Indirect DNA Transfer Without Contact of Dried Biological Materials

DEVON (ALANAH) THORNBURY

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LIST OF ABBREVIATIONS

Please note that abbreviations listed here may not appear due to text that has been removed due to copyright restrictions.

DNA Deoxyribonucleic acid

K_2EDTA Di-potassium ethylenediaminetetraacetic acid

POI Person of interest

RFU Relative florescent unit

ABSTRACT

DNA transfer is a well-recognised phenomenon impacting the probability of detecting the presence of a particular source of DNA and thus the likelihood of the evidence given considered events within forensic investigations. Indirect DNA transfer without contact is lacking comprehensive study. Additionally, the drying properties of forensically relevant biological materials are under researched despite the recognised potential for these properties to affect DNA transfer. This project investigated the drying properties and indirect DNA transfer of dried blood, saliva, semen, vaginal fluid and touch DNA without contact deposited on two different non-porous hard substrates and two different porous soft substrates by tapping (all substrates) and stretching (only soft substrates) agitations. Different drying trends were observed between the volumes, substrates and biological materials tested with substrate generally having the greatest influence. The rate and percentage of indirect transfer appeared to be dependent on agitation, substrate and biological material combination. Saliva has also received minimal focus regarding how other variables may affect its properties that impact DNA transfer. The drying properties and DNA content of saliva deposited on glass from samples collected before, during and after chewing gum were investigated. All samples except those taken during the beginning of chewing dried. Samples that dried displayed an inverse relationship between chewing and sample collection and drying time. After an initial decrease when chewing started, DNA content correlated with time between chewing and sample collection. Additionally, used clothes, pillowcases and towels with known usage and history were shaken to investigate indirect DNA transfer without contact in a slightly more realistic scenario. Transfer frequently occurred, often at levels that produced substantial quantities of DNA and/or informative profiles. The outcomes of this project may assist those evaluating the likelihood of the evidence given proposed events during activity level assessments though would benefit from further study.

STATEMENT OF AUTHORSHIP

Except where reference is made in the text of the thesis, this thesis contains no material published elsewhere or extracted in whole or in part from a thesis accepted for the award of any other degree or diploma. No other person's work has been used without due acknowledgment in the main text of the thesis. This thesis has not been submitted for the award of any degree or diploma in any other tertiary institution.

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Chapters or published works included in this thesis are as follows:

- Chapter 1: Introduction
- Chapter 2: Indirect DNA transfer without contact from dried biological materials on various surfaces (manuscript published in *Forensic Science International: Genetics*)
- Chapter 3: Drying properties and DNA content of saliva samples taken before, during and after chewing gum (manuscript submitted to *Australian Journal of Forensic Sciences,* currently awaiting response from editor)
- Chapter 4: Transfer of DNA without contact from used clothing, pillowcases and towels by shaking agitation (manuscript currently in the process of preparation for submission, intended to be submitted to either *Science & Justice* or *Australian Journal of Forensic Sciences*)
- Chapter 5: Discussion

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- Chapter 1: original draft, review and editing
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- Chapter 5: original draft, review and editing

Contribution of others to work included in this thesis, and their affiliations, are as follows:

- Dr Roland van Oorschot (supervisor)[a,c]: Chapter 1 (review), Chapter 2 (conceptualisation, methodology, resources, review and editing, supervision), Chapter 3 (conceptualisation, methodology, resources, review and editing, supervision), Chapter 4 (conceptualisation, methodology, resources, review and editing, supervision), Chapter 5 (review)
- Dr Mariya Goray (supervisor)[b]: Chapter 1 (review), Chapter 2 (conceptualisation, methodology, resources, data analysis, review and editing, supervision), Chapter 3 (conceptualisation, methodology, resources, data analysis, review and editing, supervision), Chapter 4 (conceptualisation, methodology, resources, data analysis, review and editing, supervision), Chapter 5 (review)
- Dr Kaye Ballantyne[a]: Chapter 3 (review)
- Dr Annalisa Durdle[d] & Dr Dadna Hartman[d]: Whole thesis (review, grade & examination feedback - thesis examiner)

a. Office of the Chief Forensic Scientist, Victoria Police Forensic Services Department, Macleod, Australia
b. Biometrics Division, Victoria Police Forensic Services Department, Macleod, Australia
c. School of Molecular Sciences, La Trobe University, Bundoora, Australia
d. Affiliations unknown; thesis examiner

Devon Thornbury

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CHAPTER 1: INTRODUCTION

1.1 DNA in forensic science

As early as 1920, it has been suggested an individual both deposits and takes away trace evidence from a location they were present at that could potentially link them to a crime scene or event [1, 2]. The precedence for the usage of DNA analysis in forensic science and applications of these methodologies to forensic investigations was established following the first demonstration that an individual's unique forensic profile can be obtained from samples of biological materials in the 1980s [3, 4]. Sometimes referred to as DNA fingerprints [3], forensic profiles are genetic profiles routinely used in forensic investigations [3-6]. They typically consist of the alleles of several analysed loci, exact number and loci determined by the amplification kit utilised [3, 4, 7-9], that are used to identify the number and identities of the contributors to a DNA sample based on statistical likelihood of a match to the reference profiles of suspected contributing individuals [3, 4, 7]. Since the introduction of the forensic profile, particularly over the last few decades, there has been a rapid increase in the sensitivity and discriminatory power of DNA technology, techniques and associated analytical methods [5, 10-13]. Current methods enable even very poor biological samples [14, 15] or invisible trace amounts down to 10s of picograms [15] of DNA to generate profiles [5, 13, 16], where even a single cell is sufficient to generate an individual's forensic profile [6, 7, 17]. This power has pushed DNA analysis, especially the applications of trace DNA, or 'touch DNA' [5], to the forefront of forensic investigations [12, 18]. 'Touch DNA' can be broadly considered as any DNA material deposited from a person's skin, including DNA from sloughed epithelial cells elicited by contact or natural shedding [19, 20], DNA contained in sweat and/or oils secreted from the skin [13, 21] and trace quantities of other biological materials (e.g. saliva picked up by hands [6]) present on the skin (i.e. it is not strictly only DNA produced by and shed from the skin). DNA analysis is now utilised routinely in forensic investigations ranging from criminal investigations, missing person investigations and disaster victim identifications [5], informative trace DNA being able to be retrieved from locations including clothing [22-25], and everyday handheld items [20, 26-29]. These improvements in DNA statistical interpretation techniques have significantly improved the probability of a profile generated from a sample of biological material indicating the presence of a particular individual (i.e. the donor) rather than a random individual [7, 12]. This has led to the identity of the source of the DNA generally not being disputed or given consideration for many samples (e.g. full profiles (i.e. forensic profile displaying all alleles analysed by a kit of an individual; here 40 alleles, excluding the sex chromosome Amelogenin locus)) [10, 27]. However, this increased analytical power is not without weakness [12, 30]. The difficulty of profile interpretation is increased due to background DNA, introduction of contaminating DNA by investigators [10, 31, 32] and other sources of minute quantities of DNA

located together with the DNA of interest on the sampled surface being detected and contributing to creating complex multi-person source mixed profiles [29]. Background DNA is any DNA incidentally present at the sampling location deposited before the event of interest by the person of interest (POI) or another source [12]. The increased complexity of profiles may also increase the difficulty of determination of what events, and in what order, led to different DNA sources coming to be deposited at the location of relevance, especially regarding the DNA of interest [10, 19, 27, 33-35]. Cumulatively, although there is still importance in identifying the donor of a sample, this has resulted in the focus of many investigations and court proceedings shifting away from the identity of the source of the DNA [10, 36]. Instead, when and how the biological material that was retrieved came to be located at the sampling location and the probability of any specific event or combination of events contributing to that is of greater interest [10, 19, 27, 33-36]. These are activity level assessments; the probability of the presented evidence given the proposed event [36]. These assessments frequently include properties of the DNA sample, including the phenomenon of DNA transfer as part of the assessment [36].

1.2 DNA transfer

1.2.1 Overview

The ability of trace amounts of DNA to be transferred from a person to another person or object was first demonstrated by van Oorschot et al. [29] in 1997. The research surrounding this phenomenon, termed DNA transfer, has since rapidly expanded to a seemingly endless number of possibilities [16]. The possibility of DNA transfer having occurred is predominantly considered in casework when touch DNA is involved [5, 12, 37, 38]. This is due to areas often targeted for sampling in forensic investigations being those which touch DNA may be expected to be deposited on (e.g. a knife handle [39, 40]), thus raising the question of if the DNA was directly deposited while touching the object/surface or if it came to be located at the site through indirect transfer. However, DNA transfer is also known to be possible when other forensically relevant biological materials are involved, including blood [11, 15, 30, 41-43], saliva [11, 33, 44] and semen [45, 46]], though they are generally less researched than touch.

