	17	14,16,17	11,14	16,23	14	29	12	13,14	7,9.3	20,23
Multiple	Slap	17	15,16,17	11,14	16	10,14	29		12,13,14,14.2	7,9	20,23

Table 4-5: DNA profiling results for one subject after contact, amplified using 34 cycles of PCR

4.3.1.3 Quantification results

15 Minutes post-wash

Subject	Punch (pg/ul)	Slap (pg/ul)
1	Undetected	11.90
2	Undetected	29.70
3	1.55	40.20
4	Undetected	2.33
5	2.19	8.24
6	27.00	4.62
7	1.05	32.50
8	1.95	5.64
9	1.41	34.10
10	0.99	4.44
11	Undetected	47.90
12	Undetected	22.90
13	Undetected	67.50
14	1.44	12.90
15	Undetected	Undetected

One hour post-wash

Subject	Punch (pg/ul)	Slap (pg/ul)
1	Undetected	Undetected
2	Undetected	12.30
3	14.30	11.70
4	9.42	29.90
5	5.01	Undetected
6	Undetected	39.60
7	22.40	40.00
8	Undetected	15.90
9	9.22	98.40
10	36.10	33.80
11	8.19	97.50
12	8.46	15.70
13	33.60	29.50
14	Undetected	76.60
15	Undetected	11.10

Multiple contacts

Subject	Punch (pg/ul)	Slap (pg/ul)
1	Undetected	6.61
2	6.57	11.10
3	Undetected	103.00
4	Undetected	17.90
5	Undetected	6.84
6	13.20	15.20
7	Undetected	32.00
8	5.37	10.20
9	44.30	501.00
10	3.20	92.70
11	43.20	89.00
12	29.40	5.41
13	Undetected	230.00
14	Undetected	117.00
15	4.42	31.70

Table 4-6: Quantification results for all three sampling conditions

With the exception of subject 6, 15 minutes post-wash, subjects 3, 5, 10 and 13, one hour post-wash plus subject 12, multiple contacts more DNA was sampled after slapping than after punching, as shown in Table 4-6. A variation in concentration is also shown between the subjects with several samples showing undetected levels of DNA up to 501pg/ul (Subject 9, multiple slaps). Only six of the 23 samples showing undetected levels failed to amplify and those samples with the highest levels do not have the greatest numbers of alleles in the profiles. This may be due to insufficient mixing prior to quantification or because all the samples are at pg/µl level, far below the optimum of 1ng. The IPC results indicated no PCR inhibition.

Averages of these figures indicate an increase in transfer when comparing punch to slap samples; 15 minutes post-wash a single punch transferred on average 4.7pg/µl, compared to 23.2pg/µl for a single slap. One hour after hand washing the average post-punch transfer was 16.3pg/µl and post-slap 39.4pg/µl. Multiple contacts show the same trend with three punches averaging 18.7pg/µl, with 84.6pg/µl after three slaps. These increases are consistent with the increase in the number of alleles amplified after 34 cycles of PCR. The quantification results also indicate an average increase in transfer when the time since hand washing is increased from 15 minutes to one hour as well as when contact number is increased. This is consistent with the DNA profiling results with the exception of the decrease in allele number observed after three punches compared to one.

4.3.1.4 Overall DNA profiling results

The reference profiles for all subjects can be seen in the Appendix 6.2.6.1.

All samples were first amplified using 28 cycles of PCR. Of the samples taken after punching, only one individual transferred alleles 15 minutes after hand washing, three individuals one hour after hand washing and one individual after punching the sheet three times. More alleles were observed when the subjects slapped the sheet rather than punching but under each condition less than half of the subjects transferred anything and no full profiles were seen. Therefore all samples were also amplified using the 34 cycle protocol.

Alleles highlighted in red represent those that do not match the reference profile of the subject the sample was taken from.

15 minutes post-wash – 34 cycles

	D3	VWA	D16	D2	D8	D21	D18	D19	TH01	FGA
1										
2										
3	15	16		17	12,13	30	14	12	6	
4					13					
5	15	17						13,15.2	6	
6										
7										
8										
9										
10	15								7	25
11	15	17,18		17		27			9	23,24
12								13		
13										
14		14			12			14		22
15		18			12					

Table 4-7: DNA profiling results from the 15 subjects after a single punch, applied 15 minutes after hand washing

Fifteen minutes after hand washing the 15 subjects were asked to apply a single punch to the acetate sheet. The results in Table 4-7 show that only eight transferred any DNA to the sheet, with a maximum of eight out of the possible 20 being amplified. Five of the volunteers also transferred alleles of unknown origin, a finding consistent with van Oorschot and Jones (1997), Lowe et al. (2002), Rutty (2002) and Graham and Rutty (2007). These alleles may be secondary transfer from the subject's partner/spouse or possibly transfer from communal objects.

	D3	VWA	D16	D2	D8	D21	D18	D19	TH01	FGA
1	15,15	14,17	12	19,23	13	28,32.2	11,17	14,15.2	7,9	22
2	16,17,18	16,17,18	11,13	16,17,24	12,15	28,32.2	16	13	6,9	19,21,22
3	15,16	16,18		21	13,14	29,30		12,16.2	6,7	20,22
4	18	17		17	14			14		22
5				20	14			13,14		22
6								14	9.3	
7	14,17	14,16	11,14	20,24	11,13	30,31	12	13,14,16	7,9.3	22.2,24
8	14				12			14	6	
9		15			12,13,14			13,14,15	9.3	20,21,23
10	16	14,15,19	12	19,20	10,13	29	17	12,13	9.3	21,23
11					10,13					
12	14,15,17	16,17,18	11		14	29		13,14	7,7	20,23
13	14,15	14,18	11,12	19,21	12,13	29,30	14,15	13,13	9,9.3	21,25
14	16	18			12	30		14	6	
15			9							

Table 4-8: DNA profiling results from the 15 subjects after a single slap, applied 15 minutes after hand washing

Table 4-8 displays the same sampling conditions as Table 4-7, with a single slap rather than a punch. All subjects transferred at least one allele matching their own reference profile, ranging from one to 19 out of 20. Eight also transferred unknown alleles. In two cases they outnumbered the subject's own DNA, most likely to have been 'picked up' from communal door handles, telephones, hand-shaking etc. The increase in transfer compared to that seen after punching could be due to a number of factors. Wickenheiser (2002) hypothesised that transfer could be facilitated by secretions from the skin that carried nucleated cells to the skin's surface. The palm of the hand is often moister than the back, probably due to greater concentration of sweat glands, increasing the number of cells that can be transferred. In addition he suggests that the number of transferable cells may be increased when an individual rubs their eyes or mouth, both areas with a high turnover of cells, which may happen more often with the fingers than the knuckles. Equally the higher transfer could be due to the greater surface area applied to the sheet when slapping than is observed during a punch.

One hour post-wash – 34 cycles

	D3	VWA	D16	D2	D8	D21	D18	D19	TH01	FGA
1										
2										
3	15,16									
4			11	17				13	8,9	
5			13		14			12	6	
6										
7			14					13	7	
8								13,14		
9	13,17	17			10,13			15		
10	14,15	16,17	11		11,12,13,14	28,32.2		15.2,16	9	23,25
11	17	16,17	11		10,14	29	12	13	7,7	23
12										
13	14,15	14	9,11,12	19,21	12,13	29,30		13,13	9,9.3	21
14		18	11		12					
15				23	14	30		14.2		

Table 4-9: DNA profiling results from the 15 subjects after a single punch, applied one hour after hand washing

Eleven of the 15 volunteers transferred DNA to the sheet when the time since hand washing was increased to one hour. However, with the exception of three subjects, the transfer was limited to three or four alleles. These three all show an increase in number of alleles compared to 15 minutes post-hand wash, consistent with the findings of Lowe et al. (2002). As with the previous results a number of alleles are highlighted that do not match the reference profile of the subject and were not present on the sheet prior to contact. As these are different to the unknowns observed on the knuckles 15 minutes after hand washing it is probably from normal daily contact with other people and objects.

	D3	VWA	D16	D2	D8	D21	D18	D19	TH01	FGA
1			12		14				7	23
2	17	18	11	24	13,14			14		22
3	16	18			12,13			13	7	22
4	18	14,17			11,13,14				8,9	
5	15,18	17,18	13,14	17,20	14	32,32.2	19	13,14	6,9.3	22
6		17,18	12	24			15		9.3	24
7	14,15,16	14,15,16,17			10,11,13,14,16	27,28,30		12,12.2,13,14,15	7,9.3 6,9	
8	15	15,17,18	9,12		12	30,32.2		12,14,15	9.3	20,23
9	16	17,18	12,13	19,23	10,12	28,31		15,16.2	9,9.3	20,21
10		15,16	13,14	20,24	11,12,14			12,16	7,9	
11	17,17	14,16,17	11,14	16,23	14,14	29,29	12,13	13,14	7,7	20,23
12			13	19,20	13	29		13	9,9.3	21,23
13	15	14,18	12	19	12	30	14	13,13	9,9.3	21,25
14	14,15,16,17	18	11,12		8,12	30		14,15	6,7	23
15	14,15,17	14,16,18		16	11,14	32.2	18	15.2		19,20

Table 4-10: DNA profiling results for the 15 subjects after a single slap, applied one hour after hand washing

The single-slap results one hour after hand washing, in Table 4-10, are very similar to those observed after 15 minutes, with all 15 transferring their own DNA to the sheet, although the maximum is 16 alleles rather than the 19 previously noted. As with all the single contact samples, much person-to-person variation can be seen with some subjects transferring more 15 minutes after hand washing, for example subject 13 transferred 19 alleles which decreased to 13 when the interval was one hour, whereas subject 11 only transferred two alleles initially but this increased to 16 for the one hour protocol. The unknown alleles also showed no consistency with those observed after 15 minutes.

Multiple blows, (n = 3), one hour post-wash – 34 cycles

	D3	VWA	D16	D2	D8	D21	D18	D19	TH01	FGA
1	15									
2	15	17	11		12,13,14	28		12,13,14	9	
3	15,16				13					
4	15,16	14,17							8	
5	15,18							13	6	
6	15,16		12		13	28			8,9.3	
7	14,16,17	16	14	20	15	30				22.2
8									6	
9	16	18			10,14			13,14,15,16.2	9	21
10										
11	17	17		23	10,14	29		13	7	
12	15,16	14,15,19		19,20	10	28	17	13,16.2	6,9.3	
13	14,15	14			13				9	21
14	16	15	9	25	8,12	31.2		14,14	6,7	22,23
15		15						12,16.2	9.3	

Table 4-11: DNA profiling results for the 15 subjects after three consecutive punches, applied one hour after hand washing

Only one subject did not transfer any DNA after three consecutive punches, shown in Table 4-11, despite alleles being observed in their samples after both of the single punch tests. The number of subject alleles transferred by the others ranges from one to ten, while volunteer 15 only transferred alleles that did not match their reference profile. Other than more of the subjects transferring little difference can be seen

between these results and those after a single punch when the same time interval between hand washing and contact is maintained. This is consistent with the findings of van Oorschot and Jones (1997) that transfer occurs on initial contact. The same pattern can also be seen after three consecutive slaps, as seen in Table 4-12, with all 15 subjects transfer between one and 16 alleles matching their own profile and 11 also transferring unknown alleles.

	D3	VWA	D16	D2	D8	D21	D18	D19	TH01	FGA
1	15				14	32.2		14		22
2	16,18	17,18	11		11,13			14,14	9.3	22
3	15	16,18	10,12	21	14	29,30		12,13,16.2	6,7	
4	14,16,18	14,17	12		12,13,14	31.2		12,14,13	9	22
5	15	14,17	12	16,20	10,13,16	29,32.2	12,16	12,13,14	6,9.3	22
6	17	18	11	17,24		28	15,16	13,15	9.3	20
7	14,16	14,16,17			10,12,13	30		12,13,14,17.2	4,5,6,7,9.3	
8								14		
9	16,17	17,18	12,13	19	10,12	28,31		13,15,16.2	9	20,21
10	15		13,14	20,24	12	32.2		16	9	22
11		17	11,14	16	14,14	29		12,13,14	7,7	20,23
12	14	15,16						13	6	
13	14,15	14,18	11,12		12	29,30		13	9,9.3,7	
14	14,16,18	18,18	9,11		8,12	28,30,31.2		14,14	6,7	
15	16	14,15,16			14			14,14.2	7,8,9.3	19

Table 4-12: DNA profiling results for the 15 subjects after three consecutive slaps, applied one hour after hand washing

4.3.1.5 Comparison of sampling conditions

All bar charts were generated from the data obtained from the samples after 34 cycles of PCR.

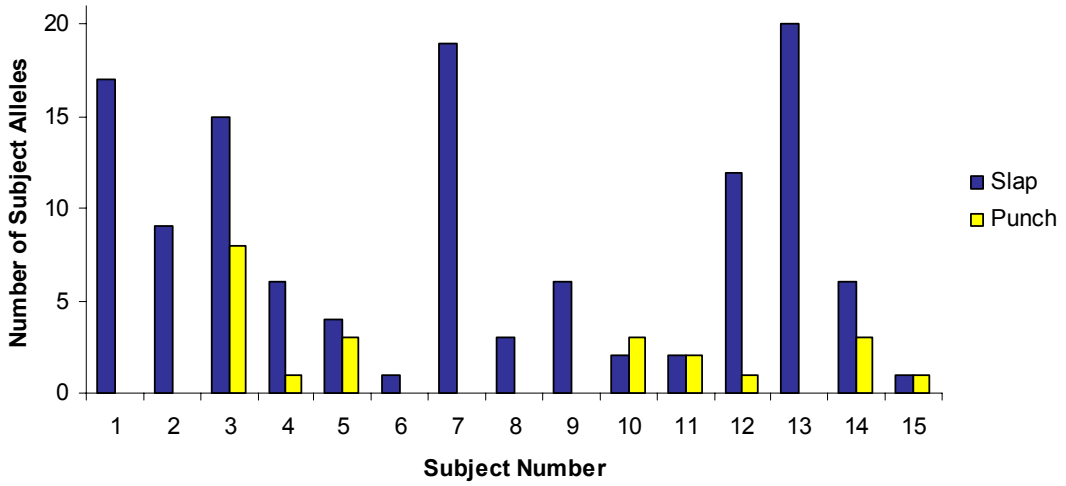


Figure 4-3: Number of subject alleles (max = 20) retrieved after a single punch or slap for each subject, 15 minutes after hand washing

Figure 4-3 summarises the data detailed in the previous Tables, highlighting the fact that 12 of the subjects transferred more of their own DNA via a single slap than by a single punch, 15 minutes after hand washing. Of the remaining three, two transferred equally with both forms of contact. The chart also highlights the person-to-person variation seen in all transfer studies.

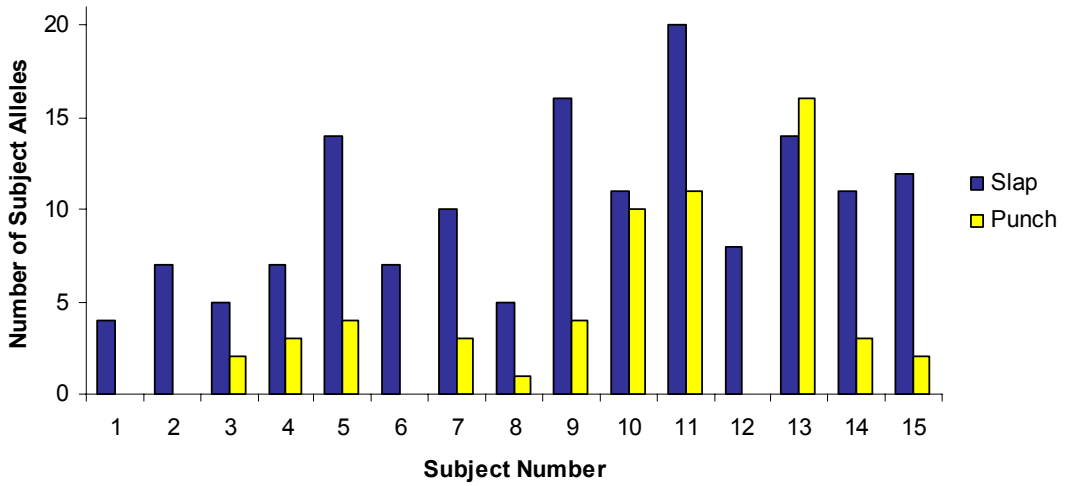


Figure 4-4: Number of subject alleles (max = 20) retrieved after a single punch or slap for each subject, one hour after hand washing

Figure 4-4 shows the results when the time interval between hand washing and contact was extended to one hour. The person-to-person variation post-slap is less dramatic than 15 minutes post-hand wash but still present, as with the post-punch transfer. All but subject 13 transferred more DNA via a single slap than by punch for this time period.

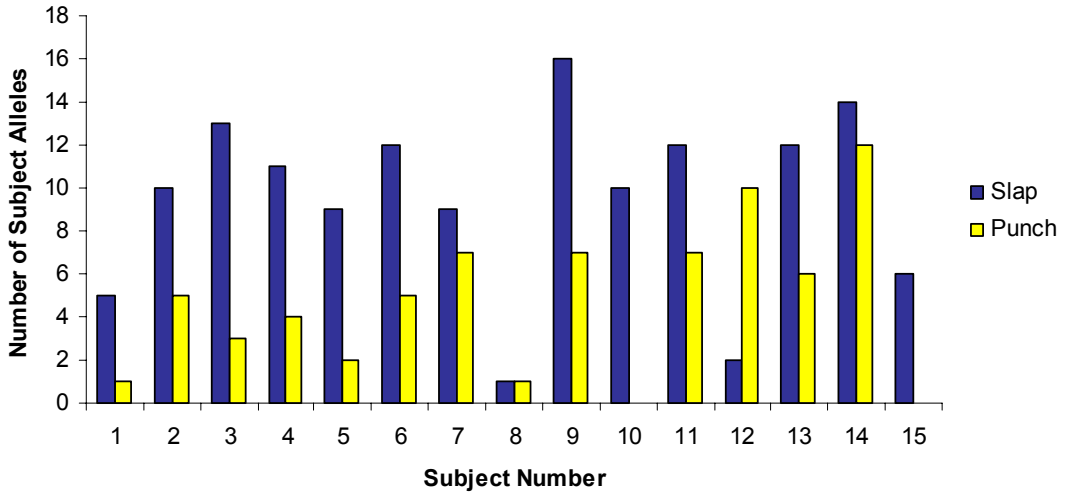


Figure 4-5: Number of subject alleles (max = 20) retrieved after three punches or slaps for each subject, one hour after hand washing

After three slaps DNA was retrieved from all of the sheets, while only two subjects failed to transfer after punching. As was noted for Figure 4-4 the person-to-person variation is less than previously but still evident. Only one subject, 12, transferred more DNA after multiple punches than after multiple slaps with subject 8 transferring equally.

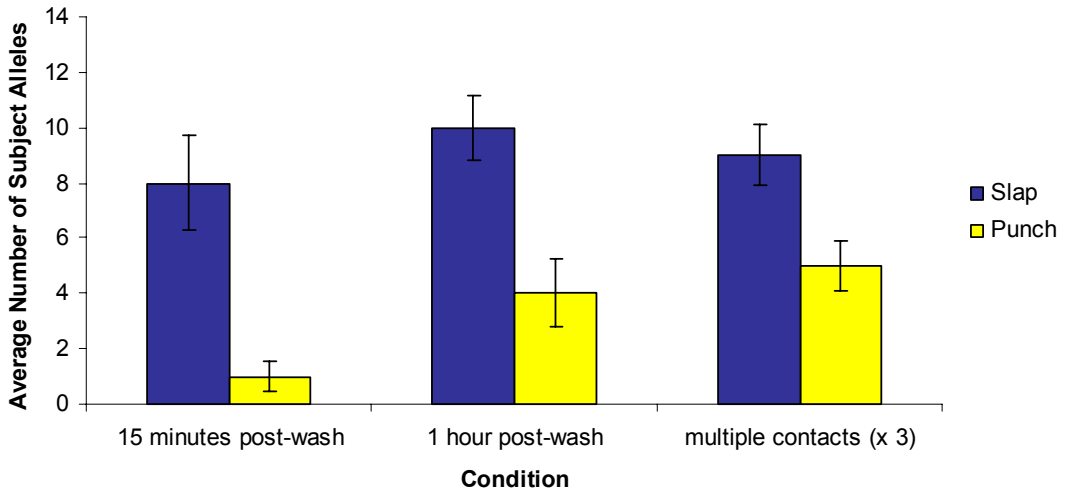


Figure 4-6: Average number of subject alleles seen after punching and slapping under each of the three sampling conditions

Figure 4-6 summarises the data previously outlined by averaging the number of subject alleles deposited on the acetate sheet after contact, when the samples were amplified using 34 cycles of PCR. Multiple contacts were applied one hour after hand washing. The chart indicates a four-fold increase in the average number of alleles transferred when the wash interval is also increased from 15 minutes to one hour. A marginal increase is shown when the sheet was punched three times rather than just once, but on average this is only by one allele. Similarly, the increase shown for single slaps when time interval between hand washing changes from 15 minutes to one hour is on average only by two alleles. In contrast an average decrease of one allele is displayed when the acetate sheet was slapped three times rather than the once.

Statistical testing was performed using the Wilcoxon Signed-Rank test and the differences between punch and slap for all three sampling conditions were found to be significant. Fifteen minutes post-wash $z = -3.05$, $p < 0.005$. One hour post-wash $z = -3.30$, $p < 0.001$. Multiple contacts $z = -2.61$, $p < 0.01$. The same test was used to determine the significance of time interval between hand washing and contact as well as single versus multiple contacts on DNA transfer. No significant difference was found between single and multiple contacts, so initial contact is likely to be responsible for the majority of transfer. The test did indicate that significantly more alleles were transferred by a single punch when the time interval between hand

washing and contact was one hour rather than 15 minutes $z = -2.01$, $p < 0.05$, which is consistent with the findings of Lowe et al. (2002). However no significant difference was found between the two time periods with regard to slapping which is not consistent with Lowe et al. (2002), possibly due to the blow and friction resulting in a sloughing of cells from the palm irrespective of the last time that washing was performed.

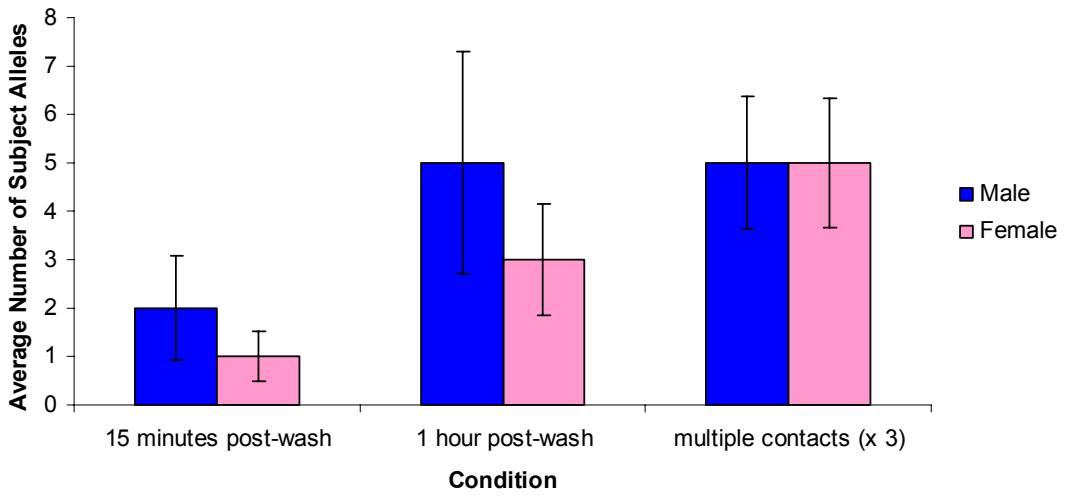


Figure 4-7: Average number of subject alleles for male (n = 7) versus female (n = 8) subjects after punching under each of the three sampling conditions

The average transfer for male versus female subjects was plotted on charts 4-7 and 4-8. In Figure 4-7 the average transfer after single punches is lower for females than for males. The averages after multiple punches are the same consistently agreeing with the premise that transfer occurs after the initial contact, with further applications of the blow having little effect. No significant difference was observed between males and females when statistical testing was performed using the Kruskal-Wallis test.

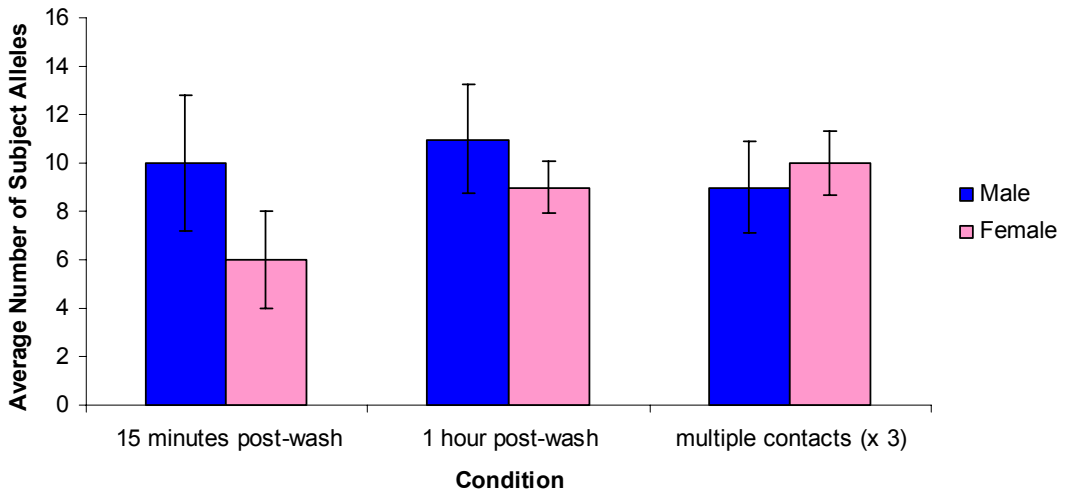


Figure 4-8: Average number of subject alleles for male (n = 7) versus female (n = 8) subjects after slapping under each of the three sampling conditions

As with Figure 4-7, the females appear to transfer less DNA after single slaps than the male subjects. This pattern does reverse, marginally, for the multiple slap results but no significant difference was observed between males and females when statistical testing was performed using the Kruskal-Wallis test.

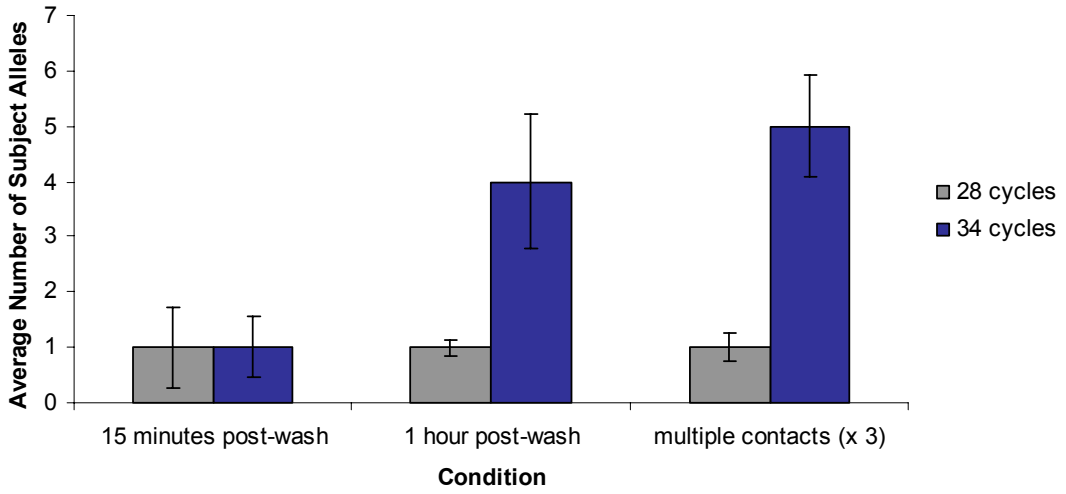


Figure 4-9: Average number of subject alleles retrieved after punching when amplified using 28 or 34 cycles of PCR

As expected more alleles were observed when the samples were amplified using 34 cycles of PCR than the standard 28, with the exception of 15 minutes after hand washing. Statistical testing indicated that the difference between the results obtained

after 28 and 34 cycles of PCR were significant for the one hour post-wash, $z = -2.94$ $p < 0.003$, and for the multiple contacts $z = -3.19$ $p < 0.001$, when using the Wilcoxon Signed-Rank test.

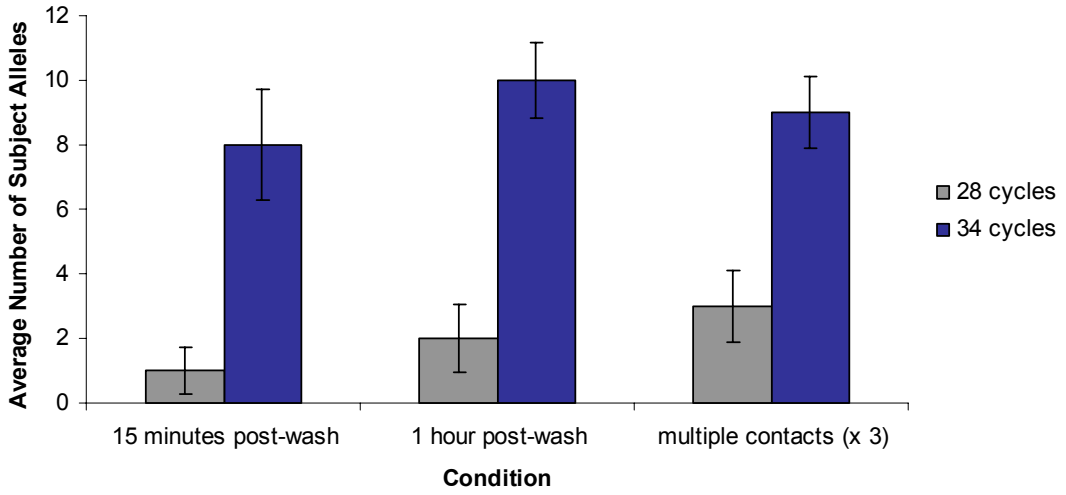


Figure 4-10: Average number of subject alleles retrieved after slapping when amplified using 28 or 34 cycles of PCR

For all three conditions, post-slap, more alleles were amplified using 34 cycles of PCR than with the standard 28, as shown in Figure 4-10. This was found to be significant using the Wilcoxon Signed-Rank test. 15 minutes post-wash $z = -3.07$ $p < 0.002$; one hour post-wash $z = -3.41$ $p < 0.001$; multiple contacts $z = -3.41$ $p < 0.001$.

4.3.2 Preliminary person-to-person investigation

The reference profiles for both subjects can be seen in the Appendix 6.2.6.2 along with the quantification results (6.2.3.2). Samples were taken from the subjects' arms and hands prior to contact and the resulting profiles are listed in the Appendix 6.2.8.

When the pre-contact samples were amplified using 28 cycles of PCR half taken from the hand of subject A successfully amplified any alleles compared to all six from subject B, with alleles of unknown origin being observed in three samples. Fewer alleles were generated from the arms of both subjects with all matching the reference profile of the subject. Increase in cycle number to 34 in allele number for both arm and hand samples taken from subject A, plus those from the arm of subject B. A decrease was however noted for the pre-contact hand samples from subject B.

4.3.2.1 28 cycles post-contact

The subjects were requested to apply the blow using their dominant hand to the non-dominant arm of their partner. Samples were then taken using a moistened cotton swab after the application of the punch or slap. Tables 4-13 and 4-14 show the DNA profiling results from the post-contact samples when amplified using 28 cycles of PCR. Alleles marked in black match the reference profile of the subject the sample was taken from, while those in blue match that of the other subject involved in the contact. The alleles in red indicate unknown origin.

Table 4-13 displays the DNA profiles retrieved from the hands of both assailants after contact was applied. Two of the repeats from the knuckles of subject A failed to amplify after 28 cycles, while the third has four alleles, two matching the subject, one of unknown origin and the fourth being possible transfer as it matches the profile of the victim. A similar pattern is observed from the same subject after slapping with alleles present in one of the three repeats, both matching the subject. No transfer was noted on the hands of subject B after contact, probably due to the high level of subject DNA retrieved.

The DNA profiles taken from the arms of both subjects, Table 4-14, all show far fewer subject alleles than were obtained from the hands, but one post-slap sample from each subject has a single allele matching the profile of the assailant indicating possible transfer. No alleles of unknown origin were amplified from any of the arm samples. Although potential transfer alleles are noted after 28 cycles of PCR the presence of alleles of unknown origin both on the hands of the assailants in this investigation, as well as in the focus pad investigation and studies by other individuals (Lowe et al. 2002; Graham and Rutty 2007) make these solitary alleles of little evidentiary value.

Blow	Repeat	Subject	D3	VWA	D16	D2	D8	D21	D18	D19	TH01	FGA
Punch	1	A										
Punch	2	A										
Punch	3	A					10,11,12,13					
Slap	1	A										
Slap	2	A										
Slap	3	A	14				13					
Punch	1	B	14,15	14,18	11,12	19,21	12,13	29,30	14,15	13,13	9,9.3	21,25
Punch	2	B	14,15	14,18	9,11,12	19,21	12,13,16	29,30	14,15	13,13	9,9.3	21,25
Punch	3	B	14	14,18	11	21	12,13	30		13,13		
Slap	1	B	14,15	18	11,12	19,21	12,13	29,30	14,15,17	13,13	9	21
Slap	2	B	14,15							13,13		
Slap	3	B	14,15	14	11,12	19,21	12,13	29		13,13	9,9.3	21,25

Table 4-13: DNA profiles from the hands of the assailants after contact

Blow	Repeat	Subject	D3	VWA	D16	D2	D8	D21	D18	D19	TH01	FGA
Punch	1	A		16	14					13		
Punch	2	A										
Punch	3	A		14	11,14	20	11					
Slap	1	A					12,13			13,14		
Slap	2	A										
Slap	3	A			11							
Punch	1	B								13		
Punch	2	B										
Punch	3	B										
Slap	1	B			11,12					13		
Slap	2	B		14						13		
Slap	3	B					11					

Table 4-14: DNA profiling results from the victims arms after contact

4.3.2.2 34 cycles post-contact

The subjects were requested to apply the blow using their dominant hand to the non-dominant arm of their partner. Samples were then taken using a moistened cotton swab after the application of the punch or slap.

Alleles marked in black match the reference profile of the subject the sample was taken from, while those in blue match that of the other subject involved in the contact. The alleles in red indicate unknown origin.