DNA transfer is conventionally divided into the different stages transfer may occur in. The initial direct deposition, or primary transfer, occurs when an individual deposits their DNA onto another person or object (e.g. a person depositing touch DNA when picking up a glass cup [27]) [12]. Subsequent transfer steps/events are collectively referred to as indirect transfer, or sometimes divided into secondary, tertiary, etc, transfer steps, where DNA is transferred from the original deposit to an area distinct from the primary surface (i.e. the donor of the DNA does not have direct contact with the location from which their DNA is collected) (e.g. a person

picking up DNA of a different individual after handling a knife previously handled by that individual [40]) [12]. DNA is able to be transferred at detectable levels between people and/or objects for multiple transfer events, dependent on conditions [42, 43].

Direct DNA transfer with contact (e.g. contact between skin and worn clothing [23]) and without contact (e.g. speaking [47]) have been demonstrated to facilitate transfer at levels enabling generation of informative forensic profiles from retrieved samples, depending on circumstances (e.g. DNA shedder status (with contact) [6, 28, 48, 49]), length of time speaking (without contact) [47]) [38]. Indirect DNA transfer has also been demonstrated to be capable of transferring detectable levels of DNA [12, 38]. However, research has currently only investigated and demonstrated the possibility of indirect DNA transfer with contact (e.g. DNA deposited on an object being transferred to a different object upon physical contact [10, 32, 42, 50, 51]). Indirect DNA transfer with contact facilitates transfer rates sufficient to produce informative partial or full forensic profiles [42] with detectable levels of DNA detected even after 6 transfer steps [16, 42], depending on conditions [6, 11, 12, 16, 42, 52, 53]. Despite the literature demonstrating the relevancy and many possibilities of indirect DNA transfer, there has been no investigation into the possibility of indirect DNA transfer without contact. Some studies have suggested indirect DNA transfer without contact as a possible mechanism for their observations [10, 32], however, none have definitively identified this form of transfer occurring. There are a number of possible scenarios where this form of transfer could occur, including: a used towel or bedsheet/blanket being shaken dislodging DNA, a clothing item being roughly removed dislodging DNA, an object with a biological material deposited on it being hit so that the deposit is dislodged, a flexible surface with a biological material deposit on it being moved in a way that causes the deposit to flake off, DNA being passively detached and falls from one surface to another, etc. This shortcoming in the existing literature is despite the great interest in expanding the knowledge base surrounding DNA transfer to enable application of transfer rates and probabilities of resulting profile types given specific event(s) occurring being considered important in casework [12, 36].

1.2.2 Variables affecting DNA transfer

1.2.2.1 Overview

The conditions affecting the possibility of DNA transfer and, if possible, the transfer rate are a result of the multitude of variables known to affect DNA transfer between any two surfaces [12]. Each variable impacts the rate of transfer differently [12, 37, 38, 52]. These variables include type of biological material [11, 43, 44] and quantity deposited [44], moisture content of biological material [11, 41, 43], types of substrate(s) and their properties [11, 18, 30, 43, 54], contact or lack of contact between involved substrate(s) [11, 42], and when contact is involved

the type of contact [11, 18, 35, 41, 43, 44, 54, 55] and number of transfer event(s) involved [44]. Additionally, other variables that impact the ability of any given DNA sample to be detected during sampling include length of time between transfer event(s) [30, 53, 56-59], exposure of the deposited biological material and/or its DNA to the elements (e.g. UV light) between transfer event(s) [40, 59-61] and the ability to recover DNA based on the efficiency of extraction and sampling methodologies from the object/surface the DNA is deposited on [30, 62].

1.2.2.2 Biological materials and related factors

Biological materials and other related factors (e.g. moisture content) impact transfer rates differently [12, 37, 38]. Though there is a much wider variety of forensically relevant biological materials, this project focused on blood, saliva, semen, touch DNA and vaginal fluid. All biological materials have distinct compositions and properties [63-65] including differences in expected DNA content (in descending order of DNA content per volume: semen, blood, saliva, touch; note that quantity recovered from any specific sample is significantly impacted by environmental factors [66]). In some biological materials these properties may vary over time within the body (e.g. salivation rate and components changes relative to when food or drink is consumed [67, 68]), once ejected (e.g. semen changes composition after ejaculation [63, 69]) or otherwise removed (e.g. blood pools clotting [70]) from the body or be altered by disease (e.g. atopic dermatitis increases DNA shedding from skin [71]). In blood, it has been found that some properties may change and/or appear different throughout the drying process and may be dependent on the substrate it is deposited on (e.g. dried blood flakes off non-porous tarpaulin while it remains adhered to porous fabric [30]) [30, 41, 72]. These drying characteristics have also been noted as impacting DNA transfer rates (e.g. dried blood transfers from tarpaulin at a higher rate than from fabric to cotton due to the difference in dried blood's adhesion to the different substrates [30]) [11, 30, 41].

Despite how differing properties, including drying times, drying characteristics and biological material composition and related properties, themselves may impact transfer rate having not been investigated, there has been research on direct and indirect DNA transfer with contact comparing different biological materials [11, 18]. Generally, touch DNA transfers at a lower rate than other biological materials, dependent on conditions (e.g. touch DNA transfers at a higher rate than blood when transfer with contact occurs from a cotton primary surface but transfers at a lower rate than blood when transfer with contact occurs from a plastic primary surface [18]) [18]. Interestingly, despite their radically different compositions and/or properties (e.g. whole blood has viscosity of 1.6-2.5mPa at 37°C [73], normal semen has viscosity of ~3.2mPa at 37°C [69], while the viscosity of saliva may be lower than these [74] it varies highly based on regularly changing conditions (e.g. flow stimulation [75]), blood and saliva transfer at similar

rates [11]. Transfer is able to be facilitated by semen (e.g. indirectly transferring DNA between clothing during laundering [34, 45, 46]), however, due to the lack of systematic studies on semen, the rate of transfer possible when semen is the biological material and how other variables may impact transfer of semen is unknown. There are also currently no studies to my knowledge investigating if and how vaginal fluid transfers.

The quantity of biological material initially deposited also affects detectability and the possibility of subsequent transfer events producing detectable quantities of DNA [12, 44]. Larger volumes of biological material increase the rate of transfer detection and/or more transfer steps due to greater DNA amounts being present in larger volumes facilitating greater detectability under the same conditions as a smaller volume [44].

In addition to the inherent variation in moisture content between biological materials, moisture content in the sense of wetness vs dryness of a particular sample has also been demonstrated to impact DNA transfer [11, 30, 41, 42]. Generally, wet samples transfer at a greater rate than dry samples, however, there may be some characteristics that enable higher transfer rates in some dry samples (e.g. dried blood powderising off fabric substrates [11, 30]) [11, 30, 41]. Thus, this also brings relevance to drying times and how environmental variables, including temperature, humidity and substrate [11, 41, 70], may impact these as an important factor to consider regarding DNA transfer. Despite how drying times and characteristics of biological materials are known to impact DNA transfer, blood is the only biological material with research focusing on drying properties.

1.2.2.3 Substrates and related factors

Substrates, the surfaces DNA is deposited on and/or transfers between, are broadly categorised based on simple characteristics and how they impact DNA transfer. Porous (e.g. cotton) substrates promote greater transfer when they are being transferred to, but do not readily release DNA to another substrate [11, 30]. Non-porous substrates (e.g. hard plastic) are the inverse; they readily release DNA to another substrate but are poor at retrieving DNA from another substrate [11, 30]. There may be variation in this relationship though [18]. For example, Goray et al. [18] proposed porous substrates absorb fluids, including liquid biological materials such as blood, thus reducing its transfer rates from porous substrates, while touch DNA does not absorb into porous substrates, thus comparatively increasing its transfer from porous substrates [18]. Therefore, it is important to investigate how a biological material will behave on a variety of substrates and not assume it will share behaviour observed in other biological materials on the same substrate. Although there has been no research on the mechanisms causing the differences in influencing DNA transfer rate in different substrates, as previously

mentioned, there have been proposed causes, including differences between physical properties (e.g. weave tightness in fabrics preventing cellular material from becoming dislodged) and chemical bonding (e.g. poor bonding between a DNA molecule's negative charge and a negatively charged surface) between different substrates and biological material combinations [11, 12, 30, 70, 76].

1.2.2.4 Contact and contactless transfer

Contact, or lack of, during transfer events between substrates also impacts DNA transfer [11, 18, 35, 41-43, 54, 55]. In direct transfer, contact generally enables greater transfer than contactless transfer [11, 42]. When contact does occur, the rate of transfer is impacted differently based on how the contact occurs [11, 18]. For example, contact with friction (i.e. the surfaces slide around over each other with weighted pressure during contact), promotes greater transfer than pressure (i.e. a weighted force presses the surfaces together during contact) than passive contact (i.e. the surfaces make light contact) [11, 18]. There are currently no studies investigating contactless indirect transfer. Although DNA or materials carrying DNA can detach from a surface (e.g. blood deposits detaching and flaking off hard non-porous substrates when drying [11, 30, 41]) resulting in transfer to another surface without contact, there are currently no studies investigating such contactless indirect transfer. Thus, it is unknown if transfer with contact occurs at a higher rate than contactless transfer when all other variables are the same when the transfer occurred indirectly.

1.2.2.5 Transfer pathways

The number of transfer steps involved in a pathway also impacts transfer [16, 42]. During each transfer step a portion of the initial DNA deposit is transferred (i.e. a portion of the target DNA remains on the previous surface; it is 'removed' from subsequent steps in the transfer pathway). Thus, even if variables involved remain the same enabling relatively similar transfer rates across transfer steps [40], at each subsequent transfer step less and less DNA will be available until the resultant quantity is less than the current detection thresholds [42, 44].

1.2.2.6 Variables impacting the detection of a DNA sample

In addition to the variables impacting the possibility and, if so, rate of DNA transfer, there are variables impacting the detectability of DNA in a resulting sample [30, 53, 56-60]. These include the ability to recover DNA based on the efficiency of extraction and sampling methodologies from the object/surface the DNA is deposited on [30, 62], the amount of time between transfer events [30, 53, 56-58] and/or environmental conditions [40, 59-61] during the transfer pathway affect both transfer rate and detection of DNA in the resulting sample [30, 53, 56-58, 60]. Some conditions may contribute to degradation of the DNA or the biological material carrying the

DNA (e.g. long-term submersion in water reduces quantity of DNA able to be retrieved from clothes [60]). Additional samples coming to be deposited at the collection site may produce more complex forensic profiles, increasing the difficulty of interpretation [29] and/or obscure the sample of interest (e.g. a larger quantity of DNA becomes deposited on top of the transferred DNA sample effectively drowning it out in forensic profiles retrieved from the sampling location [12]). Alternatively, it is possible for DNA to be removed by further transfer events to another surface away from the collection site reducing detectability of the sample of interest [57, 58]. Additionally, incidental contamination of samples by other personnel (e.g. crime scene investigators or laboratory workers who have interacted with an item of interest) may also increase the complexity of resulting forensic profiles and complicate interpretation [10, 31]. The use of staff elimination DNA databases to identify potential contaminators can alleviate this complication to an extent [10, 31].

1.2.3 DNA transfer under laboratory conditions versus realistic scenarios reflecting casework

In order to fully understand DNA transfer, studies investigating circumstances reflective of both controlled laboratory conditions and more realistic conditions are essential. There are multiple differences between how research is conducted in a controlled laboratory environment in comparison to the circumstances encountered in real forensic investigations. Laboratory conditions enable control and thus investigation of specific variables present. This enables the revelation of various 'ground truths' relating to how different combinations allow for and, if so, impact the rate of the form of DNA transfer under investigation. However, in a laboratory, there are a variety of factors or activities that are not present and/or cannot be simulated. For example, factors including aspects of various real-world background DNA situations and/or other contaminating DNA sources and environmental conditions contributing to DNA degradation (e.g. UV light) are not present. Other activities that are relevant to forensic investigations (e.g. breaking into a house) are also unable to be accurately simulated in their entirety in a laboratory. As previously discussed, these variables and/or activities may affect DNA transfer and/or the capacity of a forensic investigator to accurately and confidently interpret results [29, 40, 59, 60]. Therefore, the investigation of DNA transfer under conditions with varying degrees of realism (e.g. sampling after an agitation has been applied to a used fabric item with known history and usage in a laboratory, as opposed to depositing a known volume with approximately known DNA quantity on a DNA-free piece of material, or the item being picked up and shaken in various non-descript ways above floors and furniture within houses with various histories) and/or number of controlled variables is essential to the understanding of DNA transfer and the reliability of incorporating data generated by a study into activity level assessments. Different degrees of controlling the variables can be applied to

produce varying degrees of realism for investigations, ranging from fully controlled scenarios where one parameter is varied (e.g. laboratory conditions; DNA-free fabric swatches and known quantities of biological material) to semi-controlled scenarios (e.g. laboratory conditions; used fabric items with known history and usage) to fully uncontrolled scenarios (outside of laboratory/in the real-world; sampling of used fabric items with unknown history and usage in an occupied house), depending on the purpose and desired outcomes of the study. The massive variety of possible experimental designs regarding realism vs controlled variables enables a wide range of outcomes to be investigated in studies. Many existing studies do vary on the degree of realism and number of controlled variables [11, 12, 18, 24, 29, 37, 38, 41, 77]. However, many variables remain unexamined. More studies incorporating a wider range of variables within experimental designs can greatly expand the knowledge of how DNA may transfer in the seemingly endless possible scenarios it could occur in, greatly benefiting the range of data generated from studies that can be considered for inclusion in activity level assessments during forensic investigations [36, 78].

1.2.4 The relevance of DNA transfer research

DNA transfer has seemingly never-ending possibilities [12, 16]. Although there has been a broad coverage of many of the variables known to effect DNA transfer in the literature [5, 12, 37, 38], there are still multiple variables and/or combinations of variables that have not yet been researched as thoroughly as others (e.g. saliva is underrepresented in the literature compared to touch DNA) or have yet to be investigated (e.g. semen and vaginal fluid). There is an increasing importance of building and utilising probability-based systems to assess the relative likelihood of the evidence given different scenarios (e.g. Bayesian Networks [36, 78, 79]) during forensic investigations. These activity level assessments require knowledge of the probabilities of any given event, including DNA transfer, contributing to a particular type of profile being recovered from any given surface and a thorough understanding of how different combinations of variables impact the probabilities of that event occurring [36, 79]. Thus, further research on DNA transfer is important.

1.3 Project overview

Overall, this project aimed to generate data to improve the understanding of the possibility of indirect DNA transfer without contact. To adequately address this, the project was divided into distinct parts. Each of these parts is either presented here as its own chapter (parts three and four) or coupled with another part (parts one and two) to form a chapter. These chapters were written as self-contained studies in preparation to be published. *Chapter Two: Indirect DNA transfer without contact from dried biological materials on various surfaces* (parts one and two) has been published while *Chapter Three: Drying properties and DNA content of saliva samples*

taken before, during and after chewing gum (part three) has been submitted and *Chapter Four: Transfer of DNA without contact from used clothing, pillowcases and towels by shaking agitation* (part four) is in the process of being prepared for submission.

1.3.1 Chapter Two: Indirect DNA transfer without contact from dried biological materials on various surfaces

1.3.1.1 Part one

Part one aimed to acquire an understanding of the drying times and characteristics of a range of forensically relevant biological materials that may impact their transferability when influenced by selected variables. These properties were determined by observing blood, saliva, semen and vaginal fluid aliquots deposited on four substrates representative of substrates commonly encountered at crime scenes: two different non-porous hard substrates (melamine and glass) and two different porous soft substrates (polyester and cotton) representative of commonly encountered substrates at crime scenes. Deposits were made in 10µL and 100µL volumes at room temperature in two repeats over a 24h period. It was hypothesised that differences in drying times and characteristics would occur within each biological material on different substrates and between different biological materials on the same substrate. Additionally, it was hypothesised that drying properties would follow a trend between the two volumes tested.

1.3.1.2 Part two

Part two aimed to generate data to inform of the possibility of DNA transfer of dried biological materials from various substrates to an area distinct from the original deposition site without contact. A tapping and stretching agitation were applied to dried blood, saliva, semen, touch and vaginal fluid deposited on two different non-porous hard substrates (melamine and glass) and two different porous soft substrates (polyester and cotton) to elicit DNA transfer. It was hypothesised that indirect DNA transfer without contact would be possible via flaking off of the deposited biological material and that transfer rate would be dependent on biological material, substrate and agitation combination.

1.3.2 Part three – Chapter Three: Drying properties and DNA content of saliva samples taken before, during and after chewing gum

During part one's portion of the project, an anomaly in the results of a saliva sample collected while the donor chewed gum resulted in an additional investigation into the drying times and characteristics of saliva samples collected before, during and after chewing gum. Additionally, the DNA content of these samples was discerned. This was determined by observing 100µL aliquots of saliva deposited on glass in at least two repeats from samples taken at six timepoints before, during and after chewing gum for a minimum of 96h and quantifying the DNA content of

an aliquot. It was hypothesised samples would dry in the following order: before, after, during with the greater the time between collection and chewing the closer the drying time would be to the control (i.e. before chewing) sample. Additionally, it was hypothesised that samples taken at the longest time since chewing also most resembled the drying properties of the control sample. It was also hypothesised the DNA concentration would decrease when chewing begun as increased salivation from chewing diluted the DNA present before increasing after chewing ceased.

1.3.3 Part four – Chapter Four: Transfer of DNA without contact from used clothing, pillowcases and towels by shaking agitation

Part four aimed to further the data generated to inform of the possibility of DNA transfer by investigating its possibility with more realistic conditions, better reflecting how DNA transfer may be encountered in casework. A gentle shaking agitation was applied to used clothing, pillowcases and towels belonging to ten volunteers to elicit DNA transfer.

1.4 Chapter 1 References

[1] K. Inman, N. Rudin, Principles and practice of criminalistics: the profession of forensic science, CRC Press 2000.

[2] E. Locard, L'enquête criminelle et les méthodes scientifiques, E. Flammarion 1920.

[3] P. Gill, A.J. Jeffreys, D.J. Werrett, Forensic application of DNA 'fingerprints', Nature 318(6046) (1985) 577. DOI: 10.1038/318577a0.