Table 4-15 represents the same samples as Table 4-13 when amplified using 34 cycles of PCR. One sample post-punch and post-slap for subject A show no amplification, while the other two repeats taken after slapping show no non-subject DNA. The two post-punch repeats have partial profiles matching the subject and one has a single allele matching the victim in addition to one of unknown origin. In contrast to the results from subject A five of the six samples taken from the hand of subject B have generated full subject profiles, while amplification failed for the sixth despite no problems with the positive PCR control. One post-punch sample exhibits a single allele matching the victim, while two of the three taken post-slap each have two potential transfer alleles. Two of the three samples from subject B with potential transfer also have alleles whose origin is unknown. As mentioned, non-subject alleles in such low levels cannot be ascribed to a particular origin to any degree of certainty especially with the presence of similar levels of unknown alleles (Gill et al. 2000; Whitaker et al. 2001; Graham and Rutty 2007).

Blow	Repeat	Subject	D3	VWA	D16	D2	D8	D21	D18	D19	TH01	FGA
Punch	1	A	15,16				13	30		13,14	7,9.3	
Punch	2	A	14,17	14,16	14		11,13	30	12	13,14	9.3	22.2,24
Punch	3	A										
Slap	1	A								14		
Slap	2	A										
Slap	3	A		14			11,13	30,31		13,14	7,9.3	
Punch	1	B	14,15,18	14,16,18	11,12	19,21	12,13	29,30	14,15	13	9,9.3	21,25
Punch	2	B										
Punch	3	B	14,15	14,18	11,12	19,21	12,13	29,30	14,15	13	9,9.3	21,25
Slap	1	B	14,15	14,16,18	11,12	19,21	12,13,14	29,30	14,15	13,13	6,7,9,9.3	21,25
Slap	2	B	14,15	14,18	11,12	19,21	12,13	29,30,31	14,15	13,13	7,9,9.3	21,25
Slap	3	B	14,15	14,18	11,12	19,21	12,13	29,30	14,15	13,13	9,9.3	21,25

Table 4-15: DNA profiling results from the assailants hands after contact

Blow	Repeat	Subject	D3	VWA	D16	D2	D8	D21	D18	D19	TH01	FGA
Punch	1	A	16,17	14,16,17	11,12,14	20,24	11,13	30,31		13,14	7,9.3	
Punch	2	A										
Punch	3	A	14,16,17	14,16	11	20,24	11,13	30,31	12	13,14	7,9.3	22.2,24
Slap	1	A	14,15,16,17	14,16,18	11,12	19,21,24	11,12,13	29,30	12,13,14,15	13,14	7,9,9.3	21,24
Slap	2	A	14	14,16,18	11,12		13	29,30		13,14	7,9,9.3	
Slap	3	A	14,15	14,18	11		9,11,13	27,29,30,31	17	13,14,15	6,9,9.3	23,24
Punch	1	B	15,17	18	11,12	19	13	29,30,31	15	13	9,9.3	25
Punch	2	B	14,15	14,18	11,12	21	12,13	30	14	13	9,9.3	21,25
Punch	3	B		14			13					
Slap	1	B	14,15	14,18	11,12	19,21	12,13	29,30	14,15	13,13	7,9,9.3	21,25
Slap	2	B	14,15	14,18	11,12	19,21	12,13	29,30	14,15	13,13	9,9.3	21,25
Slap	3	B	14,15	14,18	11,12		12,13	29,30	14,15	13,13	9.3	21

Table 4-16: DNA profiling results from the victims arms after contact

More subject and non-subject alleles have been amplified from the arm samples using 34 cycles than using the standard 28, with two full subject profiles from subject B post-slap. The third is a partial with no non-subject alleles. A single transfer allele is highlighted in the first post-slap sample from this subject, while two can be seen in the first post-punch sample. Those samples taken from the arm of subject A after slapping show mixtures of both subjects indicating transfer has occurred, compared to one solitary allele post-punch. This result is consistent with the trend observed on the focus pads that slapping transfers more DNA than punching even when allowing for person-to-person variation. The results in Tables 4-15 and 4-16 also indicates that while one or maybe two alleles can be transferred from the victim's arm to the assailant's hand more are transferred in the opposite direction although this is dependent on the individual. Past testing of shedder status for other studies found that subject A was a poor shedder while subject B was a good shedder, hence the transfer only being observed on the arm of subject A post-slap.

4.3.3 Person-to-person investigation

4.3.3.1 DNA profiling results

Reference profiles for all sixteen subjects are in the Appendix 6.2.6.3 with the quantification results (6.2.3.3) and pre-contact data (6.2.8.1 and 6.2.8.3) plus the 28 cycle's post-contact profiles (6.2.8.2). Quantification indicated an average increase in DNA after contact compared to before, although the average increase is not consistent with those retrieved from the focus pad investigation.

After 28 cycles of PCR only five of the 16 subjects' arms sampled prior to contact exhibited any alleles – all partial subject DNA profiles. Two of these subjects also had alleles of unknown origin. Nine out of the sixteen pre-contact knuckle samples generated partial subject profiles, with six of these also showing the presence of alleles of unknown origin. Similar results were observed for the palms of the subjects prior to contact. Six of the subjects show partial subject profiles after amplification using 28 cycles of PCR, plus one full subject profile. Three of these seven subjects also amplified alleles of unknown origin.

As expected, 34 cycles of PCR increased the number of subject and non-subject alleles over those observed after 28 cycles. In many cases the number of non-subject alleles is greater than those matching the subject's reference profile. This differs from the results observed during the preliminary study, where the subject alleles seen varied between the two subjects, but neither show the extent of contamination seen on the arms of these 16 subjects prior to contact.

28 cycles post-contact

When amplified using 28 cycles of PCR, DNA was only retrieved from the arms of two of the 16 subjects after a single punch, in each case consisting of two subject alleles. A single allele of unknown origin is observed in the sample from the arm of subject 12. After a single slap samples from the arms of four of the subjects generated profiles consisting of between one and three subject only alleles. More alleles were amplified from the samples taken from the upper hand of three of the subjects after punching but no transfer was observed. However, post-slap two alleles were observed

in the profile generated from the palm of subject 14 that matched the profile of the subject they were paired with (subject 13) indicating possible transfer from the arm during contact.

34 cycles post-contact

In Tables 4-17 to 4-20 the alleles in black are those that match the reference profile of the subject that the sample was taken from, while blue indicates the allele matches the profile of the subject they were paired with, i.e. there was forceful contact between the two. Red highlighting marks alleles of unknown origin.

Table 4-17 shows the DNA profiling results from the samples taken from the victims' arms after a single punch. Partial subject profiles can be seen for all but two of the subjects and six also show the presence of alleles of unknown origin as was observed in the preliminary study. Potential transfer alleles can be seen in nine of the samples but as was seen in the preliminary study most consist of single alleles that could match many individuals. However the first pair, subjects one and two, exhibit evidence of transfer from the hands after punching. Both profiles are mixtures from two individuals that appear to be the two subjects applying and receiving the blow. In spite of this it cannot be determined with any certainty when the non-subject DNA was deposited there or how (Gill et al. 2000).

A similar result is shown in Table 4-18 for the second pair, subjects three and four, after a single slap was applied to the arm. The arms of both exhibit mixtures that match the profiles of the two subjects involved. In addition several alleles of unknown origin can be seen. Overall there appears to be little difference in the DNA profiles deposited on the arms after a single slap than after a single punch.

The DNA profiles obtained from the knuckles after punching are displayed in Table 4-19 with few alleles of subject or non-subject origin in evidence, as was observed in the preliminary study. Similarly, few alleles can be seen in the post-slap hand samples shown in Table 4-20. This appears to agree with the results for subject A in the preliminary study but not for subject B. From past investigations subject B was found to be a good shedder which would seem to indicate that all 16 subjects in this study

are poor shedders, despite the fact that contact was applied an hour after hand washing when shedder status has minimal effect on transfer (Lowe et al. 2002). Lowe et al. (2002) also found that approximately half of their subjects were good shedders, although Phipps and Petricevic (2006) found that the only volunteers who produced the same shedding result on five separate days were the two who transferred no DNA at all. Therefore there is a possibility that if these 16 subjects were sampled again more or less transfer may be observed even when the sampling conditions are maintained.

	D3	VWA	D16	D2	D8	D21	D18	D19	TH01	FGA
1	15,16		11	20	12,11,14	28,30	14	14,16	6	16,22
2	15,16	15,17	11,13		12,13,14	28,30	16	14	7,9.3	22,25
3		15	12		14			12,13,16.2	7,8,9.3	
4	16	17			12,14			14,16		20
5										
6	16	16,18	10		13	29,30		12,14.2,16.2	6,7	
7	14	14,15,16,17	11		13,15	28		13,16,17.2	7,9.3	22
8	15,16	16			10,14			14	9.3	
9										
10	16	14	11,14		13,13	30	15	15	7,9.3	24
11	14,16	15,16	11,12	23	13	30,31.2	12,14	12	9,9.3	23
12					12,13			12	9	
13	16	14,15,17	12	20	13,14			14	8	
14	15	16,17,18	14	17	12,14	32,32.2	12	12,13,14	6,9.3	22
15		18						12		
16					10	28,30.2		13	6,9	

Table 4-17: DNA profiling results for the samples taken from the arms of all sixteen subjects after a single punch

	D3	VWA	D16	D2	D8	D21	D18	D19	TH01	FGA
1					14			12,14		
2	15,16	15,16,18	13	24	12,14			12,15,16	7,9	25
3	15,16,18	15,18,19	12,13		14,15	27,29,31		12,13,14,16,16.2	6,8,9,9.3	
4	14,16,18	15,18,19	13	20	14,15	27,29,31		12,14,16.2	6,8,9,9.3	20
5								16.2		
6										
7	15,16	16,17			15			12,13,14,16	7,8,9,9.3	
8										
9		16	13		13	30	15	12,16		
10	16	14,16,17	11,14		13	30		11,12,15,16	6,7,9.3	
11	16	16	12		13	30,31.2		13,14,15	9.3	
12								12		
13	16,18	14,16,17	12,14	17,20	13,14	29,31.2		14	7,8,9,9.3	22
14	15,18	17,18	13,14	17	14,14	32,32.2		13,14	6,9.3	18,22
15		17			12,13,14			12,15	8,9.3	
16	15,15	14,15,17	8,9		8,13	28,29,30.2	13	13,14.2,15.2,16.2,17.2	6,6	19,25

Table 4-18: DNA profiling results for the samples taken from the arms of all sixteen subjects after a single slap

	D3	VWA	D16	D2	D8	D21	D18	D19	TH01	FGA
1	16,18	17		24	13,14			13,14,15	6,9.3	22
2	15,16	15,16	13	20,24	12,14	28,32.2		12,16	7,9	22,25
3	16	15		17	14,15	28,31		13,16.2	7,9.3	
4		18	12,13							
5										
6	15,16	16,18	10,11,12	17,21	13,14	29,30	14	12,16.2	6,7,9	20,22
7	14	16	11		15	30		13	7,9	22
8	15,16	16,17	11		10,14	31,34.2		14,14	8,9.3	22
9	15							12,16		
10						30		14		
11										
12	15,16,17	15,16	9,12		12,13	30		12,15	9,9.3	25
13	16	14			9,13,14			13	8,9	
14	14,15,18	17,18	14	17	13,14	32,32.2		13,14	6,9.3	22
15										
16	15	16,17	8,9	17,24	10,13	28,30.2	13	13,14.2	6,6	19

Table 4-19: DNA profiling results for the samples taken from the upper hand, knuckles and top of fingers, of all sixteen subjects after applying a single punch

	D3	VWA	D16	D2	D8	D21	D18	D19	TH01	FGA
1	15,16,18	15,17,18	11		13,14			14	9.3	22
2	14,15,16	15,16			12,13,14	28,32.2		12,14,16	7,9	
3		15,17			14,15			12,13,14	9.3	
4		15,18			14	27,29		14,16	8,9,9.3	23
5	16	14,15,16,18	9,10		12,13	28,29,30		12,13,14,15	6,7,9	20
6	15,16	16,18			13,14			12,16.2	6,7	
7			9		13,15				9.3	
8	15,16	16,17	11,12,13	23,24	10,11,12,13,14	29,31		13,14,15	6,8,9.3	
9								12		
10	15,16,17	16	12		10,13	30		14,15,16	6,9.3	
11		16			13				6	
12		16			12,13			12,13,15	9.3	
13	14,16,17	15,17	9		13,14	31.2		12.2,13,14	7,8,9,9.3	
14	15,18	17,18,19	13,14	17,20	14	32,32.2	12	13,14	6,9.3	22
15		17			13	28		12	7,9	
16	15	14,17	8		13,14,19	28		13,14.2	6	

Table 4-20: DNA profiling results for the samples taken from the lower hand, palm and finger pads, of all sixteen subjects after applying a single slap

4.3.3.2 Comparison of contact data

In Figures 4-12 and 4-15 to 4-21 arm 1 indicates the sample was taken from the arm post-punch while arm 2 samples were post-slap, both from the individual who had been hit. Samples labelled knuckles and palm were taken form the individual applying the forceful contact.

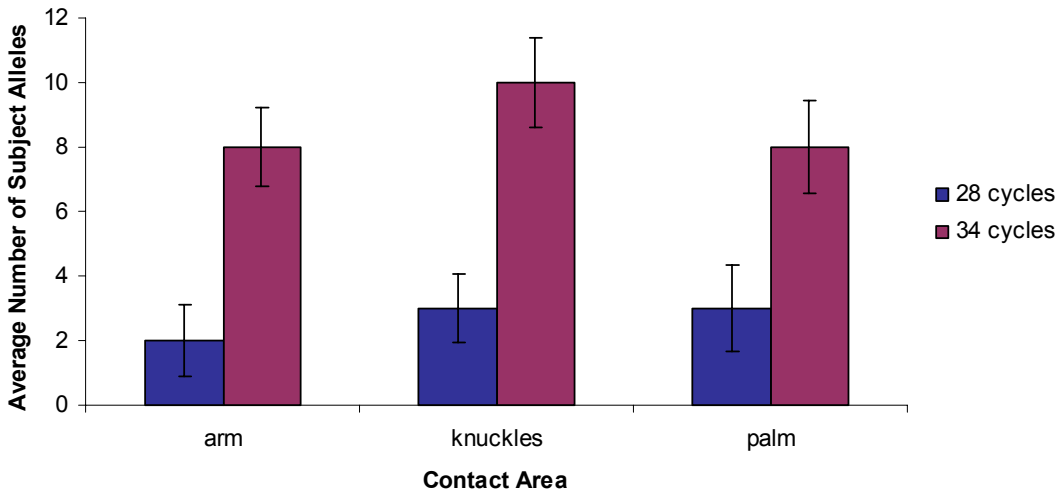


Figure 4-11: Comparison of the average number of subject alleles retrieved from the different areas prior to contact when amplified using 28 or 34 cycles of PCR

As expected the average number of subject alleles observed prior to contact, as shown in Figure 4-11, was greater for all contact areas when the samples were amplified using 34 cycles of PCR than 28. For all three sites the average at least doubled when the cycle number was increased. Statistical testing using the Wilcoxon Signed-Rank Test indicated that there is a significant difference between the results observed after 28 and 34 cycles of PCR; arm $z = -3.42$, $p < 0.001$; knuckles $z = -3.29$, $p < 0.001$; palm $z = -3.11$, $p < 0.002$.

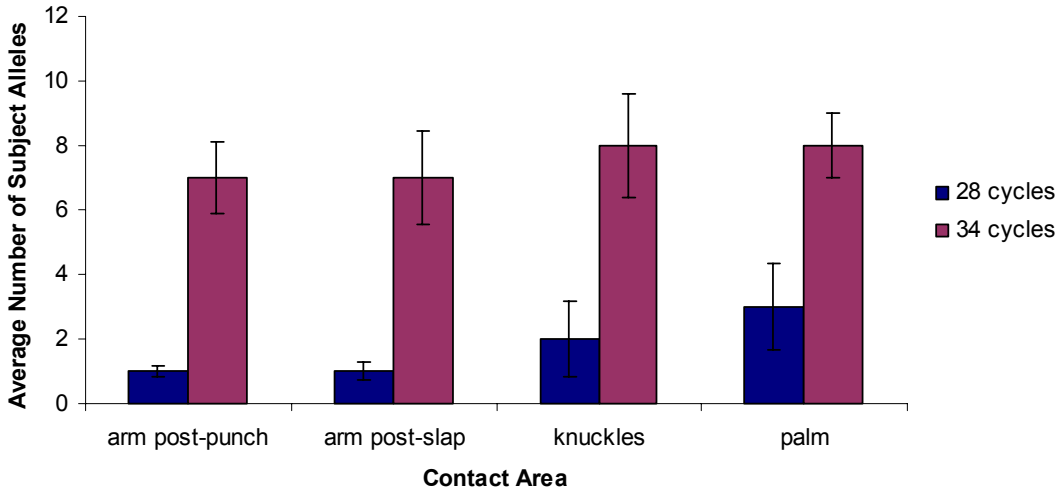


Figure 4-12: Comparison of the average number of subject alleles retrieved from the different areas after contact when amplified using 28 or 34 cycles of PCR. Arm samples were taken from the individual who had been hit. Samples labelled knuckles and palm were taken from the individual applying the forceful contact.

As with the pre-contact samples in Figure 4-11 the average number of subject alleles increased with cycle number for the post-contact samples. In this case the average triples when the LCN technique was employed. The differences shown were found to be statistically significant using the Wilcoxon Signed-Rank Test with $p < 0.002$ for the arm post-slap $z = -3.14$ and knuckles $z = -3.18$, with $p < 0.001$ for the arm post-punch $z = -3.30$ and the palm $z = -3.13$.

All of the following bar charts were generated from the data obtained from the samples after 34 cycles of PCR.

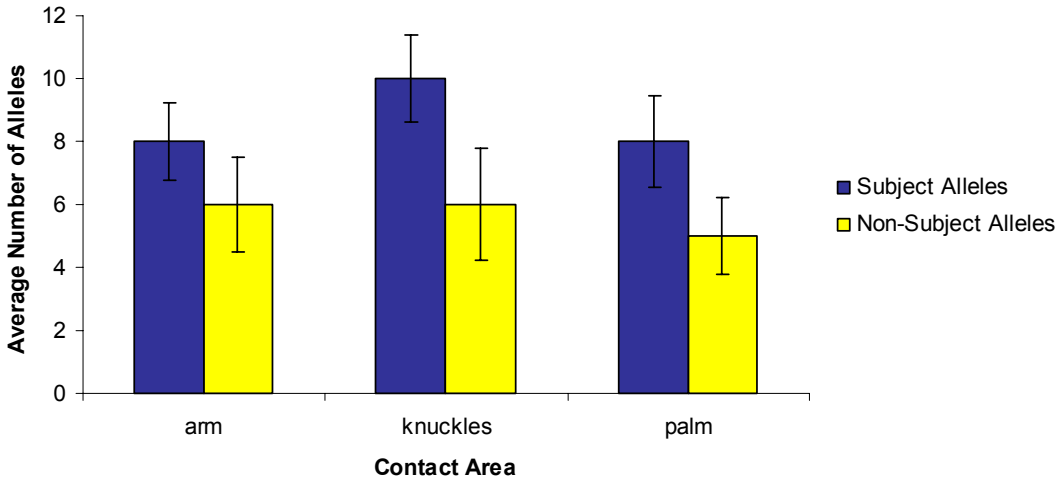


Figure 4-13: DNA profiles obtained from the palm, knuckles and arm of each of the sixteen subjects prior to contact

Figure 4-13 represents the average number of subject and non-subject alleles retrieved from the arm knuckles and palm of the sixteen prior to the application of the blow. For all three sites the average number of subject alleles is greater than non-subject. Little difference is indicated between the areas but the average is marginally higher for subject alleles from the knuckles than the palm or arm. This may correspond with the theory that drier parts of the skin's surface may shed more cells (Bright and Petricevic 2004) although other theories conclude the opposite with secretions carrying nucleated cells to the surface of the skin and aiding transfer with the sweat and oil (Wickenheiser 2002). Slightly fewer non-subject alleles were amplified from the palm of the hand than from the arm or knuckles. Statistical testing was performed using the Friedman Test and no significant difference was found between the three sites prior to contact.

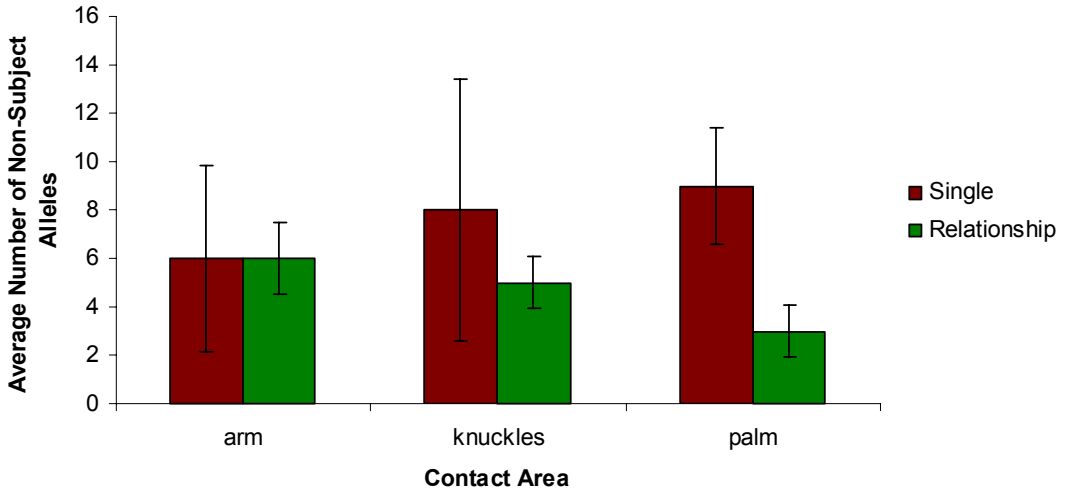


Figure 4-14: Average number of non-subject alleles retrieved from the arm, knuckles and palms of subjects who were single (n = 5) or in a relationship (n = 11), prior to contact

Figure 4-13 indicated that prior to contact similar numbers of non-subject and subject alleles were retrieved. Therefore the average number of non-subject alleles for each site was determined for subjects who were single or in a relationship at the time of sampling. The results, as shown in Figure 4-14, indicate no difference between the groups for samples taken from the arms but both knuckle and palm samples show a slightly higher average for single subjects than for those in a relationship. The Kruskal-Wallis Test was used to determine the significance of the differences observed above, with statistically significant differences only being found for the palm samples, $\chi^2 = 4.55$ $p = 0.03$. No significant difference was observed for the samples taken from the arm, which was the site more likely to maintain DNA from partners/spouses due to the lack of contact between this area and other sources of DNA. However the source of the non-subject alleles amplified from the hand samples is likely to be contact with multiple user inanimate objects (Graham and Rutty 2007; Rutty 2002) with such alleles being transferred by the subject onto their own arms.

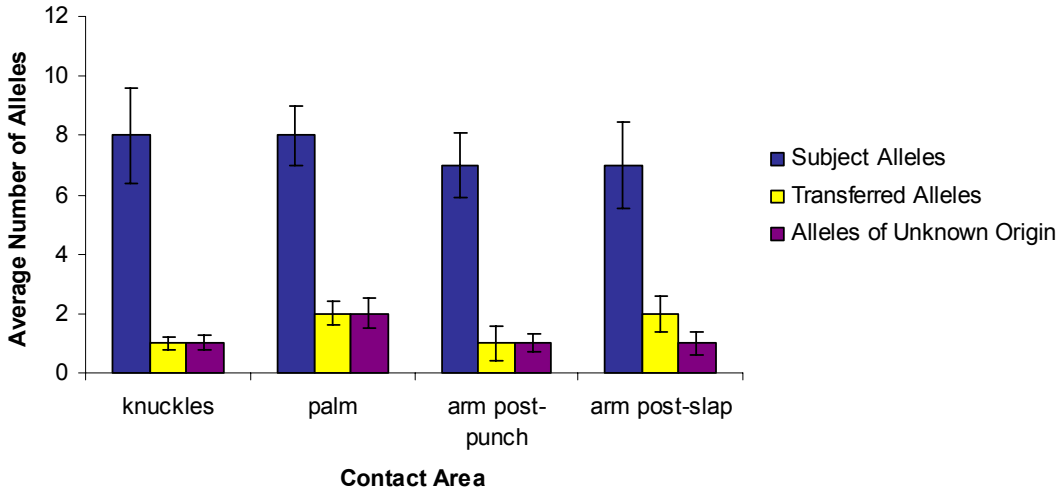


Figure 4-15: The possible origin of DNA retrieved from the hands and arms of 16 subjects after contact. Arm samples were taken from the individual who had been hit. Samples labelled knuckles and palm were taken from the individual applying the forceful contact.

Post-contact the average number of subject alleles is much higher than non-subject, with marginally higher averages for the hand samples compared to those taken from the arm. For all sites of sampling with the exception of the arm sample taken post-slap, equal numbers of alleles transferred from their test partner and those of unknown origin can be seen. The samples taken from the arms of the subjects post-slap show a higher average of transferred alleles than unknowns. Palm samples also indicate a marginally higher average of non-subject alleles than seen for the knuckles and arm post-punch. Statistical testing was performed to compare the difference in subject, transfer and unknown alleles for each of the contact areas, using the Friedman Test. No significant difference was found for subject or unknown alleles, but a significant difference was found between the transferred alleles obtained from the four sites, $\chi^2 = 8.73$ $p = 0.03$, significantly more transfer being observed post-slap than post-punch, arms and hands.

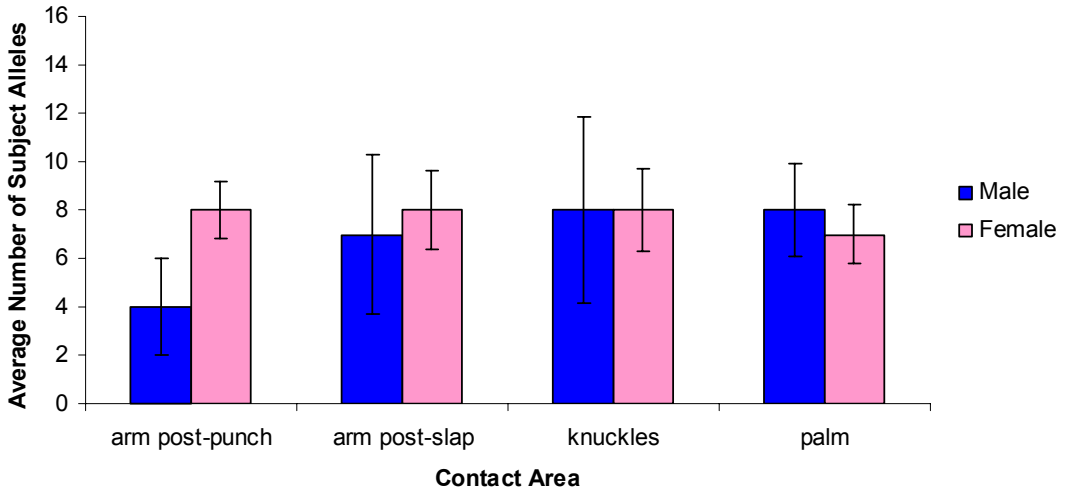


Figure 4-16: Average number of subject alleles observed for male (n = 5) versus female (n = 11) subjects after contact. Arm samples were taken from the individual who had been hit. Samples labelled knuckles and palm were taken from the individual applying the forceful contact.

Figure 4-16 aims to determine any difference between the sexes with regard to the number of subject alleles retrieved from the arms, knuckles and palms of the sixteen subjects post-contact. All four show little difference between males and females, although lower averages can be noted for the male arm samples than are displayed for the female subjects. Statistical testing using Kruskal-Wallis indicates no significant difference between males and females. The test was also used to determine any difference between males and females with regard to transferred and unknown alleles, but the results indicated no significance.

4.3.3.3 Comparison of washing techniques

Data obtained from the subjects' questionnaires were used to generate charts to compare the effects of wash techniques on the alleles retrieved from the arms and hands after contact. The data indicated that all subjects had washed their hands using water and soap with approximately the same time interval between washing and contact. Therefore the only difference in wash patterns between the subjects was the way they had washed their arms, i.e. using shower gel or soap.

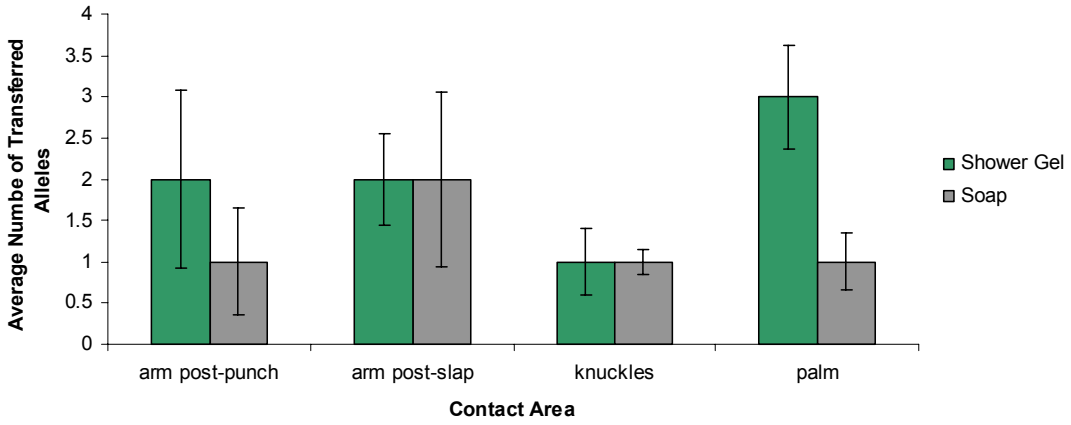


Figure 4-17: Average number of transferred alleles observed post-contact for those who had washed their arms with shower gel (n = 7) versus soap (n = 9). Arm samples were taken from the individual who had been hit. Samples labelled knuckles and palm were taken from the individual applying the forceful contact.

A greater degree of transfer can be seen on the arms of those who had washed with shower gel after a single punch than for those who had washed with soap. No difference between the two methods can be seen for transfer onto the knuckles post-punch or arm post-slap. However the average number of transferred alleles for those washing with shower gel is triple that of the soap users when the samples were taken from the palm post-slap. Statistical testing was performed using the Kruskal-Wallis test, no significant

difference was observed for either of the arm samples or those taken from the knuckles. However a significant difference was found between the number of alleles transferred to the palm when subjects washed using shower gel versus soap, $\chi^2 = 4.48$ p = 0.03. There was found to be no significant difference in the number of subject alleles that were retrieved from those washing with shower gel versus soap, using the Kruskal-Wallis non-parametric statistical test.

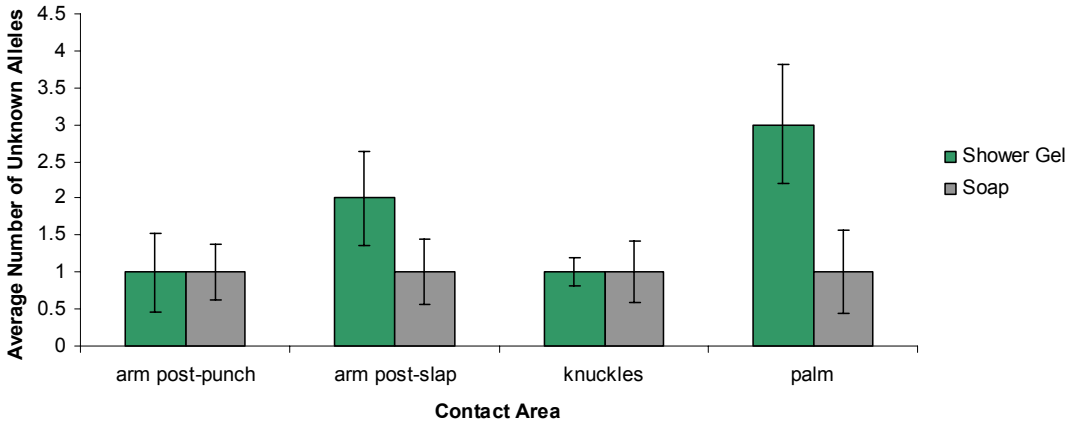


Figure 4-18: Average number of unknown alleles observed post-contact for those who had washed their arms with shower gel (n = 7) versus soap (n = 9). Arm samples were taken from the individual who had been hit. Samples labelled knuckles and palm were taken from the individual applying the forceful contact.

The final comparison of wash technique, in Figure 4-18, looks at alleles of unknown origin. Samples taken post-punch, both from the arm and knuckles show no difference between the two methods. But both sets of post-slap samples indicate a higher average of unknown alleles for those washing with shower gel. As with the transferred alleles no significant difference was observed between shower gel and soap users for the arm and knuckle samples, when using the Kruskal-Wallis test. A significant difference was found for the samples taken from the palm, $\chi^2 = 5.06$ p = 0.03.

4.3.3.4 Determination of the effects of latex gloves on DNA transfer

The data obtained from the questionnaires indicated that half of the subjects had worn latex gloves in the interval between hand washing and contact. Therefore the following charts aim to determine if the average number of alleles retrieved was affected by this fact.

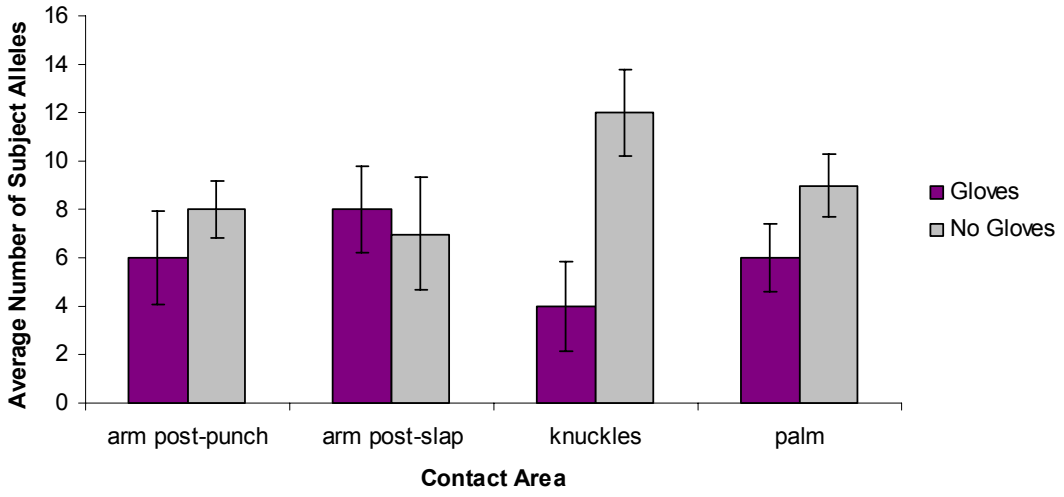


Figure 4-19: Average number of subject alleles observed post-contact for those who had (n = 8) and had not (n = 8) worn latex gloves prior to contact. Arm samples were taken from the individual who had been hit. Samples labelled knuckles and palm were taken from the individual applying the forceful contact.