[4] A.J. Jeffreys, V. Wilson, S.L. Thein, Hypervariable 'minisatellite' regions in human DNA, Nature 314(6006) (1985) 67. DOI: 10.1038/314067a0.

[5] R.A. van Oorschot, K.N. Ballantyne, R.J. Mitchell, Forensic trace DNA: a review, Investig. Genet. 1(1) (2010) 14. DOI: 10.1186/2041-2223-1-14.

[6] R.A. Wickenheiser, Trace DNA: a review, discussion of theory, and application of the transfer of trace quantities of DNA through skin contact, J. Forensic Sci. 47(3) (2002) 442-450. DOI: 10.1520/JFS15284J.

[7] V.C. Tucker, A.J. Hopwood, C.J. Sprecher, R.S. McLaren, D.R. Rabbach, M.G. Ensenberger, J.M. Thompson, D.R. Storts, Developmental validation of the PowerPlex® ESX 16 and PowerPlex® ESX 17 Systems, Forensic Sci. Int. Genet. 6(1) (2012) 124-31. DOI: 10.1016/j.fsigen.2011.03.009.

[8] H. Zhou, D. Wu, R. Chen, Y. Xu, Z. Xia, Y. Guo, F. Zhang, W. Zheng, Developmental validation of a forensic rapid DNA-STR kit: Expressmarker 16, Forensic Sci. Int. Genet. 11 (2014) 31-38. DOI: 10.1016/j.fsigen.2014.02.008.

[9] W.E. Frank, B.E. Llewellyn, P.A. Fish, A.K. Riech, T.L. Marcacci, D.W. Gandor, D. Parker, R.R. Carter, S.M. Thibault, Validation of the AmpFℓSTR™ Profiler Plus PCR Amplification Kit for Use in Forensic Casework, J. Forensic Sci. 46(3) (2001) 642-646. DOI: 10.1520/JFS15017J.

[10] A.E. Fonneløp, H. Johannessen, T. Egeland, P. Gill, Contamination during criminal investigation: detecting police contamination and secondary DNA transfer from evidence bags, Forensic Sci. Int. Genet. 23 (2016) 121-129. DOI: 10.1016/j.fsigen.2016.04.003.

[11] M. Goray, E. Eken, R.J. Mitchell, R.A. van Oorschot, Secondary DNA transfer of biological substances under varying test conditions, Forensic Sci. Int. Genet. 4(2) (2010) 62-67. DOI: 10.1016/j.fsigen.2009.05.001.

[12] R.A. van Oorschot, B. Szkuta, G.E. Meakin, B. Kokshoorn, M. Goray, DNA transfer in forensic science: a review, Forensic Sci. Int. Genet. 38 (2019) 140-166. DOI: 10.1016/j.fsigen.2018.10.014.

[13] M. van den Berge, G. Ozcanhan, S. Zijlstra, A. Lindenbergh, T. Sijen, Prevalence of human cell material: DNA and RNA profiling of public and private objects and after activity scenarios, Forensic Sci. Int. Genet. 21 (2016) 81-89. DOI: 10.1016/j.fsigen.2015.12.012.

[14] P. Wiegand, C. Heimbold, R. Klein, U. Immel, D. Stiller, M. Klintschar, Transfer of biological stains from different surfaces, Int. J. Legal Med. 125(5) (2011) 727-731. DOI: 10.1007/s00414-010-0424-x.

[15] T. Kamphausen, S.B. Fandel, J.S. Gutmann, T. Bajanowski, M. Poetsch, Everything clean? Transfer of DNA traces between textiles in the washtub, Int. J. Legal Med. 129(4) (2015) 709-714. DOI: 10.1007/s00414-015-1203-5.

[16] J. Helmus, T. Bajanowski, M. Poetsch, DNA transfer—a never ending story. A study on scenarios involving a second person as carrier, Int. J. Legal Med. 130(1) (2016) 121-125. DOI: 10.1007/s00414-015-1284-1.

[17] K. Elliott, D. Hill, C. Lambert, T. Burroughes, P. Gill, Use of laser microdissection greatly improves the recovery of DNA from sperm on microscope slides, Forensic Sci. Int. 137(1) (2003) 28-36. DOI: 10.1016/s0379-0738(03)00267-6.

[18] M. Goray, R.J. Mitchell, R.A. van Oorschot, Investigation of secondary DNA transfer of skin cells under controlled test conditions, Legal Med. 12(3) (2010) 117-120. DOI: 10.1016/j.legalmed.2010.01.003.

[19] R. Cook, I.W. Evett, G. Jackson, P.J. Jones, J.A. Lambert, A hierarchy of propositions: deciding which level to address in casework, Sci. Justice 38(4) (1998) 231-239. DOI: 10.1016/S1355-0306(98)72117-3.

[20] D.J. Daly, C. Murphy, S.D. McDermott, The transfer of touch DNA from hands to glass, fabric and wood, Forensic Sci. Int. Genet. 6(1) (2012) 41-46. DOI: 10.1016/j.fsigen.2010.12.016.

[21] S. Zoppis, B. Muciaccia, A. D'Alessio, E. Ziparo, C. Vecchiotti, A. Filippini, DNA fingerprinting secondary transfer from different skin areas: morphological and genetic studies, Forensic Sci. Int. Genet. 11 (2014) 137-143. DOI: 10.1016/j.fsigen.2014.03.005.

[22] Z. Bowman, D. Hartman, K. Mosse, A. Sungaila, R. van Oorschot, Skin-to-skin: DNA transfer, Pathol. 49 (2017) S25. DOI: 10.1016/j.pathol.2016.12.062.

[23] A.M. Magee, M. Breathnach, S. Doak, F. Thornton, C. Noone, L.G.J.F.S.I.G. McKenna, Wearer and non-wearer DNA on the collars and cuffs of upper garments of worn clothing, Forensic Sci. Int. Genet. 34 (2018) 152-161. DOI: 10.1016/j.fsigen.2018.02.011.

[24] B. Szkuta, R. Ansell, L. Boiso, E. Connolly, A.D. Kloosterman, B. Kokshoorn, L.G. McKenna, K. Steensma, R.A. van Oorschot, Assessment of the transfer, persistence, prevalence and recovery of DNA traces from clothing: an inter-laboratory study on worn upper garments, Forensic Sci. Int. Genet. 42 (2019) 56-68. DOI: 10.1016/j.fsigen.2019.06.011.

[25] J.M. Taupin, C. Cwiklik, Scientific protocols for forensic examination of clothing, CRC Press 2010.

[26] R.W. Allen, J. Pogemiller, J. Joslin, M. Gulick, J. Pritchard, Identification through typing of DNA recovered from touch transfer evidence: parameters affecting yield of recovered human DNA, J. Forensic Identif. 58(1) (2008) 33.

[27] M. Goray, R.A. van Oorschot, The complexities of DNA transfer during a social setting, Legal Med. 17(2) (2015) 82-91. DOI: 10.1016/j.legalmed.2014.10.003.

[28] M. Phipps, S. Petricevic, The tendency of individuals to transfer DNA to handled items, Forensic Sci. Int. 168(2-3) (2007) 162-168. DOI: 10.1016/j.forsciint.2006.07.010.

[29] R.A. van Oorschot, M.K. Jones, DNA fingerprints from fingerprints, Nature 387(6635) (1997) 767. DOI: 10.1038/42838.

[30] T.J. Verdon, R.J. Mitchell, R.A. van Oorschot, The influence of substrate on DNA transfer and extraction efficiency, Forensic Sci. Int. Genet. 7(1) (2013) 167-175. DOI: 10.1016/j.fsigen.2012.09.004.

[31] M. Goray, E. Pirie, R.A. van Oorschot, DNA transfer: DNA acquired by gloves during casework examinations, Forensic Sci. Int. Genet. 38 (2019) 167-174. DOI: 10.1016/j.fsigen.2018.10.018.

[32] M. Goray, R.A. van Oorschot, J.R. Mitchell, DNA transfer within forensic exhibit packaging: potential for DNA loss and relocation, Forensic Sci. Int. Genet. 6(2) (2012) 158-166. DOI: 10.1016/j.fsigen.2011.03.013.

[33] M. Goray, J.R. Mitchell, R.A. van Oorschot, Evaluation of multiple transfer of DNA using mock case scenarios, Legal Med. 14(1) (2012) 40-46. DOI: 10.1016/j.legalmed.2011.09.006.

[34] J. Helmus, M. Pfeifer, L.-K. Feiner, L.J. Krause, T. Bajanowski, M. Poetsch, Unintentional effects of cleaning a crime scene—when the sponge becomes an accomplice in DNA transfer, Int. J. Legal Med. 133(3) (2019) 759-765. DOI: 10.1007/s00414-018-1983-5.

[35] C.M. Pfeifer, P. Wiegand, Persistence of touch DNA on burglary-related tools, Int. J. Legal Med. 131(4) (2017) 941-953. DOI: 10.1007/s00414-017-1551-4.