Figure 4-19 aims to determine the effect of latex gloves on DNA transfer. The chart consists of data from the arms of the victims and hands of the assailants post-contact and in all cases the assailant is the individual who had worn/not worn latex gloves. In each

case the individual had not washed their hands after removing the gloves therefore the chart is designed to determine if the drying that latex, in some cases, can cause affected the number of alleles transferred during forceful contact. With regard to the subjects own DNA, the chart (Figure 4-19) indicates a greater average for those who had not worn gloves compared to those who had. For the knuckle samples of those who had not worn gloves the average is triple that of the subjects who had. The difference is smaller for the samples taken from the palm of the hand. Statistical testing was performed using the Kruskal-Wallis test which indicated no significant difference between the subject alleles retrieved from the arms or palm of those wearing or not wearing gloves. However a significant difference was found for the knuckle samples $\chi^2 = 6.15$ $p = 0.01$. The use of latex gloves between hand washing and contact was found to have no significant effect on the number of transferred or unknown alleles, using the Kruskal-Wallis test.

4.4 Discussion

The aim of this study was to investigate if it was possible to determine the perpetrator of physical abuse by the DNA they deposited during the forceful contact. Three phases were employed starting with the application of the blow to a clean 'DNA-free' surface attached to focus pads that are used for boxing training. Secondly, two members of the Forensic Pathology Unit were asked to punch and slap each other in order to determine if transfer was detectable before recruiting more volunteers, for Phase III, which involved eight pairs of volunteers applying a single punch or slap to their partner.

Overall the results indicated that transfer can occur and be retrieved both from clean surfaces and from a victim's skin, but this does vary from individual to individual, as found by Wiegand and Kleiber (1997) who found that 70% of their interpretable DNA profiles showed evidence of transfer after simulated manual strangulation. Rutty (2002) found transfer on the necks of seven victims when using standard PCR and all 29 when LCN was employed after simulated manual strangulation, similarly Graham and Rutty (2007) found transfer to the victim's neck in approximately 50% of cases. In the present study in some instances only single transfer alleles were noted but in others identifiable profiles could be distinguished when compared to the reference profiles of the subjects. However, in the majority the transfer was minimal and easily masked by the subject's own alleles. In addition many of the samples taken from the hands and arms of the subjects in all three phases included alleles of unknown origin, an occurrence also noted by Graham and Rutty (2007) who found that 24% of all samples exhibited between one and nine alleles of an unknown origin, while Rutty (2002) also noted that unknown third-party partial profiles could be identified from test and control sites. During the focus pad investigation some samples had more unknown than subject alleles. It is possible that the vast majority of the unknown alleles were transferred from door handles or other multiple user items such as telephones, as many were amplified from samples taken from the palm of the hand. Those found in samples taken from the arms or knuckles may have been transferred from the subject's own palm, although some may result from contact with partners/spouses, but no significant increase in unknown allele number was found for

subjects with partners or spouses over the single subjects. The presence of these unknown alleles in actual casework samples could complicate results as a good defence could argue that these alleles originated from the actual perpetrator.

All three studies indicated that slapping resulted in more transfer than punching, possibly due to the palm of the hand being moister than the knuckles with more secretions carrying cells to the skin's surface (Wickenheiser 2002) although this may be purely due to the larger surface area applying the contact. The higher transfer associated with slapping may also be a result of the contact between the palm and other areas of the body, in particular those with high cell turnover such as the eyes, nose and mouth (Wickenheiser 2002). No increase in transfer was noted when multiple contacts were applied to the surface indicating that initial contact is responsible for the transfer of DNA as observed by van Oorschot and Jones (1997). However it was also observed in the present study that the majority of individuals were not able to punch or slap the exact same spot on multiple occasions (not previously studied). Of the two who could, and did, no increase in transfer resulted.

As part of the focus pad investigation the time interval between hand washing and contact was tested, as Lowe et al. (2002) found that shedder status could be determined 15 minutes after hand washing, but after an hour most individuals shed DNA that resulted in full profiles. In the current investigation significantly more transfer occurred when punching one hour after hand washing than 15 minutes after, but no difference was noted for slapping. It may be that nervousness on the part of the subjects may have caused their palms to sweat resulting in more cells being transferred to the surface and therefore negating the effect of the increased time interval. Phipps and Petricevic (2006), like Lowe et al. (2002) found that the longer the time since washing the greater the transfer but Phipps and Petricevic (2006) also noted that no one individual produced consistent profiles on multiple occasions. Therefore it is likely that there are multiple factors influencing transfer and that nerves, with possible increased sweat production may play a part. During the focus pad and person-to-person investigations it was noted in the questionnaires that several subjects had worn latex gloves in the time interval between

hand washing and contact but this was found to have no significant effect. Other questionnaire data from the person-to-person study that were used to determine the effect on transfer was sex of subject and the methods of washing. No significant difference was observed between males and females and all subjects had washed their hands using the soap available in the laboratory toilets. However, nine of the subjects said they had washed their arms using soap while the remaining seven had washed with shower gel. Statistical testing found that significantly more transfer was observed from the arms of those washing with shower gel onto the palms of the assailant after slapping. Shower gel is expected to add moisture to skin in contrast to soap that tends to dry it. Therefore moister, softer skin may transfer more DNA than skin that is drier, as also noted when comparing the palm of the hand to the knuckles.

A big issue in this study is that the force of the blow used could not be controlled as everyone punches/slaps with differing amounts of force. In order to try to control for it as far as possible the volunteers were asked to punch/slap hard enough to redden the skin, but no harder. Overall no individual appeared to apply the same amount of force on each occasion of sampling. Additionally variables such as wearing latex gloves and washing techniques were not controlled for, while these individually had no statistically significant effect, it is possible that these effects together may result in more or less transfer than others.

Initially shedder status was tested and then repeated on a second occasion. The results from all the volunteers showed partial profiles consisting of few alleles, no more than eight alleles were observed for any one individual despite repeating the test as described by Lowe et al. (2002). By this stage the paper by Phipps and Petricevic (2006) had been published indicating that to determine shedder status as previously described was not that easy with many variables being responsible. This agreed with our results from shedder testing and both the 'normal' levels and forceful contact investigations. Further shedder status testing was therefore excluded from this work, while further research into shedder status continues within our unit.

The forceful contact study needs to be expanded before any definitive conclusions can be agreed upon. At present it does not appear that DNA transfer can be employed as means of identifying the perpetrators of abuse. Transfer varied depending on the individual, and the presence of alleles from secondary or even tertiary transfer complicate analysis. However this is a preliminary study with a small number of volunteers - sixteen volunteers are too few, so recruitment could be expanded outside the department. Gripping could be tested in addition to slapping and punching with different lengths of time for each grip. Studies by Rutty (2002) and Graham and Rutty (2007) have investigated DNA transfer during manual strangulation by gripping the upper arm and neck but specific studies into gripping as if a child was being shaken would be of use. Further time intervals between hand washing and contact in addition to those performed would provide information relevant to actual practice as would the determination of how long transferred DNA persists on the skin during normal routines. In many cases of physical abuse the blow may be from male to female or vice versa therefore the use of Y-STRs would reduce the problems associated with LCN PCR and enable quantification of the transferred DNA. Finally DNA testing of objects such as belts and coat hangers that are used to beat children could be performed in a similar manner to the toy study previously suggested.

5. Conclusion

The overall aim of my thesis was to determine if the perpetrators of physical child abuse could be identified by DNA transferred during forceful contact using existing forensic protocols for DNA extraction, quantification, amplification and electrophoresis. To establish this, the investigation was divided into two parts.

The first stage of the investigation was to identify the DNA that is present on a child's due to normal day-to-day activities. Research concluded that this had not previously been determined in either adults or children, with the exception of one paper by Graham and Rutty (2007). Further research also divulged that bruising is a common early indicator of physical child abuse and is often observed on the head and necks of children aged 0 – 5 years of age (Carpenter 1999) so this was the area that was focused on. The first part of the study involved mapping background levels of DNA present on the head and neck of children, less than five years of age, with no history of abuse or potential indicators of such. Thirty-two children were recruited, 12 areas of the head and neck were swabbed and processed using forensic DNA profiling protocols. The results provide the first data on the DNA profiles that can be retrieved from the faces of children. In spite of person-to-person variation, the majority of alleles matched the child's own reference profile, both findings consistent with previous studies on adult populations. Sex of child and the different childcare techniques employed had little impact, although a decrease in the subject's own allele number was noted as child age increased. Sixteen percent of the facial areas sampled also exhibited non-subject DNA, 15% of which consisted only of alleles that did not match the reference profiles of any individual involved in the study.

The second part was designed to determine if DNA transfer occurred during forceful contact and whether or not the perpetrator's DNA could be distinguished from the victim's own. In order to do so the study was divided into two phases beginning with the application of punches and slaps to a DNA-free plastic sheet attached to a boxing focus pad. Fifteen volunteers were recruited and asked to punch and slap the sheet once, 15 minutes after washing their hands and on a separate occasion, one hour after hand washing. On a third occasion they punched and slapped the sheet three times, one hour after hand washing. Overall the results from this phase showed that more DNA was

transferred when the subjects slapped rather than punched and that the transfer occurred during initial contact as no significant difference was observed between three versus single applications. Equally no significant difference was noted when the time interval between hand washing and contact was increased from 15 minutes to one hour. The second phase involved person-to-person contact beginning with a preliminary experiment involving two subjects to trial the protocol. After the preliminary 16 volunteers were recruited and consented to apply a single punch and on a separate occasion slap another subject. The results led to the conclusion that in some cases transferred DNA could be distinguished after a blow, from perpetrator to victim and vice-versa, with marginally more post-slap than post-punch. However, in the majority of cases transfer was minimal and associated with alleles that matched no-one involved in the study or working in the Forensic Pathology Unit. No significant difference was observed between male and female subjects with regard to transferred DNA.

A major complication of the study is the use of the LCN protocol. At the time this was deemed necessary due to the limited profiles achieved using the standard 28 cycles of PCR. However, much criticism has been levelled at the use of LCN recently due to the comments of the judge in the Omagh case last year stating that 'the technique was not yet seen to be at a sufficiently scientific level to be considered evidence' (BBC website, December 2007). Although the research outlining the protocol advised caution and the acceptance of the fact that while a profile can be achieved from as little as 100pg of DNA (Gill et al. 2000) the type of cell, such as semen, saliva etc, or time of deposition of the sample cannot be identified (Gill 2001) interpretation can be complicated, subjective and open to operator error. As a result of the Omagh trial validation and standardisation studies for the technique are in progress. In addition developments in technology such as capillary electrophoresis and computer programs for profile interpretation mean that LCN may be easier to employ in future. Other techniques such as the use of mini-STRs may eliminate the need to use LCN and as such are going to be employed in the expansion of both parts of my thesis. Two protocols currently being validated are the dilution of 34 cycle PCR products which can reduce stochastic variation and a staged approach to the 28 cycle protocol called DNA SenCE that includes post-PCR clean-up, increased sample

loading, increased injection time and increased injection voltage (Forster et al. 2008). National and international validation of such techniques, including LCN, plus rigorous monitoring of anti-contamination protocols and use of 'DNA free' consumables may ensure that such sensitive techniques may in future be employed to their fullest extent (Caddy Report, 2007).

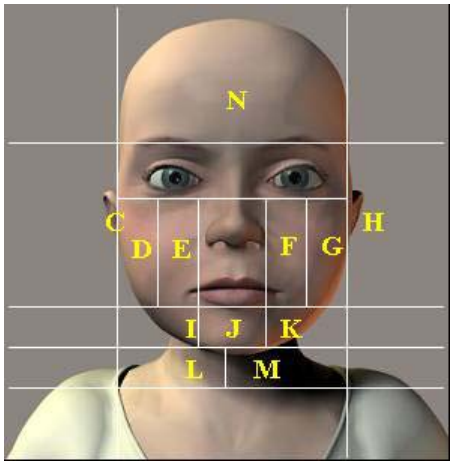
Overall the conclusion of my thesis is that while DNA transfer can occur during forceful contact and be identified this is highly dependent on the individual and in many cases would be indistinguishable from that normally observed on the faces of children. However a more violent assault may result in more transfer and an expansion of both studies may indicate otherwise. An increase in subject number would improve the statistical significance of the data and lessen the effect of the variability observed between the subjects. The number of alleles of unknown origin, most likely from secondary or even tertiary transfer, observed through both parts complicated analysis and would likely make the evidence controversial in criminal cases. Due to the person-to-person variation observed throughout further investigation is necessary before definitive conclusions can be drawn. Further studies into shedder status including ways of testing in children as well as a means of investigating DNA transfer through forceful contact without resorting to LCN protocols, such as the use of mini-STRs or possibly Y-STRs, would provide data important in the fight against physical child abuse. Validation and alternatives to LCN are currently being investigated at laboratories in the UK, while currently the Forensic Pathology Unit, Leicester is examining the questions of shedder status and expanding the studies on DNA transfer through forceful contact and mapping of children's faces.

6. Appendix

6.1 Children's faces

6.1.1 Sampling information pack

1. Take out one DNA sampling pack from bag. **DO NOT REMOVE** anything from pack at this stage.
2. Within the pack you will find the following:
    - 15 sterile blue capped swabs labeled A-O
    - 2 vials of sterile water
    - 1 pack of sterile size 7 gloves and 1 pack of sterile size 6 gloves
    - 1 face mask
    - 1 piece of Blu-tac
    - 4 spare sterile blue-capped swabs
3. Open the pack of size 6 gloves and put on **WITHOUT** touching the fingertips. **DO NOT** blow into or onto gloves.
4. Take the swabs out of the pack and lay them in a line in a suitable preparation area.
5. Take swab **A** and replace into pack with seal unbroken.
6. Break seals on all other swabs but **DO NOT** remove swab from sheath.
7. Put on facemask and then put on the other pair of gloves, again without touching the fingertips as for step 3.
8. Secure the vial of water in the Blu-tac to keep it upright and release top.
9. Take swab **B**, dip into vial of water to moisten only, shake off excess fluid and replace into sleeve. Replace swab into pack.
10. Repeat moistening of swabs **C** to **M** and sample zones on face as per diagram.



To swab the zone, press swab relatively hard onto skin and rub across area, rotating swab so that the entire swab bud contacts the skin. After swabbing each zone, place swab back into sleeve and replace into pack.

11. Take **DRY** swab **N** and firmly swab inside check of subject. Put swab back into sleeve and replace into pack.
12. Take **DRY** swab **O**, remove mask and swab inside operator's mouth as for step 11.
13. All swabs should now be back inside the pack. Remove orange sealing tape and **COMPLETELY** seal the pack, trying not to trap air in it as it is sealed.
14. Sign and date pack, in biro, in the signature box on the back of the pack.
15. Freeze entire pack as soon as possible at –70^{o}C.
16. When ready to send to Leicester, arrange same day transport to Leicester. Use enclosed address label. We **ONLY** require the pack, which is to be sent in a container containing solid CO_2 to ensure swabs remain frozen.
17. Send email to vlb8@le.ac.uk and fax to 0116 252 3274 to inform that swabs are en route.

6.1.2 Questionnaire for parents

Subject Number:	
Date:	
Time:	
Who, including yourself, has had close contact with your child for a period of more than 4 hours over the last 2 days? (include relationship to child as well as date & time of contact)	
Are any of the above contacts suffering from an upper respiratory tract infection at present?	
How many times has your child been washed today?	
What have you washed your child's face with today? a. Wet wipes b. Flannel c. Kitchen cloth d. Soap e. Baby bath product. e. g. Infacare f. Other, please give details	
What was the date and time that your child last had their face washed?	
Has any cream/ointment been applied to your child's face today?	

6.1.3 Questionnaire data

Subject	Sampling Date	Sampling Time	Age (months)	Sex	Washes	Wash Method	Time of Wash
T0021	28/10/03	11.00	50	F	1	Wet Wipes	9.00
T0022	30/10/03	11.00	38	F	1	Flannel & Water	9.00
T0023	28/10/03	11.00	55	M	1	Kitchen Roll & Water	9.00
T0024	28/10/03	11.00	36	M	1	Kitchen Roll & Water	10.45
T0026	25/11/03	10.30	13	F	3	Wet Wipes & Soap	9.00
T0027	30/10/03	14.15	8	F	3	Wet Wipes	12.00
T0029	25/11/03	10.40	6	F	1	Cotton Wool & Water	9.30
T0030	30/10/03	11.50	17	M	1	Flannel & Baby Bath Product	7.45
T0031	30/10/03	14.00	37	F	2	Flannel & Water	12.00
T0032	28/11/03	10.50	40	F	1	Soap	9.00
T0033	28/11/03	11.00	40	F	1	Soap	9.00
T0040	26/1/04	10.45	52	F	1	Flannel & Water	9.15
T0041	8/12/03	9.30	7	M	0		
T0043	8/12/03	9.10	50	F	0		
T0044	29/1/04	12.2	38	F	1	Wet Wipes	8.20
T0045	29/1/04	12.05	22	F	1	Wet Wipes	8.30

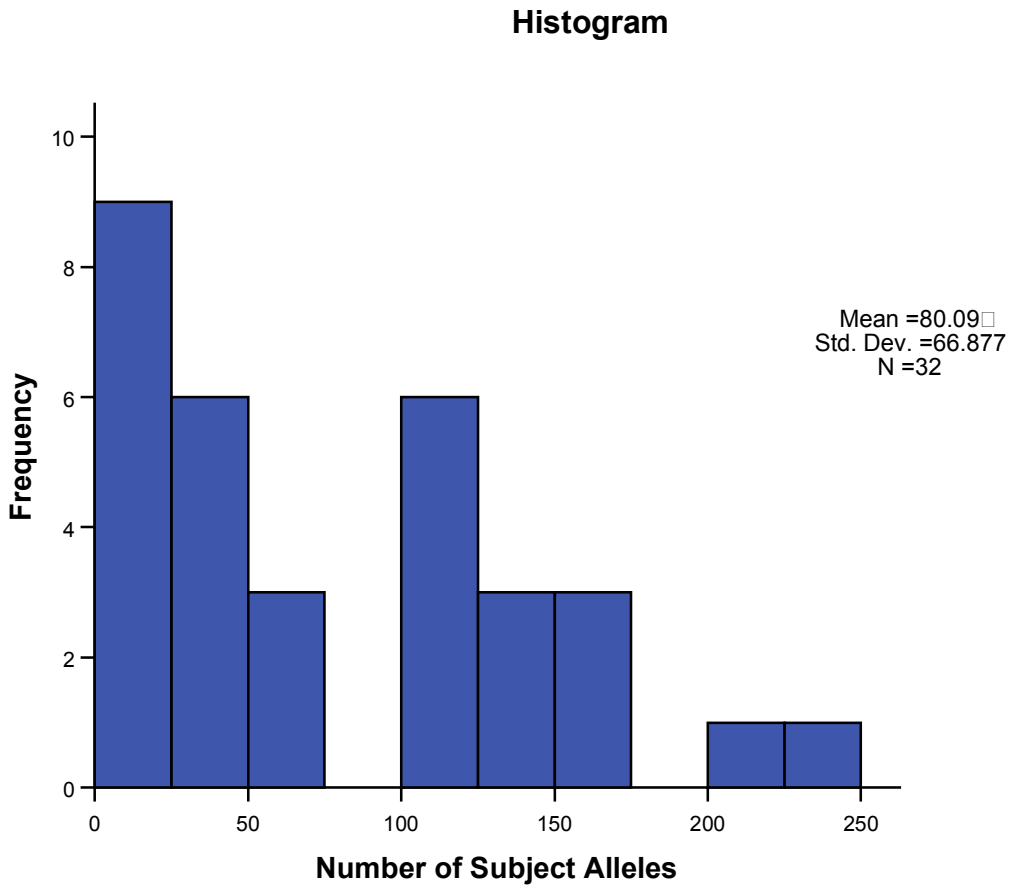
T0046	28/11/03	11.10	45	F	0		27/11/03 19.30
T0047	26/1/04	10.55	49	F	1	Flannel & Water	8.00
T0048	16/3/04	11.00	37	M	0		15/3/04 17.00
T0049	3/2/04	11.30	9	M	2	Wet Wipes	7.00
T0051	3/2/04	12.00	38	F	1	Flannel & Water	10.00
T0052	29/1/04	17.25	19	F	2	Wet Wipes	
T0054	3/2/04	11.00	16	F	1	Flannel & Water	8.45
T0057	11/5/04	16.25	13	M	4	Wet Wipes & Flannel	13.30
T0067	7/6/04	19.15	11	F	2	Wet Wipes & Flannel	18.45
T0071	15/11/04	10.00	4	M	1	Wet Wipes	7.45
T0072	5/7/04	10.45	14	M	1	Flannel & Water	8.00
T0080	21/3/05	9.50	3	F	1	Cotton Wool & Water	9.00
T0081	24/6/05	14.00	1	F	0		23/6/05 19.00
T0082	26/7/05	9.50	1	F	0		25/7/05 18.00

6.1.4 Quantification results (ng/ul)

Subject	Facial Area												Buccal	Relatives					
	C	D	E	F	G	H	I	J	K	L	M	N							
21	4.21	4.22	2.95	6.69	8.82	4.60	4.95	2.78	4.58	7.61	6.61	3.95	3.31	5.50	6.86				
22	1.64	1.47	1.67	2.55	0.58	0.27	2.27	2.28	0.84	2.23	2.08	1.20	0.45	1.52	1.86	3.49			
23	0.69	1.34	1.36	1.53	1.61	0.69	0.12	0.82	1.84	1.83	0.48	1.08	2.11	2.97	5.15	2.78			
24	2.52	2.26	2.68	3.44	2.18	1.41	3.06	2.50	2.41	2.23	3.71	5.42	7.63	4.30	4.51	2.50			
26	2.67	2.69	2.59	1.36	4.06	2.68	1.84	2.27	2.87	2.16	2.54	3.74	12.00	3.08	5.55	10.32			
27	0.32	2.60	1.09	1.92	1.31	3.30	2.13	1.70	5.36	2.45	0.95	3.47	3.36	2.94	3.23				
29	1.96	3.66	2.76	5.02	1.62	3.16	2.36	2.99	2.71	3.44	3.23	2.36	4.16	3.81	1.71				
30	2.55	2.34	2.17	2.21	2.35	2.34	3.21	3.55	1.72	1.54	2.56	1.28	2.77	2.93	2.50	3.00	5.47		
31	1.50	3.23	1.70	0.72	1.66	3.58	1.82	2.45	3.97	2.75	3.19	2.48	2.21	1.85	2.33	2.57			
32	7.11	1.71	6.29	7.44	5.28	6.41	10.98	7.53	6.10	1.39	8.40	5.18	9.05	0.66	4.14	3.05			
33	2.87	6.17	4.85	5.80	4.51	4.03	3.78	2.17	3.78	2.90	5.93	6.76	4.09	3.42	4.47	3.09			
40	4.37	4.25	3.76	2.38	4.52	6.82	3.25	2.49	1.14	5.01	6.09	2.97	5.20	3.70	5.27	3.36	5.86		
41	5.08	7.90	4.46	3.87	1.76	3.08	4.02	3.28	4.67	3.85	3.34	3.66	7.82	4.58					
43	3.26	3.49	4.05	4.38	4.54	6.17	3.48	4.94	3.11	4.52	2.31	3.24	4.83	6.13	9.12	8.99			
44	7.39	4.89	6.51	3.73	4.07	5.14	6.11	3.30	2.72	6.24	4.44	3.71	3.87	4.44	3.71	3.87			
45	1.48	3.50	4.80	3.16	11.21	7.20	3.69	12.09	8.44	9.78	1.42	7.78	3.64	5.43	4.94				
46	2.42	1.42	4.71	6.84	15.32	9.24	0.26	0.90	0.41	0.12	5.47	0.21	5.39	3.94	3.82				
47	4.29	3.64	3.89	3.21	2.40	3.87	4.23	4.36	4.75	4.21	4.92	3.91	22.58	4.21	5.02	3.07	7.17	5.23	4.47
48	1.04	0.70	1.34	2.14	2.26	1.41	1.64	1.30	1.01	1.98	2.12	0.76	1.51	2.14					
49	5.14	3.60	2.63	2.65	2.07	3.74	4.63	1.80	3.16	6.91	2.51	8.18	5.77	3.86	8.14				
50	2.89	3.30	7.49	5.22	4.99	3.95	7.21	10.12	4.79	6.16	5.39	5.28	5.44						
51	6.83	8.28	5.33	4.31	2.83	5.28	3.97	3.54	4.56	4.33	9.36	4.99	1.24	4.37	4.50				
52	7.49	9.27	12.77	5.17	9.47	2.47	8.49	8.01	11.03	1.78	4.50	2.61	11.20	7.13					
54	3.86	2.94	3.24	4.26	4.40	4.56	1.55	2.90	3.01	7.70	3.70	2.60	4.76	4.29	7.41	17.83	8.42		
57	3.41	3.84	7.51	3.85	3.69	2.37	3.19	3.48	2.82	2.90	2.28	3.48	8.52	5.03	6.04	5.22	4.71	3.46	
67	4.09	5.64	1.95	4.11	5.71	3.13	3.16	2.54	6.61	4.25	3.62	3.45	5.36	5.73	4.59				

71	3.09	5.13	2.29	3.65	4.00	4.47	5.82	2.75	3.74	4.31	5.50	5.34	4.39	7.25		
72	2.86	2.68	4.22	3.34	3.21	3.39	4.74	3.21	2.99	2.07	1.88	5.82	8.63			
80	4.09	3.94	5.38	5.97	5.34	5.50	4.98	3.27	4.56	4.77	11.88	3.42	3.44	7.50	8.46	
81	4.18	6.71	7.18	7.83	13.39	7.65	5.81	4.43	8.76	7.10	3.69	5.12	6.92	4.16	6.28	5.99
82	3.37	6.37	6.37	3.74	4.40	4.44	3.82	3.29	3.60	3.54	1.99	5.30	5.35			
83	4.05	4.36	4.79	3.06	3.21	2.89	6.16	6.74	6.07	4.45	2.81	4.12	10.67	2.99	4.25	

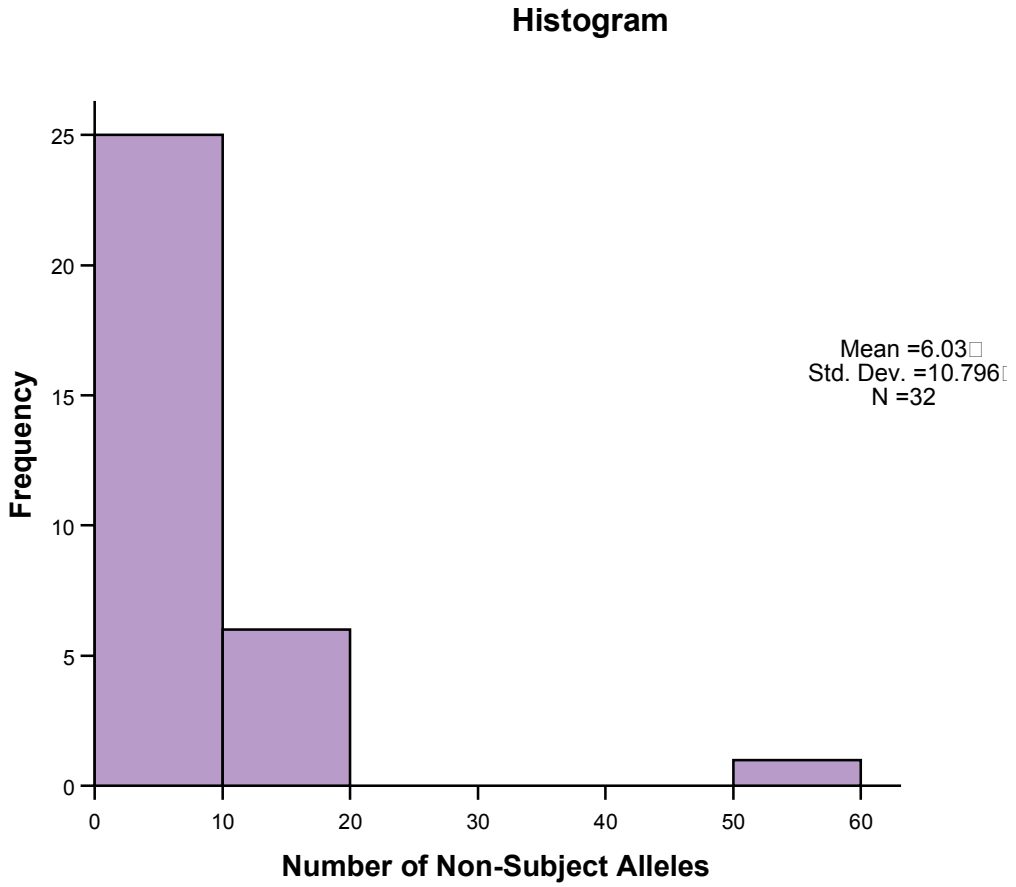
6.1.5 SPSS normal distribution test results



Tests of Normality

	Kolmogorov-Smirnov(a)			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Number of Subject Alleles	.164	32	.029	.910	32	.012

a Lilliefors Significance Correction



Tests of Normality

	Kolmogorov-Smirnov(a)			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Number of Non-Subject Alleles	.288	32	.000	.537	32	.000

a Lilliefors Significance Correction

6.1.6 DNA profiling results using standard PCR

T0024

	D3	VWA	D16	D2	D8	D21	D18	D19	TH01	FGA
Swab Control										
Water Control										
C										
D										
E										
F	15,18	17,18	11,14	18,25	13,13	31.2,33.2	12,14	13,15.2	8,9.3	21,23
G										
H										
I										
J		17,18			13,13			13		
K		18			13,13					
L										
M										
N										
Subject	15,18	17,18	11,14	18,25	13,13	31.2,33.2	12,14	13,15.2	8,9.3	21,23
Sampler	15,16	15,17	9,13	18,19	10,13	29,29	13,16	12,14	9.3,9.3	19,24
Relative	17,18	17,18	10,11	17,18	13,13	30.2,31.2	14,17	13,14	8,9.3	21,24
Relative	15,18	17,18	9,14	19,25	8,13	30,33.2	12,12	15,15.2	9.3,9.3	21,23
Relative	17,18	17,18	9,11	18,25	13,13	30,30.2	12,14	13,15	8,9.3	21,24

T0026

	D3	VWA	D16	D2	D8	D21	D18	D19	TH01	FGA
Swab Control										
Water Control										
C	14,15	18,19	9,13	18,23	14,15	28,30.2	14,15	13,14	9.3,9.3	22,24
D	14,15	18,19	9,13	18,23	14,15	28,30.2	14,15	13,14	9.3,9.3	22,24
E	14,15	18,19	9,13	18,23	14,15	28,30.2	14,15	13,14	9.3,9.3	22,24
F	14,15	18,19	9,13		14,15	28,30.2		13,14	9.3,9.3	
G		18,19	9,13		14,15					
H	14,15	18,19								
I	14,15	18,19	9,13	18	14,15	28,30.2	14,15	13,14	9.3,9.3	22,24
J	14,15	18,19	9		14,15	30.2	14	13,14	9.3,9.3	
K	14,15	18,19	9,13	18,23	14,15	28,30.2	14,15	13,14	9.3,9.3	22,24
L	14,15	18,19	9,13		14,15	28,30.2	14,15	13,14	9.3,9.3	22,24
M	14,15	18,19	9,13	18,23	14,15	28,30.2	14,15	13,14	9.3,9.3	22,24
N	14,15	18,19								
Subject	14,15	18,19	9,13	18,23	14,15	28,30.2	14,15	13,14	9.3,9.3	22,24
Sampler	15,16	15,17	9,13	18,19	10,13	29,29	13,16	12,14	9.3,9.3	19,24
Relative	14,15	18,19	12,13	23,24	13,14	29,30.2	15,16	14,14	9.3,9.3	23,24
Relative	14,17	18,18	9,12	17,18	13,15	28,30	13,14	13,15	9.3,9.3	19,22
Relative	14,16	16,18	11,11	23,23	12,13	29,32	16,16	12,14	9.3,9.3	20,24

T0033

	D3	VWA	D16	D2	D8	D21	D18	D19	TH01	FGA
Swab Control										
Water Control										
C										
D										
E	14,16	16,17	9,13		10,11	28,28		14,14		21,22
F	14,16	16,17	9		10,11	28,28		14,14	7,9	21,22
G										
H										
I										
J								14,14		
K	14,15,16	16,17	9		10,11	28,28		14,14		22
L										
M										
N								14,14		
Subject	14,16	16,17	9,13	17,20	10,11	28,28	13,16	14,14	7,9	21,22
Sampler	15,16	15,17	9,13	18,19	10,13	29,29	13,16	12,14	9.3,9.3	19,24
Relative	14,16	14,17	13,13	17,17	11,11	28,30	13,16	14,14	6,7	20,21
Relative	14,15	16,18	9,12	21,21	10,15	28,32.2	13,14	13.2,14	6,9	22,23
Relative	14,15	16,17	9,13	17,20	11,15	28,32.2	14,16	13.2,14	6,6	21,23

T0047

	D3	VWA	D16	D2	D8	D21	D18	D19	TH01	FGA
Swab Control										
Water Control										
C										
D	17,18	15,18			13			13,14		
E	17,18	15						13,14		
F	17,18	15,18			13,14	28		13,14	8,8	
G										
H										
I										
J										
K										
L										
M										
N										
Subject	17,18	15,18	13,14	17,20	13,14	28,32.2	12,18	13,14	8,8	23,25
Sampler	15,16	15,17	9,13	18,19	10,13	29,29	13,16	12,14	9.3,9.3	19,24
Relative	15,18	18,18	11,13	19,20	14,15	28,31	18,18	14,15	8,9	21,25
Relative	17,17	15,18	13,14	17,23	13,13	28,32.2	12,13	13,13	8,9.3	20,23
Relative	17,18	18,18	11,14	17,19	13,14	28,31	13,18	13,15	8,8	21,23
Relative	17,18	15,18	13,14	17,19	13,15	31,32.2	13,18	13,15	9,9.3	20,25
Relative	15,17	18,18	11,13	19,19	13,14	31,32.2	13,18	13,15	8,8	20,25
Relative	17,19	16,17	11,13	17,20	13,14	28,28	18,18	13,14	9,9.3	21,25

6.1.7 DNA profiling results using LCN PCR

T0021

	D3	VWA	D16	D2	D8	D21	D18	D19	TH01	FGA
Swab Control										
Water Control										
C					12					
D								12		
E	14,15							12,14		
F	14,15	18,19			8,13			12,14		
G	14,15							12,14		
H										
I		16	9,10		12,13	28				
J	14							12,14		
K	14,15							12,14		
L										
M	14							14		
N										
Subject	14,15	18,19			8,8	28,29	14,14	12,14	9.3,9.3	22,25
Sampler	15,16	15,17	9,13	18,19	10,13	29,29	13,16	12,14	9.3,9.3	19,24
Relative	14,17	18,19	9,11	18	8,14	30		14		22
Relative	14,15	18,19			13,14	30,30		12,14		22,25