[36] D. Taylor, B. Kokshoorn, A. Biedermann, Evaluation of forensic genetics findings given activity level propositions: a review, Forensic Sci. Int. Genet. 36 (2018) 34-49. DOI: 10.1016/j.fsigen.2018.06.001.

[37] J. Burrill, B. Daniel, N. Frascione, A review of trace “Touch DNA” deposits: Variability factors and an exploration of cellular composition, Forensic Sci. Int. Genet. 39 (2019) 8-18. DOI: 10.1016/j.fsigen.2018.11.019.

[38] G. Meakin, A. Jamieson, DNA transfer: review and implications for casework, Forensic Sci. Int. Genet. 7(4) (2013) 434-443. DOI: 10.1016/j.fsigen.2013.03.013.

[39] G.E. Meakin, E.V. Butcher, R.A. van Oorschot, R.M. Morgan, The deposition and persistence of indirectly-transferred DNA on regularly-used knives, Forensic Sci. Int. Genet. Sup. Ser. 5 (2015) e498-e500. DOI: 10.1016/j.fsigss.2015.09.

[40] G.E. Meakin, E.V. Butcher, R.A. van Oorschot, R.M. Morgan, Trace DNA evidence dynamics: an investigation into the deposition and persistence of directly- and indirectly-transferred DNA on regularly-used knives, Forensic Sci. Int. Genet. 29 (2017) 38-47. DOI: 10.1016/j.fsigen.2017.03.016.

[41] R.A. van Oorschot, R. McArdle, W.H. Goodwin, K.N. Ballantyne, DNA transfer: the role of temperature and drying time, Legal Med. 16(3) (2014) 161-163. DOI: 10.1016/j.legalmed.2014.01.005.

[42] V. Lehmann, R. Mitchell, K. Ballantyne, R. van Oorschot, Following the transfer of DNA: how far can it go?, Forensic Sci. Int. Genet. Sup. Ser. 4(1) (2013) e53-e54. DOI: 10.1016/j.fsigss.2013.10.027.

[43] R.A. van Oorschot, M. Goray, E. Eken, R.J. Mitchell, Impact of relevant variables on the transfer of biological substances, Forensic Sci. Int. Genet. Sup. Ser. 2(1) (2009) 547-548. DOI: 10.1016/j.fsigss.2009.08.105.

[44] D.H. Warshauer, P. Marshall, S. Kelley, J. King, B. Budowle, An evaluation of the transfer of saliva-derived DNA, Int. J. Legal Med. 126(6) (2012) 851-861. DOI: 10.1007/s00414-012-0743-1.

[45] H. Brayley-Morris, A. Sorrell, A.P. Revoir, G.E. Meakin, D.S. Court, R.M. Morgan, Persistence of DNA from laundered semen stains: implications for child sex trafficking cases, Forensic Sci. Int. Genet. 19 (2015) 165-171. DOI: 10.1016/j.fsigen.2015.07.016.

[46] S. Noël, K. Lagacé, A. Rogic, D. Granger, S. Bourgoin, C. Jolicoeur, D. Séguin, DNA transfer during laundering may yield complete genetic profiles, Forensic Sci. Int. Genet. 23 (2016) 240-247. DOI: 10.1016/j.fsigen.2016.05.004.

[47] N.J. Port, V.L. Bowyer, E.A. Graham, M.S. Batuwangala, G.N. Rutty, How long does it take a static speaking individual to contaminate the immediate environment?, Forensic Sci. Med. Pathol. 2(3) (2006) 157-163. DOI: 10.1007/s12024-006-0004-z.

[48] J.-A. Bright, S.F. Petricevic, Recovery of trace DNA and its application to DNA profiling of shoe insoles, Forensic Sci. Int. 145(1) (2004) 7-12. DOI: 10.1016/j.forsciint.2004.03.016.

[49] C. Ladd, M.S. Adamowicz, M.T. Bourke, C.A. Scherczinger, H.C. Lee, A systematic analysis of secondary DNA transfer, J. Forensic Sci. 44(6) (1999) 1270-1272. DOI: 10.1520/JFS14599J.

[50] B. Szkuta, M.L. Harvey, K.N. Ballantyne, R.A. van Oorschot, DNA transfer by examination tools–a risk for forensic casework?, Forensic Sci. Int. Genet. 16 (2015) 246-254. DOI: 10.1016/j.fsigen.2015.02.004.

[51] R.A. van Oorschot, S. Treadwell, J. Beaurepaire, N.L. Holding, R.J. Mitchell, Beware of the possibility of fingerprinting techniques transferring DNA, J. Forensic Sci. 50(6) (2005) JFS2004430-6. DOI: 10.1520/JFS2004430.

[52] A. Gosch, C. Courts, On DNA transfer: the lack and difficulty of systematic research and how to do it better, Forensic Sci. Int. Genet. 40 (2019) 24-36. DOI: 10.1016/j.fsigen.2019.01.012.

[53] F. Oldoni, V. Castella, D. Hall, Shedding light on the relative DNA contribution of two persons handling the same object, Forensic Sci. Int. Genet. 24 (2016) 148-157. DOI: 10.1016/j.fsigen.2016.07.002.

[54] A.E. Fonneløp, T. Egeland, P. Gill, Secondary and subsequent DNA transfer during criminal investigation, Forensic Sci. Int. Genet. 17 (2015) 155-162. DOI: 10.1016/j.fsigen.2015.05.009.

[55] S.H. Tobias, G.S. Jacques, R.M. Morgan, G.E. Meakin, The effect of pressure on DNA deposition by touch, Forensic Sci. Int. Genet. Sup. Ser. 6 (2017) e12-e14. DOI: 10.1016/j.fsigss.2017.09.020.

[56] J.J. Raymond, S.J. Walsh, R.A. van Oorschot, P.R. Gunn, L. Evans, C. Roux, Assessing trace DNA evidence from a residential burglary: abundance, transfer and persistence, Forensic Sci. Int. Genet. Sup. Ser. 1(1) (2008) 442-443. DOI: 10.1016/j.fsigss.2007.10.040.

[57] B. Szkuta, K.N. Ballantyne, B. Kokshoorn, R.A. van Oorschot, Transfer and persistence of non-self DNA on hands over time: using empirical data to evaluate DNA evidence given activity level propositions, Forensic Sci. Int. Genet. 33 (2018) 84-97. DOI: 10.1016/j.fsigen.2017.11.017.

[58] B. Szkuta, K.N. Ballantyne, R.A. van Oorschot, Transfer and persistence of DNA on the hands and the influence of activities performed, Forensic Sci. Int. Genet. 28 (2017) 10-20. DOI: 10.1016/j.fsigen.2017.01.006.

[59] R.A. van Oorschot, G. Glavich, R.J. Mitchell, Persistence of DNA deposited by the original user on objects after subsequent use by a second person, Forensic Sci. Int. Genet. 8(1) (2014) 219-225. DOI: 10.1016/j.fsigen.2013.10.005.

[60] J. Helmus, S. Zorell, T. Bajanowski, M. Poetsch, Persistence of DNA on clothes after exposure to water for different time periods—a study on bathtub, pond, and river, Int. J. Legal Med. 132(1) (2018) 99-106. DOI: 10.1007/s00414-017-1695-2.

[61] J.J. Raymond, R.A. van Oorschot, P.R. Gunn, S.J. Walsh, C. Roux, Trace evidence characteristics of DNA: a preliminary investigation of the persistence of DNA at crime scenes, Forensic Sci. Int. Genet. 4(1) (2009) 26-33. DOI: 10.1016/j.fsigen.2009.04.002.

[62] T.J. Verdon, R.J. Mitchell, R.A. van Oorschot, Swabs as DNA collection devices for sampling different biological materials from different substrates, J. Forensic Sci. 59(4) (2014) 1080-1089. DOI: 10.1111/1556-4029.12427.

[63] R. Eliasson, Biochemical analysis of human semen, J. Urol. 128(5) (1982) 1144-1144. DOI: 10.1111/j.1365-2605.

[64] G.R. Huggins, G. Preti, Vaginal odors and secretions, Clin. Obstet. Gynecol. 24(2) (1981) 355-377. DOI: 10.1097/00003081-198106000-00005.

[65] D.H. Owen, D.F. Katz, A vaginal fluid simulant, Contraception 59(2) (1999) 91-95. DOI: 10.1016/S0010-7824(99)00010-4.

[66] H.C. Lee, C. Ladd, Preservation and collection of biological evidence, Croat. Med. J. 42(3) (2001) 225-228.

[67] P.D.V. de Almeida, A. Gregio, M. Machado, A. De Lima, L.R. Azevedo, Saliva composition and functions: a comprehensive review, J. Contemp. Dent. Pract. 9(3) (2008) 72-80. DOI: 10.5005/jcdp-9-3-72.

[68] C. Yeh, D. Johnson, M. Dodds, Impact of aging on human salivary gland function: a community-based study, Aging Clin. Exp. Res. 10(5) (1998) 421-428. DOI: 10.1007/BF03339889.

[69] D.H. Owen, D.F. Katz, A Review of the Physical and Chemical Properties of Human Semen and the Formulation of a Semen Simulant, J. Androl. 26(4) (2005) 459-469. DOI: 10.2164/jandrol.04104.