T0022

	D3	VWA	D16	D2	D8	D21	D18	D19	TH01	FGA
Swab Control										
Water Control										
C	15,16	14,17	9,12	18	10,12	29	14	14	7	19,24
D	15,16				12			14		
E										
F	15,16				12			12,14		
G	15,16	17						12,14		
H	15,16							12,14		
I	15,16					29		12,14		
J						29		12		
K										
L		17				29				
M								14		
N										
Subject	15,16	17,18			12,12	28,29		12,14	9,9	24,27
Sampler	15,16	15,17	9,13	18,19	10,13	29,29	13,16	12,14	9.3,9.3	19,24
Relative	14,15	16,17	9		12,13			12		
Relative	15,16	17						14		
Relative	15,15	14,14	12,13	18,23	13,14	29,30		14,16	6,9.3	19,24

T0023

	D3	VWA	D16	D2	D8	D21	D18	D19	TH01	FGA
Swab Control										
Water Control										
C										
D										
E		18			13			13		
F					13			13		
G										
H										
I										
J		17,18			13	30		15		
K					13					
L								13		
M	17,18	17,18	9		13			15		
N	18									
Subject	17,18	17,18	9,11	18,25	13,13	30,30.2	12,14	13,15	8,9.3	21,24
Sampler	15,16	15,17	9,13	18,19	10,13	29,29	13,16	12,14	9.3,9.3	19,24
Relative	17,18	17,18	9,11	17,18	13,13	30.2,31.2	17,17	13,14	9.3,9.3	24,24
Relative	15,18	17,18	9,14	19,25	8,13	30,33.2	12,12	15,15.2	9.3,9.3	21,23
Relative	15,18	17,18	11,14		13,13	31.2,33.2	12,14	13,15.2	8,9.3	21,23

T0024

	D3	VWA	D16	D2	D8	D21	D18	D19	TH01	FGA
Swab Control										
Water Control								14		
C	15	17								
D	15,18	17,18			13	31.2,33.2	12	13,15.2	8	
E	15,18	17,18		25	13	31.2,33.2		13,15.2	8,9.3	21
F	15,18	17,18	11,14	18,25	13	31.2,33.2	12,14	13,15.2	8,9.3	21,23
G	15,18	17,18	14	18	13	31.2,33.2	12,14	13,15.2	8,9.3	21
H										
I	15,18	17,18	11,14		13	33.2	12	13,15.2	8	
J	15,18	17,18	11,14	18,25	13	31.2,33.2	12,14	13,15.2	8,9.3	21,23
K	15,18	14,17,18	11,14	18	13	31.2,33.2	12,14	13,15.2	8,9.3	21,23
L	14							14		
M		18			13					
N	15,16,18	17,18	9		10,12,13	29,33.2		13		21,23
Subject	15,18	17,18	11,14	18,25	13,13	31.2,33.2	12,14	13,15.2	8,9.3	21,23
Sampler	15,16	15,17	9,13	18,19	10,13	29,29	13,16	12,14	9.3,9.3	19,24
Relative	17,18	17,18	10,11	17,18	13,13	30.2,31.2	14,17	13,14	8,9.3	21,24
Relative	15,18	17,18	9,14	19,25	8,13	30,33.2	12,12	15,15.2	9.3,9.3	21,23
Relative	17,18	17,18	9,11	18,25	13,13	30,30.2	12,14	13,15	8,9.3	21,24

T0026

	D3	VWA	D16	D2	D8	D21	D18	D19	TH01	FGA
Swab Control										
Water Control										
C	14,15	18,19	9,13	18,23	14,15	28,30.2	14,15	13,14	9.3	22,24
D	14,15	18,19	9,13	18,23	14,15	28,30.2	14,15	13,14	9.3	22,24
E	14,15	18,19	9,13	18,23	14,15	28,30.2	14,15	13,14	9.3	22,24
F	14,15	18,19	9,13	18,23	14,15	28,30.2	14,15	13,14	9.3	22,24
G	14,15	18,19	9,13		14,15	28,30.2	14,15	13,14	9.3	22,24
H	14,15	18,19	9,13	23		28,30.2		13,14	9.3	22,24
I	14,15	18,19	9,13	18,23	14,15	28,30.2	14,15	13,14	9.3	22,24
J	14,15	18,19	9,13	18,23	14,15	28,30.2	14,15	13,14	9.3	22,24
K	14,15	18,19	9,13		14,15	28,30.2	14,15	13,14	9.3	22,24
L	14,15	18,19	9,13	18,23	14,15	28,30.2	14,15	13,14	9.3	22,24
M	14,15	18,19	9,13	18,23	14,15	28,30.2	14,15	13,14	9.3	22,24
N	14,15	18,19	9,13	18,23	14,15	28,30.2	14,15	13,14	9.3	22,24
Subject	14,15	18,19	9,13	18,23	14,15	28,30.2	14,15	13,14	9.3,9.3	22,24
Sampler	15,16	15,17	9,13	18,19	10,13	29,29	13,16	12,14	9.3,9.3	19,24
Relative	14,15	18,19	12,13	23,24	13,14	29,30.2	15,16	14,14	9.3,9.3	23,24
Relative	14,17	18,18	9,12	17,18	13,15	28,30	13,14	13,15	9.3,9.3	19,22
Relative	14,16	16,18	11,11	23,23	12,13	29,32	16,16	12,14		20,24

T0027

	D3	VWA	D16	D2	D8	D21	D18	D19	TH01	FGA
Swab Control										
Water Control										
C										
D	15,18	15,17	11,12		13	27,29		12,13	8,9	23,24
E	15,18	15,17	11,12		13	27,29	16	12,13	9	23,24
F	15,18	15,17			13	27,29		12,13	8	24
G	15,18	15,17	11,12		13	27,29	15	12,13	8,9	23,24
H	15,18	15,17			13	27,29		12,13	8	23,24
I	15,18	15,17	11		13	27,29		12,13	9	23,24
J	15,18	15,17	11,12		13	27,29	16	12,13	8,9	23,24
K	15,18	15,17			13	27,29		12,13		23,24
L	15,18	15,17			13	27,29		12,13	8,9	23,24
M								12		
N	15,18	15,17	11,12		13	27,29		12,13	8,9	23,24
Subject	15,18	15,17	11,12	17,17	13,13	27,29	15,16	12,13	8,9	23,24
Sampler	15,16	15,17	9,13	18,19	10,13	29,29	13,16	12,14	9.3,9.3	19,24
Relative	15,16	15,16	11,12	23	11,13	29,31	13,17	12,14	8,9.3	22,23
Relative	16,18	16,17	11,12	17,20	13,13	27,29	15,16	12,14	9,9	22,23

T0029

	D3	VWA	D16	D2	D8	D21	D18	D19	TH01	FGA
Swab Control										
Water Control										
C	15	16,18			16	30,31	12	14		20,23
D	14,15	16,18	9,11,12	19,22	15,16	30,31	12,16	13.2,14	7,9.3	20,23
E	15	16,18			15,16	30,31		13.2,14		20,23
F	14,15	16,18	11,12	19,22	15,16	30,31	12,16	13.2,14	7,9.3	20,23
G	14,15	16,18	12		15,16	30,31		14	7,9.3	
H	14,15	18	12		15,16	30		14		
I	14,15	16,18	11,12		15,16	30,31	16	13.2,14	7,9.3	20,23
J	14,15	16,18	12	19,22	15,16	30,31	12,16	13.2,14	7,9.3	20,23
K	14,15	16,18	11,12	19,22	15,16	30,31	12,16	14	7,9.3	20,23
L	14,15,16	16,18			15,16	30	12	14		
M	15	16,18,19			15	30,31		14,15		
N	14,15,16	16,18,19	9,11,12	19,22	14,15,16	30,31		14,15	7,9.3	
Subject	14,15	16,18	11,12	19,22	15,16	30,31	12,16	13.2,14	7,9.3	20,23
Sampler	15,16	15,17	9,13	18,19	10,13	29,29	13,16	12,14	9.3,9.3	19,24
Relative	14,16	18,19	9,12	22,23	14,15	30,30	12,18	14,15	7,9.3	23,26
Relative	15,18	14,16	11,12	19,20	10,16	30,31	16,18	13.2,15	9.3,9.3	20,24

T0030

	D3	VWA	D16	D2	D8	D21	D18	D19	TH01	FGA
Swab Control										
Water Control						28				
C		17,18			13					
D	15	16				30		12		
E	15	18			13,14			12,14	9.3	
F	15	18	12	20	13,14		14	12	9	
G	15	16								19
H	15				14			12,16		
I	15	16,18			13,14			12,15,16		19
J	15,16	16,18	12		13,14	30		12,16	9.3	19,24
K	15,16	16,17,18	12	18	13,14	29		15,16		
L	15									
M										
N	15	18	12			30		12	9,9.3	19
Subject	15,15	16,18	12,13	18,19	13,14	30,31.2	14,15	12,16	9,9.3	19,24
Sampler	15,16	15,17	9,13	18,19	10,13	29,29	13,16	12,14	9.3,9.3	19,24
Relative	15,15	14,18	9,13	17,25	12,14	29,31.2	14,15	14,16	6,9	19,24
Relative	15,16	14,16	11,12	20,23	13,14	29,30	15	12,14		23
Relative	15,15	14,16	9,12	25	13,14	29,30	16	12,14	9,9.3	19,24
Relative	15,15	14,14	12,13	18,18	13,14	29,30	15	14,16	6,9.3	19,24

T0031

	D3	VWA	D16	D2	D8	D21	D18	D19	TH01	FGA
Swab Control										
Water Control										
C										
D					13			12		
E										
F	16				13					
G										
H										
I										
J										
K										
L	16,18	16,17			13					
M										
N	15,16				13			12,14		
Subject	16,18	16,17	9,10		13,13	27,29	15,16	12,14	9,9	22,23
Sampler	15,16	15,17	9,13	18,19	10,13	29,29	13,16	12,14	9.3,9.3	19,24
Relative	18,19	16,17	12,13	20,23	12,13	27,32	16,16	12,13	9,9	22,24
Relative	15,16	15,16	11,11	17,17	13,16	29,29	13,13	12,14	8,8	22,23
Relative	15,18	15,17	11,11		13,13	27,29	15,16	12,13	7,9	23,24

T0032

	D3	VWA	D16	D2	D8	D21	D18	D19	TH01	FGA
Swab Control										
Water Control										
C	14,15	16,17						13.2,14		
D	14,15	16,17	9,13	19,20	11,15	28,32.2	14,16	13.2,14	6	21,23
E	14,15	16,17	9,13	19,20	11,15	28,32.2	14,16	13.2,14	6	21,23
F	14,15	16,17			11,15	32.2		13.2,14		
G	14,15	16,17								
H	15									
I	14,15	16,17			11,15	28,32.2		13.2,14		
J	14,15	16,17	9		11,15	28,32.2		13.2,14	6	21,23
K	14,15	16,17	9,13	19,20	11,15	28,32.2	14,16	13.2,14	6	21,23
L	15									
M										
N	14,15	16,17			11,15	28,32.2		13.2,14		
Subject	14,15	16,17	9,13	19,20	11,15	28,32.2	14,16	13.2,14	6,6	21,23
Sampler	15,16	15,17	9,13	18,19	10,13	29,29	13,16	12,14	9.3,9.3	19,24
Relative	14,16	14,17	13,13	17,19	11,11	28,30	13,16	14,14	6,7	20,21
Relative	14,15	16,18	9,12	20,21	10,15	28,32.2	13,14	13.2,14	6,6	22,23
Relative	14,16	16,17	9,13	17,20	10,11	28,28	13,16	14,14	7,9.3	21,22

T0033

	D3	VWA	D16	D2	D8	D21	D18	D19	TH01	FGA
Swab Control										
Water Control	13									
C										
D	14,15,16	16,17	9		10,11,15	28		14	6	21,23
E	14,16	16,17	9,13		10,11	28		14	7,9	21,22
F	14,16	16,17	9,13	17	10,11	28	13	14	7,9	21,22
G	14,15,16	16,17	9		10,11,15	28,32.2		14	6,9	21
H										
I	14,16	16,17			10,11	28		14	7,9	21
J	14,16	16,17	9,13	20	10,11	28	13,16	14	7,9	21,22
K	14,16	16,17	9,13		10,11,15	28		14	7,9	21,22
L	14,15,16	16,17	13		10,11	28		14	7	
M		16			10,11			14		
N	14,15,16	16,17	9		10,11,15	28,32.2		14	6,7,9	21,22
Subject	14,16	16,17	9,13	17,20	10,11	28,28	13,16	14,14	7,9	21,22
Sampler	15,16	15,17	9,13	18,19	10,13	29,29	13,16	12,14	9.3,9.3	19,24
Relative	14,16	14,17	13,13	17,17	11,11	28,30	13,16	14,14	6,7	20,21
Relative	14,15	16,18	9,12	21,21	10,15	28,32.2	13,14	13.2,14	6,9	22,23
Relative	14,15	16,17	9,13		11,15	28,32.2	14,16	13.2,14	6,6	21,23

T0040

	D3	VWA	D16	D2	D8	D21	D18	D19	TH01	FGA
Swab Control										
Water Control										
C										
D	17,19	17				28		13,14		
E					13,14			14		
F	17,19	16,17			13,14	28		13,14	9	21
G	17	17						13		
H		17			13					
I	17	16				28		13,14		
J	17									
K										
L	19					28		13		
M		17				29				
N	17,19	16			13	28		13,14		
Subject	17,19	16,17	11,13	17,20	13,14	28,28	14,18	13,14	9,9.3	21,25
Sampler	15,16	15,17	9,13	18,19	10,13	29,29	13,16	12,14	9.3,9.3	19,24
Relative	17,17	16,18	11,13	18,20	13,14	28,31.2	14,14	14,14	9,9.3	21,22.2
Relative	16,19	17,17	12,13	17,22	13,13	28,28	18,18	13,13	9,9	22,25
Relative	16,17	16,17	11,12	20,22	13,13	28,31.2	14,18	13,14	9,9.3	22,22.2
Relative	17,18	15,18	13,14	17,20	13,14	28,32.2	12,18	13,14	8,8	23,25

T0041

	D3	VWA	D16	D2	D8	D21	D18	D19	TH01	FGA
Swab Control										
Water Control										
C										
D										
E										
F	15,17	16						14		
G	15,17	16,18	11,14	17,20	12,13	28,30	14,15	14	7,8	21,25
H										
I					13			14		
J										
K	15,17	16,18	11		12,13	28,30		14	8	21,25
L										
M										
N	15,17	18						14		
Subject	15,17	16,18	11,14	17,20	12,13	28,30	14,15	14,14	7,8	21,25
Sampler	15,16	15,17	9,13	18,19	10,13	29,29	13,16	12,14	9.3,9.3	19,24
Relative	15,15	15,18	11,12	17,25	13,14	28,29	13,15	12,14	7,8	20,25

T0043

	D3	VWA	D16	D2	D8	D21	D18	D19	TH01	FGA
Swab Control										
Water Control										
C										
D										
E										
F	15,17	14,16		23	14,15	29,30		12,15.2	9	
G										
H										
I										
J										
K										
L										
M										
N										
Subject	15,17	16,18	12,12	17,23	12,13	28,31.2	14,14	12,14	7,7	20,21
Sampler	15,16	15,17	9,13	18,19	10,13	29,29	13,16	12,14	9.3,9.3	19,24
Relative	15,15	15,18	11,11	25,25	13,14	28,29		12,14	8,8	20,25
Relative	15,17	16,18			12,13	28,30	14,14	12,13		23,25
Relative	16,18	16,18	11,14	17	12,13	28,30	14,15	15,15	7,8	21,25

T0044

	D3	VWA	D16	D2	D8	D21	D18	D19	TH01	FGA
Swab Control										
Water Control										
C								13,14		
D								13,14		
E					11			13,14		
F	16,17				11			13,14,17		
G								13,14		
H								13,14		
I								13,14		
J								13		
K								13		
L								13,14		
M								13,14		
N	16,17	15			11			13,14,17		
Subject	17,18	15,17	11,12	20,20	11,11	28,32.2	12,13	14,17	7,9.3	20,22
Sampler	15,16	15,17	9,13	18,19	10,13	29,29	13,16	12,14	9.3,9.3	19,24
Relative	16,17	15,17	10,12	17,24	11,12	30,32.2	12,13	13,17	6,9.3	19,20
Relative	16,18	17,18	11,12		11,15	28		14	6	20,22
Relative	16,17	15,17	10,12	17	11	28,30,32.2	12	13,14	6,9.3	19,22

T0045

	D3	VWA	D16	D2	D8	D21	D18	D19	TH01	FGA
Swab Control										
Water Control										
C								13		
D								13		
E					11			13		
F								13		
G								13		
H					11			13		
I								13		
J								13		
K								13		
L								13		
M								13		
N								13,17		
Subject	16,17	15,18	10,12	24	11,11	28,30		13,14	9,9.3	20,22
Sampler	15,16	15,17	9,13	18,19	10,13	29,29	13,16	12,14	9.3,9.3	19,24
Relative	16,18	17,18			11,15	28		14		20,22
Relative	16,17	15,17	10,12	24	11,12	30,32.2		13,17	6,9.3	19,20

T0046

	D3	VWA	D16	D2	D8	D21	D18	D19	TH01	FGA
Swab Control										
Water Control										
C										
D										
E	16							14		
F								14		
G										
H										
I										
J	16,17	17,18			12,17	29		14.2		23
K										
L										
M										
N	16,17	17,18	12		12,17			14		
Subject	16,17	17,18	12,12		12,17	29,30		14,14.2	6,9.3	23,23
Sampler	15,16	15,17	9,13	18,19	10,13	29,29	13,16	12,14	9.3,9.3	19,24
Relative	14,16	14,18			17	30		14		
Relative	14,17	15,17	11,12		11,12	29,30.2	13,17	14,14.2	9.3	23,25

T0047

	D3	VWA	D16	D2	D8	D21	D18	D19	TH01	FGA
Swab Control										
Water Control										
C		17,18			13			13,14		
D	17,18	15,18	13,14	17	13,14	28,32.2	12,18	13,14	8	23,25
E	17,18	15,18	13,14		13,14	28,32.2		13,14	8	23,25
F	17,18	15,18	13,14	17,20	13,14	28,32.2	12,18	13,14	8	23,25
G	17,18	15,18	13,14	17,20	13,14	28,32.2	12,18	13,14	8	23,25
H										
I	17	15			14	32.2		13,14		23
J	17	15,18						13,14		
K										
L	18	15,17,18			13,14			13,14		
M										
N	17,18	15,18	13,14		13,14	28,32.2	12	13,14	8	23,25
Subject	17,18	15,18	13,14	17,20	13,14	28,32.2	12,18	13,14	8,8	23,25
Sampler	15,16	15,17	9,13	18,19	10,13	29,29	13,16	12,14	9.3,9.3	19,24
Relative	15,18	18,18	11,13	19,20	14,15	28,31	18,18	14,15	8,9	21,25
Relative	17,17	15,18	13,14	17,23	13,13	28,32.2	12,13	13,13	8,9.3	20,23
Relative	17,18	18,18	11,14	17,19	13,14	28,31	13,18	13,15	8,8	21,23
Relative	17,18	15,18	13,14	17,19	13,15	31,32.2	13,18	13,15	9,9.3	20,25
Relative	15,17	18,18	11,13	19,19	13,14	31,32.2	13,18	13,15	8,8	20,25
Relative	17,19	16,17	11,13	17,20	13,14	28,28	18,18	13,14	9,9.3	21,25

T0048

	D3	VWA	D16	D2	D8	D21	D18	D19	TH01	FGA
Swab Control										
Water Control										
C	14,16	18,19	10	17	14	27,32.2	10,12	13,15	6,10	24
D	14,16	18,19	10,11	20	14	27,32.2	10,12	13,15	6	24,26
E	14,16	18,19	10,11	17,20	14	27,32.2	10,12	13,15	6,10	24,26
F		19						13		
G	14,16	18,19			14	27,32.2		13,15		24
H	14,16	18,19	10,11	17,20	14	27,32.2	10,12	13,15	6,10	24,26
I	14,16	18,19	10	17	14	27,32.2	10	13,15	10	24,26
J	14,16	18,19			14	27		12,13,15		24
K	14,16	18,19	11		14	27,32.2	10,12	13,15		24,26
L	14,16	18,19	10,11	17	14	27,32.2	10,12	13,15	6,10	24,26
M	14,16	18,19	10		14	27,32.2	10,12	13,15	10	
N	14,16	18,19	10,11		14	27,32.2	10,12	13,15	6,10	24,26
Subject	14,16	18,19	10,11	17,20	14,14	27,32.2	10,12	13,15	6,10	24,26
Sampler	15,16	15,17	9,13	18,19	10,13	29,29	13,16	12,14	9.3,9.3	19,24
Relative	14,14	16,18	11,12	17,25	11,14	30,32.2	12,15	12,13	9,10	21,24

T0049

	D3	VWA	D16	D2	D8	D21	D18	D19	TH01	FGA
Swab Control										
Water Control										
C	16,18	14,15	10		13,16		16	14.2,16	9,9.3	19,24
D	16,18	15,17			13,16	32.2		14.2,16		19
E	16,18	14,15			13,16	30,32.2		14.2,16	9	19,24
F	16,18	15			13,16	30,32.2		14.2,16	9	19,24
G	16,18	14,15	12		13,16	30,32.2		14.2,16		19,24
H	16,18	15						14.2		
I	16,18	14,15			13,16	30,32.2		14.2,16		19,24
J	16,18	14,15	10,12		13,16	30,32.2		14.2,16	9,9.3	19,24
K	16,18	14,15			13,16	32.2		14.2,16		19,24
L	16,18	15,18			13,16	30,32.2		14.2,16	9,9.3	19,24
M	16,18	14,15			13,16			14.2,16		
N	16,18	14,15,17	12		13,16	30,32.2		14.2,16	7,9	19,24
Subject	16,18	14,15	10,12	17,17	13,16	30,32.2	16,21	14.2,16	9,9.3	19,24
Sampler	15,16	15,17	9,13	18,19	10,13	29,29	13,16	12,14	9.3,9.3	19,24
Relative	14,16	14,17	10,10	17,21	15,16	30,33.2	12,16	14,14.2	7,9.3	19,22
Relative	16,18	14,15	10,12	21,24	13,15	30,32.2	16,21	14,14	7,9.3	19,25

T0050

	D3	VWA	D16	D2	D8	D21	D18	D19	TH01	FGA
Swab Control										
Water Control										
C										
D										
E										
F										
G										
H								14		
I										
J										
K										
L										
M										
N										
Subject	14,18	14,16	9,10	20,23	12,13	28,31	12,15	14,15	7,9.3	24,26
Sampler	15,16	15,17	9,13	18,19	10,13	29,29	13,16	12,14	9.3,9.3	19,24

T0051

	D3	VWA	D16	D2	D8	D21	D18	D19	TH01	FGA
Swab Control										
Water Control										
C										
D										
E	14							15		
F								15		
G										
H								12,15		
I										
J	15	14,19				28		15		
K										
L								15		
M										
N								14,15		
Subject	14,15	15,19	11,11		14,14	30,31.2		14,15	9.3,9.3	20,24
Sampler	15,16	15,17	9,13	18,19	10,13	29,29	13,16	12,14	9.3,9.3	19,24
Relative	15,17	15,17			13,14	31.2		15		20,21
Relative	14,17	18,19	11,11	18,23	12,14	28,31.2	14,16	14,15	7,7	22,24

T0052

	D3	VWA	D16	D2	D8	D21	D18	D19	TH01	FGA
Swab Control										
Water Control										
C										
D										
E										
F										
G										
H										
I										
J										
K										
L		17								
M										
N										
Subject	17,18	16,17	11,13	18,24	10,12	29,30	13,19	13,14.2	7,9	24,25
Sampler	15,16	15,17	9,13	18,19	10,13	29,29	13,16	12,14	9.3,9.3	19,24
Relative	16,18	17	11,13	24	12,15	29,30	12,19	14,14.2	7,9	24,27

T0054

	D3	VWA	D16	D2	D8	D21	D18	D19	TH01	FGA
Swab Control										
Water Control										
C								14		
D	16,18	15,17			12			14		
E	16,18	15,17			12,13	30,31.2		14		23,26
F	14,16,18	15,17			12,13	30,31.2	16	14	9.3	23,26
G	16,18	15,17	9		12,13	30,31.2		14	9.3	23,26
H	16,18	15						14		
I								14		
J	16	15,17						14		
K	16,18	15,17			12,13			14		
L	16,18							14		
M								14		
N	16,18	14,15			12,13	30		14	8	23
Subject	16,18	15,17	9,12	17,19	12,13	30,31.2	16,16	14,14	8,9.3	23,26
Sampler	15,16	15,17	9,13	18,19	10,13	29,29	13,16	12,14	9.3,9.3	19,24
Relative	17,18	17,18	12,13	17,17	13,15	29,29	16,20	13,14	8,8	23,24
Relative	16,16	15,17	9,13	17,19	12,15	28,31.2	15,16	14,15	6,9.3	19,26
Relative	16,18	15,18	9,13	17,19	15,15	29,31.2	16,20	14,15	6,8	19,23
Relative	16,17	17,18	9,12	17,19	15,15	28,29	15,16	14,15	6,8	23,26

T0057

	D3	VWA	D16	D2	D8	D21	D18	D19	TH01	FGA
Swab Control	16,17		10		12			14		
Water Control		18			13	29,30		14		
C								14	7	
D	15,17	14,18						16		
E		18	9		13	28,30		14	7,9.3	
F	16,17	15,16,17	9	17	12,13	28,29,30		14	7	
G	17									
H	16,17	15,16,17,18	10		10,11,12,13	28,29,30		12,14	7,9.3	
I	15,16,17	16			12			14	9.3	
J	16	15								
K								14,15		
L	15,16,17	16,17,18			12,13	28,29,30		12,14,17		
M	17	16,17,18		20	12,13	30	12	14	7	
N	15,16,17	14,16,18	9,10,11,12,14		13	28,29,30		12,13,14,16	7,9.3	
Subject	15,17	18,19	9,12	16,20	13,14	27,29	12,17	16,16	7,9.3	23,23
Sampler	15,16	15,17	9,13	18,19	10,13	29,29	13,16	12,14	9.3,9.3	19,24
Relative	14,15	16,19	11	20,24	12,13	27,30	17	14,16	7,9.3	20,23
Relative	17,17	16,18	9,10	16,25	11,13	29,31	12,16	15,16	6,9.3	18,23
Relative	14,17	16,18	12,13	16,20	13,14	27,29	16,17	14,15	6,9.3	23,23
Relative	15,17	16,18	12,13		12,13	27,29	12	14,15	7,9.3	18,20
Relative	15,16	14,15	12,12	22,25	14,15	30,31.2	15,15	14,15	9,9.3	22,24

T0067

	D3	VWA	D16	D2	D8	D21	D18	D19	TH01	FGA
Swab Control										
Water Control										
C										
D	15									
E	15									
F	15	17,18			13,14	30		12,14		22
G	15	17						14		
H										
I										
J	15									
K	15				13	30		12,14		
L	15									
M					13			12,14,15		
N	14,15							14		
Subject	15,15	17,18	9,10	20,24	13,14	30,32.2	13,19	12,14	6,9.3	22,23
Sampler	15,16	15,17	9,13	18,19	10,13	29,29	13,16	12,14	9.3,9.3	19,24
Relative	15,17	17,17	9,13	17,20	13,14	29,32.2	16	14,15	6,6	23,24
Relative	14,15	17,18	9,11	18,24	10,14	28,30	13,16	12,15	8,9.3	22,23

T0071

	D3	VWA	D16	D2	D8	D21	D18	D19	TH01	FGA
Swab Control										
Water Control										
C	16,18	17,18		20	12	28		14		20
D	16,18	17,18	10,11	17,20	12,15	28,30	13,16	14,17	7,9.3	20
E	16,18	17,18	10,11		12,15	28,30	16	14,17	7	20
F	16,18	17,18	10,11	17	12,15	28,30	13	14,17	7	20
G	16,18	17,18	10,11		12,15		13	14,17	9.3	20
H	16,18	18	12	17	12,15		13	14,17		20
I	14,16,18	17,18	10,11,12		12,15	28,30		14,17	7	20,22
J	16,18	17	10		15			14,17		
K	16,18	17,18	10,11	17,20	12,15	28,30	13,16	14,17	7,9.3	20
L	16	17				30				20
M	18									
N	16,18	17,18	10,11	17,20	12,15	28,30	13,16	14,17	7,9.3	20
Subject	16,18	17,18	10,11	17,20	12,15	28,30	13,16	14,17	7,9.3	20
Sampler	15,16	15,17	9,13	18,19	10,13	29,29	13,16	12,14	9.3,9.3	19,24
Relative	16,17	15,17	10,12	17,24	11,12	30,32.2	12,13	13,17	6,9.3	19,20

T0072

	D3	VWA	D16	D2	D8	D21	D18	D19	TH01	FGA
Swab Control										
Water Control										
C	17	16,18	11	17,20	12,13	28		14		21
D	15,17	16,18	11,14		12,13	28,30	15	14	7,8	21,25
E	15,17	16,18	11		12,13	28,30		14	7	21,25
F	15,17	16,18	11,14		12,13	28,30		14	7,8	21,25
G	15,17	16,18	11		12,13	28,30		14	7,8	21,25
H								14		
I	15,17	16,18			12,13	30				21
J	15,17	14						14,15		
K	15,16,17				12,13					
L	15							14		
M	15,17							14		
N	15,16,17	14,16,18,19	11,14		10,12,13	28,30,31.2		14,15	7,9	21,25
Subject	15,17	16,18	11,14	17,20	12,13	28,30	14,15	14,14	7,8	21,25
Sampler	15,16	15,17	9,13	18,19	10,13	29,29	13,16	12,14	9.3,9.3	19,24

T0080

	D3	VWA	D16	D2	D8	D21	D18	D19	TH01	FGA
Swab Control	15,16				12,13	28		14		
Water Control	16	16								
C	16,18	15,18			12,14,15	31.2	14	14	6	23
D	16	15,18	12,13		14	30				
E	16,18	15,18	12,13	24,25	12,14	30,31.2	14,16	13,14,15	6,8	20,23
F	15,16,18	15,18	12		12	28,30		13,14,15		
G	16,18	15,18	12,13	24,25	12,14	30,31.2	14,16	13,14	6,8	20,23
H	16,18	15,18	12,13	24,25	12,14	30,31.2	16	13,14	6,8	20,23
I	16		12,13		12,14			13,14		23
J	16	15,18	12		13,14		14	13	8	
K	14,16	18			14	30		12,13,14,15		
L	16,18	18	9,13	25	12,14	31.2		12,13,14		
M	18	15,18	12,13	24,25	12,14	30,31.2		13,14,15	6	
N	16,18	15,18	12,13	24,25	12,14	30,31.2	14,16	13,14	6,8	20,23
Subject	16,18	15,18	12,13	24,25	12,14	30,31.2	14,16	13,14	6,8	20,23
Sampler	15,16	15,17	9,13	18,19	10,13	29,29	13,16	12,14	9.3,9.3	19,24
Relative	18,18	17,18	12,12	17,25	12,13	28,30	14,18	14,14	6,8	20,23
Relative	15,16	15,18	11,13	20,24	14,15	30,31.2		13,14	6,8	22,23

T0081

	D3	VWA	D16	D2	D8	D21	D18	D19	TH01	FGA
Swab Control										
Water Control			9,10		12			14		
C										
D		17	11		13	28		14	8	
E	17,18	17	11	23	13	28	15	14	8	24
F	15	16,17	11	17,23	11,13	28,30.2,33.2		13,14	8	
G		16	9,10					12,14,15		
H					13			14		
I								14		
J			11	17		28		12,14		
K		16,17	11	17	13	28		13,14		
L		17	11	17,23	13	28		14	8	
M										
N	17,18	17	11	17,23	13	28	14,15	14	8	22,24
Subject	17,18	17,17	11,11	17,23	13,13	28,28	14,15	14,14	8,8	22,24
Sampler	15,16	15,17	9,13	18,19	10,13	29,29	13,16	12,14	9.3,9.3	19,24
Relative	15,17	16,17	11,12	17,23	13,13	28,30.2	14,15	14,16	8,9.3	21,24
Relative	15,18	15,17	11,11	17,24	13,15	28,32.2	15,15	14,14	6,8	22,23
Relative	15,18	16,17	11,11	17,23	11,13	30.2,33.2	14,15	13,14	6,8	19,21

T0082

	D3	VWA	D16	D2	D8	D21	D18	D19	TH01	FGA
Swab Control										
Water Control										
C	14,16	16,18	12,13	24,25	10,14	28,29	12,13	14,15	7,8	21,25
D	14,16	16,18	12,13	24,25	10,14	28,29	12,13	14,15	7,8	21,25
E	14,16	16,18	12,13	24,25	10,14	28,29	12,13	14,15	7,8	21,25
F	14,16	16,18	12,13	24,25	10,14	28,29	12,13	14,15	7,8	21,25
G	14,16	16,18	12,13	24,25	10,14	28,29	12,13	14,15	7,8	21,25
H	14,16	16,18	12,13	24,25	10,14	28,29	12,13	14,15	7,8	21,25
I	14,16	16,18	12,13	24,25	10,14	28,29	12,13	14,15	7,8	21,25
J	14,16	16,18	12,13	24,25	10,14	28,29	12,13	14,15	7,8	21,25
K	14,16	16,18	12,13	24,25	10,14	28,29	12,13	14,15	7,8	21,25
L	14,16	16,18	12,13	24,25	10,14	28,29	12,13	14,15	7,8	21,25
M	14,16	16,18	12,13	24,25	10,14	28,29	12,13	14,15	7,8	21,25
N	14,16	16,18	12,13	24,25	10,14	28,29	12,13	14,15	7,8	21,25
Subject	14,16	16,18	12,13	24,25	10,14	28,29	12,13	14,15	7,8	21,25
Sampler	15,16	15,17	9,13	18,19	10,13	29,29	13,16	12,14	9.3,9.3	19,24

T0083

	D3	VWA	D16	D2	D8	D21	D18	D19	TH01	FGA
Swab Control										
Water Control										
C	17	14								
D	17	14,18			12,15	27,29		13,14	6,9	
E	17	14,18	11,12	23,24	12,15	27,29		13,14	6,9	21
F	17	14,18			12,15			13,14	6	
G	17	14,18	11,12	23,24	12,15	27,29		13,14	6,9	21
H										
I	17	14,18	11,12	23,24	12,15	27,29		13,14	6,9	21
J	17	14,18	11,12	23,24	12,15	27,29		13,14	6,9	21
K	17	14,18			12,15			13,14		
L	17									
M	17				12					
N	17	14,18	11,12	23,24	12,15	27,29		13,14	6,9	21
Subject	17,17	14,18	11,12	23,24	12,15	27,29	12,16	13,14	6,9	21,21
Sampler	15,16	15,17	9,13	18,19	10,13	29,29	13,16	12,14	9.3,9.3	19,24
Relative	16,17	15,18	8,12	21,24	12,15	27,30	12,16	13,14	6,6	21,23
Relative	15,17	14,19	11,11	17,23	11,12	27,29		13,15.2	9,9.3	21,23

6.2 Forceful contact

6.2.1 Questionnaire

Study Number:	
Subject Number:	
Date:	
Contact Type:	
Relationship Status:	
Any clothing worn over the top of the arm?	
Time since washing arms:	
Products used:	
Any contact between partner/spouse and the upper arm?	
If yes, how long since contact?	
Time since washing hands:	
Products used:	
Have latex gloves been worn since hand washing?	