[70] N. Laan, F. Smith, C. Nicloux, D. Brutin, Morphology of drying blood pools, Forensic Sci. Int. 267 (2016) 104-109. DOI: 10.1016/j.forsciint.2016.08.005.

[71] T. Kamphausen, D. Schadendorf, N. von Wurmb-Schwark, T. Bajanowski, M. Poetsch, Good shedder or bad shedder—the influence of skin diseases on forensic DNA analysis from epithelial abrasions, Int. J. Legal Med. 126(1) (2012) 179-183. DOI: 10.1007/s00414-011-0579-0.

[72] F. Ramsthaler, P. Schmidt, R. Bux, S. Potente, C. Kaiser, M. Kettner, Drying properties of bloodstains on common indoor surfaces, Int. J. Legal Med. 126(5) (2012) 739-746. DOI: 10.1007/s00414-012-0734-2.

[73] O. Peschel, S. Kunz, M. Rothschild, E. Mützel, Blood stain pattern analysis, Forensic Sci. Med. Pathol. 7(3) (2011) 257-270. DOI: 10.1007/s12024-010-9198-1.

[74] S. Govindaraj, M.J. Daniel, S.S. Vasudevan, J.V. Kumaran, Changes in salivary flow rate, pH, and viscosity among working men and women, Dent. Medical Res. 7(2) (2019) 56. DOI: 10.4103/dmr.dmr_20_19.

[75] P.J. Rantonen, J.H. Meurman, Viscosity of whole saliva, Acta Odontol. Scand. 56(4) (1998) 210-214. DOI: 10.1080/00016359850142817.

[76] L. Pauchard, M. Adda-Bedia, C. Allain, Y. Couder, Morphologies resulting from the directional propagation of fractures, Phys. Rev. E 67(2) (2003) 027103. DOI: 10.1103/PhysRevE.67.027103.

[77] A. Sparer, B. Serp, L. Schwarz, U. Windberger, Storability of porcine blood in forensics: How far should we go?, Forensic Sci. Int. (2020) 110268. DOI: 10.1016/j.forsciint.2020.110268.

[78] P. Gill, T. Hicks, J.M. Butler, E. Connolly, L. Gusmão, B. Kokshoorn, N. Morling, R.A. van Oorschot, W. Parson, M. Prinz, DNA commission of the International society for forensic genetics: Assessing the value of forensic biological evidence-Guidelines highlighting the importance of propositions. Part II: Evaluation of biological traces considering activity level propositions, Forensic Sci. Int. Genet. 44 (2020) 102186. DOI: 10.1016/j.fsigen.2019.102186.

[79] D. Taylor, A. Biedermann, L. Samie, K.-M. Pun, T. Hicks, C. Champod, Helping to distinguish primary from secondary transfer events for trace DNA, Forensic Sci. Int. Genet. 28 (2017) 155-177. DOI: 10.1016/j.fsigen.2017.02.008.

CHAPTER 2: INDIRECT DNA TRANSFER WITHOUT CONTACT FROM DRIED BIOLOGICAL MATERIALS ON VARIOUS SURFACES

Chapter 2: Indirect DNA transfer without contact from dried biological materials on various surfaces is presented as it has been published in *Forensic Science International: Genetics* with the addition of numbered headings and inclusion of the chapter number in figure and table titles to unify the thesis' presentation and enable navigation of the thesis. The manuscript's citation is provided in Appendix A.1. Please refer to *Statement of Authorship* for contributions by parties other than the candidate to this chapter.

The contents of *Chapter 2: Indirect DNA transfer without contact from dried biological materials on various surfaces* have been removed due to copyright restrictions. Please refer to the following for the entirety of the chapter's content: D. Thornbury, M. Goray, R.A.H. van Oorschot, Indirect DNA transfer without contact from dried biological materials on various surfaces, Forensic Sci. Int. Genet. 51 (2021) 102457. DOI: 10.1016/j.fsigen.2020.102457.

2.9 Appendix A. Supplementary data

For the purposes of this thesis, supplementary materials can be found in *Appendix A.2 Chapter 2 Supplementary Data*.

CHAPTER 3: DRYING PROPERTIES AND DNA CONTENT OF SALIVA SAMPLES TAKEN BEFORE, DURING AND AFTER CHEWING GUM

Chapter 3: *Drying properties and DNA content of saliva samples taken before, during and after chewing gum* is presented as it has been submitted to *Australian Journal of Forensic Sciences* with minor formatting adjustments to unify the thesis' presentation and enable navigation of the thesis (e.g. addition of numbered headings and inclusion of chapter number in figure and table titles) and minor amendments as requested by thesis examiners. Proof of this manuscript's status is provided in Appendix B.1. Please refer to *Statement of Authorship* for contributions by parties other than the candidate to this chapter.

Additionally, due to *Chapter 3: Drying properties and DNA content of saliva samples taken before, during and after chewing gum* being presented as it has been submitted to *Australian Journal of Forensic Sciences* and the word limit this journal imposes on submissions, some details of the Materials and Methods (section 3.3) have been omitted from the main portion of this chapter. For this information, please refer to *3.11. Chapter 3 Additional Materials and Methods*.

The majority of the contents of *Chapter 3: Drying properties and DNA content of saliva samples taken before, during and after chewing gum* have been removed due to copyright restrictions.

3.9 Appendix B. Supplementary Data

For the purposes of this thesis, supplementary materials can be found in *Appendix B.2 Chapter 3 Supplementary Data*.

3.11 Chapter 3 Additional Materials and Methods

All participants were instructed not to eat or drink anything besides water for approximately one hour prior to sample collection and to only chew the provided single piece of gum during collection.

All samples were fresh when deposited; all sample depositions were completed within 40min (av. 24.5min, range 17-39min) of the sample set being received immediately after the collection of the final sample. Immediately prior to depositions, samples were homogenised via pipetting up and down. All depositions were made approximately $\leq$3mm from the substrate surface. Samples were deposited onto individual glass slides set up on a bench in an enclosed laboratory room only accessed by the researcher with constant environmental conditions (e.g. lighting, reduced air movement) where they remained undisturbed for the duration of the experiment.

Dryness tests utilising filter paper were only performed after an initial visual assessment of dryness determined the photographed sample dry. When the dryness test utilising filter paper

was applied to the corresponding drying test sample, visual assessment of transfer to the filter paper was used to determine if a sample was dry; if transfer to the filter paper occurred it was still wet, if transfer did not occur it was dry. Drying test samples were only tested once. If a sample was determined to be wet that sample would be discarded and the subsequent test would be performed on a different repeat.

CHAPTER 4: TRANSFER OF DNA WITHOUT CONTACT FROM USED CLOTHING, PILLOWCASES AND TOWELS BY SHAKING AGITATION

Chapter 4: *Transfer of DNA without contact from used clothing, pillowcases and towels by shaking agitation* is presented as its current state in the process of preparing it for submission, intended to be submitted to either *Science & Justice* or *Australian Journal of Forensic Sciences*, with minor formatting adjustments to unify the thesis' presentation and enable navigation of the thesis (e.g. addition of numbered headings and inclusion of chapter number in figure and table titles). Please refer to *Statement of Authorship* for contributions by parties other than the candidate to this chapter.

The contents of *Chapter 4*: *Transfer of DNA without contact from used clothing, pillowcases and towels by shaking agitation* have been removed due to copyright restrictions.

4.9 Appendix C. Supplementary data

For the purposes of this thesis, supplementary materials can be found in *Appendix C Chapter 4 Supplementary Data*.

CHAPTER 5: DISCUSSION

5.1 Analysis and significance of the results

5.1.1 Overall

Overall, the data this project generated demonstrated indirect DNA transfer without contact, a form of DNA transfer previously lacking targeted research, is possible, and dependent on circumstances. Thus, this form of transfer must be considered as a potential mode of deposition of the DNA sample of interest at the crime scene during forensic investigations alongside other forms of DNA transfer. Here, the key results of the project will be highlighted. Only their principal implications will be briefly discussed to avoid excessive repetition of the deeper discussions of the results previously presented in the individual chapters for each part of the project.

5.1.2 Part one: drying times and characteristics

Part one observed the drying times and characteristics of blood, saliva, semen and vaginal fluid. Of these biological materials, these properties have only been previously researched in blood despite the recognised potential for drying times and characteristics to impact forensically relevant phenomena including DNA transfer [1-3]. The substrate seemed to impact both drying properties investigated. This was especially noticeable for drying times, where substrate dictated drying order in both volumes tested: cotton deposits dried first followed by glass deposits, then those on polyester and melamine dried at a relatively similar rate. In contrast, drying characteristics seemed to be more dependent on the combination of biological material, substrate and volume. This tendency for drying characteristics to differ between substrates has been observed in blood deposits in prior studies [2, 3]. Therefore, a thorough understanding of the drying properties of a biological material in a variety of conditions is essential as observations of one biological material under one circumstance will not necessarily be able to be accurately extrapolated to other biological materials or even the same biological material under a different circumstance. These differences are likely due to different biological materials having noticeably different compositions, thus different properties [4-8], and these elements interreacting differently with the surfaces of different substrates resulting in the variations in the drying properties observed. Targeted investigation into these interactions may provide deeper insight into the drying properties of different biological materials beyond observations like those made in this project.

5.1.3 Part two: indirect DNA transfer without contact under laboratory conditions

Part two demonstrated indirect DNA transfer without contact to be possible under controlled laboratory conditions and dependent on the biological material, substrate and agitation combination. In some combinations, the possibility of transfer and/or the transfer rate could be

explained by the drying characteristics observed in part one (e.g. the detachment and flaking from the substrate caused higher transfer rates of blood deposited on melamine). This relationship between drying characteristics and transfer behaviour has also been noted in prior studies [1, 3]. Thus, the drying characteristics of a biological material under specific circumstances may provide insight into the possibility of this form of DNA transfer occurring and/or may further the understanding of the mechanisms of this form of transfer. Similarly to how different combinations of variables impacted the drying properties observed in part one, here it was found that the possibility of transfer and/or transfer rates observed differed between each combination of variables. Thus, how transfer behaves for any given combination of variables cannot be assumed to be reflective of transfer for other combinations of variables. The possibility and/or rate of indirect DNA transfer without contact being dependent on the specific combination of variables present during the event is therefore similar to the impact different variable combinations have on other forms of DNA transfer [1-3, 9-12].

5.1.4 Part three: drying properties and DNA content of saliva samples taken before, during and after chewing gum

Drying times and characteristics of saliva samples taken before, during and after chewing gum were observed and the DNA content of the samples determined in part three. Interestingly, samples collected during chewing after 2 minutes of chewing did not dry, however, all other samples did. Control (i.e. before chewing) samples dried first, followed by the post-chewing samples where an inverse relationship was observed for period of time after chewing ceased and drying time for the three samples (collected at 5 minutes, 25 minutes and 1 hour post-chewing). Then the later stage of chewing sample (collected during chewing after 20 minutes of chewing) dried last. It is proposed that gum-derived agents present in the saliva, initially in higher concentrations then being diluted and/or consumed as chewing progressed, caused this. Although drying characteristics were relatively similar to what was observed in part one, there were some differences. The closer a sample taken during chewing was to the beginning of chewing when collected, the more prominent a web-like texture was in the deposit. This was not observed in any of the control samples. It is proposed that the presence of gum-derived agents in the saliva, increased salivation rate due to stimulation [4, 13, 14] and/or sample collection time (e.g. a longer sample collection time may be a result of lower salivation rate and/or have different concentrations of gum-derived agents of component of saliva than a shorter collection time) caused this. Additionally, samples collected an hour after chewing ceased dried in a different pattern; uniformly instead of the outside-inside drying pattern observed in other saliva deposits and most other biological materials observed in part one. It is unknown what caused this deviation from previous observations. Overall, DNA content initially

decreased when chewing began before slowly increasing and eventually exceeding the control's DNA content in post-chewing samples. It is proposed that variations in salivation rate due to stimulation or lack of [4, 13, 14], thus diluting or concentrating the DNA present in the saliva, caused this. Thus, forensic investigators must be conscious of possible deviations in traits that may impact other forensically relevant phenomena and/or influence interpretations, including drying properties and/or DNA content of biological materials, caused by natural variation and/or variation caused by agents present.

5.1.5 Part four: Indirect DNA transfer without contact in more realistic conditions

Indirect DNA transfer without contact under more realistic conditions reflective of casework was investigated for part four. This part of the project demonstrated that DNA present on personal used fabric items can transfer with relatively minimal agitation to a secondary surface without contact. Substantial quantities of DNA were transferred and the resulting profiles from this transferred DNA were generally informative partial or full profiles. The known user of the item (i.e. the donor) was almost always the major contributor on both the primary and secondary surface. The number of donor alleles, average RFUs and DNA quantity of the primary surface profile and secondary surface profile were often similar. It would thus be difficult to readily distinguish the DNA that has been transferred indirectly during contact with an item, from DNA that has been transferred indirectly during agitation of an item without contact being made (e.g. distinguishing the profile generated from a sample collected from a floor or table after a used clothing item, pillowcase or towel directly contacting the floor or table, from a profile of a sample collected from these surfaces after such items were shaken above these surfaces but not contacting them). There are many possible activities and sources that can be responsible for creating background DNA on items and surfaces, the extent to which DNA collected from a surface can be said to have more likely come from direct contact, indirect contact, or indirectly without contact, will require further investigation. These findings coupled with those from part two demonstrated the possibility of indirect DNA transfer without contact under various conditions. Therefore, this form of transfer must be considered as a possible means for transfer to occur when possible transfer pathways are being assessed during forensic investigation. Additionally, the possibility of profiles resulting from indirect DNA transfer without contact may be of higher quality (i.e. high RFUs and/or full profile) than normally expected of profiles resulting from indirect transfer [15, 16] and appear similar to the profiles of primary deposits. Thus, caution must be taken when assessing the probability of a profile being from an indirect transfer event based off the quality of the profile.

5.2 Limitations of the project and recommendations for future studies

In addition to the limitations and recommendations for future studies raised in the individual chapters, there were some general limitations and areas that would benefit from further investigations relevant to the overall project. To expand the knowledge base of indirect DNA transfer without contact and associated variables (e.g. drying properties of biological materials) it would be desirable to address these issues in future studies.

The samples sizes used in this project were relatively small and/or were derived from samples donated from only a small number of participants. As a result of this, statistical analysis was only able to be performed for part four and many aspects of the project were only able to have apparent trends rather than statistically based trends considered when analysing the results and discussing their implications. It is also unknown if seemingly atypical results (e.g. person D's vaginal fluid sample that flaked off melamine in part two unlike other samples observed) are expected or an unusual occurrence not generally seen from samples donated from the general populace given this limitation. This negatively impacted the ability to draw conclusions and reduced the reliability of comparisons to the trends found in other studies. This project demonstrated the possibility of indirect DNA transfer under a variety of variable combinations. In further research, expansion of the sample size and/or pool of participants donating samples would be beneficial. When expanding the pool of participants donating samples it would be beneficial to utilise random sampling and/or include a range of ages, sexes, diseases states or lack of, biogeographical ancestry, etc to produce data representative of the population, as some of the listed variables influence the composition and/or characteristics of some biological materials [4-6, 13, 14, 17]. Accommodating for this in future studies would enable statistical analysis and determination of trends and probabilities of transfer when involving a specific set of variables.

This project's primary focus was investigating the possibility of indirect DNA transfer without contact and some variables relating to it. Two parts of this project, parts two and four, focused on this form of transfer in laboratory and more realistic conditions, respectively. It was demonstrated that transfer was possible for both the highly controlled laboratory setting, dependent on variables, and more realistic conditions. However, the experimental design for both parts were independent and not designed with consideration of how the results of each part may be compared. Thus, the results of the study were only able to be compared as binaries of the possibility of transfer rather than the comparison of transfer rates. Additionally, the agitations and items/substrates investigated were not particularly analogous of each other in different degrees of realism. In future, methodologies designed to better enable comparison between more controlled 'idealistic' laboratory conditions and less controlled more realistic

conditions should be considered to gain greater insight into how different variables impact transfer. For example, for comparison of transfer rates, introduction of a known volume of a biological material, thus approximate DNA content based on the material's general DNA content can be extrapolated by quantitating the DNA of an aliquot of the sample, to a DNA-free everyday item and an agitation applied to elicit indirect transfer from the item could be performed as opposed to utilising items with unknown DNA content. Alternatively, for comparison of agitations, for example a new agitation type of 'airflow', a controlled known air movement over the top of a sample in a laboratory could be compared to samples collected from various locations throughout a room or vehicle after deposition of a biological material near air conditioning units or open windows.

DNA transfer among other forensically relevant concepts (e.g. DNA recovery) is impacted by the drying times and characteristics of the involved biological material as observed in the results of this project and prior studies [1-3]. There are many variables that have been observed affecting these properties in blood including temperature [2, 3, 18, 19], humidity [18, 20], substrate [2, 21], some medications [22], as well as other agents in saliva as observed in part three. However, the influence of many of these variables have yet to be thoroughly investigated in forensically relevant biological materials their effects may be relevant to (e.g. the effects of temperature, something that impacts drying time of fluids, has not been investigated in saliva, semen and vaginal fluid). Further investigation into how different variables impact drying times and characteristics of biological materials would be valuable. In addition, it would be interesting to test for variations in DNA content caused by any of these variables as significant deviations in expected DNA content could impact interpretations in forensic investigations.

Additionally, several planned components that expanded on parts three and four were unable to be performed due to COVID-19 restrictions preventing sample donation and laboratory access to the researcher since March 2020. These plans were presented as recommendations for possible future studies in their respective chapters (e.g. investigating the humectant glycerol as the causing agent of the results observed for part three, investigating more item types and the possibility of transfer by different agitations (e.g. walking past or over a DNA-free sampling area) for part four).