6.2.2 Questionnaire data

6.2.2.1 Focus pad investigation

Subject	Force	Relationship Status	Time since wash	Products Used	Gloves worn
1	Punch	Partner	15 minutes	Soap	N
2	Punch	Married	15 minutes	None	N
3	Punch	Married	15 minutes	Soap	N
4	Punch	Partner	15 minutes	Soap	N
5	Punch	Single	15 minutes	Soap	N
6	Punch	Single	15 minutes	Soap	N
7	Punch	Single	15 minutes	None	N
8	Punch	Single	15 minutes	Soap	N
9	Punch	Partner	15 minutes	Soap	N
10	Punch	Married	15 minutes	Soap	N
11	Punch	Married	15 minutes	Soap	N
12	Punch	Partner	15 minutes	Soap	N
13	Punch	Married	15 minutes	Soap	N
14	Punch	Partner	15 minutes	Soap	N
15	Punch	Single	15 minutes	Soap	N
1	Slap	Partner	15 minutes	Soap	N
2	Slap	Married	15 minutes	Soap	N
3	Slap	Married	15 minutes	Soap	N
4	Slap	Partner	15 minutes	Soap	N
5	Slap	Single	15 minutes	Soap	N
6	Slap	Single	15 minutes	Soap	N
7	Slap	Single	15 minutes	Soap	N
8	Slap	Single	15 minutes	Soap	N
9	Slap	Partner	15 minutes	Soap	N
10	Slap	Married	15 minutes	Soap	N
11	Slap	Married	15 minutes	Soap	N
12	Slap	Partner	15 minutes	Soap	N
13	Slap	Married	15 minutes	Soap	N
14	Slap	Partner	15 minutes	Soap	N
15	Slap	Single	15 minutes	Soap	N

Subject Force Relationship Status Time since wash Products Used Gloves worn

1	Punch	Partner	1 hour	Soap	Y
2	Punch	Married	1 hour	Soap	Y
3	Punch	Married	1 hour	Soap	N
4	Punch	Partner	1 hour	Soap	N
5	Punch	Single	1 hour	Soap	N
6	Punch	Single	1 hour	Soap	N
7	Punch	Single	1 hour	Soap	N
8	Punch	Single	1 hour	Soap	N
9	Punch	Partner	1 hour	Soap	N
10	Punch	Married	1 hour	Soap	N
11	Punch	Married	1 hour	Soap	N
12	Punch	Partner	1 hour	Soap	N
13	Punch	Married	1 hour	Soap	N
14	Punch	Partner	1 hour	Soap	N
15	Punch	Single	1 hour	Soap	N
1	Slap	Partner	1 hour	Soap	Y
2	Slap	Married	1 hour	Soap	Y
3	Slap	Married	1 hour	Soap	N
4	Slap	Partner	1 hour	Soap	N
5	Slap	Single	1 hour	Soap	N
6	Slap	Single	1 hour	Soap	N
7	Slap	Single	1 hour	Soap	N
8	Slap	Single	1 hour	Soap	N
9	Slap	Partner	1 hour	Soap	N
10	Slap	Married	1 hour	Soap	N
11	Slap	Married	1 hour	Soap	N
12	Slap	Partner	1 hour	Soap	N
13	Slap	Married	1 hour	Soap	N
14	Slap	Partner	1 hour	Soap	N
15	Slap	Single	1 hour	Soap	N

Subject Force Relationship Status Time since wash Products Used Gloves worn

1	Punch x 3	Partner	1 hour	Soap	Y
2	Punch x 3	Married	1 hour	Soap	N
3	Punch x 3	Married	1 hour	Soap	N
4	Punch x 3	Partner	1 hour	Soap	N
5	Punch x 3	Single	1 hour	Soap	Y
6	Punch x 3	Single	1 hour	Soap	N
7	Punch x 3	Single	1 hour	Soap	N
8	Punch x 3	Single	1 hour	Soap	Y
9	Punch x 3	Partner	1 hour	Soap	N
10	Punch x 3	Married	1 hour	Soap	N
11	Punch x 3	Married	1 hour	Soap	N
12	Punch x 3	Partner	1 hour	Soap	Y
13	Punch x 3	Married	1 hour	Soap	N
14	Punch x 3	Partner	1 hour	Soap	N
15	Punch x 3	Single	1 hour	Soap	N
1	Slap x 3	Partner	1 hour	Soap	Y
2	Slap x 3	Married	1 hour	Soap	N
3	Slap x 3	Married	1 hour	Soap	N
4	Slap x 3	Partner	1 hour	Soap	N
5	Slap x 3	Single	1 hour	Soap	Y
6	Slap x 3	Single	1 hour	Soap	N
7	Slap x 3	Single	1 hour	Soap	N
8	Slap x 3	Single	1 hour	Soap	Y
9	Slap x 3	Partner	1 hour	Soap	N
10	Slap x 3	Married	1 hour	Soap	N
11	Slap x 3	Married	1 hour	Soap	N
12	Slap x 3	Partner	1 hour	Soap	Y
13	Slap x 3	Married	1 hour	Soap	N
14	Slap x 3	Partner	1 hour	Soap	N
15	Slap x 3	Single	1 hour	Soap	N

6.2.2.2 Preliminary investigation

Subject	Force	Relationship Status	Clothing	Time since washing hands	Products	Gloves	Time since washing arms	Products	Contact with Spouse/Partner	Time since contact
A	Punch	Partner	Football Shirt	1 hour +	N	Y	8 hours	Shower Gel	N	N/A
A	Punch	Partner	N	1 hour +	Soap	N	2 hours	Shower Gel	N	N/A
A	Punch	Partner	Shirt	1 hour +	N	Y	9 hours	N	N	N/A
A	Slap	Partner	T-shirt	1 hour +	Soap	Y	15 hours	Shower Gel	N	N/A
A	Slap	Partner	T-shirt	1 hour +	Soap	Y	5 hours	Shower Gel	Y	5 hours
A	Slap	Partner	N	1 hour +	N	Y	5 hours	N	Y	10 hours
B	Punch	Married	Polo shirt	1 hour +	Soap	N	24 hours	Soap	N	N/A
B	Punch	Married	Polo shirt	1 hour +	Soap	N	48 hours	Soap	N	N/A
B	Punch	Married	Polo shirt	1 hour +	Soap	N	17 hours	Soap	N	N/A
B	Slap	Married	Polo shirt	1 hour +	Soap	N	24 hours	Soap	N	N/A
B	Slap	Married	Polo shirt	1 hour +	Soap	N	48 hours	N	N	N/A
B	Slap	Married	Polo shirt	1 hour +	Soap	N	24 hours	Soap	N	N/A

6.2.2.3 Person-to-person investigation

Subject	Clothing	Relationship Status	Time since washing arms	Products	Time since washing hands	Products	Gloves
1	Cardigan	Married	1 hour	Soap	1 hour	Soap	N
2	Cardigan	Married	5 hours	Shower gel	1 hour	Soap	Y
3	N	Single	14 hours	Soap	1 hour	Soap	Y
4	Jumper	Partner	12 hours	Soap	1 hour	Soap	N
5	T-shirt	Married	6 hours	Soap	1 hour	Soap	Y
6	Shirt	Married	14 hours	Soap	1 hour	Soap	N
7	Jumper	Married	8 hours	Shower gel	1 hour	Soap	N
8	Jumper	Single	7 hours	Shower gel	1 hour	Soap	N
9	T-shirt	Married	2 hours	Soap	2 hours	Soap	Y
10	T-shirt	Married	5 hours	Shower gel	2 hours	Soap	Y
11	Jumper	Married	8 hours	Soap	2 hours	Soap	Y
12	Jumper	Married	8 hours	Soap	1 hour	Soap	N
13	Jumper	Single	7 hours	Shower gel	1 hour	Soap	Y
14	N	Single	8 hours	Shower gel	1 hour	Soap	N
15	T-shirt	Single	8 hours	Soap	1 hour	Soap	Y
16	T-shirt	Partner	24 hours	Shower gel	2 hours	Soap	N

6.2.3 Quantification results

6.2.3.1 Focus pad investigation

Subject	Condition	Punch (pg/ul)	Slap (pg/ul)
1	15 minutes	Undetected	11.90
2	15 minutes	Undetected	29.70
3	15 minutes	1.55	40.20
4	15 minutes	Undetected	2.33
5	15 minutes	2.19	8.24
6	15 minutes	27.00	4.62
7	15 minutes	1.05	32.50
8	15 minutes	1.95	5.64
9	15 minutes	1.41	34.10
10	15 minutes	0.99	4.44
11	15 minutes	Undetected	47.90
12	15 minutes	Undetected	22.90
13	15 minutes	Undetected	67.50
14	15 minutes	1.4	12.90
15	15 minutes	Undetected	Undetected
1	1 hour	Undetected	Undetected
2	1 hour	Undetected	12.30
3	1 hour	14.30	11.70
4	1 hour	9.42	29.90
5	1 hour	5.01	Undetected
6	1 hour	Undetected	39.60
7	1 hour	22.40	40.00
8	1 hour	Undetected	15.90
9	1 hour	9.22	98.40
10	1 hour	36.10	33.80
11	1 hour	8.19	97.50

12	1 hour	8.46	15.70
13	1 hour	33.60	29.50
14	1 hour	Undetected	76.60
15	1 hour	Undetected	11.10
1	Multiple	Undetected	6.61
2	Multiple	6.57	11.10
3	Multiple	Undetected	103.00
4	Multiple	Undetected	17.90
5	Multiple	Undetected	6.84
6	Multiple	13.20	15.20
7	Multiple	Undetected	32.00
8	Multiple	5.37	10.20
9	Multiple	44.30	501.00
10	Multiple	3.20	92.70
11	Multiple	43.20	89.00
12	Multiple	29.40	5.41
13	Multiple	Undetected	230.00
14	Multiple	Undetected	117.00
15	Multiple	4.42	31.70

6.2.3.2 Preliminary investigation

Prior to contact

Subject	Force	Repeat	Hand (ng/ul)	Arm (ng/ul)
A	Punch	1	0.90	1.55
A	Punch	2	1.86	1.97
A	Punch	3	2.19	1.40
A	Slap	1	1.28	1.95
A	Slap	2	1.53	2.47
A	Slap	3	2.14	3.87
B	Punch	1	1.18	1.12
B	Punch	2	2.59	1.62
B	Punch	3	1.82	1.89
B	Slap	1	1.93	1.51
B	Slap	2	4.49	2.54
B	Slap	3	2.50	2.60

After contact

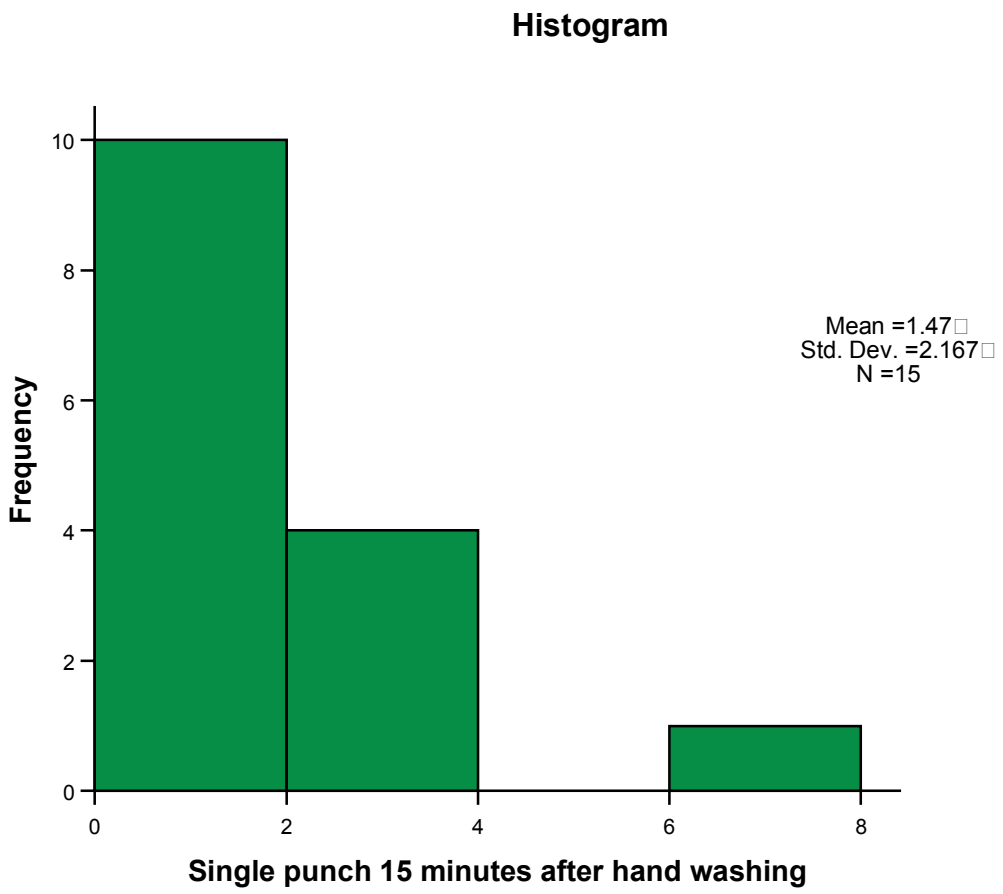
Subject	Force	Repeat	Hand (ng/ul)	Arm (ng/ul)
A	Punch	1	1.49	1.27
A	Punch	2	1.28	2.43
A	Punch	3	2.62	1.23
A	Slap	1	1.77	1.67
A	Slap	2	2.80	2.42
A	Slap	3	2.54	3.47
B	Punch	1	0.54	2.77
B	Punch	2	2.32	1.32
B	Punch	3	1.56	1.19
B	Slap	1	1.53	2.25
B	Slap	2	2.59	2.09
B	Slap	3	2.40	1.95

6.2.3.3 Person-to-person investigation

Subject	Force	Hand (pg/ul)	Arm (pg/ul)
1	Punch	6.40	17.40
2	Punch	175.00	20.80
3	Punch	15.10	48.50
4	Punch	4.90	39.00
5	Punch	65.10	32.90
6	Punch	490.00	25.00
7	Punch	11.30	36.80
8	Punch	209.00	19.30
9	Punch	9.68	23.90
10	Punch	17.30	47.00
11	Punch	5.07	47.30
12	Punch	17.40	16.20
13	Punch	13.70	62.90
14	Punch	36.50	10.30
15	Punch	6.84	10.60
16	Punch	21.20	12.00
1	Slap	23.00	7.65
2	Slap	64.00	57.90
3	Slap	25.30	26.70
4	Slap	97.70	63.20
5	Slap	55.70	138.00
6	Slap	731.00	24.60
7	Slap	25.80	40.30
8	Slap	253.00	28.90
9	Slap	49.60	29.40
10	Slap	36.70	47.00
11	Slap	10.40	45.50
12	Slap	37.80	16.30
13	Slap	27.50	41.90
14	Slap	112.00	53.60
15	Slap	8.80	15.00
16	Slap	22.20	38.20

6.2.4 SPSS normal distribution test results (34 cycle data)

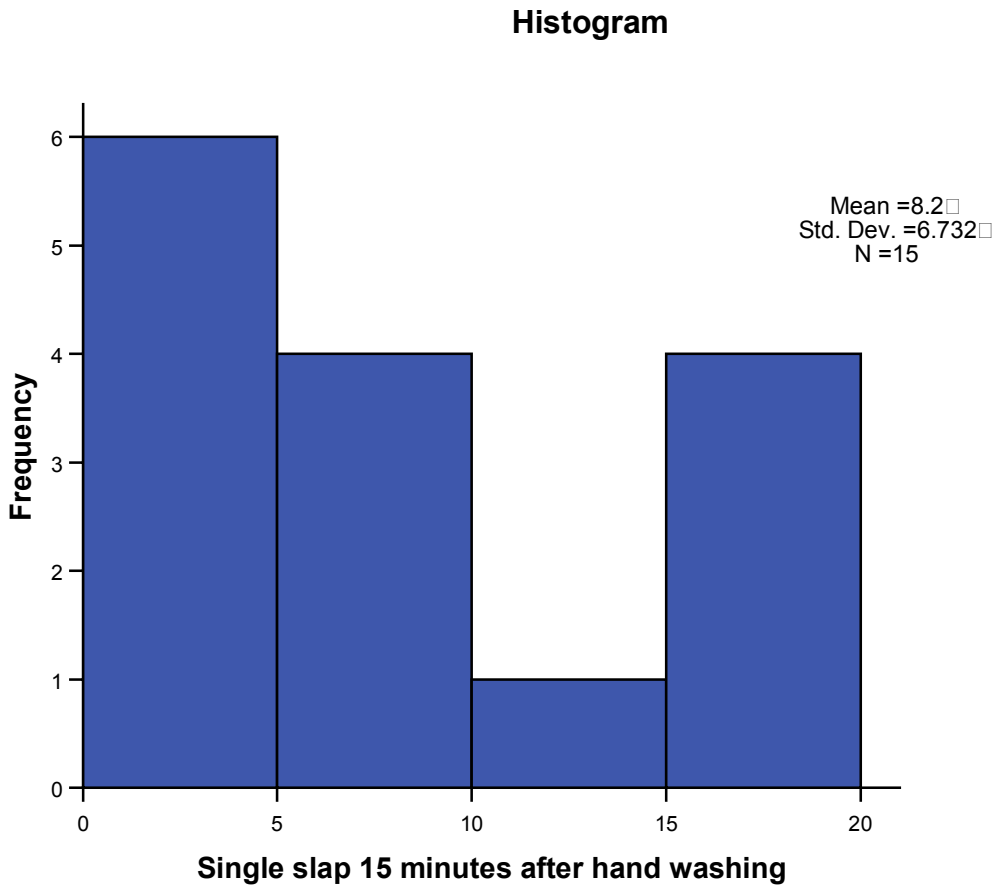
6.2.4.1 Focus pad investigation – transferred alleles



Tests of Normality

	Kolmogorov-Smirnov(a)			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Single punch 15 minutes after hand washing	.252	15	.011	.709	15	.000

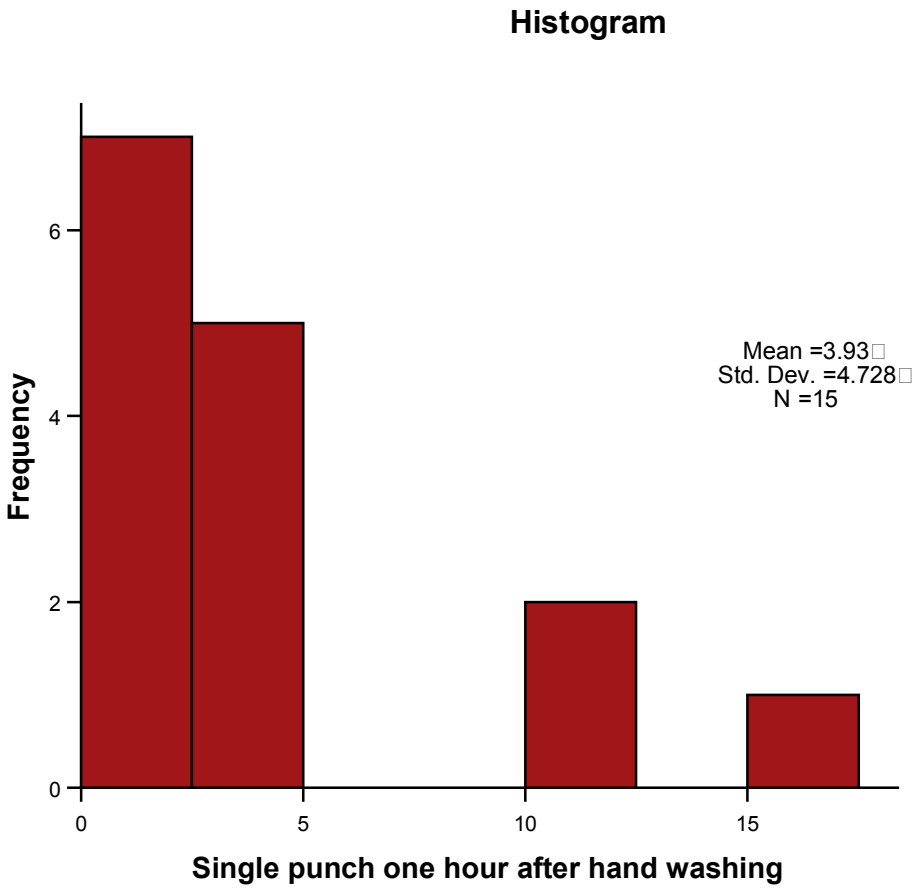
a Lilliefors Significance Correction



Tests of Normality

	Kolmogorov-Smirnov(a)			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Single slap 15 minutes after hand washing	.228	15	.035	.876	15	.042

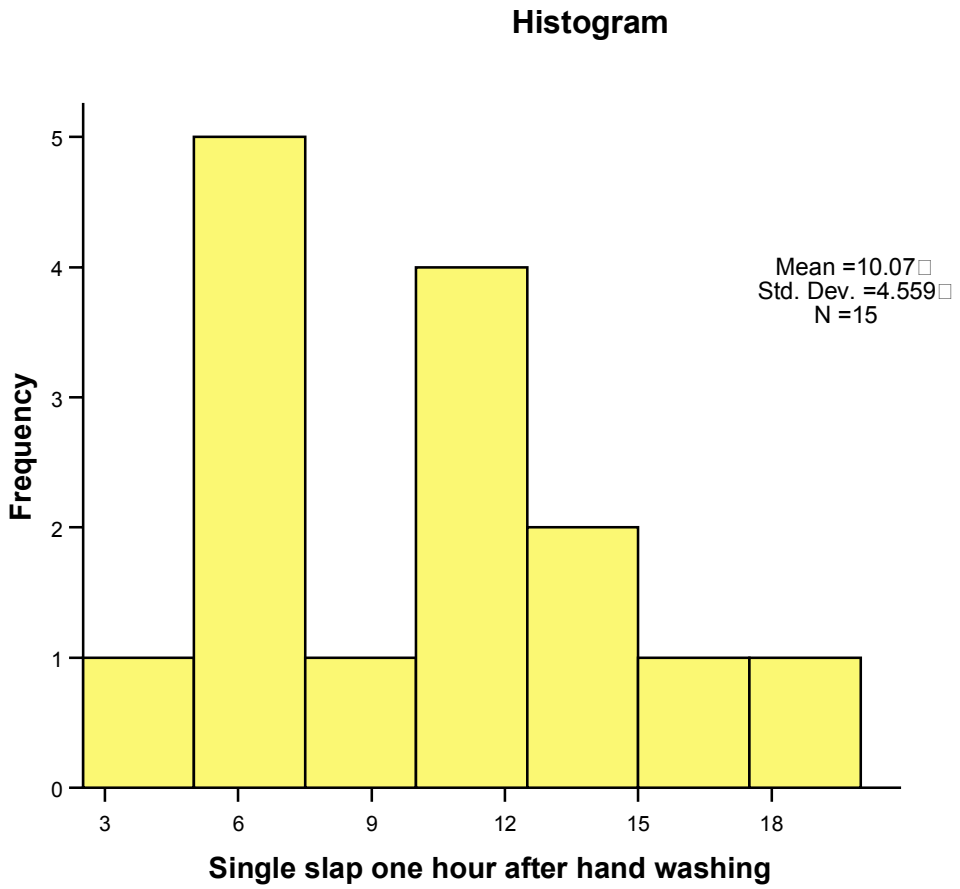
a Lilliefors Significance Correction



Tests of Normality

	Kolmogorov-Smirnov(a)			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Single punch one hour after hand washing	.294	15	.001	.784	15	.002

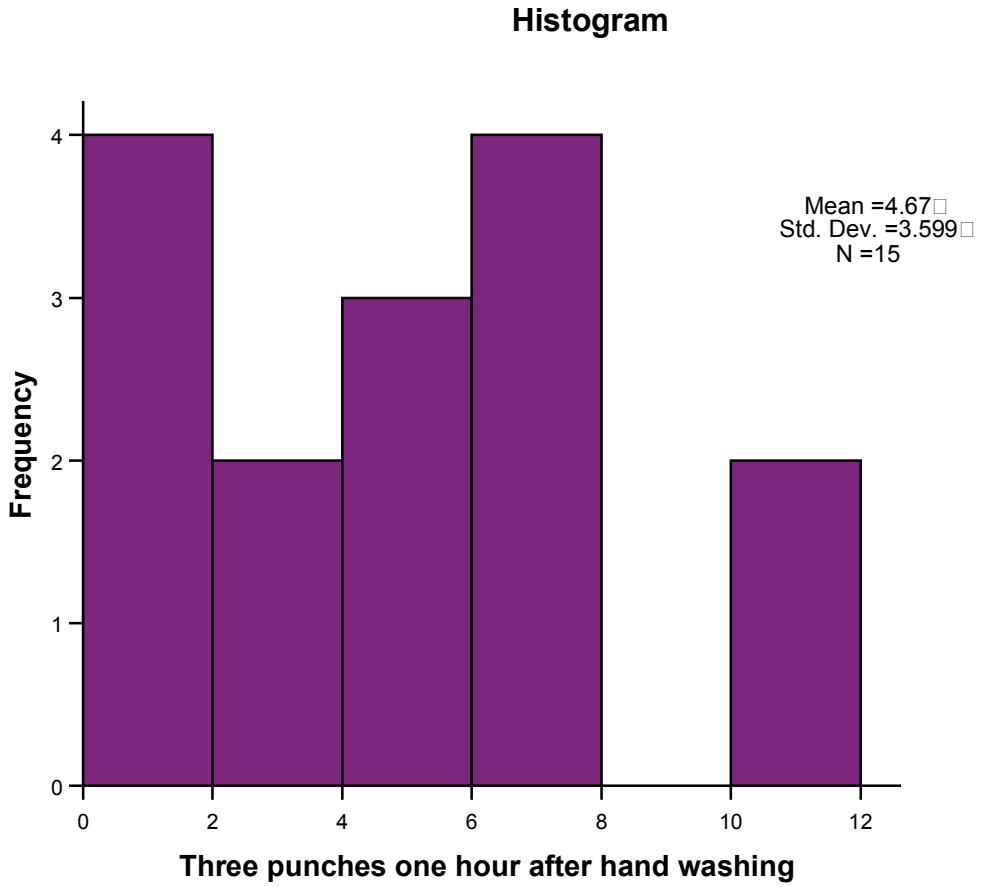
a Lilliefors Significance Correction



Tests of Normality

	Kolmogorov-Smirnov(a)			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Single slap one hour after hand washing	.149	15	.200(*)	.947	15	.474

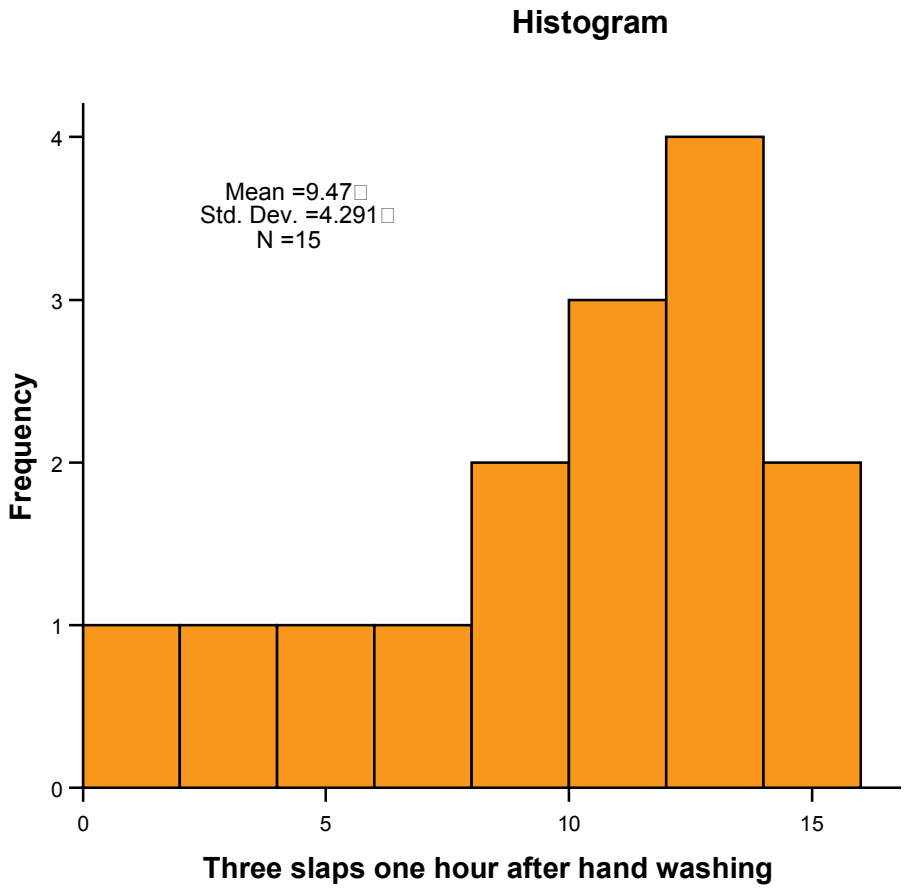
* This is a lower bound of the true significance.
a Lilliefors Significance Correction



Tests of Normality

	Kolmogorov-Smirnov(a)			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Three punches one hour after hand washing	.125	15	.200(*)	.946	15	.460

* This is a lower bound of the true significance.
a Lilliefors Significance Correction

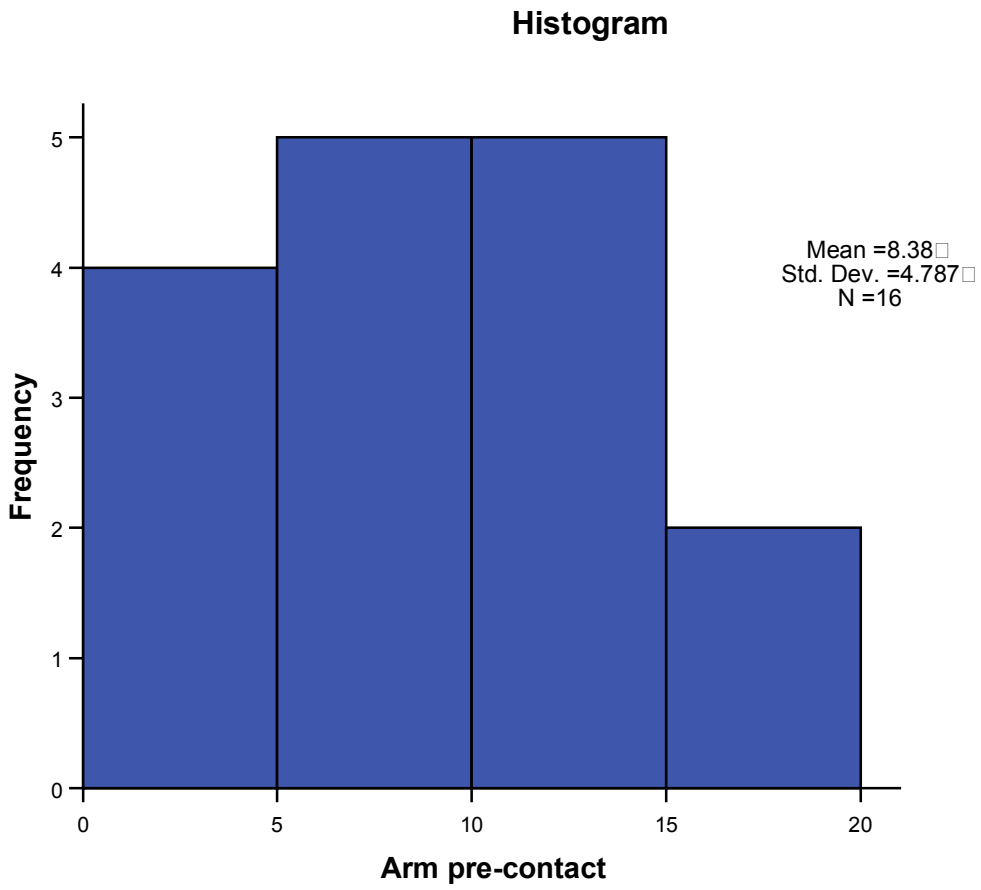


Tests of Normality

	Kolmogorov-Smirnov(a)			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Three slaps one hour after hand washing	.190	15	.150	.936	15	.331

a Lilliefors Significance Correction

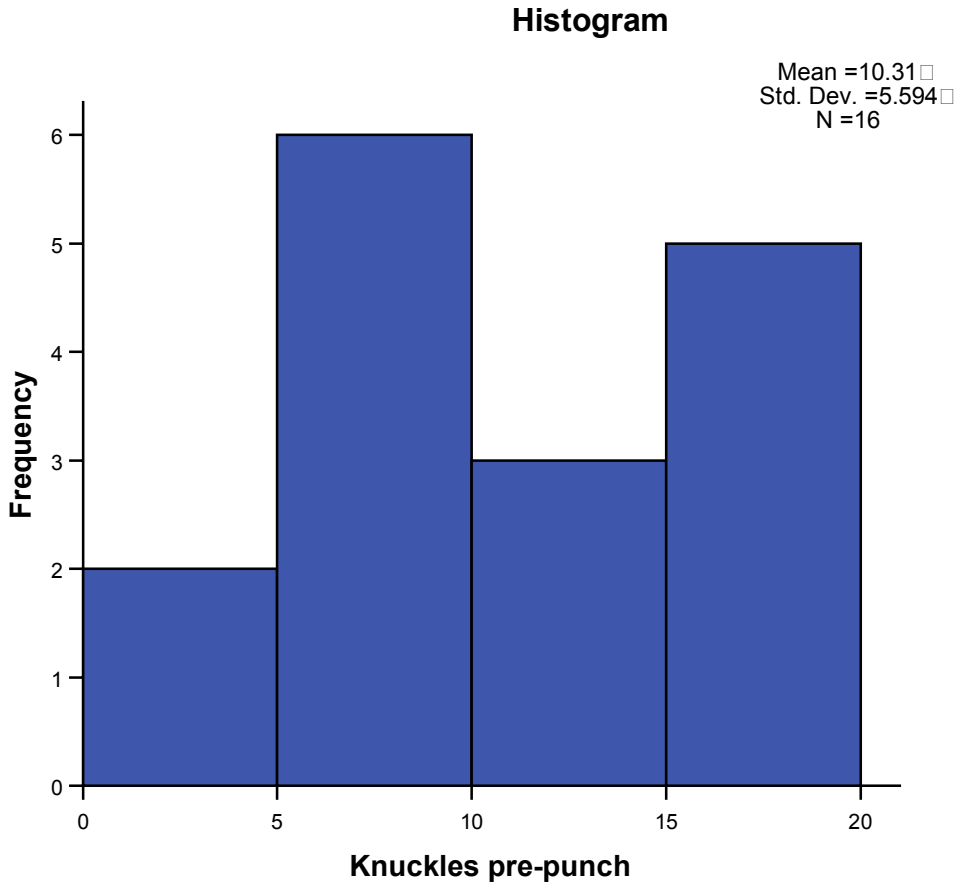
6.2.4.2 Person-to-person investigation – subject alleles



Tests of Normality

	Kolmogorov-Smirnov(a)			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Arm pre-contact	.167	16	.200(*)	.946	16	.424

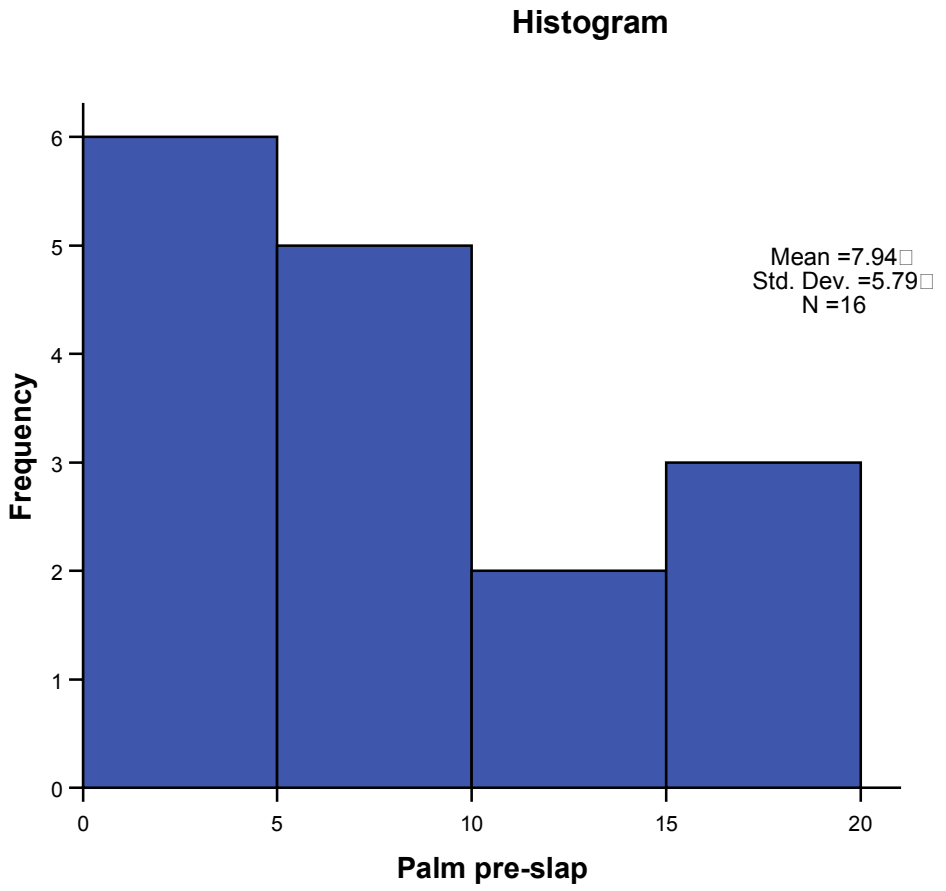
* This is a lower bound of the true significance.
a Lilliefors Significance Correction



Tests of Normality

	Kolmogorov-Smirnov(a)			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Knuckles pre-punch	.122	16	.200(*)	.952	16	.521

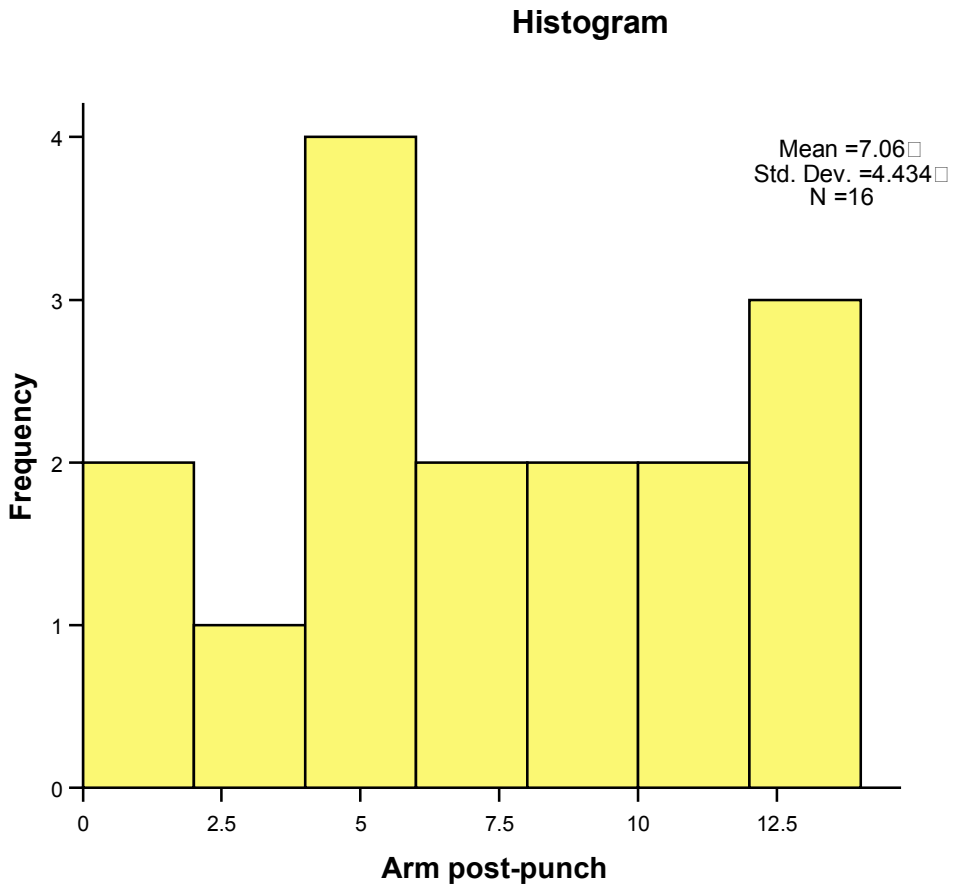
* This is a lower bound of the true significance.
a Lilliefors Significance Correction



Tests of Normality

	Kolmogorov-Smirnov(a)			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Palm pre-slap	.132	16	.200(*)	.938	16	.328

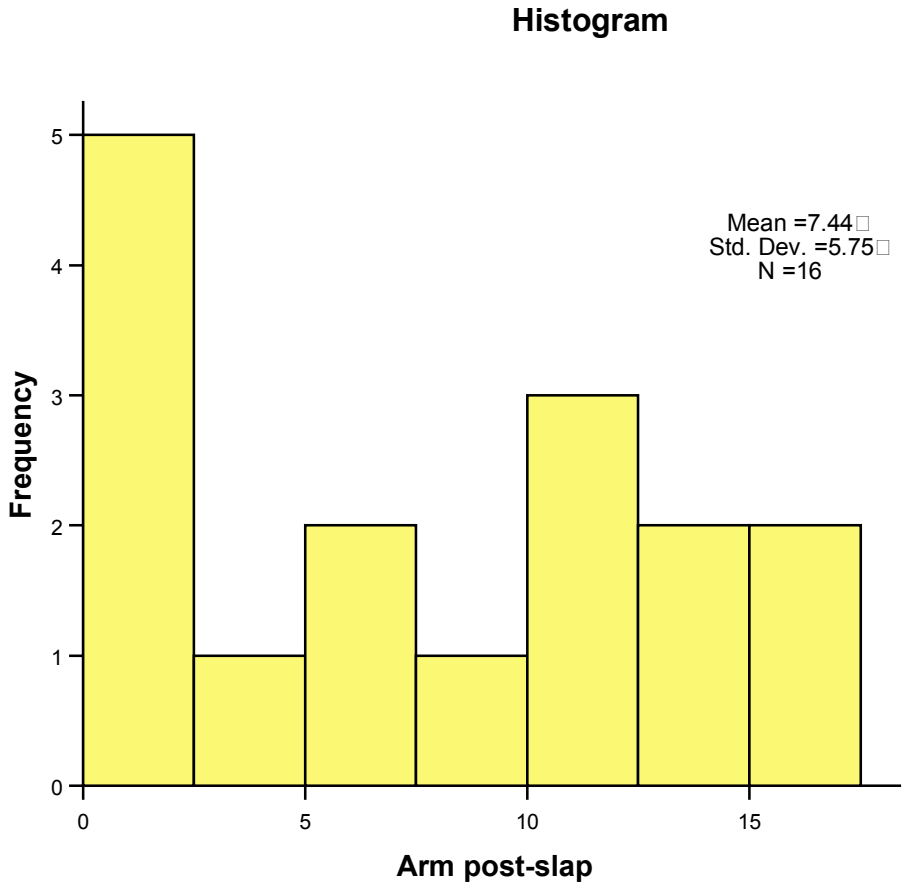
* This is a lower bound of the true significance.
a Lilliefors Significance Correction



Tests of Normality

	Kolmogorov-Smirnov(a)			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Arm post-punch	.125	16	.200(*)	.953	16	.532

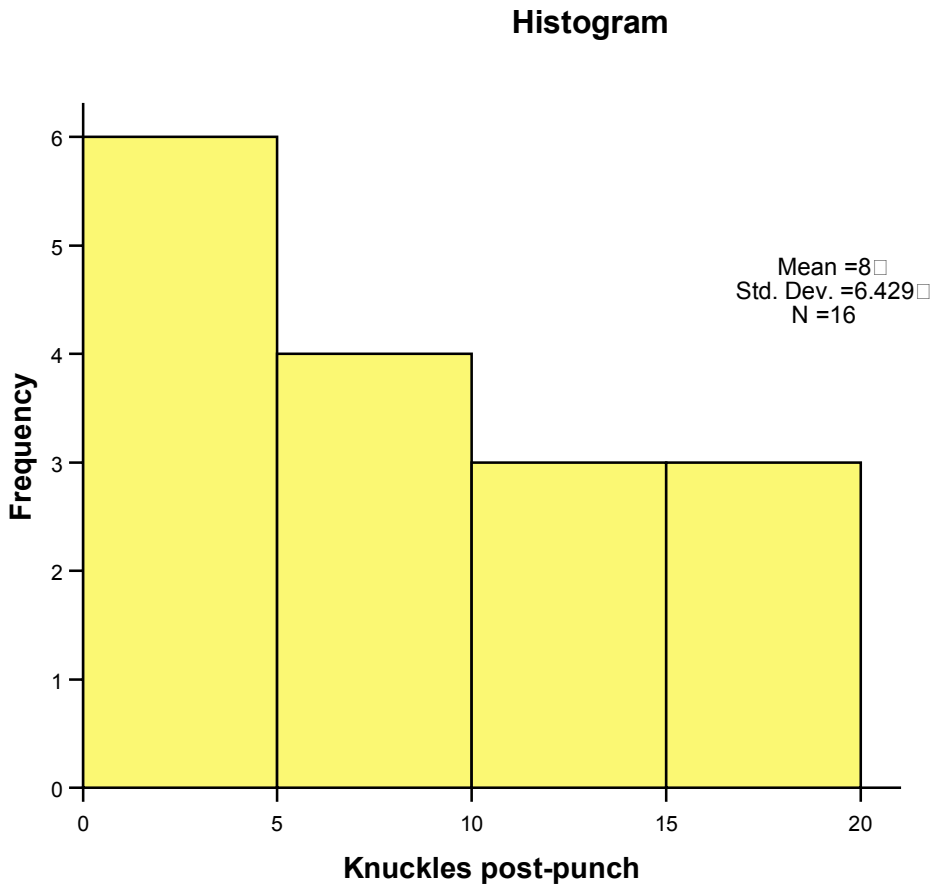
* This is a lower bound of the true significance.
a Lilliefors Significance Correction



Tests of Normality

	Kolmogorov-Smirnov(a)			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Arm post-slap	.170	16	.200(*)	.901	16	.083

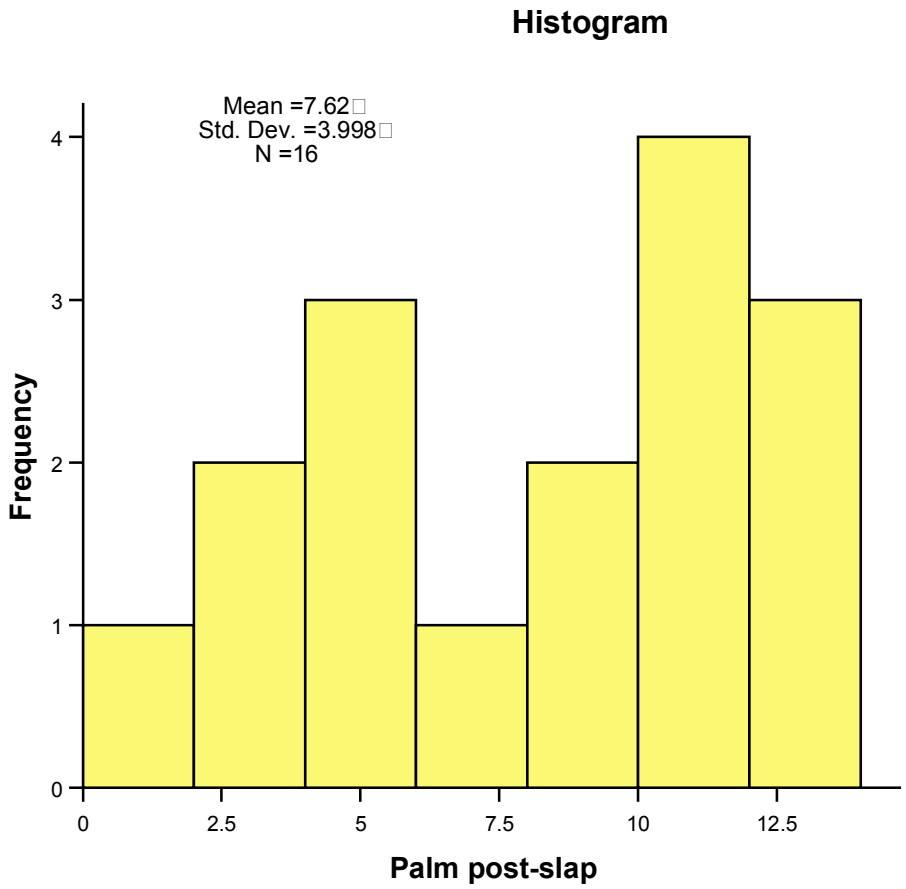
* This is a lower bound of the true significance.
a Lilliefors Significance Correction



Tests of Normality

	Kolmogorov-Smirnov(a)			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Knuckles post-punch	.157	16	.200(*)	.920	16	.170

* This is a lower bound of the true significance.
a Lilliefors Significance Correction

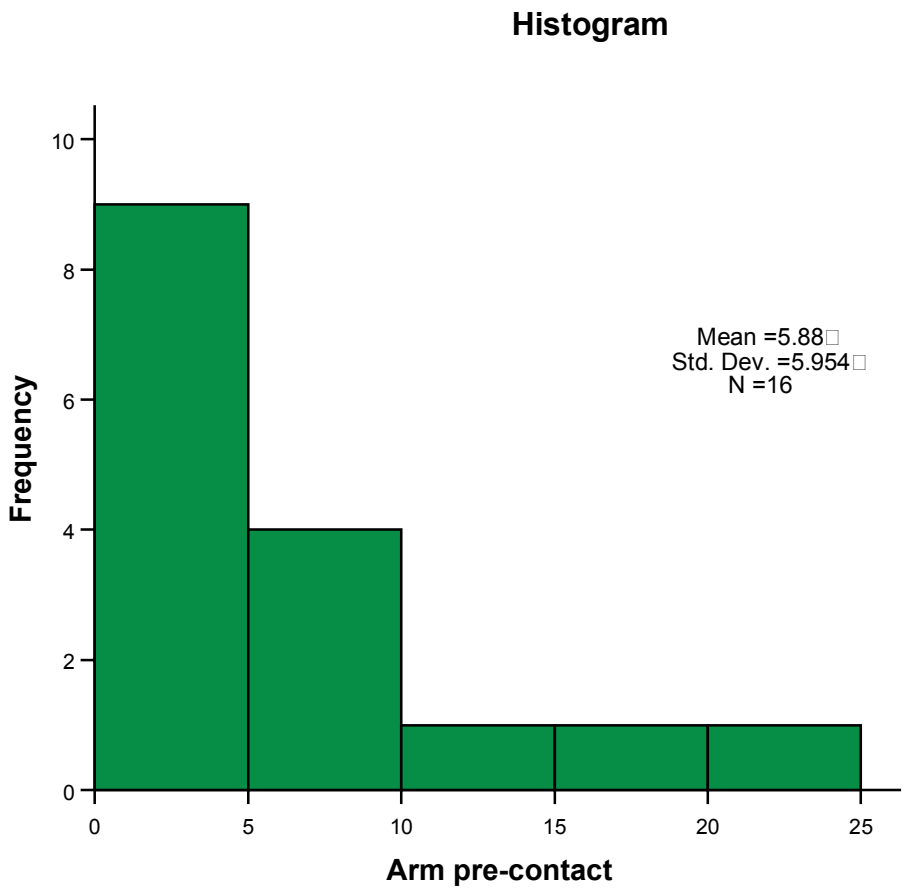


Tests of Normality

	Kolmogorov-Smirnov(a)			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Palm post-slap	.161	16	.200(*)	.942	16	.377

* This is a lower bound of the true significance.
a Lilliefors Significance Correction

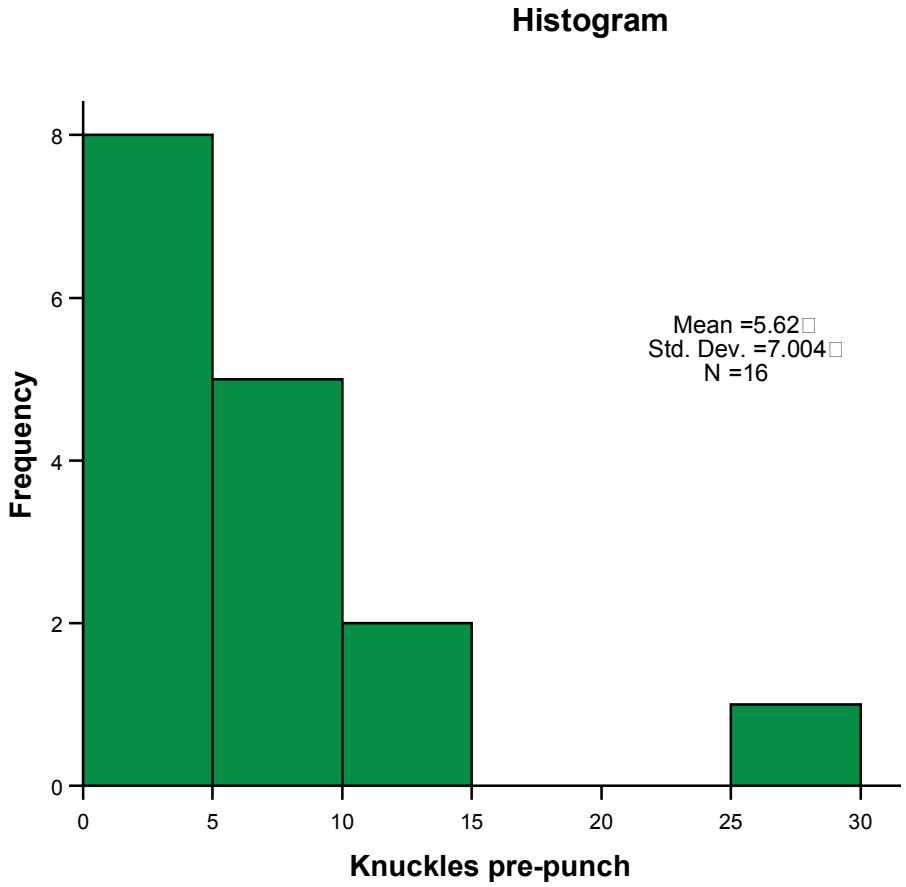
6.2.4.3 Person-to-person investigation – non-subject alleles



Tests of Normality

	Kolmogorov-Smirnov(a)			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Arm pre-contact	.246	16	.011	.806	16	.003

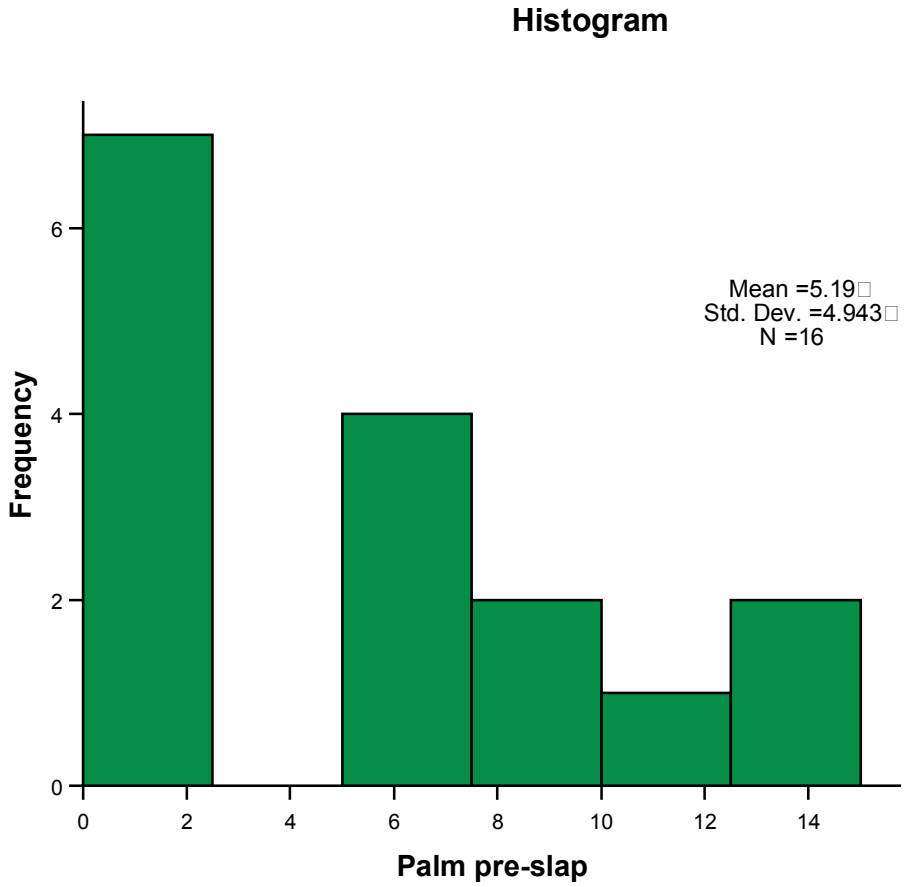
a Lilliefors Significance Correction



Tests of Normality

	Kolmogorov-Smirnov(a)			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Knuckles pre-punch	.211	16	.055	.742	16	.001

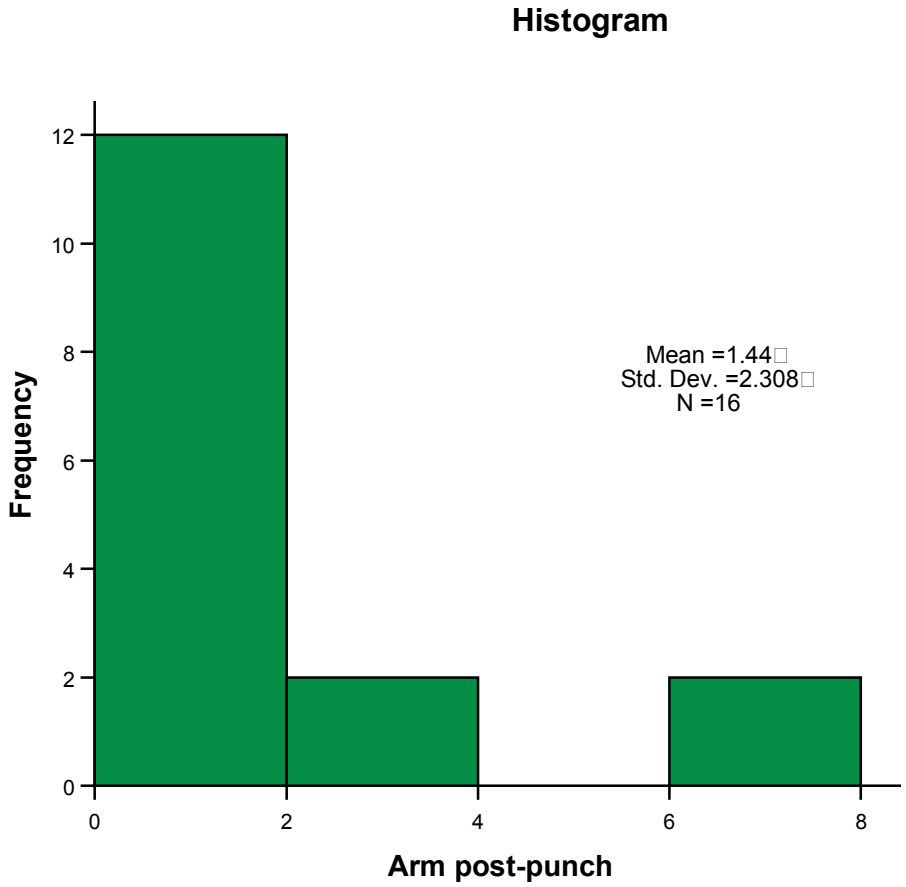
a Lilliefors Significance Correction



Tests of Normality

	Kolmogorov-Smirnov(a)			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Palm pre-slap	.178	16	.187	.894	16	.065

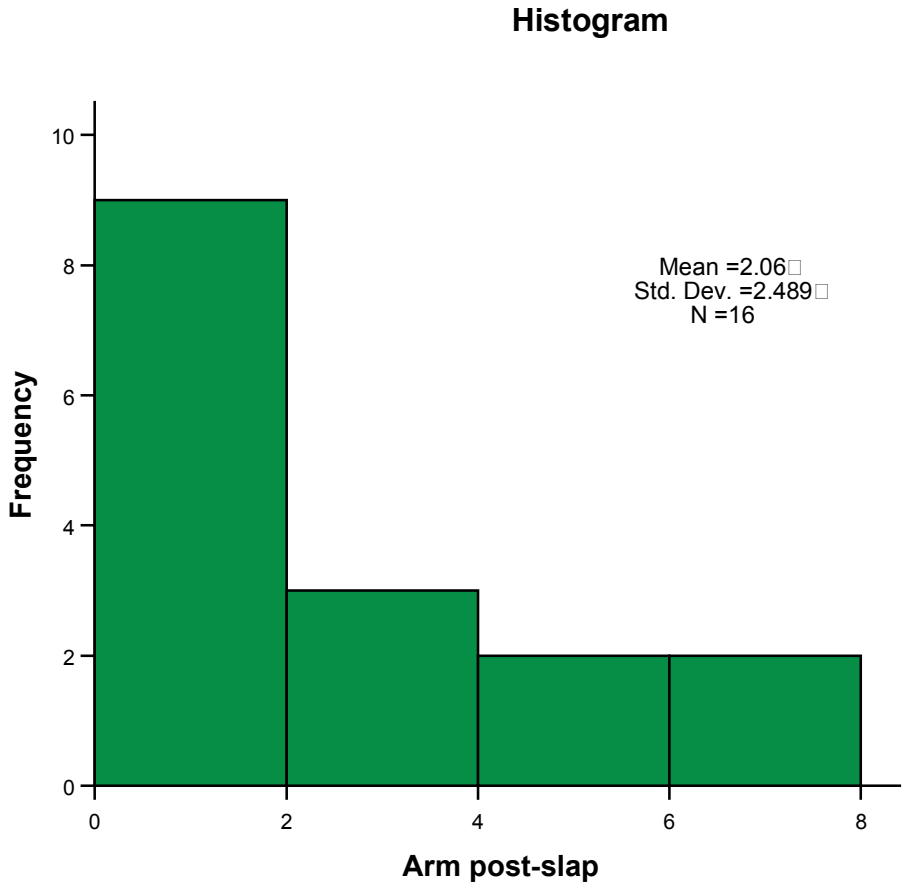
a Lilliefors Significance Correction



Tests of Normality

	Kolmogorov-Smirnov(a)			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Arm post-punch	.325	16	.000	.652	16	.000

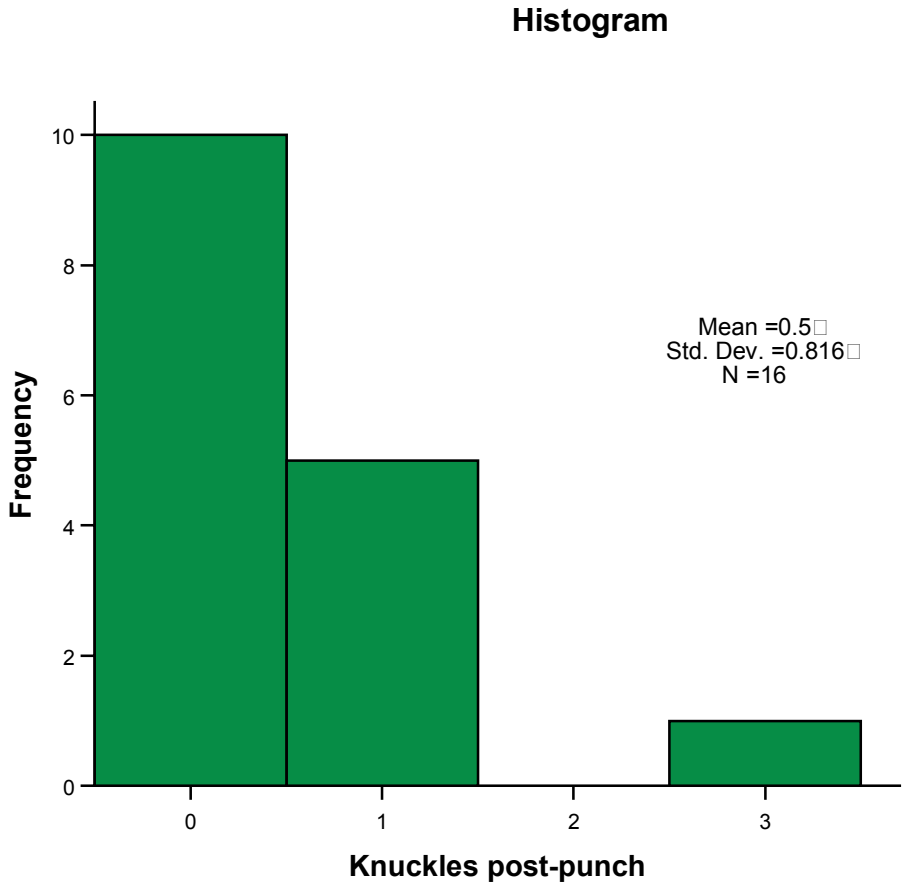
a Lilliefors Significance Correction



Tests of Normality

	Kolmogorov-Smirnov(a)			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Arm post-slap	.260	16	.005	.792	16	.002

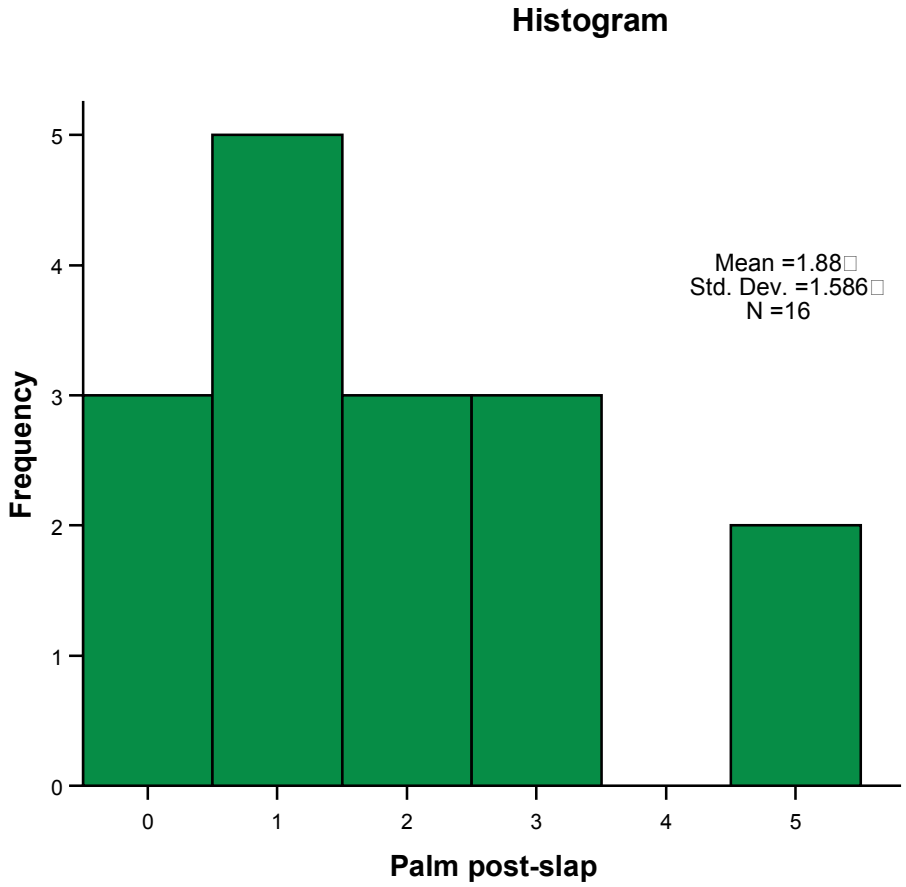
a Lilliefors Significance Correction



Tests of Normality

	Kolmogorov-Smirnov(a)			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Knuckles post-punch	.355	16	.000	.644	16	.000

a Lilliefors Significance Correction



Tests of Normality

	Kolmogorov-Smirnov(a)			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Palm post-slap	.209	16	.059	.887	16	.050

a Lilliefors Significance Correction

6.2.5 Peak height/area data – focus pad investigation, subject 11

Peak height – 28 cycles

Contact Type	Variable	Before or after contact	Loci								
			D3	VWA	D16	D2	D8	D21	D18	D19	TH01	FGA
Punch	15 mins	Before										
Punch	1 hour	Before										
Punch	multiple	Before										
Slap	15 mins	Before										
Slap	1 hour	Before										
Slap	multiple	Before										
Punch	15 mins	After										
Punch	1 hour	After					59					
Punch	multiple	After										
Slap	15 mins	After	75,115	162,202			52,186			173	137	
Slap	1 hour	After	215	147,127	86,82		179	110		88,95	188	
Slap	multiple	After	79	56			61			52	113	