5.3 Conclusions

This project aimed to generate data to improve the understanding of the possibility of indirect DNA transfer without contact in both controlled laboratory and more realistic conditions to address the current lack of targeted research on this form of DNA transfer. Additionally, other variables, primarily the drying times and characteristics of forensically relevant biological

materials, that have also been insufficiently researched despite the recognised impact they have on DNA transfer were also investigated. The results demonstrated indirect DNA transfer without contact was possible, dependent on the combination of variables involved, and can produce informative partial or full profiles in some of the circumstances tested. These findings demonstrated this form of DNA transfer should be considered as a potential variable factor in proposed transfer pathways by forensic investigators. While the drying times of the biological materials investigated were most influenced by substrate, varying drying characteristics were generally observed for different combinations of variables. These drying characteristics were able to explain transfer possibility and/or rate in some variable combinations. It was also found that chewing gum altered the drying properties and DNA content of saliva samples, dependent on when the sample was collected in relation to when chewing was started or stopped. Thus, how the drying properties and possible differentiation in the DNA content of the involved biological materials may impact possible transfer during these forensic investigations should also be considered. Due to this project primarily being proof of concept many aspects of it were limited in scope. For example, the small sample sizes precluded statistical analysis thus preventing the application of the data to activity level assessments. Therefore, it would be highly beneficial to the understanding of indirect DNA transfer without contact and influential variables to investigate these elements in further studies.

5.4 Chapter 5 References

[1] M. Goray, E. Eken, R.J. Mitchell, R.A. van Oorschot, Secondary DNA transfer of biological substances under varying test conditions, Forensic Sci. Int. Genet. 4(2) (2010) 62-67. DOI: 10.1016/j.fsigen.2009.05.001.

[2] R.A. van Oorschot, R. McArdle, W.H. Goodwin, K.N. Ballantyne, DNA transfer: the role of temperature and drying time, Legal Med. 16(3) (2014) 161-163. DOI: 10.1016/j.legalmed.2014.01.005.

[3] T.J. Verdon, R.J. Mitchell, R.A. van Oorschot, The influence of substrate on DNA transfer and extraction efficiency, Forensic Sci. Int. Genet. 7(1) (2013) 167-175. DOI: 10.1016/j.fsigen.2012.09.004.

[4] P.D.V. de Almeida, A. Gregio, M. Machado, A. De Lima, L.R. Azevedo, Saliva composition and functions: a comprehensive review, J. Contemp. Dent. Pract. 9(3) (2008) 72-80. DOI: 10.5005/jcdp-9-3-72.

[5] R. Eliasson, Biochemical analysis of human semen, J. Urol. 128(5) (1982) 1144-1144. DOI: 10.1111/j.1365-2605.

[6] G.R. Huggins, G. Preti, Vaginal odors and secretions, Clin. Obstet. Gynecol. 24(2) (1981) 355-377. DOI: 10.1097/00003081-198106000-00005.

[7] D.H. Owen, D.F. Katz, A vaginal fluid simulant, Contraception 59(2) (1999) 91-95. DOI: 10.1016/S0010-7824(99)00010-4.

[8] D.H. Owen, D.F. Katz, A Review of the Physical and Chemical Properties of Human Semen and the Formulation of a Semen Simulant, J. Androl. 26(4) (2005) 459-469. DOI: 10.2164/jandrol.04104.

[9] J. Burrill, B. Daniel, N. Frascione, A review of trace “Touch DNA” deposits: Variability factors and an exploration of cellular composition, Forensic Sci. Int. Genet. 39 (2019) 8-18. DOI: 10.1016/j.fsigen.2018.11.019.

[10] A. Gosch, C. Courts, On DNA transfer: the lack and difficulty of systematic research and how to do it better, Forensic Sci. Int. Genet. 40 (2019) 24-36. DOI: 10.1016/j.fsigen.2019.01.012.

[11] G. Meakin, A. Jamieson, DNA transfer: review and implications for casework, Forensic Sci. Int. Genet. 7(4) (2013) 434-443. DOI: 10.1016/j.fsigen.2013.03.013.

[12] R.A. van Oorschot, B. Szkuta, G.E. Meakin, B. Kokshoorn, M. Goray, DNA transfer in forensic science: a review, Forensic Sci. Int. Genet. 38 (2019) 140-166. DOI: 10.1016/j.fsigen.2018.10.014.

[13] S.P. Humphrey, R.T. Williamson, A review of saliva: normal composition, flow, and function, J. Prosthet. Dent. 85(2) (2001) 162-169. DOI: 10.1067/mpr.2001.113778.

[14] C. Yeh, D. Johnson, M. Dodds, Impact of aging on human salivary gland function: a community-based study, Aging Clin. Exp. Res. 10(5) (1998) 421-428. DOI: 10.1007/BF03339889.

[15] G.E. Meakin, E.V. Butcher, R.A. van Oorschot, R.M. Morgan, The deposition and persistence of indirectly-transferred DNA on regularly-used knives, Forensic Sci. Int. Genet. Sup. Ser. 5 (2015) e498-e500. DOI: 10.1016/j.fsigss.2015.09.

[16] G.E. Meakin, E.V. Butcher, R.A. van Oorschot, R.M. Morgan, Trace DNA evidence dynamics: an investigation into the deposition and persistence of directly- and indirectly-transferred DNA on regularly-used knives, Forensic Sci. Int. Genet. 29 (2017) 38-47. DOI: 10.1016/j.fsigen.2017.03.016.

[17] T.G. Cooper, E. Noonan, S. von Eckardstein, J. Auger, H.W.G. Baker, H.M. Behre, T.B. Haugen, T. Kruger, C. Wang, M.T. Mbizvo, K.M. Vogelsong, World Health Organization reference

values for human semen characteristics*‡, Human Reproduction Update 16(3) (2009) 231-245. DOI: 10.1093/humupd/dmp048.

[18] N. Laan, F. Smith, C. Nicloux, D. Brutin, Morphology of drying blood pools, Forensic Sci. Int. 267 (2016) 104-109. DOI: 10.1016/j.forsciint.2016.08.005.

[19] F. Ramsthaler, P. Schmidt, R. Bux, S. Potente, C. Kaiser, M. Kettner, Drying properties of bloodstains on common indoor surfaces, Int. J. Legal Med. 126(5) (2012) 739-746. DOI: 10.1007/s00414-012-0734-2.

[20] W.B. Zeid, D. Brutin, Influence of relative humidity on spreading, pattern formation and adhesion of a drying drop of whole blood, Colloids Surf. A Physicochem. Eng. Asp. 430 (2013) 1-7. DOI: 10.1016/j.colsurfa.2013.03.019.

[21] D. Thornbury, M. Goray, R.A.H. van Oorschot, Indirect DNA transfer without contact from dried biological materials on various surfaces, Forensic Sci. Int. Genet. 51 (2021) 102457. DOI: 10.1016/j.fsigen.2020.102457.

[22] F. Ramsthaler, A.-K. Kröll, M. Verhoff, C.G. Birngruber, M. Kettner, Effect of anticoagulation therapy on drying times in bloodstain pattern analysis, Int. J. Legal Med. 131(4) (2017) 955-961. DOI: 10.1007/s00414-017-1599-1.

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APPENDICES

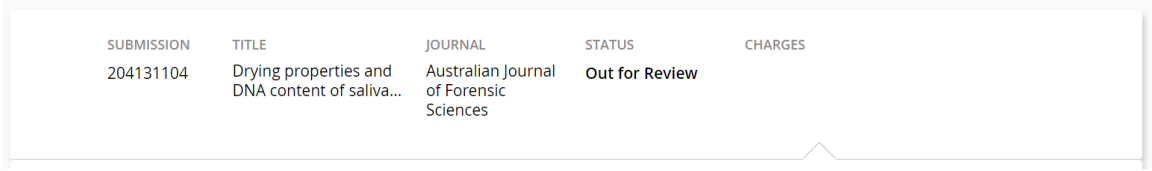
Appendix A.1 Manuscript citation for Forensic Science International: Genetics

D. Thornbury, M. Goray, R.A.H. van Oorschot, Indirect DNA transfer without contact from dried biological materials on various surfaces, Forensic Sci. Int. Genet. 51 (2021) 102457. DOI: 10.1016/j.fsigen.2020.102457.

Appendix A.2 Chapter 2 Supplementary Data

The contents of *Appendix A.2 Chapter 2 Supplementary Data* have been removed due to copyright restrictions. Please refer to the supplementary materials of the following for this appendix's content: D. Thornbury, M. Goray, R.A.H. van Oorschot, Indirect DNA transfer without contact from dried biological materials on various surfaces, Forensic Sci. Int. Genet. 51 (2021) 102457. DOI: 10.1016/j.fsigen.2020.102457.

Appendix B.1 Proof of submission to Australian Journal of Forensic Sciences



SUBMISSION	TITLE	JOURNAL	STATUS	CHARGES
204131104	Drying properties and DNA content of saliva...	Australian Journal of Forensic Sciences	Out for Review	

Appendix B.2 Chapter 3 Supplementary Data

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Appendix C Chapter 4 Supplementary Data

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