Peak area – 28 cycles

Contact Type	Variable	Before or after contact	Loci								
			D3	VWA	D16	D2	D8	D21	D18	D19	TH01	FGA
Punch	15 mins	Before										
Punch	1 hour	Before										
Punch	multiple	Before										
Slap	15 mins	Before										
Slap	1 hour	Before										
Slap	multiple	Before										
Punch	15 mins	After										
Punch	1 hour	After					537					
Punch	multiple	After										
Slap	15 mins	After	812,1251	1575,1936			514,1827			1426	1244	
Slap	1 hour	After	1985	1286,1150	820,805		1664	914		739,772	1599	
Slap	multiple	After	668	532			573			436	991	

Peak height – 34 cycles

Contact Type	Variable	Repeat	Loci									
			D3	VWA	D16	D2	D8	D21	D18	D19	TH01	FGA
Punch	15 mins	1		100,111			61,262			99,166		
Punch	15 mins	2										
Punch	1 hour	1		1497,620		252	475				79	
Punch	1 hour	2		552	479	460			80,61	279	148	
Punch	multiple	1		1167			436,152,1095			1120	1454	
Punch	multiple	2	666,596,894					768		522	1217	
Slap	15 mins	1	1000	3124,1924	531,269		2180,2052	1096,690	50,55	3013	814	702
Slap	15 mins	2	510									
Slap	1 hour	1										520,232
Slap	1 hour	2			671	315,538,188,79	2061		57		1152	91
Slap	multiple	1	813,201	877,1118	226		1842			1884,391,967	2082,874	95
Slap	multiple	2	561,910	1041,882		105,163	616,2219					
Punch	15 mins	1	1846	1914,3404	1744,873	169		1381		2169,21014	653,587	589,117
Punch	15 mins	2	231	435,1611,2305		1045	1145,1486,2665,478	805,653			327,989,187	255,342
Punch	1 hour	1	1975	958,849	310	737,159	85,334	879,285	201,330,80	593,1400,1105	276,1853	214,231,190
Punch	1 hour	2	1723,1075	1064,1815,974,903	1068,697	463,795	86,61,3748	1774	435	675	3932	735,278
Punch	multiple	1	1133,3085	896,1542	187,310,446	161	1144,160,905	373	68	3274	2206,997	366
Punch	multiple	2	1475	738,135		270,77,339	79,338,889	304,1306,178		233,704,419	86,972,132	57
Slap	15 mins	1	473,273,316,2073	1620,1644,1053	1225,422	557,136,136	1126,1453,3479	167,871,374		2333,1697,863	2706,653,153	75,155,52
Slap	15 mins	2	2243		1077		1856,7690	462,2533,887	400,490			643,382
Slap	1 hour	1	4315	147,568,3698,2114	1566,790	730,438	6676	2725	283,449	4576,3802,94	4478	685,607
Slap	1 hour	2	3821	56,4092,4202	1072,468	873,514	5897	1544	140,125	2120,2031	5066,84,243	273,336
Slap	multiple	1	3653	1714,4613,2180	1453,616	110,92	3440,283	2180	84	3145,2775	4943,229	176,108
Slap	multiple	2	1163	440,1080,1557	339,295	67	243,2106	1128		632,2740,3002,156	3395,81	54,50

Peak area – 34 cycles

Contact Type	Variable	Repeat	Loci									
			D3	VWA	D16	D2	D8	D21	D18	D19	TH01	FGA
Punch	15 mins	1		881,1009			673,2783			799,1315		
Punch	15 mins	2										
Punch	1 hour	1		12282,5019		2351	4022				686	
Punch	1 hour	2		4616	4167	4142			705,531	2161	1171	
Punch	multiple	1		10114			3807,1256,9325			8918	11757	
Punch	multiple	2	5269,4976,7099					6002		3887	9405	
Slap	15 mins	1	10213	29961,18538	5382,2761		25044,23173	10081,6410	608,594	25787	7372	6823
Slap	15 mins	2	5186									
Slap	1 hour	1										4343,2027
Slap	1 hour	2			5816	2923,4830,1773,763	17213		520		9244	812
Slap	multiple	1	7452,1751	7827,9873	2017		18212			15063,3146,7813	17108,7042	854
Slap	multiple	2	6028,8018	8975,7639		1027,1674	5698,20799					
Punch	15 mins	1	16560	15901,28704	14961,7589	1598		11209		18399,2654	5391,4817	5093,1009
Punch	15 mins	2	2300	4340,15993,23557		12342	11322,14962,26553,4663	7746,6374			3191,9611,1781	2709,3683
Punch	1 hour	1	17742	8259,7299	2671	6817,1460	684,2970	7194,2390	1815,3020,734	4614,10928,8555	2306,14853	1789,1972,1701
Punch	1 hour	2	14231,9214	8814,14808,8068, 7631	9084,6076	4149,7417	739,531,31890	14155	3824	5266	30646	6199,2425
Punch	multiple	1	10237,28417	7854,13674	1658,2811,4075	1621	10009,1469,8136	3155	681	252941	17882,8102	3235
Punch	multiple	2	12918	6213,1124		2545,696,3025	719,2925,7658	2342,10227,1457		1743,5295,3157	687,7689,1055	497
Slap	15 mins	1	5491,3497,3529, 23376	15328,16142,10093	12893,4614	7004,1658,1804	13940,17324,42671	1638,8210,3514		20638,15059,7578	25217,6136,1412	759,1546,485
Slap	15 mins	2	24335		11077		22222,99834	4174,22991,7972	4333,5302			6302,3746
Slap	1 hour	1	39355	1216,4926,3624, 18167	13184,6874	7960,5111	70648	21703	2823,4486	35599,29182,745	35839	5934,6200
Slap	1 hour	2	34768	462,34394,35350	9241,4063	9451,5614	61049	12451	1419,1193	16495,15976	40350,633,1975	2270,2878
Slap	multiple	1	33389	14912,40066,19125	12483,5395	1248,997	35358,3035	17602	803	24468,21535	40015,1872	1480,933
Slap	multiple	2	9801	3672,8990,13008	2778,2424	719	2180,22334	8606		4680,20332,22060,1179	26249,635	452,440

6.2.6 Reference profiles

6.2.6.1 Focus pad investigation

	D3	VWA	D16	D2	D8	D21	D18	D19	TH01	FGA
1	15,15	14,17	11,12	19,23	13,14	28,32.2	11,17	14,15.2	7,9	22,23
2	16,18	17,18	11,11	16,24	13,14	30,30	15,16	14,14	9.3,10	22,25
3	15,16	16,18	10,12	17,21	13,14	29,30	14,15	12,16.2	6,7	20,22
4	16,18	14,17	12,12	17,20	13,14	29,31.2	15,18	14,14	8,9	22,24
5	14,17	17,18	13,14	17,20	14,14	32,32.2	12,19	12,13	6,9.3	22,22
6	15,17	17,18	11,12	17,24	11,13	28,28	15,16	13,15	9.3,9.3	20,24
7	14,17	14,16	11,14	20,24	11,13	30,31	12,18	13,14	7,9.3	22.2,24
8	16,17	15,17	10,12	17,17	12,14	28,28	15,16	14,14	6,7	22,25
9	16,17	17,18	12,13	19,23	10,12	28,31	14,15	13,15	9,9.3	20,21
10	14,15	15,16	13,14	20,24	12,14	28,32.2	12,14	11,16	7,9	22,25
11	17,17	16,17	11,14	16,23	14,14	29,29	12,13	13,14	7,7	20,23
12	16,18	15,19	9,12	19,20	10,13	28,29	14,17	13,13	9,9.3	23,25
13	14,15	14,18	11,12	19,21	12,13	29,30	14,15	13,13	9,9.3	21,25
14	14,16	18,18	9,11	25,25	8,12	30,31.2	17,17	14,14	6,7	22,23
15	15,17	14,18	9,14	16,18	11,14	32.2,33.2	17,18	14.2,15.2	8,9	19,20

6.2.6.2 Preliminary investigation

	D3	VWA	D16	D2	D8	D21	D18	D19	TH01	FGA
A	14,17	14,16	11,14	20,24	11,13	30,31	12,18	13,14	7,9.3	22.2,24
B	14,15	14,18	11,12	19,21	12,13	29,30	14,15	13,13	9,9.3	21,25

6.2.6.3 Person-to-person investigation

	D3	VWA	D16	D2	D8	D21	D18	D19	TH01	FGA
1	16,18	17,18	11,11	16,24	13,14	30,30	15,16	14,14	9.3,10	22,25
2	14,15	15,16	13,14	20,24	12,14	28,32.2	12,14	11,16	7,9	22,25
3	16,16	17,19	12,13	17,20	14,15	29,31	16,16	12,16.2	6,9.3	19,24
4	16,18	15,18	12,13	17,20	14,14	27,29	13,14	14,16	8,9	20,23
5	15,16	16,18	9,13	20,24	12,13	29,29	13,14	12,14	6,9	20,22.2
6	15,16	16,18	10,12	17,21	13,14	29,30	14,15	12,16.2	6,7	20,22
7	14,18	16,17	11,12	16,18	13,15	28,30	12,13	13,16	7,9	21,22
8	15,16	16,17	11,11	23,24	10,14	31,34.2	13,18	14,14	9.3,9.3	22,24
9	15,18	16,16	12,13	17,21	13,17	28,30	15,17	12,16	6,9.3	21,22
10	16,16	14,17	11,14	17,23	13,13	30,31	14,15	11,15	7,9.3	19,24
11	14,16	15,16	11,12	23,24	13,13	30,31.2	12,14	13,15.2	9.3,9.3	23,23
12	16,17	15,16	9,12	19,23	12,13	30,31	12,14	12,15	9,9.3	21,25
13	16,18	14,17	12,12	17,20	13,14	29,31.2	15,18	14,14	8,9	22,24
14	14,17	17,18	13,14	17,20	14,14	32,32.2	12,19	12,13	6,9.3	22,22
15	15,17	16,18	11,13	17,24	13,14	28,32.2	12,13	12,17.2	7,9	21,24
16	15,15	14,17	8,9	17,24	10,13	28,30.2	13,13	13,14.2	6,6	19,25

6.2.7 DNA profiling results – focus pad investigation

6.2.7.1 Pre-contact 28 cycles

Subject	Variable	Blow	D3	VWA	D16	D2	D8	D21	D18	D19	TH01	FGA
1	15mins	slap										
2	15mins	slap								14		
3	15mins	slap										
4	15mins	slap										
5	15mins	slap										
6	15mins	slap										
7	15mins	slap										
8	15mins	slap										
9	15mins	slap										
10	15mins	slap										
11	15mins	slap										
12	15mins	slap										
13	15mins	slap										
14	15mins	slap										
15	15mins	slap										
1	15mins	punch										
2	15mins	punch										
3	15mins	punch										
4	15mins	punch										
5	15mins	punch										
6	15mins	punch										
7	15mins	punch										
8	15mins	punch										
9	15mins	punch										
10	15mins	punch										
11	15mins	punch										
12	15mins	punch										
13	15mins	punch										
14	15mins	punch										
15	15mins	punch										

Subject	Variable	Blow	D3	VWA	D16	D2	D8	D21	D18	D19	TH01	FGA
1	1 hour	slap										
2	1 hour	slap										
3	1 hour	slap										
4	1 hour	slap										
5	1 hour	slap										
6	1 hour	slap										
7	1 hour	slap										
8	1 hour	slap										
9	1 hour	slap		16								
10	1 hour	slap										
11	1 hour	slap										
12	1 hour	slap										
13	1 hour	slap										
14	1 hour	slap										
15	1 hour	slap										
1	1 hour	punch										
2	1 hour	punch										
3	1 hour	punch										
4	1 hour	punch										
5	1 hour	punch										
6	1 hour	punch										
7	1 hour	punch										
8	1 hour	punch										
9	1 hour	punch										
10	1 hour	punch										
11	1 hour	punch										
12	1 hour	punch										
13	1 hour	punch										
14	1 hour	punch										
15	1 hour	punch										

Subject	Variable	Blow	D3	VWA	D16	D2	D8	D21	D18	D19	TH01	FGA
1	multiple	slap										
2	multiple	slap										
3	multiple	slap										
4	multiple	slap										
5	multiple	slap										
6	multiple	slap										
7	multiple	slap										
8	multiple	slap										
9	multiple	slap										
10	multiple	slap	16	15,16,17,18			13			12,13,14	6,9.3	
11	multiple	slap										
12	multiple	slap										
13	multiple	slap										
14	multiple	slap										
15	multiple	slap										
1	multiple	punch										
2	multiple	punch										
3	multiple	punch										
4	multiple	punch										
5	multiple	punch										
6	multiple	punch										
7	multiple	punch										
8	multiple	punch		16								
9	multiple	punch										
10	multiple	punch										

11	multiple	punch
12	multiple	punch
13	multiple	punch
14	multiple	punch
15	multiple	punch

6.2.7.2 Post-contact 28 cycles

Subject	Variable	Blow	D3	VWA	D16	D2	D8	D21	D18	D19	TH01	FGA
1	15mins	slap										
2	15mins	slap	17,18	18			12	28				
3	15mins	slap										
4	15mins	slap										
5	15mins	slap										
6	15mins	slap										
7	15mins	slap	17									
8	15mins	slap										
9	15mins	slap										
10	15mins	slap					13			13		
11	15mins	slap	14,17	16,17			13,14			13	7,7	
12	15mins	slap										
13	15mins	slap	14,15	14,18	12	19	12			13,13		
14	15mins	slap										
15	15mins	slap										
1	15mins	punch										
2	15mins	punch										
3	15mins	punch										
4	15mins	punch										
5	15mins	punch										
6	15mins	punch	15,17	17,18	11,12		11,13			13	9.3,9.3	
7	15mins	punch										
8	15mins	punch										
9	15mins	punch										
10	15mins	punch										
11	15mins	punch										
12	15mins	punch										
13	15mins	punch										
14	15mins	punch										
15	15mins	punch										

Subject	Variable	Blow	D3	VWA	D16	D2	D8	D21	D18	D19	TH01	FGA
1	1 hour	slap										
2	1 hour	slap										
3	1 hour	slap										
4	1 hour	slap										
5	1 hour	slap										
6	1 hour	slap										
7	1 hour	slap	14,17	16						13		
8	1 hour	slap	15				13				9.3	
9	1 hour	slap	16,17	17			10			13,15	9,9.3	
10	1 hour	slap					12,14					
11	1 hour	slap	17,17	16,17	11,14		14,14	29,29		13,14	7,7	
12	1 hour	slap										
13	1 hour	slap								13,13		
14	1 hour	slap										
15	1 hour	slap										
1	1 hour	punch										
2	1 hour	punch										
3	1 hour	punch										
4	1 hour	punch										
5	1 hour	punch										
6	1 hour	punch										
7	1 hour	punch										
8	1 hour	punch										
9	1 hour	punch										
10	1 hour	punch		15			14					
11	1 hour	punch					14					
12	1 hour	punch										
13	1 hour	punch						27				
14	1 hour	punch										
15	1 hour	punch										

Subject	Variable	Blow	D3	VWA	D16	D2	D8	D21	D18	D19	TH01	FGA
1	multiple	slap										
2	multiple	slap										
3	multiple	slap	15				13			16.2		
4	multiple	slap										
5	multiple	slap					14,14					
6	multiple	slap										
7	multiple	slap										
8	multiple	slap										
9	multiple	slap	16,17	17,18			10,12	28		13,15	9,9.3	
10	multiple	slap										
11	multiple	slap	17,17	17			14,14			13	7,7	
12	multiple	slap										
13	multiple	slap	14,15	14,18			12,13			13,13	9,9.3	
14	multiple	slap	14,16	18,18	11		8			14,14	7	
15	multiple	slap										
1	multiple	punch										
2	multiple	punch										
3	multiple	punch										
4	multiple	punch										
5	multiple	punch										
6	multiple	punch										
7	multiple	punch										
8	multiple	punch										
9	multiple	punch										
10	multiple	punch										
11	multiple	punch										

12	multiple	punch										
13	multiple	punch										
14	multiple	punch		18,18						14,14		
15	multiple	punch										

6.2.7.3 Pre-contact 34 cycles

Subject	Variable	Blow	D3	VWA	D16	D2	D8	D21	D18	D19	TH01	FGA
1	15mins	slap										
2	15mins	slap	14,15	15	12		13,14	30	17	14	9.3	23
3	15mins	slap										
4	15mins	slap					13					
5	15mins	slap								15		
6	15mins	slap										
7	15mins	slap										
8	15mins	slap			14					13	9	
9	15mins	slap	15									
10	15mins	slap										
11	15mins	slap	15									
12	15mins	slap										
13	15mins	slap		17					15,16			25
14	15mins	slap										
15	15mins	slap		16								
1	15mins	punch										
2	15mins	punch	15		12		10,16	30,32.2	16	12,13		21
3	15mins	punch								13		
4	15mins	punch	15			17				12,13		
5	15mins	punch					12			12		
6	15mins	punch										
7	15mins	punch										
8	15mins	punch	14,15	20	9	17	14	28		12,14	9.3	22
9	15mins	punch										
10	15mins	punch										
11	15mins	punch										
12	15mins	punch	14,15	15,18	12	17	13,14	29	17,18	14	9.3	20,23
13	15mins	punch	17									
14	15mins	punch	14,15	17,18	11,11	17,17	9,13	27,30	13,17	12,13,15	6,9	23,24
15	15mins	punch		16			13			12,13,15		

Subject	Variable	Blow	D3	VWA	D16	D2	D8	D21	D18	D19	TH01	FGA
1	1 hour	slap										
2	1 hour	slap										
3	1 hour	slap									9.3	
4	1 hour	slap	16									
5	1 hour	slap								12		
6	1 hour	slap	15			17	9,13			13		
7	1 hour	slap						36				
8	1 hour	slap	14				13,14	33.2				
9	1 hour	slap	14,17	14,16						12,13		
10	1 hour	slap					10,13					
11	1 hour	slap										
12	1 hour	slap										
13	1 hour	slap	14				13					
14	1 hour	slap										
15	1 hour	slap								12		
1	1 hour	punch	16	16,16								
2	1 hour	punch	17									
3	1 hour	punch		17						12,15	6	
4	1 hour	punch										
5	1 hour	punch	14				13	29				20
6	1 hour	punch										
7	1 hour	punch	14	16								
8	1 hour	punch		16								
9	1 hour	punch			11		16				6	
10	1 hour	punch	16	15,18	12		9	27,30		12,13,15	7	
11	1 hour	punch										
12	1 hour	punch										
13	1 hour	punch		18		16						23
14	1 hour	punch					13					
15	1 hour	punch	14									

Subject	Variable	Blow	D3	VWA	D16	D2	D8	D21	D18	D19	TH01	FGA
1	multiple	slap	16		12							23
2	multiple	slap										
3	multiple	slap	16	15			11,13					
4	multiple	slap					10	29			9.3	
5	multiple	slap					14				7	
6	multiple	slap	15				10,13					
7	multiple	slap	15									
8	multiple	slap										
9	multiple	slap		16							9.3	
10	multiple	slap	14,16	14,15,16,17	11		10,11,13,14,15	30		12,13,14	6,7,9.3	
11	multiple	slap	16,17	15,16			12					
12	multiple	slap										
13	multiple	slap			9		10,13			12,13		
14	multiple	slap		16			13			12		
15	multiple	slap	15,15				8					
1	multiple	punch										
2	multiple	punch	16									
3	multiple	punch										
4	multiple	punch				24	13			13	7,9.3	
5	multiple	punch	14	16,17,18			13,14	30		12		
6	multiple	punch						30		12,13	7	
7	multiple	punch										
8	multiple	punch	17	16,18	11		13,14			12	7,9	
9	multiple	punch	17								9.3	
10	multiple	punch	16	16			13				9	
11	multiple	punch										
12	multiple	punch	14	16			7			15.2		
13	multiple	punch	16							12,13		
14	multiple	punch	14,15	17,18	11	17,20	9,10,13	27,30	13	13,15		24
15	multiple	punch	14,15	17,18	11	17	9,11,13	27,30		13,15	6,9	23,24

6.2.8 DNA profiling results – preliminary investigation

6.2.8.1 Pre-contact 28 cycles

Hand

Subject	Force	Repeat	D3	VWA	D16	D2	D8	D21	D18	D19	TH01	FGA
A	Punch	1										
A	Punch	2										
A	Punch	3								12,14		
A	Slap	1		14,16						13,14		
A	Slap	2										
A	Slap	3	14,17	14,16	11,14	2,24	10,11,12,13	30,31	12,18	13,14	7,9.3	22.2,24
B	Punch	1	14,15	14,18	11,12	19,21	12,13	29,30	14,15	13,13	9,9.3	21,25
B	Punch	2	14,15	14,18	11,12	19,21	12,13	29,30	14,15	13,13	9,9.3	21,25
B	Punch	3	14,15	14,18	11,12	21	12,13	30	14	13,13		21
B	Slap	1	14,15	14,18	11,12	19,21	12,13	29,30	14,15	13,13	9,9.3	21,25
B	Slap	2	14,15,16	14,18	11,12	19,21	8,12,13	29,30	14	13,13,14	9,9.3	21,25
B	Slap	3	14,15	14,18	11,12	19	13	29	14	13,13	9,9.3	21,25

Arm

Subject	Force	Repeat	D3	VWA	D16	D2	D8	D21	D18	D19	TH01	FGA
A	Punch	1	14,17	14,16		24	11	30,31			7	
A	Punch	2	14	14								
A	Punch	3										
A	Slap	1										
A	Slap	2					11,13					
A	Slap	3										
B	Punch	1		14,18	12		12	30		13		
B	Punch	2										
B	Punch	3										
B	Slap	1										
B	Slap	2	14		11	19	12			13	9	
B	Slap	3	14		11,12		12	29	14,15	13,13		

6.2.8.2 Pre-contact 34 cycles

Hand

Subject	Force	Repeat	D3	VWA	D16	D2	D8	D21	D18	D19	TH01	FGA
A	Punch	1										
A	Punch	2	14,17	14,16	11,14		11,13	30,31	12	13,14	7,9.3	24
A	Punch	3										
A	Slap	1		14,16			11,13			13,14	9.3	
A	Slap	2								15	7	
A	Slap	3	14,17	14,16	11,14		11,13	30,31		13,14	7,9.3	
B	Punch	1	14,15	14,18	11,12	19,21	12,13	29,30	14,15	13	9,9.3	21,25
B	Punch	2	14,15	14,18	11,12	19,21	12,13	29,30	14,15	13	9,9.3	21,25
B	Punch	3	14,15	14,18	12	19	12,13	30		13,14,15	9	
B	Slap	1	14,15	14,18	11,12	19,21	12,13	29,30	14,15	13	9,9.3	21,25
B	Slap	2		14,18	11,12	19,21	12,13	29,30			9,9.3	21
B	Slap	3		14			12,13			13		

Arm

Subject	Force	Repeat	D3	VWA	D16	D2	D8	D21	D18	D19	TH01	FGA
A	Punch	1	14,17	14,16	9,11,12,14	20,24	11,13	30,31	12	13,14	7,9.3	22.2,24
A	Punch	2	14,17	14,16	11,14	20,24	11,13	30,31	12,18	13,14	7,9.3	22.2,24
A	Punch	3										
A	Slap	1	14	14,16	11,14		11	30,31		13,14	7,9.3	
A	Slap	2	17	14,16	11,14		11,13	30,31		13,14	7,9.3	
A	Slap	3										
B	Punch	1	14,15	14,18	11,12	19,21	12,13	29,30	14,15	13	9,9.3	21,25
B	Punch	2	14,15	14,18	11,12	19,21	12,13	29,30	14,15	13	9,9.3	21,25
B	Punch	3	15	14,18	11		12	29,30		13		21,25
B	Slap	1	15		12		13			13,15	9.3	21
B	Slap	2	14,15	14,18	11,12	19,21	12,13	29,30	14,15	13	9,9.3	21,25
B	Slap	3	14,15	14,18	11,12	19,21	12,13	29,30	14,15	13	9,9.3	21,25

6.2.9 DNA profiling results – person-to-person investigation

6.2.9.1 Pre-contact 28 cycles

Arm

	D3	VWA	D16	D2	D8	D21	D18	D19	TH01	FGA
1										
2										
3										
4										
5										
6	15,16	16,18	9,10,12	17	13,14	29,30		12,16.2	6,7	20,22
7										
8		17								
9		16								
10										
11										
12										
13		14								
14	15	17,18	13,14	17	14			13,14	6	
15										
16										

Knuckles

	D3	VWA	D16	D2	D8	D21	D18	D19	TH01	FGA
1								14		
2	14,15,16	15,16	11	20	12,14	28		12,16	7,9.3	
3	16									
4	14,15,16	15,17,18	9	17	12,13			12,13,15	9.3	
5										
6	15,16	16,18	10,12		8,13,14	29,30		12,16.2	6,7	20
7										
8										
9										
10	14				13					
11										
12		16			13					
13										
14	15	17,18			14			13,14		
15	13,14,15,16	12,17,18			8,11,13	24,27,28				
16										

Palm

	D3	VWA	D16	D2	D8	D21	D18	D19	TH01	FGA
1	16							14		
2										
3	16	15								
4										
5										
6	15,16	16,18	10,12	17,21	13,14	29,30	14,15	12,16.2	6,7	20,22
7										
8	14,16	15,16,17,18	9	17	10,12,13,19	30,31		12,13,14,15	9.3	20
9	15	16	12		13,17	30		16	6	
10										
11										
12										
13										
14	15,18	17,18	13,14	20	14					
15										
16	15	14,17			10	28		13,14.2	6	

6.2.9.2 Post-contact 28 cycles

Arm after a single punch

	D3	VWA	D16	D2	D8	D21	D18	D19	TH01	FGA
1										
2										
3										
4										
5										
6										
7										
8										
9										
10										
11										
12	15		9		13					
13		14			14					
14										
15										
16										

Arm after a single slap

	D3	VWA	D16	D2	D8	D21	D18	D19	TH01	FGA
1										
2										
3					14					
4										
5	16							12		
6										
7										
8										
9										
10										
11										
12										
13					13			14		
14		18			14					
15										
16										

Knuckles

	D3	VWA	D16	D2	D8	D21	D18	D19	TH01	FGA
1										
2	15				12,14			12,16		
3										
4										
5										
6	15,16	16,18	10,12	17	13,14	29,30		12,16.2	6,7	
7										
8	15,16	17	11		10,14	31		14	9.3	
9										
10										
11										
12										
13										
14										
15										
16										

Palm

	D3	VWA	D16	D2	D8	D21	D18	D19	TH01	FGA
1										
2										
3										
4										
5										
6	15,16	16,18			13,14	29		12,16.2	6,7	
7										
8	15	16,17	11		10,12,14			14	9.3	
9										
10										
11										
12										
13					14					
14	15,18	17,18	13,14	17,20	14	32,32.2		13,14	6,9.3	22
15										
16	15	14,17			10			13,14.2	6	

6.2.9.3 Pre-contact 34 cycles

Arm

	D3	VWA	D16	D2	D8	D21	D18	D19	TH01	FGA
1	18				13	30		14		
2	12,14,15,16	15,16,17	8,9	24	10,12,17	28.2,32,32.2		10,11,12,12.2,13.2,16	6,7,8,9,9.3	
3		15,17,22	13	20	11,14	29,31		12.2,13	6,7,9.3	
4	16,18	15,18	13		14	27,29		13,14,15	9,9.3	
5	14,15,16		12,13	24	13	29	13,14	12,13,14,15		
6	15,16	16,18	10,12	17,21	13,14	27,29,30		12,14,15,16.2	6,7,9.3	20,22
7	14,16				14,15	29.2		13,16		
8	14,15,16	12,16,17	10,11,13	16,17,18,23,25	12,14	26,29,31,32,35,36		13,14	5,6,8,9	
9	15,16	16,18	13	21	9,10,13,15,17	28,30		12,16	6	
10	16	13,14,17	10		11,15	25		13,15	6,7	
11	14	16,18	11		13			10,13	9,9.3	
12		15			8,10,11,12,13	30.2			8,9	
13	16				13,14			14	8,9	
14	15,18	17,18	13,14	17,20	14	28,32,32.2	12,19	12,13,14	6,9.3	22
15										
16	15,16	12,13,14,17,20	8,9,12		10,12,13,14	27,28		13,14.2	5,6,9	

Knuckles

	D3	VWA	D16	D2	D8	D21	D18	D19	TH01	FGA
1	16,18	17	11		13,14	30		14	9.3,10	22,25
2	14,15,16	15,16	9,11,13,14	20,24	12,14	28,29,32.2		12,13,16	7,9,9.3	
3	14,15,16	15,17,18,19	11,12,13		14,15	27,31		12,13,15,16.2	6,7,9.3	19
4	15,18	18	12	21	12,14	30		16	6,9	20
5		16	9		12,13	28,29		12,14,14.2	6,7,9	
6	15,16	16,18	10,12	17,21	13,14	29,30		12,14,16.2	6,7	20,22
7	14,18	11,16,17	11,12	16	12,13,14	28,30		12,13,16	7,9	21
8	15,16	14,16,17	11		10,14	31,34.2		14	9.3	
9	15	16,17,18	11	17	9,13	27,28		13,15	6,9	
10	14,15	17,18	11	17	9,13	27,30		13,15	6,9	23,24
11								13		
12	14,16,17	15,16	9,11,12	19,23	12,13	30,31	12	12,13,15	6,7,9,9.3	21,25
13	16		12	17	13,14	31.2				22
14										
15	12,13,14,15,16	13,14,15,16,17			8,9,10,11,12,13,14,15	24,25,26,27,28		10,11,12,13,14,15	6,7,8,9,9.3	
16	15	14,17	8,9,11		9,10,13	28,30,30.2	13	13,14.2,15.2	6,8,9	19,25

Palm

	D3	VWA	D16	D2	D8	D21	D18	D19	TH01	FGA
1		12,14,17			9,10,11,14			12,14	9.3	
2	14,15,16	15,16	11,13,14	20,24	9,12,14	28,32.2		12,13,16	5,7,9	
3	14,15,16	15,16,19	12,13		11,13,14,15	27,30.2,32.2		12,13,16	6,7,9.3	22
4								14,16		
5					13			12,13,14	6,7	
6	15,16	16,18	10,12	17,21	13,14	29,30		12,16.2	6,7	20,22
7	15,16	16	11		10,12,14	28		12,13,14,16	7,9.3	
8	15,16,17	16,17	9,10,11,12,13	23,24	10,12	29,31,32.2,34.2		13,14,15	8,9.3	22,24
9		16	12	17	13	28,30		16	6,9.3	
10										
11										
12	16,17	15,16,17			9,13	28		11,12,13,14,15	6,8,9,9.3	
13	15,16	12,14,16			11,12,13	28		14,15,17	4,5,6,7,9.3	
14	15,18	14,17,18	13,14		14	27,29,32,32.2		13,14	6,9.3	22
15	15							12,13,17.2	6,7,9	
16	15	14,16,17	8,9,11	17	10,13,14	30.2	13	12,13,14.2	6,7	19

6.3 List of publications

6.3.1 First authored publications

Bowyer, V.L., E.A.M. Graham, and G.N. Rutty, *Forensic web watch – DNA in Forensic Science*. Journal of Clinical Forensic Medicine, 2004. **11**: p. 271 – 273.

Bowyer, V.L., *DNA Review: Real – Time PCR*. Forensic Science Medicine and Pathology, 2007. **3**(1): p. 61-64.

6.3.2 Co-authored publications

Port, N.J, **V.L. Bowyer**, E.A.M. Graham, M.S. Batuwangala, and G.N. Rutty, *How long does it take a static speaking individual to contaminate their immediate environment?* Forensic Science Medicine and Pathology, 2006. **2**(3): p. 157-164.

Maguire, S., B. Ellaway, **V.L. Bowyer**, E.A.M. Graham, and G.N. Rutty, *Retrieval of DNA from the faces of children aged 0-5 years: A technical note*. Journal of Forensic Nursing - accepted for publication January 2007.

Graham, E.A.M., **V.L. Bowyer,** V.J. Martin, and G.N. Rutty, *Investigation into the usefulness of DNA profiling earprints*. Science and Justice, 2007. **47**(4): p. 155-159.

6.3.3 Abstracts

Bowyer, V.L., Graham, E.A.M., Rutty, G.N., Maguire, S and Ellaway, B. (2005) Determination of the distribution of DNA to the faces of children aged 0 – 5 years due to normal day–to–day interaction between the child and carers (B72). *Proceedings American Academy of Forensic Sciences* **11**: p66.

Port, N, **Bowyer, V.L**., Batuwangala, M., Graham, E.A.M. and Rutty, G.N. (2005) How long does it take to contaminate a scene by talking? *17th Meeting of the International Association of Forensic Sciences* Abstract No. A0583.

Maguire, S., Ellaway, B., **Bowyer, V. L**., Graham, E.A.M., Sibert, J. Kemp, A., and Rutty, G.N. (2006) Measurement of DNA found on the faces of 30 children aged 0-5 years: a pilot study relevant to child protection assessments. *Royal College of Paediatrics and Child Health 10th Spring Meeting* Abstract No. G93.

Graham, E.A.M., **Bowyer, V.L** and Rutty G.N. (2006) DNA analysis of a mass fatality incident. *XXth Congress of the International Academy of Legal Medicine.*

Bowyer, V.L., Graham, E.A.M., Maguire, S and Ellaway, B and Rutty, G.N. (2006) Determination of the distribution of DNA to the faces of children due to normal day–to–day interaction between the child and carers. *XXth Congress of the International Academy of Legal Medicine.*

Bowyer, V.L., Graham, E.A.M., and Rutty, G.N. DNA transfer through forceful contact (B113). *Proceedings American Academy of Forensic Sciences* **13**: p98.

Bowyer, V.L., Graham, E.A.M., and Rutty, G.N. Investigation into DNA transfer through forceful contact. *Proceedings American Academy of Forensic Sciences 2008.*

6.3.4 Presentations

Bowyer, V.L., Graham, E.A.M., Maguire, S and Ellaway, B and Rutty, G.N. (2006) Determination of the distribution of DNA to the faces of children due to normal day–to–day interaction between the child and carers. *XXth Congress of the International Academy of Legal Medicine, Budapest, Hungary,* August 2006.

Bowyer, V.L., Graham, E.A.M., and Rutty, G.N. DNA transfer through forceful contact (B113). *59th Annual Meeting of the American Academy of Forensic Sciences, San Antonio, Texas, USA*, February 2007.

6.4 Publications

6.4.1 Forensic web watch

Bowyer, V.L., E.A.M. Graham, and G.N. Rutty, *Forensic web watch – DNA in Forensic Science*. Journal of Clinical Forensic Medicine, 2004. **11**: p. 271 – 273.

Abstract

In 1923, within the Manual of Police technique, Edmond Locard published what is commonly known as the Doctrine of Exchange; a series of rules related to the exchange of trace evidence between the victim and offender. Although at the time of publication these rules principally applied to trace evidence related to print (for example finger print or shoeprint), fiber and blood, today one can add the very substance that defines each human being – DNA. Since the first use of DNA evidence to help identify an offender in the Pitchfork Murders of 1986, the use of DNA within forensic science has developed from its humble days within a single experimental laboratory at the University of Leicester to a multi-million pound industry. It thus seems fitting that this forensic web watch should originate from the very University where the use of DNA in forensic science was conceived, drawing the readers attention to a number of sites which can be used as an introduction to the concept of the use of DNA in forensic science today.

Keywords: Internet; DNA; STR; Profiling

1. Introduction

It has been almost 20 years since the concept of DNA fingerprinting was first introduced to the scientific community in the form of a seemingly insignificant research article in the Journal Nature by the now Professor Sir Alec Jeffreys. This paper identified regions of repetitive DNA in the human genome that upon length analysis appeared to show an 'individual' pattern for all humans, except monozygotic twins. It did not take long for the potential forensic application of DNA fingerprinting, or DNA profiling as it is now known, to be recognised and grow into the industry that it is today.

The fact that DNA is present in al bodily fluids and cells, with the exception of red blood cells means that it is theoretically virtually impossible for an individual to enter a crime scene without leaving some form of biological trace evidence. This may be in the form of blood, hair or even as single cells deposited with fingerprints. Major advances in both molecular biology and computer technology that have occurred over the last 20 years are now allowing investigators to obtain DNA profiles from smaller and smaller starting quantities of biological material, reinforcing the importance of Edmond Locard's principles to criminal investigation. It must however be noted that as techniques become ever more sensitive as many additional problems are created as may be solved. This is due to sample contamination resulting in the generation of confusing mixed profiles. In the context of DNA profiling contamination can be caused by as innocent an act as an investigator breathing over a body at a crime scene. It is therefore essential in situations where samples may be taken for DNA profiling that all investigators are properly trained in anti contamination techniques.

Many commercial kits used for DNA profiling, which are based on repetitive regions of the human genome known as short tandem repeats (STRs), are now available. They are designed to analyse up to 16 separate regions of DNA in a single reaction, providing 'chance match' probabilities which far exceed the present human population. Systems of this kind not only give the investigator confidence in the individuality of the profile but are also optimised to be initiated from minute starting quantities of DNA. Thus as new techniques for the extraction, quantification and analysis of samples are

developed, this will enable faster results from even the smallest and most degraded of samples to be obtained. It also will shortly be possible to routinely generate a profile from as little starting material as a single cell.

Because of the commercial industry that has grown up around DNA the majority of websites related to DNA in forensic science are those that are advertising products or books on the subject. However, a number of different search engines (including Google, Altavista, Sciseek, Ask Jeeves and Yahoo) revealed that there are hundreds of thousands of websites containing information regarding DNA in general as well as in relation to forensics. This review has identified a number of these sites, which contain useful information both for the beginner and more experienced scientist to help understand how DNA is or can be used in forensic investigations.

1.1 DNA in general

There are thousands of websites that delve into the extremely broad topic of DNA to varying degrees. One of the best is the DNA from the Beginning website created by the Dolan DNA learning centre at Cold Spring Harbour Laboratory. This site provides a detailed description of DNA, chromosomes and Mendelian inheritance that is very easy to read. Additionally, the website includes brief biographies of important individuals involved in DNA as well as pictures and animations that further illustrate the points being made. Similarly the Tech website provides a simple overview of what DNA is in an easy to read, step-by-step slide show without the site visitor being overloaded by too much technical detail.

2. DNA profiling

Human DNA profiling has many uses from solving crime to determining the paternity of a child. Most of these are covered in various websites that are offering products and services in each of these areas, but most do not cover the methods of how the techniques are performed. Information on the principles of DNA profiling can be found on the American Chemical Science and Technology website in the form of a PDF 'slideshow'. The information is laid out in a basic point form that provides the visitor with essential information on DNA profiling in human identification: both crime and paternity testing. The only draw back to such a site is that some prior knowledge of DNA and the techniques used, such as polymerase chain reaction (PCR), are necessary though, in order to fully appreciate the site.

Complementary to this is the website prepared by Thomas Curran which provides a more detailed account of DNA profiling that includes both RFLP analysis as well as STRs with an excellent explanation of the PCR. There is also a thorough overview of DNA itself that includes a description of RFLPs, VNTRs, STRs and mitochondrial DNA as well as general information on genes and chromosomes. A similar site, without the background DNA information, is the Dumfries and Galloway Council Information Service website. Presented here is DNA fingerprinting as initially discovered by Sir Alec Jeffreys plus detailed descriptions of DNA isolation, electrophoresis and PCR.

Although, www. Karisbale.com is a website that mainly deals with the telling of true crime stories, there is a decent section on DNA and forensics with a useful slideshow that briefly outlines the steps involved in creating a DNA profile. There is also a timeline of key dates in the development of forensic science, plus a database of all aspects of forensic science. An interactive game allows you to test your skills as a detective and the true crime stories show just how DNA is used in practice. Among the many links found at this site is to the website of Kate Brinton and Kim-An Lieberman of Washington University which covers the basics of DNA fingerprinting. The site, however, is quite difficult to read due to the choice of background wallpaper used. However, the 'DNA

101' section gives a very simple look at what DNA is plus short sections on the uses and problems of DNA fingerprinting.

The University of Utah website is an excellent site that simply covers the main points of interest in DNA fingerprinting with additional pages that cover the basics. Tour of the basics includes a superb animation showing the location and structure of DNA, genes and chromosomes as well as further animations on hereditary traits.

Another outstanding site is the NBII Genetic Biodiversity webpage. It is, as the site title suggests, mainly concerned wit diversity but by following the other topics links it leads to pages on basic genetics with very simple, easily interpreted diagrams that help the reader to fully understand the site text, no matter how much or how little they already know of the subject. Genetic Analysis in the Lab gives a similarly easy to read/understand overview of the procedures involved in DNA fingerprinting, such as extraction and PCR further supplemented with appropriate diagrams. This site is ideal for individuals who are just starting to learn about DNA and forensics.

3. STRs and mitochondrial DNA

Some websites are focused on more specific areas of DNA profiling, particularly on STRs and other identification markers. A good site for information on STRs is the American Chemical Science and Technology lab's 'STR base'. This website provides excellent background information on STRs as well as more detailed fact sheets on specific loci. The site was created by John Butler who is a leading figure in this area and contains a number of links to useful journal articles on different STRs. Overall this is an excellent site that supplies all the essential information necessary on the STRs in use today.

Information on mitochondrial DNA is provided by sites, such as that of Mitotyping Technologies LLC. Although the content of the site is limited, it does give a

concise account of mitochondrial DNA and its uses in forensic science. For additional information the bibliography page presents a number of useful references.

3. Conclusion

The sites listed are just a few of thousands of related websites on the subject or commercial businesses related to DNA and forensic science that are available on the internet. At the end of the day, as is the theme of the internet, most are company related but there are still numerous sites on the topic of DNA with more being added as more is discovered about the human genome. Whatever knowledge is needed by the user, there will be a website covering it, although to find specific information more specific, limited searches will be needed to be performed than just using the simple term 'DNA'.

Dolan DNA Learning Centre www.dnaftb.org/dnaftb

The Tech www.thetech.org/exhibits/online/genome/overview.html

Chemical Science and Technology Lab US (1) www.cstl.nist.gov/biotech/strbase/ppt/intro.pdf

Thomas Curran www.parl.gc.ca/information/library/PRBpubs/bp443-e.htm

Dumfries and Galloway Council
www.dumgal.gov.uk/Services/depts/educate/genetics/genetics.htm

Kari and associates www.karisable.com/crdna1.htm

Kate Brinton and Kim-An Lieberman www.biology.washington.edu/fingerprint/dnaintro.html

University of Utah www.gslc.genetics.utah.edu/features/forensics

NBII www.genetics.nbii.gov/forensics.html

Chemical Science and Technology Lab US (2) www.cstl.nist.gov/biotech/strbase

Mitotyping technologies LLC www.mitotyping.com/dna.htm

6.4.2 DNA review

Bowyer, V.L., *DNA Review: Real – Time PCR*. Forensic Science Medicine and Pathology, 2007. **3**(1): p. 61-64.

Abstract

Research into the field of DNA has increased dramatically in the years since DNA profiling was first identified. Since then techniques have been developed to identify individuals from DNA samples equivalent to a single cell. These techniques have meant that the ability to accurately quantitate the DNA in such samples has had to evolve at an equal rate. As a result there are a variety of quantification protocols available including the picogreen assay, slot blot systems and real-time PCR. Real-Time PCR is a relatively recent development but promises to be the most sensitive and accurate technique available.

Keywords: Forensic science; DNA profiling; real-time PCR; quantification

Introduction

Over the past 21 years some of the most important discoveries in the world of forensics have been in the field of DNA. Most notable are those of the techniques known as the polymerase chain reaction, identified in 1985 by Kary Mullis and the Human Genetics Group at the Cetus Corporation (1) and DNA profiling described by Alec Jeffreys in 1985 (2). The combination of these two techniques now form the basis of forensic DNA testing.

Developments in both techniques over the years has made it possible to obtain full DNA profiles from minute quantities of DNA even single cells (3). These developments have resulted in commercial kits being developed and produced for both PCR and DNA profiling by several companies to ease both processes. Such kits include the AmpFlSTR SGM plus® PCR amplification kit from Applied Biosystems and Powerplex 16 from Promega. Amplification kits like these work best when the concentration of the template DNA that is added is within a specific, but narrow range. If too much DNA is added to a PCR reaction split peaks can be seen for some alleles, as a result of incomplete 3′ A nucleotide addition on the end of the PCR product. The DNA polymerase is overwhelmed by the excess template so that the extra A nucleotide fails to be added. If this happens to some alleles but not others accurate genotyping can be made more complicated than normal. Excess template can also result in off scale alleles. These manifest as flat topped peaks for that particular allele, but also results in 'pull up' peaks. Pull up peaks can be seen masking peaks of the same size but labelled with a different fluorescent dye to the off scale allele, so the actual allele of interest is difficult to determine. If too little DNA is added to the PCR reaction allele dropout can occur, i.e. with insufficient template two alleles in a heterozygote can be amplified unequally resulting in the formation of a false homozygote peak (4)

As a result is important that the amount of DNA added to the PCR reaction falls within the optimal range recommended by the manufacturer of that particular DNA amplification kit. Quantification is therefore an essential part of any forensic DNA protocol and as such, there are many different methods available. Those most commonly

used today include the picogreen microtiter assay (Forensic Science Service), slot blot quantification, spectrophotometry, the AluQuant system (5) and end-point PCR assays. However the focus of this review is a relatively new system of quantification known as Real-Time PCR.

Real-Time PCR

Real-time PCR otherwise known as quantitative PCR or kinetic analysis is so called as the process involves instrumentation that measures DNA concentration during PCR, as the template is amplified. The basis of this process is the polymerase chain reaction itself as qPCR incorporates fluorescent dyes into the reaction and measures the fluorescence that is produced as the quantity of DNA product increases cycle by cycle. The fluorescent signal is directly proportional to the product produced.

The main advantages to Real-Time PCR over and above other types of quantification include the species specificity enabled by accurate probe design and the sensitivity provided by measurement of PCR product as it is formed cycle by cycle. As qPCR measures the PCR product this process provides the concentration of amplifiable DNA in the sample rather than total DNA. This means that the minimum amount of template can be added to downstream PCR applications (of the range recommended by manufacturers), limiting the effect of any PCR inhibitors present within the sample and ignoring the presence of any degraded DNA that fails to amplify (6).

Real-time PCR can be divided into four separate phases; baseline, exponential, linear and the plateau. Baseline is the phase during which the fluorescent signal is consistent with that of the normal background levels. Once the fluorescent signal increases beyond this level the process enters the exponential phase of PCR. The point at which the exponential phase starts is also known as the cycle threshold (CT); the number of cycles that have been completed at this point is inversely related to the template concentration at the beginning of the qPCR process. During the exponential phase of PCR all reagents are at their optimum levels, the dye, primers, magnesium and polymerase are present in

sufficient quantities for amplification to continue efficiently. However as the PCR product increases reagents do begin to decrease, especially the DNA polymerase, resulting in the slowing down of the formation of new product. Eventually the polymerase and reagents are fully depleted and PCR enters the plateau phase as the product concentration remains constant.

Of the four phases the exponential is the one at which PCR product formation is the most consistent between samples due to the optimum concentrations of reagents. Hence the cycle threshold (when PCR enters the exponential phase) is the point at which the fluorescence versus cycle number is measured.

A standard curve is generated using the cycle threshold values of a series of standards of known concentration. The CT values of the samples are then compared to this standard curve in order to determine the starting concentration. (4 & 7)

At present there are two main approaches to real-time PCR; fluorogenic 5′ nuclease assay with Taqman probes and using an intercalating dye such as SYBR Green.

Fluorogenic 5′ Nuclease Assay (Taqman)

One of the most common methods of real- time or qPCR involves a fluorescent reporter dye and a quencher dye that are attached to the 5′ (reporter) and the 3′ (quencher) of a probe (Taqman). The probe anneals to a specific target on the template DNA between the forward and reverse primers so that during PCR it is cleaved by the DNA polymerase, releasing the two dyes so that the reporter dye is able to fluoresce freely. Hence the fluorescent signal increases as more copies of the template are created and the probes are cleaved releasing more reporter dye (8). The main advantage of this system, in addition to the ease of use and fast throughput, is that the process can be species specific – the probe can be designed to target a sequence specific to the species of interest. For example the Alu repeat sequences in humans (9).

The Quantifiler™ real-time PCR kits from Applied Biosystems are examples of fluorogenic 5′ nuclease assays. Quantifiler human targets the human telomerase reverse transcriptase locus on chromosome 5, while Quantifiler Y targets the sex reversal locus (SRY) on the Y chromosome. i.e. Quantifiler human detects total human DNA while Quantifiler Y is male specific. These assays during validation studies (10) have been shown to be reliable and comparable to other forms of quantification while only requiring 2µl of the original template and are less time consuming. Quantifiler Y also enables the measurement of male DNA in samples that are mixtures of male and female, such as in rape cases that require downstream processing of male only DNA that is otherwise difficult to quantitate (10). Another advantage of the quantifiler kits is the inclusion of an internal PCR control that labelled with a different reporter dye to the probe and hybridises with a synthetic template added to each reaction. This ensures that all aspects of the reaction are working correctly.

Similarly Horsman (11) described a technique for simultaneous quantification of total DNA and male DNA, in a multiplex reaction rather than separate singleplexes. This multiplex consumes less template, is cheaper and takes less time than the Quantifiler singleplexes while maintaining the accuracy, specificity and lower limits of detection. This multiplex targets the TPOX locus for total human DNA and the sex reversal locus (SRY) on the Y chromosome plus the Taqman probes.

SYBR Green Assay

This assay simply works by measuring Taq polymerase activity with the interchelating dye SYBR Green that binds to the minor groove of all double stranded DNA present in a sample. SYBR Green is a highly specific, double stranded DNA binding dye that detects the PCR product as it is formed. As the polymerase amplifies the target DNA sequence double stranded PCR products are formed, to which the SYBR Green binds. Hence as the PCR product increases the fluorescent signal increases. However, unlike the Taqman probes SYBR Green will bind to all double stranded DNA in a sample, including non-specific binding products resulting in false positives. The use of dissociation curve

analysis will allow for detection of these products, especially primer dimmers as they have a lower melting temperature than the target DNA sequence (12). As this system does not require a target specific probe like the Taqman assays, it is as a result slightly cheaper and more flexible (12).

Conclusion

Accurate quantification is an essential part of any forensic DNA procedure and many techniques have been identified for this purpose. The most recent development in this area is that of Real-Time PCR involving the measurement of PCR product as it is formed using fluorescent dyes that are incorporated into the reaction. While this process has been shown to be accurate, less time consuming than other processes and fairly easy to perform it is more expensive during the initial set-up as it requires specialist equipment. However this initial expense seems well worth it when considering the accuracy, the sensitivity (validation of the Quantifiler kit from ABI shows this kit can detect as little as 32pg of DNA (10)) and the species specificity of the procedure.

Educational Message

1. As DNA profiling techniques have become more and more sensitive over the past decade it has become essential to quantitate the DNA present in the sample.
2. There are many different techniques available to quantitate the DNA in a sample, including spectrophotometry, slot blot assays, end point PCR and real-time PCR.
3. There are two main real-time PCR assays: the fluorogenic 5′ nuclease assay (Taqman) and SYBR Green.
4. Real-time has been shown to be cost effective, high throughput, easy to use, accurate, sensitive and species specific.
5. Before choosing a quantification type for your lab it is important to investigate the aspects of each type as some may be more suitable than others. For example some assays may require the purchase of new equipment (such as the real-time PCR assays) while others may be too time consuming for higher throughput labs (Slot blot assays).

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6.4.3 Technical note

Maguire, S., B. Ellaway, **V.L. Bowyer**, E.A.M. Graham, and G.N. Rutty, *Retrieval of DNA from the faces of children aged 0-5 years: A technical note*. Journal of Forensic Nursing - accepted for publication January 2007.

Abstract

Approximately 21% of children suffer from some form of physical abuse. It is hypothesised that when an individual hits a child some of that person's DNA will be deposited onto the child's skin. As yet, no-one has reported a method of sampling DNA from the skin of this vulnerable group of individuals. We have sampled DNA from several facial areas of 30 children aged 5 years of age and under. The results show that it is possible to swab the faces of this age group without distressing them or contaminating the samples. Additionally the results indicate that the DNA obtained is almost entirely that of the subject, with little non-donor DNA being observed.

Introduction

Research carried out by the National Society for the Prevention of Cruelty to Children has shown that 21% of children experience some form of physical abuse (NSPCC Inform, 2006). The most common injury in physical abuse is bruising with studies showing that up to 79% of children dying from non-accidental head injury have at least one area of bruising (Atwal, Carter, Rutty & Green, 1998). However this is the extreme end of the spectrum with many cases of physical abuse going unnoticed or undiagnosed. The difficulty in diagnosing abuse comes in distinguishing between bruises caused during the normal day-to-day activities of the child and those of non-accidental injury: 50% to 75% of the latter are found to the mouth, face and head with accidental bruises located to the bony areas and front of the body (Carpenter, 1999; Naidoo, 2000; Wedgwood, 1990). The age of the children most at risk (0-5 years) results in them being either incapable of defending themselves, or verbalizing what has taken place. Lucas et al (2002) have shown that 55% of fatally abused infants have previously suffered physical trauma (Lucas et al, 2002). If we could identify the infants when they present with soft tissue injuries, we might possibly be able to prevent further serious injury or death. Information gathered by Childline (NSPCC Inform, 2006) has indicated that the majority of abuse is delivered within the home environment, by the carer of the child. However in the absence of any witnesses, an estimate of the likelihood of abuse comes down to the professional opinion of the doctors involved, based on the history offered. In the current climate that is often not enough for the investigating agencies or the courts although, unfortunately, there are at present no scientific tests which may aid this assessment.

In 1923 Locard observed that “every contact leaves a trace” (Locard, 1923). Although originally related to fibres, hairs, fingerprints and biological fluids for example blood or semen, since 1985 one can add DNA to this list. The recovery of DNA from crime scenes or from the victims of crime, living or dead, has become routine. A number of authors have shown that DNA may be recovered from the site of physical contact between an

offender and a victim. This has resulted in the practice of swabbing all exposed areas of skin of the victim of a homicide in an attempt to identify the assailant (Banaschak, Moller & Pfeiffer, 1998; Ladd, Adamowicz, Bourke, Scherzinger & Lee, 1999; Rutty, 2002; Wickenheiser, 2002; Wiegand & Kleiber, 1997). However, this has led to problems distinguishing between innocent DNA transference during normal every day activities and that deposited during a crime as well as issues concerning contamination (Lowe, Murray, Whitaker, Tully & Gill, 2002; Rutty, Hopwood & Tucker, 2003). We have hypothesised that if an offender gripped, slapped or punched a child then offender DNA may be transferred to the site of impact and victim DNA to the offender. Thus if the child presents with a fresh bruise, the site of the injury could be sampled for exogenous DNA, which may help to distinguish an accidental from an intentional cause. However, before initiating this, we need to establish the following; 1) as the face is the most common target of physical abuse, how does one sample it in this age group, 2) will the examiner contaminate the child and 3) what is the normal distribution of exogenous (non-self) DNA at the site of sampling? As the carer of the child may also be the offender, one has to consider the distribution of exogenous DNA on the face due to day-to-day play, feeding, kissing and handling by the carer, if one is to attempt to distinguish such innocent transfer of DNA from an assault.

In this preliminary report we describe our experience in using an ethically acceptable, child friendly approach to sampling the face of a child for the investigation of the presence of exogenous DNA.

Methods

Ethics

Ethical permission was granted by the MultiCentreEthics Committee, Cardiff, 18th March 2003 (MREC number 03/9/29) to recruit children into a study to investigate the 'normal' distribution of DNA on their faces, with parental or guardian consent. The samples were anonymised at source, prior to transport to the laboratory in Leicester. All samples were destroyed at the end of the study, and witnessed confirmation of same was sent to parents or guardians.

Experimental design

To date there are no published papers concerning how to retrieve DNA from the skin surface of a child aged five years or under. We opted for the use of moistened cotton wool swabs (which are used for adults) as we considered that they would be acceptable to both child and carer alike and the methods for DNA extraction from such swabs are known. We used a single swab technique rather than the double swab technique as used on deceased adults (Sweet, Lorente, Lorente, Valezuela & Villanueva, 1997), not only because our experience indicates double swabbing for DNA retrieval may be unnecessary, but we also felt that infants would not tolerate the repeated application of wet and dry swabs to their face.

A face map (figure 1) was also designed in order to divide the face of each child into areas of suitable size for easy swabbing. In addition these areas also represent the different areas in which abusive bruises are commonly observed (Carpenter, 1999; Naidoo, 2000; Wedgwood, 1990).

Contamination prevention

A priority in undertaking this study was to obtain accurate and uncontaminated samples in an ethically acceptable and child friendly manner. In adult forensic practice, to minimise contamination the examiner would usually require full gown or scene suit and mask, including head covering and gloves. This however would clearly be a very frightening approach to a young infant. Therefore we modified current sampling methods by wearing long sleeves and double latex gloves rather than gowns. As we felt a face mask would also be disturbing to children we omitted it, so to avoid oral contamination we did not speak during sampling (Port, Bowyer, Graham, Batuwangala & Rutty, 2006). The child or infant was normally seated on the parent's knee. However, older children typically sat alone during the procedure.

Recruitment

Thirty children were recruited into the study, their ages ranging from zero to five years of age, with 20 females and 10 males. All families were invited to take part by posters and approached directly, both methods being approved by the Ethics committee. Families were then given a full written and verbal explanation, and after being given some days to reflect on this they were again approached. In those families with more than one child less than five years of age, all suitable children were recruited. Any family could withdraw at any time, but all families completed the study. Many families who agreed to participate then introduced the research nurse to further families who may be interested in participating. Children were excluded if there were any bruises to the face, any dermatological disorders or if any proprietary skin products were being used.

Development of sampling technique

A total of 12 swabs were taken from several areas of the face (figure 1), plus a single buccal swab from the inner cheek of the child. This buccal sample provides the child's DNA profile which acts as a reference for the facial swabs to be compared to. The swabs used for sampling the face were first moistened with sterile water before being rubbed firmly across the appropriate facial area. All children completed full swabs sets. The

preparation for the sampling took five to ten minutes and was undertaken by a single operator.

It was quickly apparent that some of the children were wary of the examiner when they approached with a swab without speaking. As such, using previous published work concerning how far DNA may be projected in front of a speaking adult, we amended the protocol, whereby we did speak while swabbing, to reassure the child, but maintained one metre distance between the operator and the child when doing so (Port et al, 2006; Rutty et al, 2003).

DNA Preparation and Analysis

All samples were stored in a -70°C freezer within two hours of sampling and were later sent to Leicester, packaged in dry ice, where they were stored at -20°C until use.

DNA was extracted from the swabs using the Chelex 100 (BioRad, Hercules, CA, USA) method as described by Walsh, Metzger & Higuchi (1991). The DNA extracted from the swabs was then quantified using the Oligreen fluorescent dye from Molecular Probes (Oregon, USA) as per manufacturer's instructions. Lambda DNA (Molecular Probes, Oregon USA) was used to create a series of standards 0-100ng of DNA per well to act as a standard curve. These standards were diluted in 1xTE. 100µl of each standard was placed in the wells of the top row of a 96 well nunc plate (Nunc Corporation), in duplicate. For the rest 90µl of 1xTE and 10µl of the sample were added to the wells. The oligreen was diluted 200 fold in a beaker and mixed before 100µl was added to each well. Foil was used to cover the plate for approximately five minutes before the fluorescence was read using a cytofluor automated multiwell plate reader (Series 4000 Per Septive Biosystems).

The extracted DNA was amplified using the polymerase chain reaction (PCR) and the SGM plus® amplification kit from Applied Biosystems. This kit amplifies 10 regions of the human genome known as short tandem repeats (STR's) including: D3S1358, vWA, D16S539, D2S1338, D8S1179, D21S11, D18S51, D19S433, TH01, FGA and the

Amelogenin locus. The PCR reaction was set-up as per manufacturer's instructions, using 34 cycles as well as the recommended 28. The amplified DNA was then visualised using the 377 DNA sequencer, Genescan and Genotyper software (all from Applied Biosystems) as per manufacturer's instructions.

Results

All DNA profiles generated from the facial swabs of the children were compared to the buccal (reference) samples in order to determine the origin of the DNA gathered from the face.

Of the 30 children tested, all showed varying levels of self DNA across the face with minimal non-self DNA being observed. Figure 2 shows the percentage of subjects and the type of self DNA profile generated from each facial swab. A full DNA profile indicates that all 10 regions of DNA investigated have successfully amplified during the polymerase chain reaction. For a partial profile to be observed at least one of these regions must be observed. The graph shows that for all facial areas no more than 20% of the subjects' exhibit full DNA profiles that match their own reference profile, but at least 55% show at least a partial profile.

Figure 3 represents the non-subject DNA that was observed on the faces of the subjects. In all cases, across all facial areas, no full profiles were observed that did not match the subject. At least 70% of all subjects in all subject areas show no non-donor DNA whatsoever. In the remaining cases the non-donor DNA observed consisted of only one or two of the regions under investigation. In order for an accurate determination of the origin of a DNA sample to be determined at least six of the 10 need to be observed, as this increases the probability of the profile matching only one individual.

Discussion

We have demonstrated for the first time that it is possible to swab for DNA from the faces of young children and infants in a way that is acceptable to children and families, while minimizing operator contamination. Although in adults it is recommended that gowns/scene suits, face masks/visors and gloves should be worn this presents an impractical, frightening environment for the impressionable child, especially if the child is the victim of abuse. If one loses the confidence or interest of the child then the technique will fail and further examinations may be inappropriate. A distressed child is likely to push the operator away, and as such is almost certain to contaminate the swabs with which you are sampling. If one considers previous work in this area it relates to the contamination of cadaver at the crime scene rather than the examination of the living within an examination room. Thus this previous work is not applicable to the clinical paediatric practise. Contamination from shed cells from operator's clothing is considered unlikely to be an issue and thus the need to wear gowns or suits is unnecessary. Similarly the need for head protection is also considered unnecessary. This only leaves the question of DNA contamination projected from the speaking operator's mouth. Although the use of masks was considered, even with shapes or designs these were rejected as being intimidating to a child. Although the option of not speaking to the child to stop contamination was also tried this was found impractical. Thus we opted for no mask and speaking but kept the distance between the operator and the child of one metre as this has previously been shown to be a distance within which oral contamination can occur under specific conditions (Rutty et al, 2003).

This preliminary work has also indicated that DNA obtained from the face of children is mostly that of the subject and little DNA from carers can be observed. The non-donor DNA that is collected is of insufficient quantity to identify the individual it originated from. If forceful contact transfers sufficient DNA for identification of the perpetrator then it may be possible to distinguish this from innocent non-donor DNA present. However a number of further studies need to be performed before this can be put into practice. For instance is DNA actually transferred between individuals during forceful contact, and if

so how long does it persist on the skin for? Such studies are at present being investigated and will hopefully indicate whether DNA can be used as a means of protecting infants and potentially adults from further physical abuse.

Implications for Clinical Forensic Nursing Practice

With increasing emphasis on the need for a higher standard of evidence gathering in Child Protection cases, it is inevitable that questions will be asked as to whether the use of DNA techniques are appropriate. Before any attempt can be made to attempt to identify a perpetrator of physical abuse by DNA transferred to a child during an assault, it is essential to establish firstly an acceptable method of DNA swabbing, and secondly to ensure that any DNA retrieved has not been transferred innocently. In this study, we have shown that modifying current forensic DNA collection practices to a more child and family centred approach is feasible, and does not reduce the standard of evidence obtained. It is vital that the nurse collecting such samples maintains a relationship with both the child and their carer, as this may be the first part of a longer investigation, which will be extremely difficult if the child is distressed and alienated. Also, it is important to bear in mind that a number of families undergoing these investigations will not be guilty of any crimes. As such we must avoid alienating any family from the services which are aiming to help all children. We have demonstrated some early data to suggest that innocent contamination appears minimal in this group, but clearly this needs further larger scale studies to confirm this.

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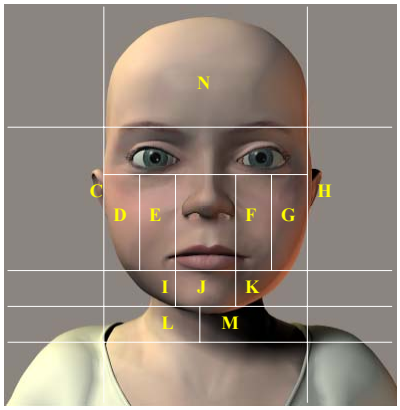

Figure 1. Facial map representing the 12 areas that were swabbed by the operator.

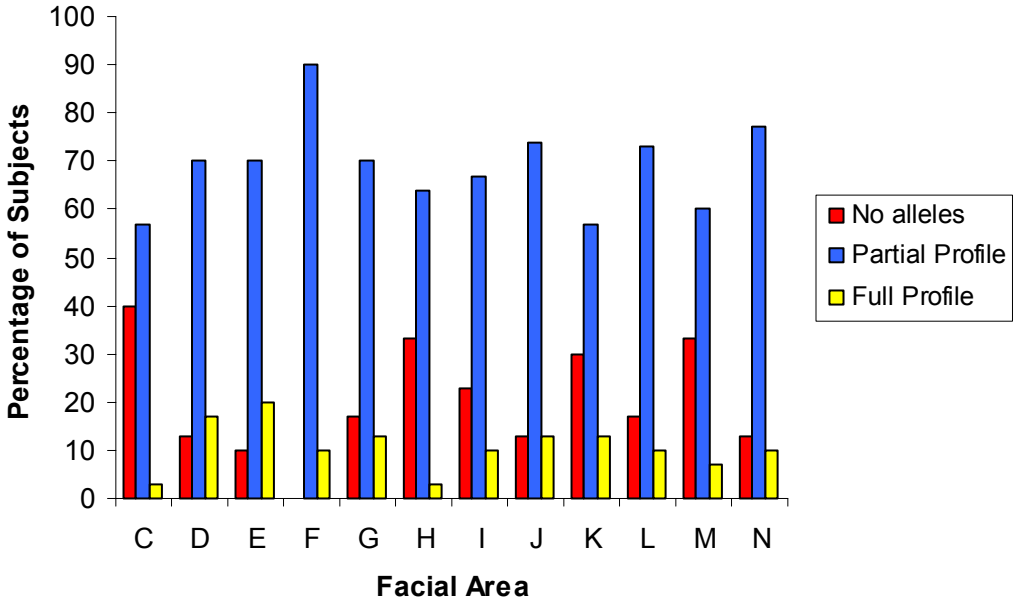

Figure 2. Bar chart representing the donor DNA profiles observed for each facial area for the 30 subjects sampled.

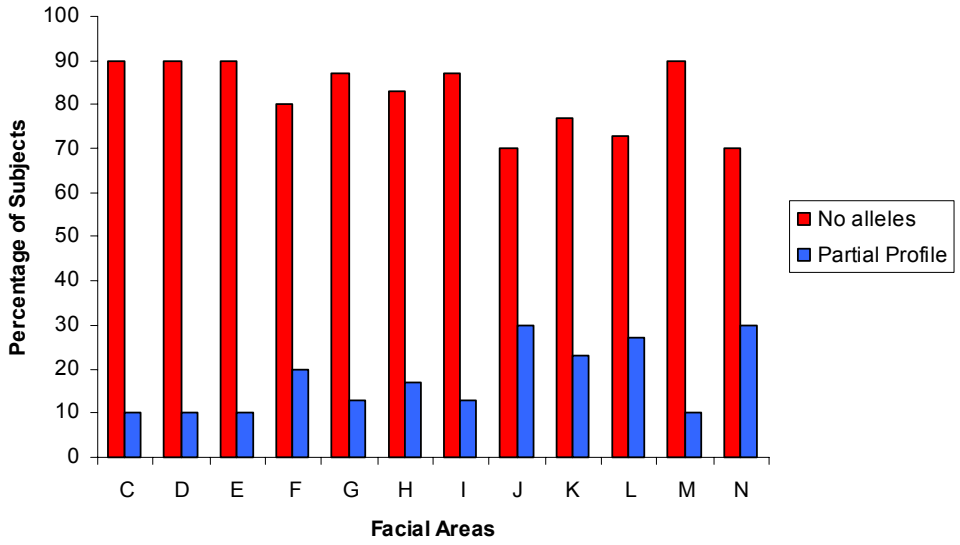

Figure 3. Bar chart representing the non-donor DNA profiles observed across the facial areas of the 30 subjects sampled.

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